

Prediction of Aerosol Viability From Experiments on Surfaces (PAVES): Program Summary Report

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14. ABSTRACT This report describes the Prediction of Aerosol Viability from Experiments on Surfaces (PAVES) program after completion of its third year. PAVES includes both experimental and computational modeling tasks. Experimental determination of bacterial particle viability loss (biological decay) due to radiation exposure forms the main body of work. The research embodies two parallel investigations on particles comprising <i>Bacillus anthracis</i> spores (Sterne strain). One study exposes spores while suspended as aerosols and the other after deposition onto surfaces. Both use two commercially available ultraviolet (UV) radiation sources: a low-pressure mercury vapor discharge germicidal lamp and a solar simulator (SS) lamp. Survival fractions are determined from plating assays as a function of radiation exposure time (dose) and particle size. This report presents the final core experimental data, a comprehensive description of the program, and a comparative analysis between aerosol- and surface-exposed radiation effects on spore viability.					
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EXECUTIVE SUMMARY

This document provides a detailed report for the Prediction of Aerosol Viability from Experiments on Surfaces (PAVES) program after completion of its third year (01 February 2019 to 31 January 2020). Earlier progress reports periodically submitted to the sponsor provide the overall program organization, technical approaches, and task descriptions in a historical and progressive context. This report emphasizes refined procedures and presents the final core experimental data for *Bacillus anthracis* Sterne strain spores on filter surfaces and as aerosols. An attempt is made to provide a complete and comprehensive description of the program as well as an initial comparative analysis. Additional background information has been added for context. The PAVES program includes both experimental and computational modeling tasks, and each of these activities was distributed at multiple geographical locations. Consequently, the program required close coordination of the different team activities. This document attempts to combine the progress from each of the team groups on their respective tasks into a cohesive summary.

Experimental determination of bacterial particle viability loss (biological decay) due to radiation exposure forms the main body of work. The research embodies two parallel investigations on particles composed of *Bacillus anthracis* spores (Sterne strain). One study exposes spore particles while suspended as aerosols, and the other exposes similar particles following deposition onto surface substrates. Both investigations use two commercially available ultraviolet (UV) radiation sources for sample exposure: a low-pressure Hg vapor discharge germicidal lamp and a solar simulator (SS) lamp. In both studies, survival fractions are determined as a function of radiation exposure time (dose) for samples of particles collected from aerosols and resuspended in aqueous solutions. In order to ensure that the results of these two investigations could be directly compared, significant efforts were made to maintain major experimental and procedural elements in common.

Details of the experimental apparatus and procedures are described below. Measurement results and comparative analysis are presented and conclusions discussed. Background content from earlier reports appears in appendices as needed. In addition, data obtained from aerosol and surface exposures appears in Appendices D–G. The technical content is organized into seven report sections:

1. Introduction
2. Common Experimental Methods and Coordination
3. Surface Exposure Experimental Approach
4. Aerosol Exposure Experimental Approach
5. Survival Fraction Results and Decay Rate Analysis
6. Comparison of Surface and Aerosol Degradation and Discussion
7. Computational Model Development and Analysis of Optical Properties for Random Clusters of Primary Particles

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PREDICTION OF AEROSOL VIABILITY FROM EXPERIMENTS ON SURFACES (PAVES): PROGRAM SUMMARY REPORT

1. INTRODUCTION

This report summarizes technical progress on the Prediction of Bacterial Agent Aerosol Degradation Using Surface Deposition Data program primarily during the third performance year (01 February 2019 to 31 January 2020). Informally the program is referred to as Prediction of Aerosol Viability from Experiments on Surfaces (PAVES). The PAVES goal is to develop and validate an ability to predict biological decay of bacterial agent aerosols exposed to environmental stressors using viability data of similar particles exposed on a surface. Here, “similar” means particles produced from the exact same sample material, aerosolized using similar methods and at the same concentrations to achieve similar particle size distributions, and then exposed to optical radiation from the same types of sources under similar environmental conditions. The use of live agent samples to develop and evaluate experimental methods and procedures would have been prohibitively expensive. Consequently, a genetically close simulant at the BSL-2 level was chosen for method development. Experiments determined the loss of viability due to solar and narrow-band UV radiation exposure for particles consisting of *Bacillus anthracis* Sterne strain spores. Samples were irradiated while suspended in air or after deposition onto filters and other surfaces. This report summarizes all the simulant data obtained.

An outline of the experimental approach follows:

- Generate simulant spore aerosols in three notional sizes: (a) “single” spores, (b) small clusters, and (c) large clusters, comprising 1–5, 10–17, and 44–60 spores per particle, respectively.
- Expose samples of spore particles to solar radiation either as suspended aerosols or as deposits of similarly sized particles on surfaces (e.g., polycarbonate filters).
- Expose similar spore samples to a standard UV radiation lamp (Hg atomic line at 254 nm) both as suspended aerosol and as particles on surfaces as a procedural verification of the experimental apparatus.
- Determine biological decay rates of each spore cluster size as a function of radiation dose for both light sources and for both suspended aerosol and surface deposition exposures.
- Develop and evaluate a detailed, physics computational model for radiation exposure. The model should evaluate the distribution of incident light at different wavelengths within cluster particles with a specified number of spores either freely suspended or in contact with a specified surface material.
- Analyze data from aerosol and surface exposures to evaluate correlations between the two exposure scenarios for future predictive capability.

As originally proposed, experimental determination of bacterial particle viability loss (biological decay) due to radiation exposure forms the main body of work. The research embodies two parallel investigations on particles comprising *Bacillus anthracis* spores (Sterne strain). One study exposes spore particles while suspended as aerosols, and the other exposes similar particles following deposition onto surface substrates. Both investigations use two commercially available ultraviolet (UV) radiation sources for sample exposure: a low-pressure Hg vapor discharge lamp, and a solar simulator (SS) lamp. Survival fractions are determined for collected samples of particles in both studies as a function of radiation exposure time (dose). In order to ensure that the results of these two investigations could be directly compared, the team expended significant efforts to hold major experimental and procedural elements in common:

1. Spore sample stock material — provided by CCDC-CBC Edgewood, Maryland (Dr. Rastogi).
2. Sample recovery and handling procedures and culture protocols for determining survival fractions.
3. Radiation sources with respect to spectral distribution and overall intensity.
4. Particle generation method — microdroplet generation from liquid sample with solvent evaporation.
5. Particle characterization — narrow distribution targets, controlled to produce two specific “sizes,” as reflected by mean spore cluster number, with known identical composition.
6. Controlled or constant environmental conditions (temperature, relative humidity).

Section 2 describes these features in detail. The remaining sections summarize progress on all aspects of the program. Experimental apparatus and methods for both surface and aerosol exposures are presented in Sections 3 and 4, respectively. Section 5 captures graphically the results of those experiments. Comparative analyses between the aerosol and surface spore particle data from 254 nm and simulated solar (SS) exposures appear in Section 6. The latest developments in the computational radiation exposure model are covered in Section 7. The report concludes with final observations and recommendations in Section 8.

2. COMMON EXPERIMENTAL METHODS AND COORDINATION

2.1 Sample Selection

A principal program objective was to evaluate two forms of bacterial organisms: an endospore and a vegetative cell species. We proposed to use genetically closely related, but low-pathogenic, organisms as agent simulants in order to validate the experimental methods and procedures, and subsequently to verify simulant results using the associated live agent analog. This approach has a major advantage of significant reduction in the overall cost and schedule compared to conducting the entire program with agent samples. Of course, this approach minimized overall safety risks as well.

For the spore sample, the only choice for agent was *Bacillus anthracis*, since no other bacterial endospore-forming organism is on the CDC Bioterrorism agent category A or B lists. The Ames strain was chosen out of convenience. For its near-neighbor simulant, the vaccine strain, *B. anthracis* Sterne, was selected. Spore formation (*sporulation*) is relatively rare among bacterial genera. The vast majority of bacterial species do not possess this capability. Greater detail can be obtained easily elsewhere,¹ but briefly, spore formation is an evolutionary adaptation for preservation and propagation in which the live cell is triggered by external cues to shut down active metabolism and to encase its DNA and other essential biomolecules in a dense, layered matrix. The resulting metabolically inactive (dormant) cell form possesses several qualities that make it especially attractive for bioaerosol investigations. *Bacillus* spores in general have a characteristically rigid, oblate spheroidal shape and size. *B. anthracis* endospores, in particular, are highly resilient, surviving extremes of temperature and low-nutrient environments over decades or even centuries, as well as surviving many chemical sterilants. Favorable environmental conditions (e.g., host presence) may trigger spores to revert to the active cell form (germination) that can lead to host infection. Partly because of these features, the history of *B. anthracis* spores as a potential biological warfare (BW) agent is well-documented.²

Since selection of the first sample type was effectively predetermined, this program began with an experimental effort based on spore samples, while simultaneously evaluating selection of the vegetative cell species as a first-year task. From the CDC bioterrorism lists, there are two bacterial vegetative cell candidates in category A and five in category B. These seven species were evaluated in terms of (a) current relevance, (b) level of difficulty in preparation, (c) prior experience in aerosolization, and (d) identification of and familiarity with a genetically close simulant species. Based on these criteria, agent candidates were narrowed down to *Francisella tularensis* and *Burkholderia pseudomallei*. As part of this evaluation process, a workshop was held near the end of the first year in which various research scientists with backgrounds in microorganism aerosolization were invited to attend and participate. Unlike *F. tularensis*, *Burkholderia* species showed^{3,4,5} significant longevity in pure water samples. For reasons explained in more detail in Appendix A, this feature became a key deciding factor for the choice of the vegetative cell pathogen.

Following this meeting, *B. pseudomallei* and *B. thailandensis* were selected as the vegetative cell agent-simulant pair. While we were aware of prior experience in successful aerosolization of the agent material *B. pseudomallei*,⁶ we could not find prior reports on aerosolization of the simulant *B. thailandensis*. Unfortunately, initial tests with *B. thailandensis* showed approximately a two-log reduction in sample viability simply by spotting the suspension on filters and allowing the sample to air dry. Appendix A.2 summarizes this evaluation. Additionally, preliminary attempts to aerosolize the simulant sample indicated that an additional two logs of viability loss occurred promptly as the aerosol droplets dried. A viability loss of roughly four orders of magnitude, resulting from simply converting the sample suspension to an aerosol, would be a significant barrier to the determination of environmental degradation of aerosol samples because of the reduction of experimental dynamic range. For example, a measurement of a reduction of two orders of magnitude in sample viability would require about 10^3 viable particles. But if, at time zero, only one in 10^4 particles is viable, then the starting particle population would need be at least about 10^7 . However, such large

populations of aerosols were beyond our experimental capability. Three possible avenues for mitigation of this situation presented themselves: (1) evaluate alternative droplet generation methods, (2) evaluate additives that would protect the vegetative cells as aerosol particles, or (3) select a different sample organism that was more amenable to aerosolization. Unfortunately, at the time of this evaluation, any of these options would involve an extension to the program. Ultimately, the sponsor decided to reduce the program scope and to keep the original schedule since the vegetative cell evaluation was not a threshold requirement. Consequently, all the radiation damage data obtained in this effort is from *B. anthracis* spore samples.

2.2 Spore Samples: Preparation, Storage, and Culture Protocols

During Year 1, two batches of *B. anthracis* Sterne strain simulant stocks were prepared and delivered to the surface and aerosol experimental groups. Appendix B describes the typical protocol. The two batches had been washed and titered at 1.25×10^8 and 7.5×10^8 cfu/ml, respectively. It was established that they could be stored at 4 °C with little or no degradation over extended periods ranging up to months. The stocks contain a small amount of surfactant (0.01% (v/v) polysorbate 80, also known as Tween 80) to prevent spore aggregation and surface adhesion during storage. Stock solutions are periodically titered to identify any degradation in storage.

Although quality control evaluations included microscopic imaging on each stock, no direct dimensional analysis was performed for these samples. Characteristic *Bacillus* spore sizes have been previously published.⁷ Table 1 compares the dimensions of *B. anthracis* Sterne strain spores to those of a common simulant, *B. atrophaeus* (“BG”).

Table 1—Dimensions⁷ of Two Species of *Bacillus* Spores

Spore	Mean L (μm)	Mean W (μm)	Mean V ^a (μm ³)	Aspect Ratio
BG ^b	1.22	0.65	0.273	1.85
Sterne	1.49	0.85	0.569	1.75

^a. BG stands for *Bacillus atrophaeus* subsp. *globigii*, formerly known as *B. subtilis* var. *niger* and *B. globigii*.

^b. V is computed as the volume of an ellipsoid with length (L) and width (W) from $V_{\text{spore}} = (\pi/6)LW^2$.

Using dimensions from Table 1, we see that 1 ml of the higher-concentration *B. anthracis* Sterne stock has a total volume of (viable) spores of $[(7.5 \times 10^8 \text{ spores/cm}^3) \times (0.569 \times 10^{-12} \text{ cm}^3)] = 4.3 \times 10^{-4} \text{ cm}^3$. Thus, the volume fraction of the target organism in the highest-concentration stock is approximately 0.04% (v/v).

Both experimental groups generate aerosols by droplet generation of spore sample suspensions at agreed upon concentrations in order to achieve similar particle sizes at both sites. The sample concentration and initial microdroplet size (volume) determine the resultant particle size distribution. (See Section 2.5 for details.) By convention, all sample suspensions for particle generation maintain a Tween 80 concentration of 0.001% (v/v). For reasons discussed below, the Tween 80 concentration was reduced tenfold compared to stocks in order to limit residual Tween 80 content in particles but still to prevent spore aggregation and surface adhesion.

2.3 Sample Extraction, Recovery, and Survival Fraction Determination

The basic sample extraction and recovery procedure for both surface and aerosol experimental efforts has been discussed in previous reports. In its original conception, entrained aerosol particles would be drawn through polycarbonate membrane filters to create a sample, which is referred to as *sample collection*. The

process by which the biological particles were extracted from the membranes for analysis is referred to as *sample recovery*. Sample recovery was achieved by placing a sample filter into a centrifuge tube containing a standard recovery fluid of phosphate buffered saline (PBS) with 0.01% (CBC) or 0.1% (NRL) (v/v) Tween 80 (polysorbate 80), and then vortexing to extract particles from the filter into the recovery fluid. For the surface experiments, 47-mm-diameter filters were placed into 50-ml tubes with 20 ml of recovery fluid and were vortexed for 10 min. For the aerosol experiments, initially, 13-mm filters were placed into 1.5-ml tubes with 1 ml of fluid and were vortexed; however, subsequent adjustments to this procedure became necessary during this report period, as described in Section 4.3.

Analysis of the recovered samples was achieved by plating aliquots of the sample fluid onto tryptic soy agar (TSA) plates, incubating the plates overnight in a humidified oven at 37 °C (14–20 hours), and counting colonies. Plate counts from 25 to 300 colonies were accepted by both groups. CBC accepted plate counts as low as 1 colony if no other plated dilutions displayed colony-forming units (CFU) in that range. NRL recorded plate counts below 25 colonies but excluded them from calculations of biological decay rates. “Damaged” colonies that appeared significantly smaller than normal at the initial count were excluded from the recovery assay for the purposes of comparing surface and aerosol results. (See Section 4.3.1 for further detail on this aspect of colony-count procedure.)

2.4 Radiation Exposure Sources

Both surface and aerosol exposure studies used two radiation sources that emitted wavelengths short enough to result in damage to the biomolecular structure of the sample organisms (spores). Care was taken to ensure that the mechanism of damage was photochemically induced reactions rather than thermal damage. The main objective of this study was to assess decay that could occur under operational conditions, meaning radiation from natural sunlight. For controlled laboratory experiments, both the surface and aerosol simulant investigations purchased the same commercial solar simulator (SS). In order to establish a point of reference that enables comparison of our results to previous studies on biological radiation damage on other organisms, a standard germicidal lamp was also used. This low-pressure mercury vapor lamp provides radiation predominantly at a single Hg atomic emission line at 253.7-nm wavelength (i.e., 254 nm).

While the SS data was of primary interest for application purposes, analysis of that data may be complicated because the damage to exposed sample spores is caused by a continuous range of radiation over the solar UV wavelengths. The complication results from the wavelength-dependent mechanisms of radiation damage. Photochemical reactions in which the fundamental unit of radiation, a photon, is absorbed by a material and induces a molecular chemical change in that material will depend on (a) the rate at which photons are incident on the material (flux), (b) the material absorption coefficient (probability of photon absorption) of the material, and (c) the probability that the energy of the absorbed photon induces a chemical reaction (reaction rate) rather than just excess thermal energy deposition in the material. For the SS spectrum, (a), the photon flux, is wavelength-dependent. In general, factors (b) and (c) are both material-specific and wavelength-dependent. Consequently, the net probability of causing damage in spore samples under SS irradiation is a convolution integral of the product of all three wavelength-dependent factors over the relevant spectral range. While some data from independent measurements of the absorption coefficient of spores at specific wavelengths exist in published literature, their spectral absorption profile is not quantitatively well-established (results vary with each study). Also, an independent quantitative measure of the wavelength-dependent probability of reaction (usually referred to as an “action” spectrum) has not been published. Nevertheless, a lower bound to the photon energy necessary to induce genetic damage in biological organisms (due to the activation energy necessary to break a DNA bond) can be estimated. Photon energies lower than this minimum will not directly result in any chemical reaction no matter how intense the photon flux is (disregarding any temperature increase in the material). In our case, a photon wavelength of 400 nm ($E \approx 3.1$ eV) is a conservative upper bound to the effective spectral range. For a lower wavelength bound, solar intensities decrease steeply (by more than 10^{10}) as wavelengths approach 280 nm ($E \approx 4.4$ eV).

2.4.1 UV Germicidal Lamp

Earlier PAVES reports have described the models of commercially available germicidal lamps⁸ used for both the surface deposition and aerosol investigations. While the lamps were not identical, their spectral output is the same (253.7 nm). During this report period, the UV radiometers used by each group were taken to the National Institute for Standards and Technology (NIST) in Gaithersburg, Maryland, for an informal calibration so that a correction factor for each group could be employed to ensure that measurements from the two groups have the same fluence scale and that the fluence values were reasonably accurate on an absolute basis. These correction factors have been applied to the 254-nm data shown in Section 5.

2.4.2 Solar Simulator

At the beginning of the PAVES program, two Oriel solar simulators⁹ were selected and purchased, one for the surface study and one for the aerosol study. These units are Class ABB solar simulators certified to IEC 60904-9, edition 2 (2007), JIS 8904-9 (2017), and ASTM E927-10 (2015) standards, using a 1,000 W xenon arc lamp with proprietary filters to reliably meet performance parameters of nominally a 1 sun output power over a beam focal area of 5 x 5 cm (2 x 2 in.). Generally, two aspects of solar simulator (SS) radiation should be addressed to achieve reliability and reproducibility for reporting the results of this study: total output light intensity (W/m^2 , or equivalent) and its spectral distribution. Throughout the 3-year PAVES program, different pieces and aspects of our SS radiation sources have been included in interim reports and presentations, but at this point, it is appropriate to provide a summary overview of this key component of the PAVES program.

The original sponsor solicitation for the PAVES program required relevance to operational environmental conditions by stipulating that bioaerosol degradation be determined as a result of solar radiation. At that time, an earlier, but still-ongoing, program (DEGRADE program sponsored through DTRA ReachBack) had a strong topical overlap with the PAVES program, and it was decided that the PAVES program should adopt some of the DEGRADE approaches and standards to ensure easy comparison. One of those “inherited” aspects was a decision to follow the ASTM G177 standard for ultraviolet solar radiation, which is specified over the spectral range of 280 to 400 nm. Consequently, “bare bulb” output of the PAVES SS light sources was measured at the beginning of the program, and custom spectral filters were specified, ordered, and installed to shape the spectral distribution from our SS lamp to conform as closely as possible to the G177 distribution and intensity. Additionally, an effort was made to confirm absence of even trace levels of radiation at shorter wavelengths. (See Appendix C for an excerpt from the “4th Quarter Summary (1st Year) Technical Progress Report” submitted February 28, 2018, to DTRA-JSTO-CBD.) Since the genesis of these standards and their adoption were essentially passed on from an earlier program, their significance has not been previously discussed within the context of the PAVES program. In order to provide background and context for what the PAVES SS output represents, a detailed synopsis of the solar radiation spectral standards follows.

Solar irradiance at any given location and moment on the Earth’s surface is highly variable, depending on time of day, season, and weather. Consequently, establishing a standard for solar irradiation starts just above the Earth’s atmosphere. The sun’s radiant energy output is relatively constant in time and primarily consists of thermal blackbody radiation. Figure 1a¹⁰ shows measurements of the sun’s radiant energy spectrum on a log-log plot to capture the range in both wavelength and intensity. Figure 1b¹¹ presents a series of computed blackbody radiation spectra for selected temperatures, and one can see that the emitted continuum of thermal radiation increases rapidly in power (intensity), and shifts toward shorter wavelengths as the emitter temperature increases. By fitting the measured solar spectrum (for wavelengths longer than 150 nm) to the blackbody formula, the sun’s surface temperature is determined to be 5,778 K.

(This value is frequently rounded to 5,800 K in many general texts and lay references.) At wavelengths shorter than 150 nm, Fig. 1a shows relatively small amounts of vacuum ultraviolet and soft X-rays that arise from a combination of atomic hydrogen and high-temperature plasma interactions in the sun's atmosphere (corona). Consequently, they are not part of its surface thermal radiation.

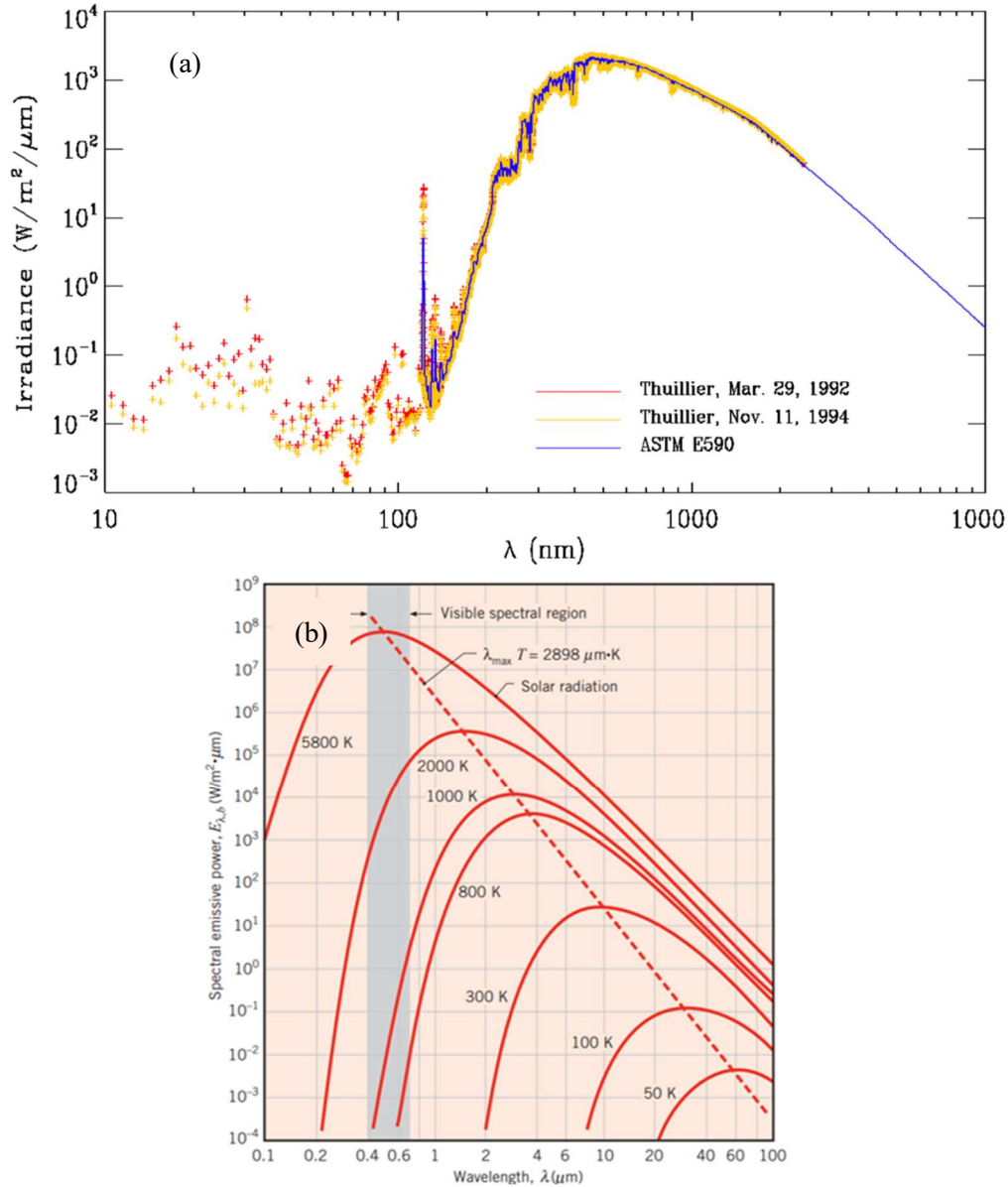


Fig. 1—(a) Absolute solar radiation spectrum at a distance of the Earth's orbit for which thermal blackbody radiation emission curve fit (blue)¹⁰ yields a surface temperature of 5,778 K. (b) Ideal blackbody emission spectra¹¹ for a series of temperatures from 50 to 5,800 K overlaid with the visible spectral region (shaded).

The Earth's orbit around the sun is very nearly circular, with an eccentricity of about 0.0167, equivalent to its orbital major axis, about 3% longer than its minor axis. Therefore, the radiant energy reaching the Earth from the sun is very uniform throughout the Earth's orbit, with an accepted value of $1.361 \text{ kW}/\text{m}^2$, referred to as the solar constant. At the top of the Earth's atmosphere, the solar radiation in both intensity and spectral distribution is reasonably invariant. However, because of the approximate 23.5° tilt of the Earth's rotational

axis with respect to the orbital plane, there is seasonal variation of the sun's radiation reaching the Earth's surface throughout its complete orbit. Consequently, standards for solar irradiance at the Earth's surface are most easily defined in terms of an annual average. As radiation at the top of the Earth's atmosphere propagates to its surface, it undergoes attenuation due to scattering and absorption. Both of these effects have strong dependencies on radiation wavelength.

Figure 2 shows computational results of annual total solar radiation intensity on a Mercator projection map of the Earth¹². The upper plot shows solar irradiation, or insolation, at the top of the atmosphere, while the lower plot shows the reduction in total intensity at the Earth's surface after passing through the atmosphere. The radiation intensity for both plots is color-coded as shown by the scale bar between them. In the upper plot, dependence of average radiant flux on latitude is evident due to the curved surface of the planet. Only the equatorial region has the sun directly overhead, or with an average zenith angle (z , or latitude) of zero. At all other latitudes, the parallel rays of the sun's radiation strike at an angle with respect to the local zenith, so that the effective power per unit area is reduced by $\cos(z)$. Over an annual average, the upper and lower hemispheres appear symmetric, with each pole receiving the minimum radiant energy. In the lower plot, total radiant energy arriving at the surface is further reduced by roughly a factor of two due to mostly atmospheric absorption by water vapor (in the infrared) and oxygen (in the UV), but also to some extent to scattering effects. In the model results of Fig. 2, surface reflectivity (albedo) and absorption are also included so that increased insolation over northern Africa and the equatorial Pacific Ocean are discernable.

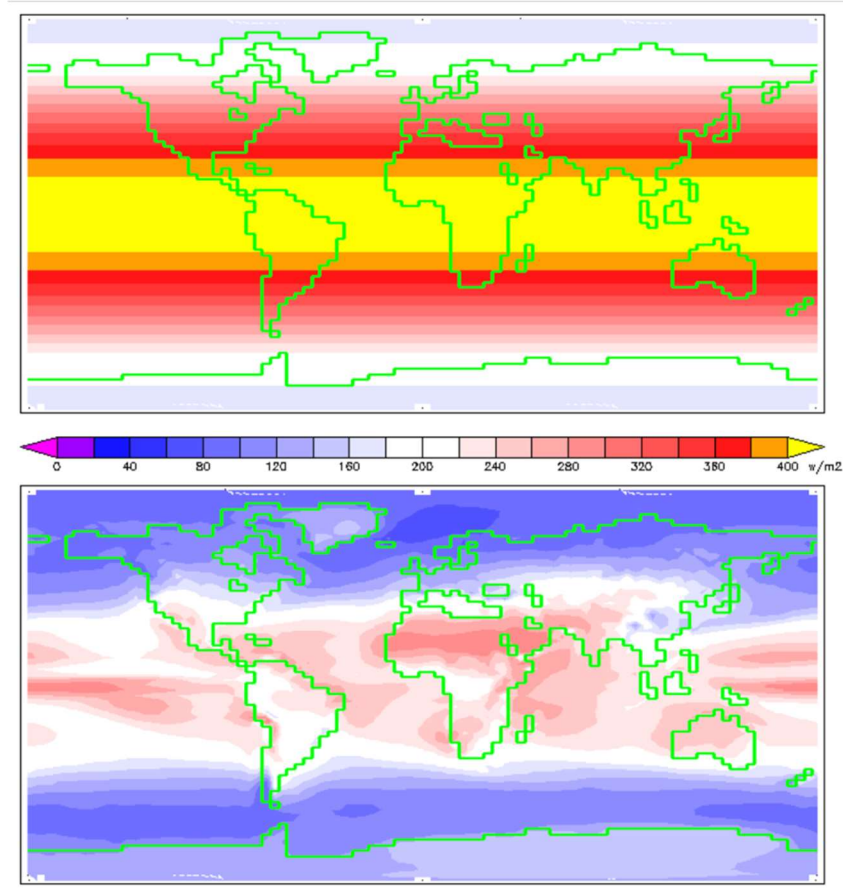


Fig. 2—Annual average insolation in W/m^2 modeled¹² at the top of the atmosphere (top) and the surface (bottom)

In terms of spectral distribution, the American Society for Testing and Materials (ASTM) has established standards for annual solar radiation averaged over the continental United States. The standard has been periodically updated, with the latest revision in 2008. The active version,¹³ known as G173-03, includes three spectra: incident radiation at the top of the atmosphere, total radiation at sea level averaged over the continental US, and direct radiation (including coronal disk). Since the average latitude of the continental US is 37° , the averaged total insolation is referred to as “global tilt 37.” Radiation that propagates from the sun (sun’s image) directly to the Earth’s surface is referred to as “direct circumsolar” and is also corrected for a 37° latitude, but does not include contributions from all parts of the sky. The global tilt 37 model is intended to include all radiation incident on a flat area in the tangent plane at any US location integrated over a $2\text{-}\pi$ solid angle, so that it includes not only the direct solar radiation, but also radiation that is scattered by the atmosphere onto that area. These spectra are based on a computational model known as SMARTS2 (version 2.9.2, Simple Model for Atmospheric Transmission of Sunshine of Gueymard).¹⁴ The surface spectra are also sometimes referred to as “air mass 1.5,” which refers to the thickness of the atmosphere that is input to the SMARTS2 calculation (air mass 0.0 refers to the incident radiation spectrum at the top of the atmosphere).

Figure 3 shows the three G173 spectral standards with absolute intensity ($\text{W}/\text{m}^2\text{-nm}$) on the vertical axis versus wavelength in nanometers (nm) on the horizontal axis. The upper spectrum (black) is the extraterrestrial (ETR) incident solar profile, the middle spectrum (blue) is the global tilt 37 profile, and the lowest spectrum (red) is the direct circumsolar profile. One can readily see large chunks of the incident radiation are removed in the two surface spectra by atmospheric absorption for wavelengths between about 800 and 3,000 nm (by mostly water vapor and carbon dioxide), as well as UV radiation from about 250 to 280 nm (mostly by oxygen in the upper atmosphere). Integration of the area under the global tilt 37 spectrum results in a mean total incident fluence of $1,000 \text{ W}/\text{m}^2$, also known as 1.0 “sun” in some literature.

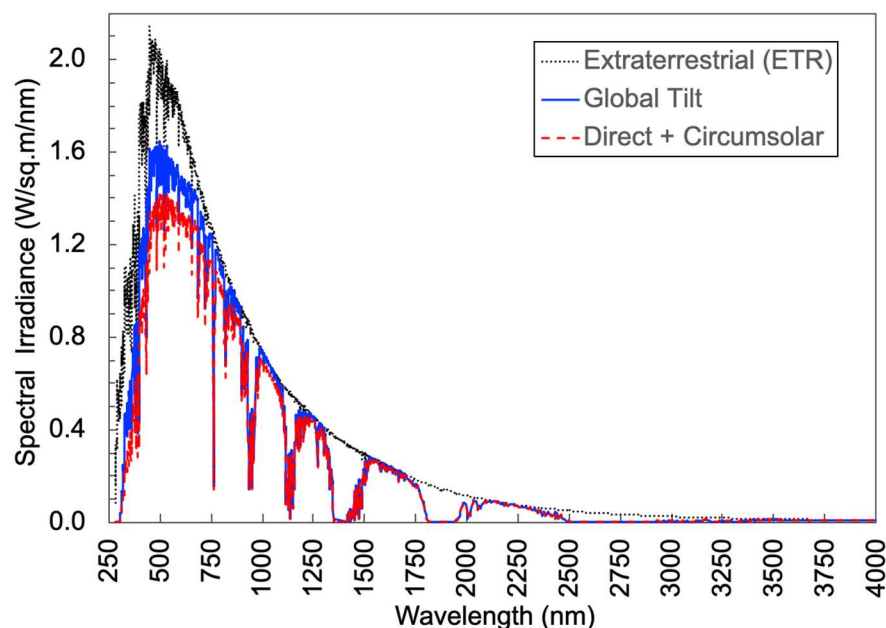


Fig. 3—The ASTM G173-03 solar radiation standards¹³ for annual average solar spectral distributions over the continental US. “ETR” represents incident solar radiation at the top of the Earth’s atmosphere and the remaining two spectra show radiation profiles at the Earth’s surface. The plot was re-created from data retrieved from reference ¹³.

However, the G173 standard is inadequate for applications that are sensitive to UV radiation, such as material degradation or direct conversion of sunlight to electricity. Consequently, the ASTM G177 standard was established (2015) to compensate for the mean continental US elevation above sea level. A shorter path length through the atmosphere reduces absorption at all wavelengths, but it makes a critical difference in the UV region. The G177 standard is sometimes referred to as air mass (AM) 1.05 for this reason. Since the objective of this standard is the UV spectral region, it was defined only from 280 to 400 nm. Figure 4 compares the G177 and G173 spectral standards in absolute intensity over this band. The integrated total radiant flux under these two standards is 64.2 and 46.4 W/m², respectively (roughly 6.4% and 4.6% of the G173 global tilt 37 total). The higher spectral intensity of the reduced air mass model extends to all wavelengths, and it is not clear how much effect this would have for the rest of the spectrum at longer wavelengths. In our opinion, the G177 standard should be extended to the same spectral range as the G173, permitting a comparison of the total solar radiant energy between them. Currently, it is unclear how close the spectral intensities of G177 and G173 will approach at longer wavelengths.

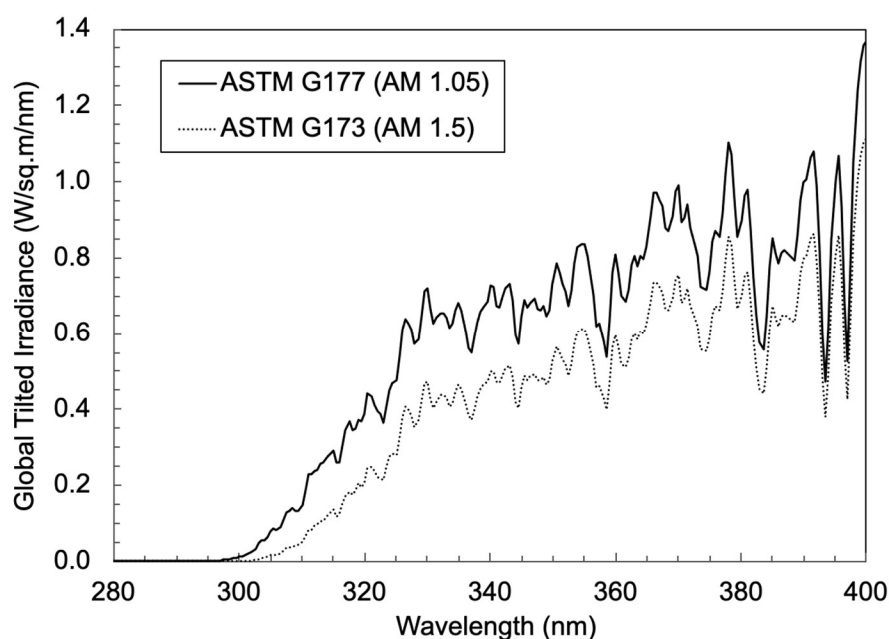


Fig. 4—Global irradiance on a 37° tilt: comparison of ASTM absolute reference spectra in the UV from G173 and G177 solar radiation standards

The solar simulator lamps used for the PAVES investigation (described at the beginning of this section), result principally from thermal blackbody radiation of a high-pressure Xe plasma arc, typically at an apparent temperature of about 6,200 K. Consequently, output from these lamps can be a good approximation to solar radiation except that, even for high-pressure lamps, prominent emission lines are superimposed on the thermal emission continuum. The bare-bulb, or unfiltered, absolute output spectrum from a 1,000-W Xe arc solar simulator¹⁵ appears in Fig. 5. This spectrum is from the same lamp manufacturer (Oriel) with nearly the same specifications as the one used in the PAVES program, albeit an earlier model. Consequently, the spectrum is representative of the PAVES SS, but may not be exactly the same. In Fig. 5, the absolute spectral intensity is on a linear vertical scale and the wavelength axis runs from 300 nm to 2,500 nm. One can easily see the underlying blackbody radiation continuum has strong, pressure-broadened Xe emission lines superimposed throughout, but especially between 800 and 1,100 nm.

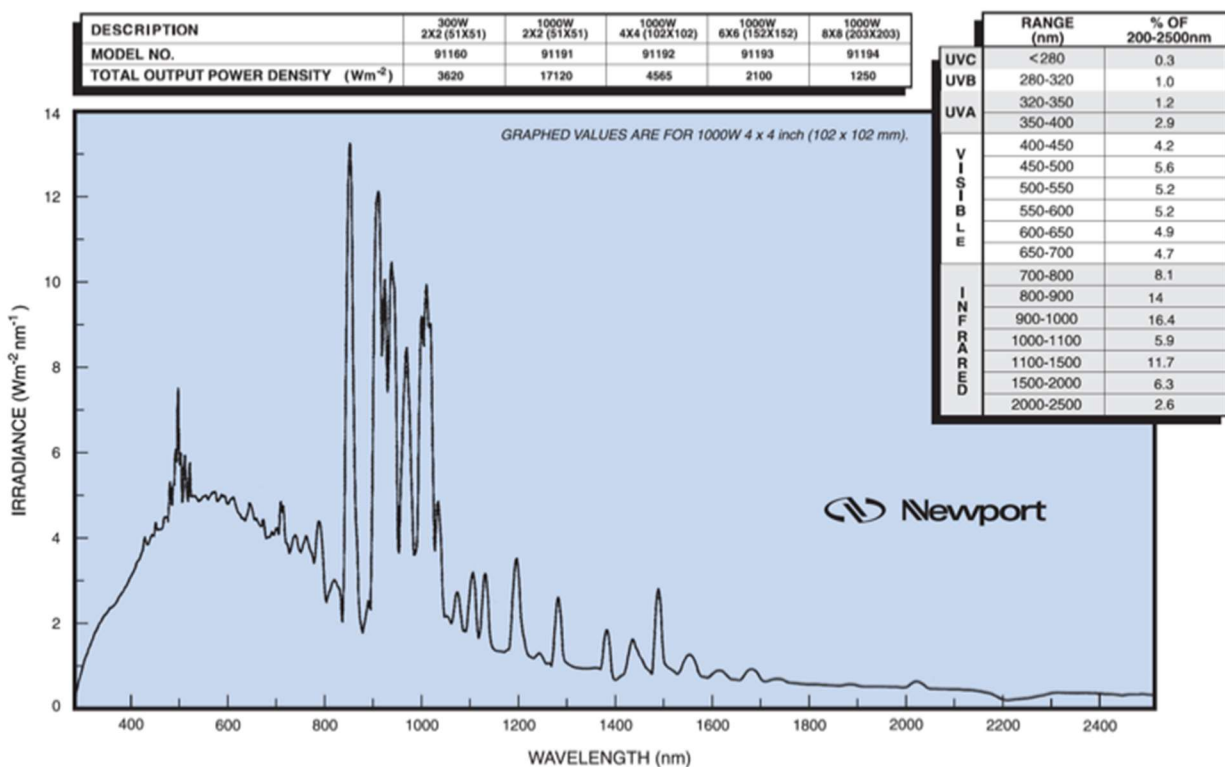


Fig. 5—The unfiltered output spectrum of an Oriel Model 91192, 1,000-W Xe arc SS lamp¹⁵

As part of our radiation calibration process, and to document experimental control, SS spectral intensities were recorded for each experiment of the CBC surface exposure study using an Ocean Optics (OO) model USB2000 fiber-coupled spectrometer with factory-calibrated intensity output. Figure 6 (top) shows the full mean spectrum (blue trace) calculated from 12 spectra measured on randomly selected dates between February 2018 and May 2019. The solid, blue trace shows the mean spectrum, and the mean plus and minus the sample standard deviation appears as red, dashed plots above and below. One can readily appreciate that the standard deviation spectra are barely distinguishable from the mean spectrum. A slight decrease in output over the 15 months could be discerned as a trend, and this accounts for much of the standard deviation shown in Fig. 6 (top). However, this decrease was judged not sufficiently significant to warrant time-dependent correction factors for lamp output. An additional indication of the PAVES SS reproducibility derives from computation of the integrated total energy flux for each of the same 12 spectra over two wavelength ranges. The 15-month average integrated irradiance was (651 ± 13) W/m² and (58.34 ± 1.8) W/m² over the 280-to-928-nm and 280-to-400-nm spectral windows, respectively. These values correspond to very low coefficients of variation of 2.1% over the wider spectral range and 3.1% over our region of interest for photon damage to spores.

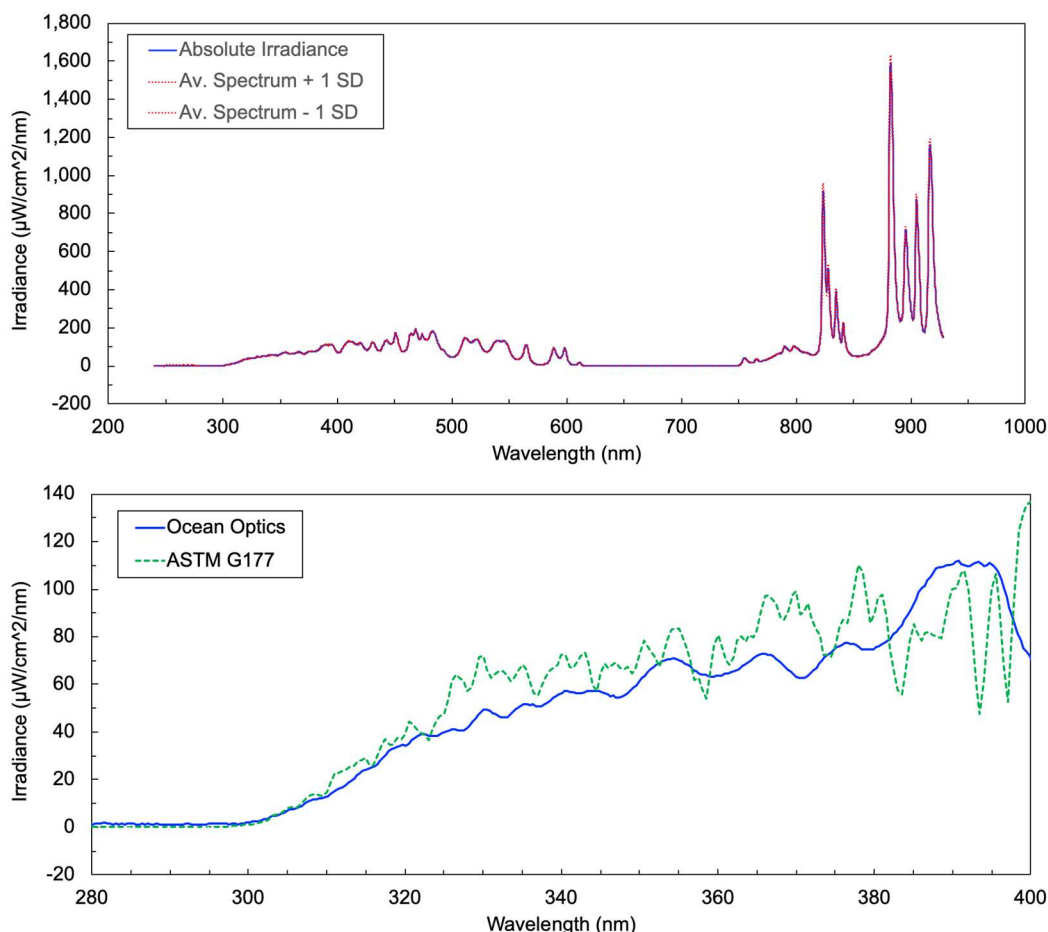


Fig. 6—Top: filtered absolute spectral output of the 1,000W Xe arc SS lamp used in the PAVES program. The spectrum is dominated by near infrared Xe atomic emission lines (800–950 nm). Bottom: expanded view from 280 to 400 nm of the mean SS output in absolute intensity (blue, solid line) compared with the G177 standard spectrum (green, dashed line). The integrated intensities of these two spectra are: 64.2 W/m^2 (G177) and 58.34 W/m^2 (SS output).

Figure 6 (top) also shows that our SS lamp output spectrum differs significantly from the representative unfiltered SS spectrum in Fig. 5. This dissimilarity arises primarily from the use of custom filters to emphasize the UV region of the output spectrum. An expanded view of our mean SS output in Fig. 6 (bottom) shows the relevant spectral range of 280 to 400 nm (solid, blue curve) compared to the G177 standard (dashed, green curve). One can see that generally, the custom filters appear to provide a close similarity of the SS output to the G177 spectrum. However, on a linear scale, this appearance is superficial. In the region between 300 and 280 nm, the G177 spectral intensity decreases by 10 orders of magnitude. In order to capture that large dynamic range, a logarithmic scale is required (*vide infra*). This topic will be explored further in the next section.

For the purpose of computing survival fraction data as a function of solar simulator fluence, we adopted a convention to use the integrated radiant energy from 280 to 400 nm as measured by a calibrated Ocean Optics (OO) spectrometer. To obtain the exposure dose (fluence) of the spore samples for this SS output radiation, the integrated total flux value of 58.34 W/m^2 is simply multiplied by the exposure time ($\text{W}/\text{m}^2 \times \text{s} = \text{J}/\text{m}^2$). Total SS output was also measured with a calibrated photovoltaic silicon radiometer (Oriel [Newport] model 91150V) with a quartz window. The spectral response of this device is broad, from about 290 to 1150 nm, and its output therefore corresponds to the entire spectrum as shown in the top graph in Fig. 6. A value

of 1.43 Sun (1430 W/m²) was determined for the surface studies SS unit. This same radiometer was brought to the aerosol facility, where a mean value of 1.28 Sun (1280 W/m²) was determined for that system. In each case, the photometer was placed at the location of the sample measurement for SS illumination. The slightly lower value for the aerosol study is due primarily to the fact that the aerosol chamber had to be physically located slightly farther away from the SS lamp to accommodate the chamber enclosure. The ratio of these values (1.28/1.43 = 0.895) provides a normalization factor for the aerosol study fluence dose, so the scale factor for the aerosol data becomes 58.34 x 0.895 = 52.21 W/m².

These UV fluence calibration factors (58.34 for the surface study and 52.21 for the aerosol study) permit placement of the two sets of measurements of survival fraction data on the same scale of absolute solar fluence for direct comparison of their respective decay rates.

2.4.3 Comparison of PAVES Solar Simulator Output to ASTM G177

Addressing the issue of how well our SS spectral output matches the G177 standard requires some sort of evaluation of how effective the different wavelengths of the radiation continuum are with respect to causing damage to the spores. Fortunately, a recent article has compiled data that permits an approximate approach for assessing relative efficacy of radiation damage as a function of wavelength.¹⁶ Figure 7 is a reproduction of Fig. 1 from that paper showing prior data of first-order decay rates (k, in m²/J) from multiple published studies of *Bacillus subtilis* spores due to exposure of narrowband radiation at specific wavelengths. The reproduced image has been modified by overlaying a continuous response curve (red, dotted line) comprising three connected line segments to outline the general shape, or trend, of the plotted experimental data. One can readily see that decay rates increase rapidly from 400 to roughly 270 nm, but then appear to level off and remain fairly constant from 270 to 200 nm. The composite red, dashed line represents a visually estimated average dependence of decay rate (E) as a function of radiation wavelength (λ). Each segment is algebraically represented by a linear equation:

$$\log(E) = m \lambda + b \quad (1)$$

where λ is wavelength (nm). Values for m and b are provided in Table 2 along with the parameters of wavelength ranges and end-point values. While we appreciate that an absolute representation of radiation damage could be subject to significant error, our intent is to crudely capture the very strong dependence of the onset of radiation damage capability starting near 400 nm. The data in Fig. 7 imply the damage efficiency per photon increases by approximately a factor of 4x10⁵ as wavelengths decrease from 400 nm toward 250 nm. For this purpose, we judge the absolute values and fine details of the red, dashed curve shown in Fig. 7 are not critical. The use of a graphical visual approximation for the number of line segments and their endpoint values should be adequate to illustrate the general effect.

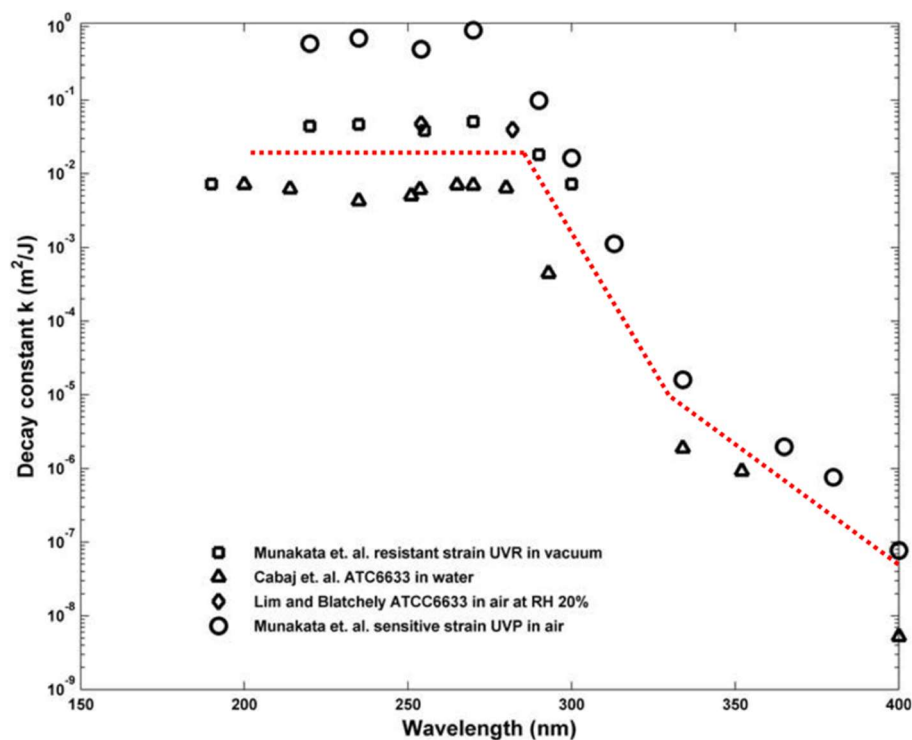


Fig. 7—A plot¹⁶ summarizing published values of first-order decay rate constants for *Bacillus* spores measured at specific wavelengths. A crude estimate of the efficacy for spore damage that has been constructed as the red, dashed line in this image can be inferred from this data.

Table 2—Relative Damage Effectiveness Function $[E(\lambda)]$ Values Derived from Fig. 7

Wavelength Range (nm)	Endpoint Value (k)	Relative Value	Segment Slope (m)	Y-Intercept (b)
200–285	-0.02	400,000	0.0	-0.02
285–330	1.0×10^{-5}	200	-0.0746	19.625
330–400	5.0×10^{-8}	1	-0.0329	5.752

The physical mechanism responsible for this behavior is the onset of sufficiently energetic photons to drive photochemical degradation of the organisms' DNA. In general, the rate of photochemical damage is proportional to the product of three basic factors: the flux of photons, the absorption coefficient (probability), and the reaction rate (probability) for altering the absorbing molecule. The constructed efficacy curve ($E(\lambda)$) in Fig. 7 is an empirical measure of the product of the joint probabilities of photon absorption and photochemical reaction. This is an inherently quantum mechanical process that “turns on” as the photon energy becomes comparable to the chemical reaction activation energy for any available damage reaction pathways. Therefore, in order to apply this as an empirical efficacy, or weighting function, to the incident spectral radiation intensities on the spore samples, the first-order decay constants must also be converted to units of numbers of photons rather than total radiant energy by equating the incident radiation intensity to the photon flux times the energy per photon. This conversion results in an additional scaling factor proportional to wavelength, or $E(\lambda) \cdot \lambda$.

Capturing the important features of the G177 standard solar spectrum, the SS output spectrum, the efficacy weighting factor, and the resultant weighted standard and output spectra requires a dynamic range of 10^{18} or more using a logarithmic vertical scale, as shown in Fig. 8. The G177 (UV enhanced) solar spectral standard (shown as a red, dashed line) decreases rapidly starting around 295 to 300 nm and drops by a factor of $\approx 10^{12}$ from there to 280 nm. The black, dotted curve “SS Output (direct)” is the OO spectrometer-measured SS output. These two spectra can be contrasted with their representations on a linear scale shown in Fig. 6 (bottom). The somewhat constant intensity of the OO SS averaged spectra at a value of about $0.01 \text{ W/m}^2\text{-nm}$ between 280 and 297 nm is clearly not due to actual radiation at those wavelengths. (For reference, this value continues on at almost the same level to 250 nm.) Rather, this feature represents an effective background caused by either the limited dynamic range of the OO focal plane array detector system, or low levels of scattered light reaching the detector inside the single-pass monochromator, or some combination of both. During the first year of the PAVES program, an effort to accurately measure the SS output in this spectral region was made and the result is plotted as the blue, dashed curve labeled “SS Output (indirect).” Compared to the G177 standard, the SS output achieves reasonable agreement over the region from about 290 to 300 nm, but it shows substantially higher intensities from 280 to 290 nm. At the top of the graph, the gray, dashed curve depicts the complete weighting factor $E(\lambda) \cdot \lambda$. It shows its most rapid rate of change over 285 to 330 nm, beyond which it declines more slowly with increasing wavelength. The solid curves show the results of convolving this weighting factor with the G177 (red) and our SS output (blue) spectra. It's evident that the competition between their respective decreasing intensities and the increasing damage sensitivity (represented by the weighting factor) results in maximum values in both spectral responses near a wavelength of 310 nm.

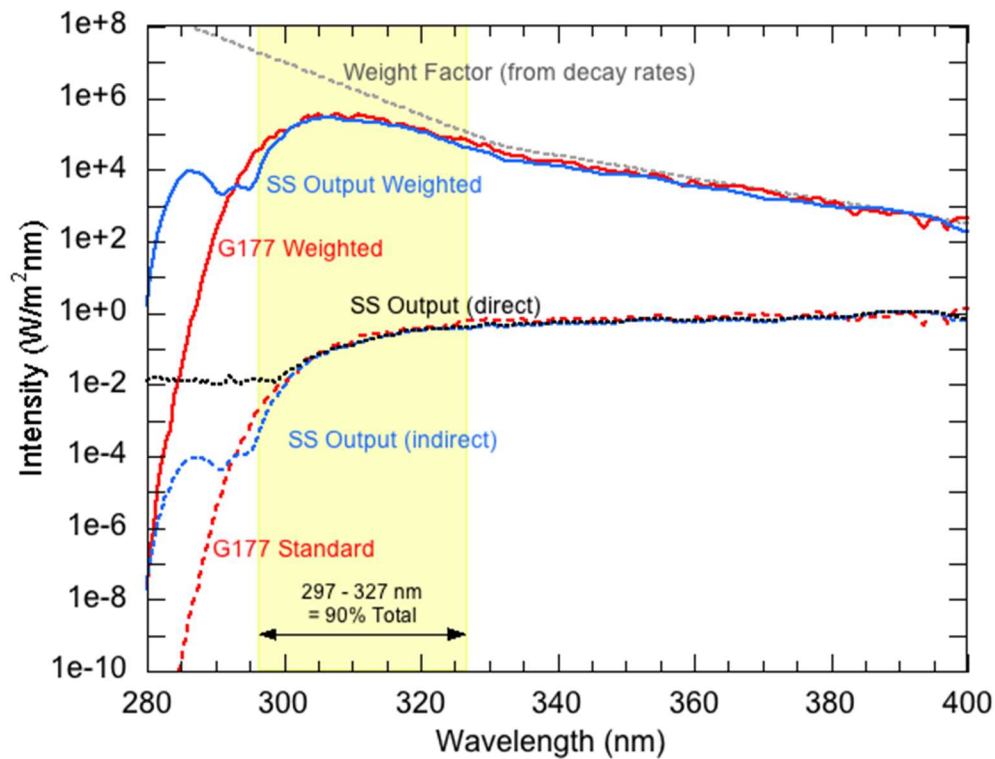


Fig. 8—This plot summarizes the spectral intensities of the PAVES SS output measured by the OO direct method (black dotted) and a more accurate indirect measure (blue dashed) are compared to the G177 standard (red dashed). The total spectral efficacy (see Fig. 7) is shown at the top (gray dashed). The resultant convolution of the SS output and G177 weighted by the efficacy are shown as the blue and red solid curves, respectively.

To appreciate these features better, these two weighted spectra are shown on a linear scale in Fig. 9. When normalized on a linear scale, one can see clearly the formation of a localized peak for both the G177 and SS output response spectra. About 90% of the integrated solar inactivation of our spore samples occurs between 297 and 327 nm as indicated by the yellow shaded regions in Fig. 8 and Fig. 9. When seen through this process of photochemical deactivation efficacy, the PAVES SS spectral output is essentially identical to the G177 solar standard. Thus, concern over slight discrepancies between the two occurring at wavelengths shorter than 295 nm or longer than 330 nm can be allayed. As a final point, a weighted version of the G173 spectrum is also shown in Fig. 9. One can readily contrast the difference between the G173 and G177 standards as having a spectrally integrated difference of approximately 3x in terms of spore deactivation effectiveness. This significant distinction between the two standards is not readily apparent in a simple comparison of spectral intensity as shown in Fig. 4.

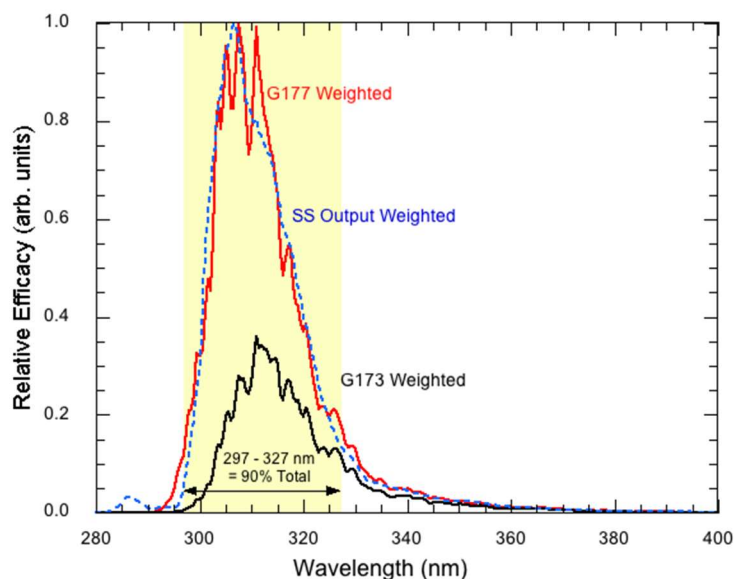


Fig. 9—The SS output and G177 weighted by the efficacy are shown on a linear scale as the blue dashed and red solid curves, respectively (from Fig. 8). Nearly all (90%) of the integrated spectral contribution for both of these curves occurs between 297 and 327 nm.

2.5 Spore Aerosol Generation and Characterization

2.5.1 Droplet Aerosol Generation

An overarching feature of this program was that aerosol and surface-exposure experiments were carried out at different geographic locations (NRL and CBC). Descriptions of experimental details of the surface and aerosol exposures appear in Sections 3 and 4, respectively. In order to be able to compare results from these two separate studies, it was necessary for them to each prepare the spore samples with procedures as closely matched as possible. Moreover, measurement of organism survival places special constraints on the approach and methods used to carry out these experiments. As an example, the process of aerosolization itself can be potentially a significant source of microbial sample degradation. Depending on the extent of this damage, subsequent determination of environmental effects on aerosol sample mortality becomes proportionately more difficult. Another consideration is that, for a given particle composition, organism survival as an aerosol under ambient conditions is likely dependent on particle size. Consequently, particle samples should be prepared with narrow size distributions to permit determination of size-dependent cofactors. Generation of uniform,

monodisperse aerosol samples with control over the resulting particle size, particle composition, and particle production rate would be ideal. However, in practice, technological limitations, fundamental constraints, and inherent statistical variability force trade-offs between performance and experimental control. Consequently, choices for approach and method for particle generation were carefully considered with regard to experimental objectives as well as practical constraints. Some details of specific aerosol generation methods for this effort have been previously published.¹⁷ Although a comprehensive review of bioaerosol generation is beyond the scope of this report, a brief overview is given below to provide a context for the rationale for the specific systems that were ultimately used.

At the most fundamental level, the type of aerosol dispersion method for microbial organisms is dictated by whether the sample material is a liquid suspension or a dry powder. Sample materials containing biological organisms are commonly and most easily prepared as liquid suspensions that can be then aerosolized as spray droplets. Conversion of microbial liquid suspension samples to powder form typically imposes a mortality penalty on the target organism^{18, 19} and introduces additional complexity and sources for experimental error. Additionally, liquid droplet generation offers a wider range of options with typically greater control over the dispersed droplets. For these reasons, we chose to prepare samples as liquid suspensions (as described in Section 2.2) and to disseminate microdroplets as the means for aerosolization.

In scientific literature, one can find a large number of references that cover a very wide range of commercially available devices or descriptions for device fabrication that achieve small droplet dissemination. Most of these methods will produce droplets with a broad range of diameters, but some may associate a limited range of droplet sizes with specific operating conditions. Devices, such as pneumatic sprayers, that disperse a broad continuum of initial droplet diameters frequently can be well-characterized as log-normal²⁰ distributions. When considering microorganisms as sample material, a key concern is potential damage to target microorganisms caused by mechanical or other stresses^{21, 22} inherent in the droplet generation process itself. Additionally, there are a number of methods (and some commercial devices) designed to provide very narrow, even monodisperse (geometric mean standard deviation less than 1.25 μm) initial droplets. This feature usually permits a degree of control over the nominal droplet size.^{23, 24, 25} Regardless of droplet generation method, the initial droplet dissemination is followed by a transient period in the aerosol flow where the solvent (typically water) evaporates from the droplet and a stable residue particle is formed. For droplets less than 100 μm , evaporation time in normal room air is rapid, for example, less than 5 s for a 60 μm diameter droplet at 50% relative humidity (RH) and 20 °C.²⁶ However, evaporation times are sensitive also to the rate of spray generation²⁷ due to the local increase in RH. So, rapid evaporation times may only be achieved by mixing the spray with large volumes of clean (particle-free) dry air, a process that contravenes the use of a pneumatic spray for obtaining high concentrations. For relatively small total mass ejection rates into an air or gas flow, this process can work sufficiently well to produce equilibrated residue particle aerosols.

The overall transformation from liquid suspension to droplet aerosol and then to residual aerosol particles is depicted in Fig. 10. For the purposes of a general concept, the microbial sample suspension includes predominantly target microorganisms (red dots) and potentially other suspended solids (green squares) such as cellular debris or other undissolved solids from spent culture media. In addition, dissolved nonvolatile or low-volatility compounds persist as liquid residue (yellow fill) in the final particle after solvent equilibrium evaporation occurs.

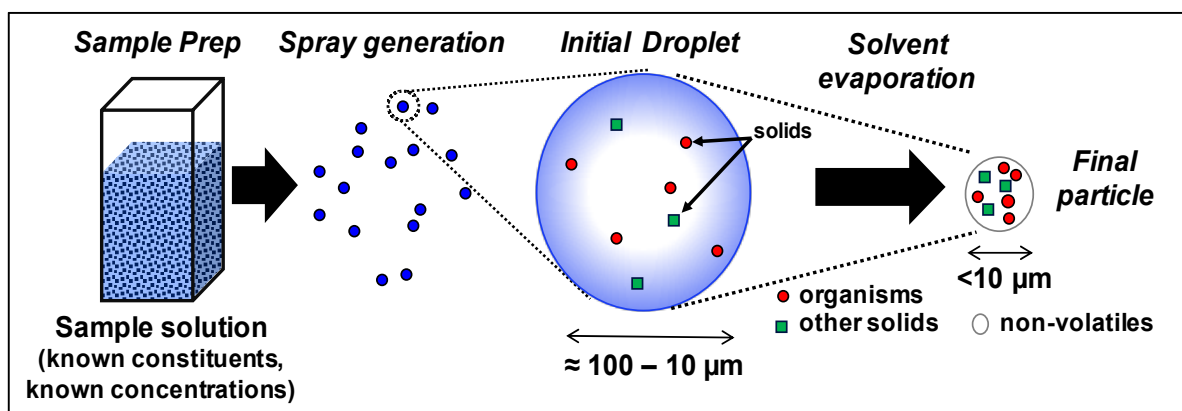


Fig. 10—The process of bioaerosol generation from a liquid sample microdroplet dispersion is depicted schematically from left to right. Droplets formed from a homogeneous suspension result in individual particle compositions that reflect the relative proportions of the original sample.

Many microorganisms do not survive well in pure water due to osmotic pressure across the cell membranes and require additives to remain viable. As a specific example, phosphate buffered saline (PBS) solution is a non-nutritive diluent that is widely used in microbiological research. In principle, an initial sample suspension might consist of only target organisms in PBS, but in practice, there are nearly always small quantities of other solids (such as cell fragments from the sample growth process). Additionally, after droplets are first formed and the solvent (typically water) starts to evaporate, the concentration of dissolved constituents (e.g., salts) increases. At equilibrium with the environment, such additives may deliquesce (precipitate) to form new solids inside the droplet. The green squares in Fig. 10, then, represent either of these potential sources of particulate materials (other than the target organisms) within the droplet as it shrinks due to evaporation. If there are any soluble compounds that are not volatile, they, too, will be concentrated and remain behind in the residue particle after equilibrium is achieved. This example brings out an additional point, that sample additives initially beneficial to the microorganism and necessary to maintain its viability in suspension can become deleterious (toxic) to the organism during a rapid change in concentration as the initial droplet evaporates. Two key aspects of aerosol generation by droplet evaporation illustrated in this example are: first, the resulting residue particle size and its composition can be predicted using simple mass balance if the initial sample composition and the initial droplet size are known. Second, the viability of the target organism must be considered in terms of the residue particle equilibrium composition. Consequently, control over both the initial sample composition and the initial droplet size provides control of the resulting residue particle size and composition.

Two top candidates for droplet-on-demand generation devices included a piezoelectric (PZT) capillary and an inkjet printer head, since they both had an ability to provide highly repeatable and reproducible primary droplets of uniform size, and also due to prior familiarity. Both systems use an electrical pulse to trigger generation of an individual droplet. In the case of the PZT, the mechanism is a small mechanical displacement of the sample fluid inside a quartz capillary caused by the deformation of the attached piezoelectric quartz crystal due to an applied voltage pulse. For the inkjet printer head adaptation, known as the Ink Jet Aerosol Generator (IJAG), the mechanism of droplet expulsion is due to rapid heating in a sample microchamber due to current flowing through a resistor element embedded in the top wall of the microchamber. The flash heating vaporizes liquid sample adjacent to the wall element, and this bubble expansion forces a sample droplet to be ejected through an orifice on the opposite side. Between the two devices, the PZT system is potentially less violent. Additionally, although the inkjet droplet-generation method was known to work satisfactorily for spore samples,²⁸ we anticipated that vegetative cell samples could be much more sensitive to rapid and high-temperature changes than spore samples. Even as a fast, transient event, thermal flash heating of sample liquid

could have unknown consequences for viable biological samples generally. Consequently, we initially chose the PZT droplet generator system^{29, 30} to use for both the aerosol and surface investigations. However, during the execution of the program, this initial plan was forced to be modified as described below.

The PZT droplet generator was available with optional capillary diameters, and we chose one of the larger sizes at 70 μm inside diameter, hoping to avoid potential complications of clogging. In its most stable, desirable mode of operation, the PZT system produced droplets nominally the same diameter as the capillary. As a concrete numerical example, assume an initial droplet diameter is 70 μm yielding a spherical drop volume of $[(\pi/6) \times (70 \mu\text{m})^3 \times (10^{-12} \text{cm}^3/\mu\text{m}^3)]$, or approximately $1.8 \times 10^{-7} \text{cm}^3$. As described in Section 2.2, spore stock suspensions were prepared and distributed by CCDC-CBC. The stocks were diluted at each location as needed to achieve residue particles of three nominal sizes. At the highest concentration, the expected mean number of viable spores per droplet would be about

$$7.5 \times 10^8 \text{ spores/cm}^3 \times 1.8 \times 10^{-7} \text{ cm}^3 \approx 135 \text{ spores/droplet.} \quad (2)$$

At a lower concentration of $1.4 \times 10^7 \text{ spores/cm}^3$, the average number of spores per droplet would be about 22.

As droplets move through the system, their solvent evaporates away. Ultimately, the residue particle consists of the same number of spores, and consequently the same spore mass, as initially contained in the wet droplet. Thus, the mass of spores per particle is proportional to the spore concentration and the initial droplet volume of the sample suspension. Furthermore, using the Tween 80 concentration of the stock solution of 0.01% (v/v) and assuming that the surfactant was uniformly distributed throughout the sample volume, the mass of pure surfactant in a 70- μm droplet would be equivalent to that of a sphere of approximately 3.25 μm in diameter. This level of mass balance between the target organism and the surfactant would not be satisfactory for generating residue particles equivalent to single spores, since the equivalent volume of the residual Tween 80 would be about 30-fold larger than the volume of a single spore. However, the surfactant volume concentration could be significantly lower than this simple estimate due to its inherent preference to accumulate at interfaces. Consequently, a study was conducted to determine how much the Tween 80 concentration could be reduced while still maintaining its effectiveness to prevent spore aggregation in the sample suspension and spores sticking to container walls. As a result, it was determined that a tenfold reduction in Tween 80 concentration to 0.001% would not alter its surfactant capability significantly. (Appendix D summarizes this study.) Consequently, all sample solutions for particle generation maintained a Tween 80 concentration of 0.001% (v/v) for both the surface deposition and the aerosol investigations. At this concentration, the equivalent volume of Tween 80 residue from a nominally 70 μm droplet is reduced to approximately $1.8 \mu\text{m}^3$. Although this quantity is still greater than the volume of a single spore, further reductions in the surfactant concentration would begin to compromise its function. Note that the true per-particle volume of the surfactant could be lower than this estimate (vide supra). Although the true value is difficult to estimate, the reduction is sufficient for the spore mass to be dominant for most of the spore cluster residues. Clearly, as the spore concentration increases to make larger residue particles, the mass fraction of Tween 80 becomes much less significant.

Initially, both CCDC-CBC and NRL planned to generate bacterial aerosol particles from the same batch of spore stock using essentially the same PZT device (same nozzle tip from the manufacturer) for the surface and aerosol studies. Three nominal particle sizes would then be achieved by standardizing three dilutions of stock and maintaining fixed initial PZT droplet volumes. For a 65- μm droplet diameter, these low, middle, and high concentrations of *B. anthracis* suspensions were calculated to provide particles of approximately 1, 2.5, and 4 μm diameter, respectively. However, after the PZT droplet generator systems were set up in both locations, a problem was encountered in its application for the surface exposure study. In that effort, the number of organisms deposited on the filter surface was experimentally determined by the number of droplets generated multiplied by the average number of organisms (spores) per droplet. The number of droplets was

determined by precisely timing the flow of the droplets generated onto the filter substrate. The PZT droplet generator was run in a continuous-pulse mode at a fixed frequency, nominally 2 kHz. Consequently, the number of organisms deposited onto the filter was the product of the average number of organisms per drop, the PZT droplet generation frequency, and the total time of deposition. In order to obtain a measurement with reasonable accuracy, a minimum number of organisms was required to be deposited onto the filter surface, typically on the order of 5×10^5 to 5×10^6 . In cases where the targeted residue spore cluster size was small (a goal of one spore/microdroplet corresponding to a 1- μm -diameter “cluster”) the total number of droplets is so large that the operation times of the PZT could increase to 10–30 minutes for loading samples onto a filter substrate because each data point required replicate filters (three for measurements and three for controls). Unfortunately, they were unable to reliably operate the PZT system for such long periods.

For the aerosol exposure experiments at NRL, relatively small quantities of initial droplets (on the order of 10^3 to 10^4) were necessary to fill the aerosol trapping and confinement system. Thus, the relatively low output rate of the PZT droplet-on-demand generator was adequate, with operation times of a few seconds per fill. However, the surface study required higher rates of droplet generation, which was too time-consuming and difficult to execute using PZT system, and alternative methods of microdroplet generation were employed. Ultimately, the CBC team used a combination of two systems: an IJAG and a CAD system based on an ultrasonic vibrating reed (modified Sono-Tek™) droplet generator (see Section 3.1 for detail). Potential differences in bacterial survival that arise from using different droplet-generation methods were nevertheless mitigated to some extent by referencing the viability assays of exposed samples to controls produced by the same method. Furthermore, all of these methods were based on the same fundamental mechanism of microdroplet generation of known sample suspensions followed by subsequent evaporation to provide the spore cluster aerosol as described by this section.

2.5.2 Cluster Size Distribution

Cluster “size” in terms of number of spores per particle is an important parameter to characterize in this project. Ideally, the configuration of the spores within each cluster would be desirable in order to analyze experimental data and to accurately model the UV inactivation of the spores inside clusters. However, there was no experimental means by which individual particle sizes and shapes could be directly measured. For the CBC surface-deposited samples in which aerosolized clusters were collected onto polycarbonate filters, population distributions of the dried clusters were routinely monitored using an aerodynamic particle sizer (APS). This instrument measures the aerodynamic particle diameter (D_{ae}) of individual particles as they flow through the device. Aerodynamic diameter is defined as the diameter of a sphere (of a specified density) that has the equivalent terminal velocity of the measured particle. CBC did not provide primary data for these measurements to include here. However, for the sake of consistency in day-to-day or run-to-run operations, they standardized their cluster-generation process to always obtain a size distribution mode of either 2.4 or 4.0 μm when preparing filter substrate samples from mid- and high-concentration suspensions, respectively.

Starting with a sample suspension of a known concentration of essentially identical spores, spore cluster generation was achieved via microdroplet generation and permitting the water to evaporate away, leaving a suspended spore cluster as a residue. Microdroplets are generated by extruding the suspension fluid through a nozzle of a known diameter. Due to surface-tension forces, the column of extruded suspension fluid subsequently breaks into droplets of a uniform diameter at a rate determined by adjusting the volume flow of the fluid through the nozzle and the driving voltage frequency of the PZT attached to the nozzle. The underlying assumption is that the spores are independent of each other, and consequently, their number concentration in any small hypothetical volume in the suspension is described as a stochastic variable with a uniform probability distribution. Since the total spore volume fraction is very small compared to the total fluid volume, individual spore motion can be described as diffusive. Moreover, we assume that the process of fluid extrusion and droplet formation do not influence local spore concentration. Therefore, we describe the number of spores in any given droplet as equivalent to a hypothetical subvolume in the fluid in which spores are free

to diffuse into and out of the subvolume as a function of time, or equivalently, subdividing the entire fluid volume into an ensemble of a large number of subvolumes at any specific instant of time. Under these conditions, the number of particles in a subvolume (droplet) would be described by a Poisson distribution in which the probability P of an event is defined by two parameters, the mean number of events per interval, λ , and the specific number of events, k , as:

$$P_{(k \text{ events in interval})} = e^{-\lambda} \frac{\lambda^k}{k!} \quad (3)$$

In generation of “single organism” aerosols using a droplet generator, the suspension concentration will be lowered to achieve an expected value of 1 organism per droplet (e.g., at a spore concentration $\approx 3.7 \times 10^6/\text{ml}$). However, the Poisson distribution shows that the occurrence of doublets, triplets, and higher-multiple spore clusters would be a significant fraction ($>50\%$) of the total number of particles generated. In order to reduce the occurrence of such multiple-spore particles, the average concentration would need to be further reduced. Unfortunately, a consequence of lower-spore concentrations is that an increased fraction of droplets would contain no organisms. In the case where organisms were suspended in only pure water, this would not create any new problems: the water-only droplets would evaporate completely and only spore-containing residue particles would remain. However, since a small mass fraction of surfactant is also present in the suspension, then evaporation of droplets that don't contain any organisms will still create small particles of surfactant residue. Hence, the correlation between total particle count and bacteria-containing particle count would be lost and interpretation of plating results with respect to exposure effects for both the aerosol and surface experiments would become much less useful.

The cluster residues produced by droplet drying will have a variability that is governed by the same statistical mechanics that describe Brownian motion of small particles in liquid suspension even when the size of the droplet is fixed. The actual number of spores or vegetative cells per droplet can be modeled as a Poisson distribution since the probability of locating a spore is spatially uniform in the sample fluid. *In order to generate three cluster sizes (“singles,” mid-sized, and large), CCDC-CBC and NRL agreed on three target solution concentrations derived from the “as received” stocks of the B. anthracis Sterne spores. Dilution of the highest-concentration stock by 50-, 10-, and 2.5-fold would produce the smallest, midsized, and largest clusters, respectively.* The expected results from these three concentrations are depicted in three graphs shown in Fig. 11. These calculations assume a droplet diameter of 69 μm . Distributions for high, medium, and low concentrations are shown in blue, red, and black, respectively. The mode of each of the three distributions is determined by taking the known suspension concentration (spores/ml) and multiplying by the droplet volume. For the high concentration, the cluster size distribution appears as an almost Gaussian curve for which the standard deviation of the cluster size is nearly symmetrically centered on the distribution mode at about (52 ± 7) spores per droplet (mode $\approx 134/2.5$). The medium-concentration distribution also appears by eye to be symmetric, with a mode at approximately (13 ± 4) spores per droplet (mode $\approx 134/10$). However, for the lowest concentration, with a mode at about 2.6 spores per cluster (mode $\approx 134/50$), the distribution is clearly no longer symmetric. The latter shows a nearly 8% probability of having a droplet with no spores and a standard deviation of less than 2. While lower sample concentrations would produce even lower cluster-size distributions, the probability of having “empty” droplets with no spores will steadily increase as the mean size falls below 1 spore/cluster. This situation complicates the experimental interpretation of results since many of the aerosol particles that are generated will essentially be residues of Tween 80 as just discussed above.

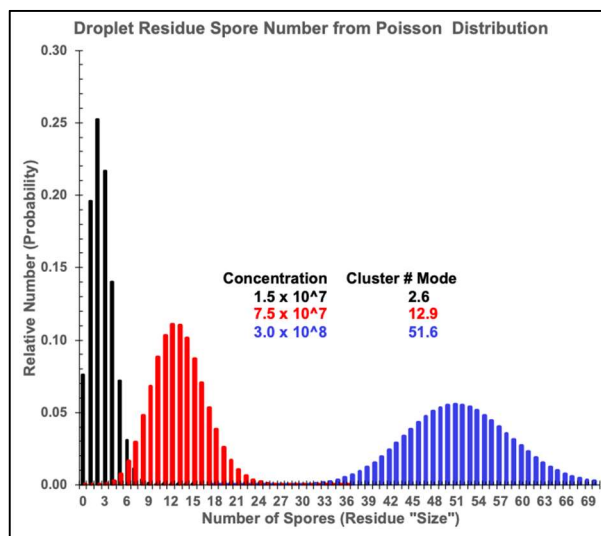


Fig. 11—Distribution of spore cluster sizes formed from 69- μ m-diameter droplets at concentrations of $1.5 \times 10^7/\text{cm}^3$ (50x dilution), $0.75 \times 10^8/\text{cm}^3$ (10x dilution) and $3.0 \times 10^8/\text{cm}^3$ (2.5x dilution) yields mean (expected) cluster sizes of about 3, 13, and 52 spores per particle, respectively

While the analysis above is useful for determining the number of organisms per residue particle from microdroplet generation and estimating the quantity of other materials such as surfactant, etc., that will also compose the residue particle, it does not directly address the topic of particle size. We could use the foregoing model results to estimate a lower bound to the residue particle size by assuming that the bacterial organisms are optimally packed and that other materials such as surfactant completely filled the gaps between the organisms, but without additional direct experimental investigation, we do not know a priori that these densities will occur as part of the natural process from droplet evaporation.

2.5.3 Splat Test

CCDC-CBC obtained cluster aerodynamic diameter measurement data using a TSI APS instrument on droplets after evaporative drying had occurred, but before they impacted collection surfaces. In addition, the number of organisms per particle was counted by generating droplets and allowing them to impact the surface of aluminum-coated glass slides before drying. This process is referred to as a “splat test.” The impacted droplets were visualized and enumerated by microscopy. Individual organisms within each impact splat were visually counted under a 40X objective to determine the number of organisms per initial droplet. Based on the counting of organisms for different-sized particles, the equation indicating the total number of organisms in a cluster can be provided in a format shown below. A statistical model analysis of the splat test data to evaluate the reliability of the number of spores per droplet from the IJAG for a known concentration is described in detail below.

B. anthracis Sterne spores were counted in micrographic images of liquid droplets deposited onto an aluminum-coated glass slide in which deposition occurred before significant evaporation could occur. However, in some of the images, it appeared that multiple droplets may have landed in overlapping locations, resulting in a range from 33–148 spores per image in a total of 60 images. Close examination of images suggested that multiple droplets occurred in some images. The number of droplets appeared to range from single droplets (singlets) to at least triplets. However, it was difficult to tell with certainty the exact combination state (singlets vs. multiples) from the micrographs alone. In order estimate the number of spores per singlet, we assumed that the spore count within singlets varied according to a Poisson distribution with expected value λ and that droplets combined with independent probability p .

Using the axioms of probability theory, a probability space for the combination of independent events, each with probability p , can be defined. For example, let the probability that droplets are combined into doublets (or higher) equal p , then the probability that a droplet is a singlet is $1 - p$. The probability that droplets are combined at least twice into triplets (or higher) is then p^2 , and the probability that a droplet is a doublet is $p - p^2$. Triplets occur at $p^2 - p^3$ (Fig. 12). This reasoning can be extended to an arbitrary number of combinations.

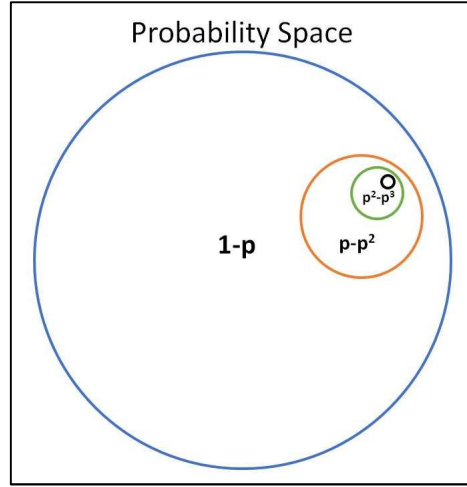


Fig. 12—Probability space for combinations up to triplets. The blue circle has area 1, the orange circle has area p , and the green circle has area p^2 . The probability for each combination state is the area of the circle minus the area of the next smaller circle.

To estimate the probability of observing a particular count, the probability mass for that count must be included, along with the probabilities of alternative combination states. The probability mass function for the Poisson distribution is

$$\frac{\lambda^k e^{-\lambda}}{k!} \quad (4)$$

or, in short,

$$\text{dpois}[\lambda, k] = \frac{\lambda^k e^{-\lambda}}{k!}, \quad (5)$$

where λ is the expected value for the count and k is the observed count.

If only singlets and doublets are possible, the probability of observing a particular count k is the combined probability that the count derives from a singlet, or that k derives from a doublet. This probability can be expressed as

$$\text{dpois}[\lambda, k, p] = \left[\frac{\lambda^k e^{-\lambda}}{k!} * (1 - p) \right] + \left[\frac{2\lambda^k e^{-2\lambda}}{k!} * p \right] \quad (6)$$

or

$$\text{dpois}[\lambda, k, p] = [\text{dpois}[\lambda, k] * (1 - p)] + [\text{dpois}[2\lambda, k] * (p)]. \quad (7)$$

However, if combination states of higher orders than doublets are possible, these must also be considered. For example, if singlets, doublets, triplets, and quartets are possible,

$$\text{dpois}[\lambda, k, p; p^4] = [\text{dpois}[\lambda, k, p; (1-p)]] + [\text{dpois}[2\lambda, k, p; (p-p^2)]] + [\text{dpois}[3\lambda, k, p; (p^2-p^3)]] + [\text{dpois}[4\lambda, k, p; (p^3-p^4)]] \quad (8)$$

In our data set, combinations of orders higher than quartets are extremely unlikely. Furthermore, no counts large enough to expect higher-order combinations were observed ($k_{\max} = 148$).

The likelihood for all n observations is the product of the individual count probabilities:

$$L[\text{data}_{(1:n)}] = \prod_1^n \text{dpois}[\lambda_{1:5}, k, p; p^5] \quad (9)$$

Note that the likelihood can be a very small number, which necessitates restrictive computational limits.

We programmed an optimization algorithm in the computational language R (R Core Team 2013) to assign a range of values to λ and p , then to find that combination that resulted in the highest-likelihood value. This provided maximum-likelihood estimates of λ and p .

For our data, λ , the expected count of spores within a single droplet was estimated to be 54, and p was estimated to be 0.2. The Poisson distribution with $\lambda = 54$ has 95% confidence intervals between 42 and 66. This represents a frequentist confidence interval where we would expect 95% of observed counts to fall within this range, assuming $\lambda = 54$. However, we are probably more interested in a range for the expected value λ itself, given the data, which is a philosophically Bayesian interpretation. An accepted method for estimating the confidence interval of λ using the likelihoods we calculated and including the uncertainty associated with estimating p uses an interval of -2 log-likelihoods (Edwards 1992), and resulted in a confidence interval for λ between 42 and 57 (Table 3). This represents the uncertainty in our estimate of the parameter λ , rather than an expected range of data values in an arbitrarily large sample.

Table 3—Estimated Singlet Values from Spore Counts

λ	p	Log-likelihood
54 (Expected Value)	0.2	-297.1149
42 (Lower 95% C.I.)	0.36	-299.0527
57 (Upper 95% C.I.)	0.14	-298.9345

Results obtained through maximum-likelihood estimates compare favorably to results obtained through other methods. We have archived R codes for the method and will continue to look for means of validation so that it can be applied to other organisms, other conditions, and other droplet sizes.

2.5.4 Electron Microscopy of Spore Clusters

Near the beginning of 2019, the CCDC-CBC team also produced spore sample droplets using the IJAG, and deposited spore clusters (after droplet evaporation) onto sample stubs for scanning electron microscopy (SEM) analysis. The sample solution used to produce these clusters was the high (stock) concentration, and typical results are shown in Fig. 13 and Fig. 14. Figure 13 is a relatively low-magnification image (2000X) that shows reasonably uniform (narrow) cluster size distribution and approximately spherical cluster shapes in a population of roughly 80 clusters. Individual spores are clearly resolved in the two images shown in Fig. 14 at higher magnification (>20,000X). Here, the individual spores can be resolved and appear to have aspect ratios typical of the *Bacillus* genera. Moreover, the diameter of the clusters can be estimated visually from these images as roughly 6–8 spore diameters (minor axis widths). As a crude estimate, the number of spores per cluster can be obtained by dividing the volume of a single spore into the volume of a minimal enclosing sphere, corrected by a nominal packing fraction. Since the SEM images are not calibrated for absolute scale,

the spore volume mean value of $0.57 \mu\text{m}^3$ was taken from an earlier study by Carrera, et al.⁷ The reported spore measurements reported used images obtained by transmission electron microscopy (TEM) on samples fixed with formaldehyde/glutaraldehyde. Since both transmission and scanning electron microscopy require relatively high vacuum to function, it is possible that some systematic dimensional differences between the spore clusters used in our experiments and the analysis of SEM/TEM results due to additional water removal. However, only a rough estimate is sought for our purposes here. Taking $7 \times 0.85 = 5.95 \mu\text{m}$ as the diameter of an enclosing sphere, we get a cluster volume of $\approx 110.3 \mu\text{m}^3$. For a spore packing fraction, it is important to distinguish between a volumetric density and packing within a given spherical enclosure. The former can yield relatively high packing fractions (≈ 0.7) depending on the aspect ratio of the ellipsoid, but the latter is a significantly different problem and typically yields fractions less than 0.5.³¹ For the purposes of this estimate, we take 0.6 as an upper bound to the packing fraction of ellipsoids in a sphere. Consequently, a typical number of spores contained the clusters shown in Fig. 13 and Fig. 14 is roughly estimated at 116 ($0.6 \times 110.3/0.57$). Since the spore sample solution used for the cluster SEM images was the high stock solution, it is instructive to compare this number to Eq. (2) which calculated the expected number of spores in a $70\text{-}\mu\text{m}$ -diameter droplet as 135. The IJAG device has been characterized with a slightly lower droplet diameter of about $67 \mu\text{m}$ ³². Correcting for the smaller initial droplet volume ($(67/70)^3 = 0.877$) provides an expected number of spores from the IJAG of 118. Given the approximations that have gone into this simple “back-of-the-envelope” analysis, the agreement in the number of spores per cluster between the SEM images and the computed value of the stock solution concentration and IJAG droplet volume is remarkably encouraging (116 vs. 118).

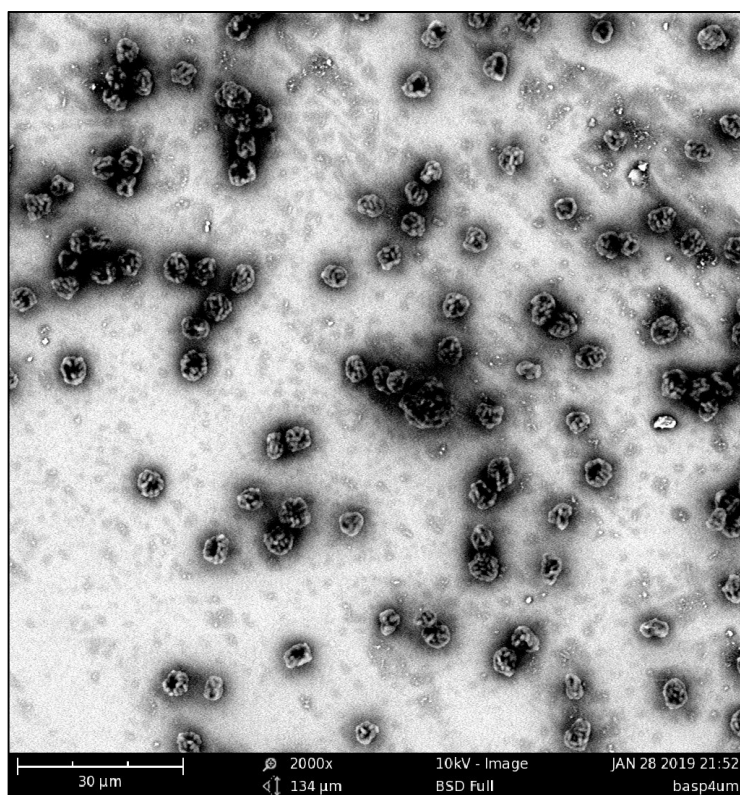


Fig. 13—This SEM image shows spore clusters from the IJAG droplet generator using a high spore concentration that produced a distribution aerodynamic diameter mode of $4.0 \mu\text{m}$ using the TSI APS instrument for “dried” (in air) cluster measurement.

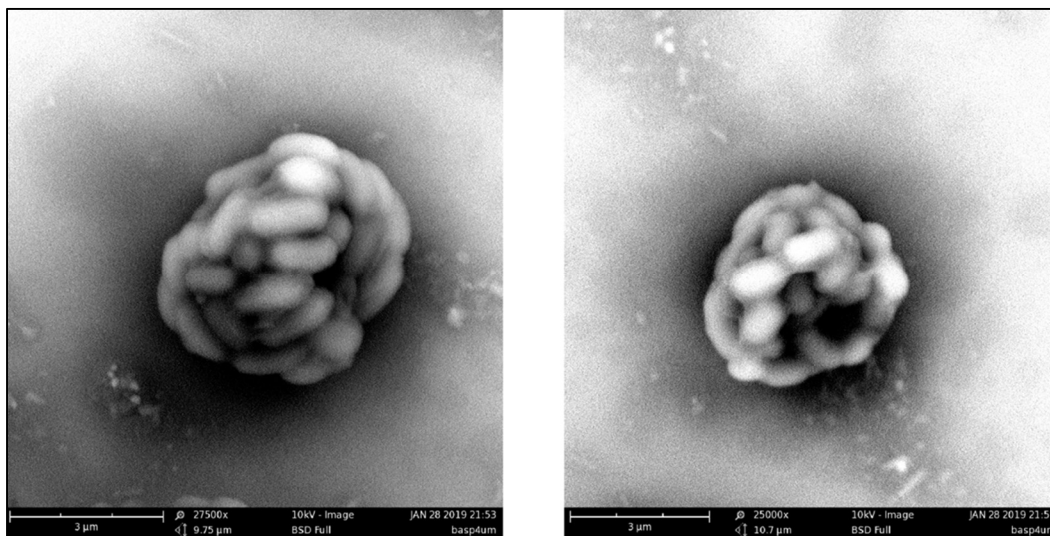


Fig. 14—These SEM images show spore clusters from the IJAG droplet generator using a high spore concentration that produced a nominal 4.0 μm aerodynamic diameter particle as measured by the APS instrument. Both images have dark, indented regions that suggest that the spore cluster residues may have formed in a way that left voids in their interiors.

2.5.5 Summary of Spore Cluster Characterization

Results of three different analyses regarding spore cluster sample generation and characterization have been presented above: (a) (Section 2.5.2) calculations based on measured initial droplet sizes (discussed in detail in Section 4.1 and in reference 17) and measured spore sample concentrations, (b) (Section 2.5.3) direct enumeration of spores contained in droplets by microscopy, and (c) (Section 2.5.4) estimation of spores per cluster from SEM images. All three approaches provide very good agreement.

At this point, it is appropriate to reiterate a main objective, which was to establish uniformity and commonality between the surface sample experiments and the suspended aerosol experiments conducted by CBC and NRL, respectively. With regard to the spore sample generation procedure, it is important to appreciate that both research efforts shared the same stock spore suspensions and produced aerosol clusters by the same method of microdroplet generation and subsequent evaporation of the water solvent. The primary experimental control over the resultant spore cluster “size” distribution is the initial spore sample suspension concentration, which was standardized by both groups. Starting with the same stock solution, “large” clusters are associated with a dilution factor of 2.5x, “intermediate” clusters with a dilution of 10x, and the “small” clusters (targeting single-spore aerosol particles) with a dilution of 50x. Because of differences between the specific methods for each group, it turned out not to be practical to maintain the exact same microdroplet generation device. The aerosol exposure experiments were able to maintain the Microdroplet Inc. droplet-on-demand device because their experiments required relatively small numbers of spore particle/clusters. The surface deposition and exposure experiments needed total sample numbers 2–4 orders of magnitude larger and were forced to adopt the IJAG device for the intermediate and large cluster samples and another device (a Sono-Tek-based CAD apparatus) for small cluster production.

Additionally, in order to establish run-to-run control for experimental reproducibility, each group was able to insert a real-time measurement capability to monitor the droplet-generation process and to establish a de facto reference that permitted intercomparison of replicate measurements. For the surface deposition and exposure effort, this capability was provided by a TSI APS for the large numbers of spore particles that were needed. This instrument provided a measure of the aerodynamic diameter distribution for the spore sample

cluster population that was being produced and monitored it in real time. For them, the “large” cluster size was standardized to provide a 4.0- μm aerodynamic distribution mode, while the “intermediate” cluster size resulted in a 2.5- μm aerodynamic distribution mode and the “small” spore cluster size was characterized by a 1.5- μm distribution mode. Run-to-run consistency was achieved by making slight adjustments in the sample concentration. Appendix E provides typical APS acquisition data for spore clusters generated at the three aerodynamic diameters depending on their suspension sample concentration. For the aerosol confinement and exposure effort, real-time monitoring was achieved by direct imaging and video recording of each initial microdroplet as it was launched from the PZT nozzle and ultimately was standardized to 67 ± 2 microns. Adjustments to achieve reproducibility from one experiment to the next could be achieved by tweaking the Microdroplet operating parameters (principally pressure and drive frequency).

Given these necessary differences between the two experimental efforts, it is gratifying to see such good agreement between them. The calculated mean spore cluster count for the three sample concentrations shown in Fig. 11 are 52, 13, and 2.6, corresponding to the 2.5x, 10x, and 50x stock dilution for a 69- μm -diameter initial droplet size. In the splat test (Section 2.5.3), the expected number of spores per droplet from the IJAG (65- μm diameter) at the 2.5x dilution is 54 with a 95% confidence interval. Finally, with SEM evaluation of clusters from the IJAG, we see that an estimated number of spores from the IJAG at the stock concentration was 116, in reasonable agreement with the predicted number of 118. Moreover, this number would be equivalent to a value of 46.4, as the expected number of spores from the IJAG at the 2.5x dilution.

3. SURFACE EXPOSURE EXPERIMENTAL APPROACH

3.1 Surface Spore Deposition and Exposure Methodology

In this study, *B. anthracis* Sterne was grown, processed, and aerosolized to provide similar particle sizes for parallel experiments in which particles either remained suspended in LEQ traps or were deposited on surfaces. In all of these studies, spore aerosol particles were produced with the same basic mechanism: first generating microdroplets from the spore sample liquid suspension and then allowing for subsequent evaporation of the solvent liquid. The size distribution of dried spore particles was controlled by the spore concentration in the starting liquid and was checked by measurement with an Aerodynamic Particle Sizer (TSI APS Model 3321). In addition, liquid suspensions were pipetted directly onto polycarbonate membrane filters to provide non-aerosolized single spores on surfaces.

For the surface studies, aerosols were generated using either an Ink Jet Aerosol Generator (IJAG) for larger spore cluster aerosols, or a Controllable Aerosol Dispenser (CAD) for single-spore aerosols. The IJAG was developed at CBC and has extensive historical documentation, but was unable to generate sufficient quantities of droplets to satisfy the single-spore-aerosol challenge. The CAD provided enough single-spore particles at a sufficient rate to make surface exposures feasible. It was built around a commercial droplet generator known as the Sono-Tek, which was operated at 120 kHz. It also supplied a gentler aerosol generation and delivery system for use with vegetative bacteria that, being more fragile than spores, are more likely to be killed by the stress of aerosol generation. (See Appendix A.) An illustration of the CAD system is provided in Fig. 15. A Sono-Tek aerosol generator was selected to provide less stress to the organisms during the aerosol generation. A heated tube increases the temperature of the air (170–180 °F) to dry the generated particles before the particles exit the CAD system. A filter holder is attached to the exit of the CAD to collect the dry particles for exposure studies.

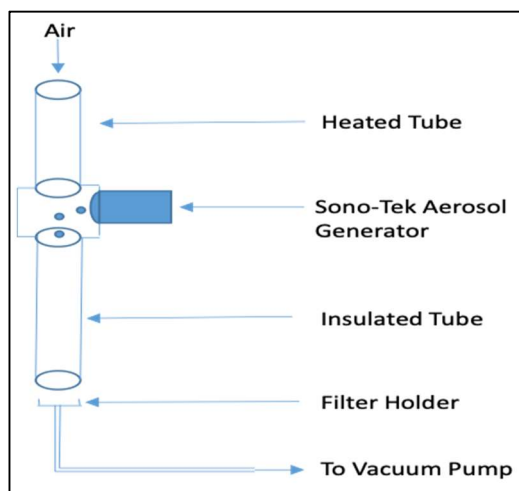


Fig. 15—A Controllable Aerosol Dispenser (CAD) system

Both IJAG- and CAD-generated particles were deposited onto polycarbonate membrane filters by air flow directly through the filters to minimize the collection time and to assure 100% deposition. The IJAG output tube was moved uniformly over the filter surface during deposition to prevent clusters from collecting on top of each other, ensuring that the particle deposition on the surface was uniform and particles were not shielded by each other. The quantity of sample material deposited on each filter was controlled by the amount of deposition time.

A narrow-band UV-C light source and a solar radiation simulator were implemented to expose particles to radiation in chambers as shown in Fig. 16 and Fig. 17. Filters containing the spores were placed into filter holders and were inserted into the exposure site, either on the bottom of the chamber that contained the UV-C lamp or into the custom-built exposure stand for the solar simulator. For the UV-C source, a low-pressure 15 W Hg UVC lamp (Sankyo Denki Co. LTD, Japan, G15T8) producing a wavelength of 253.7 nm was used. It was mounted in a 0.88 m X 1.03 m X 1.21 m plexiglass box as illustrated in Fig. 16. The bulb was unshielded and mounted at the top of the box so that the distance from the lower lamp surface from the sample holder in the bottom of the box was approximately 1 m. A manually operated shutter controlled the exposure duration and allowed exposures to proceed without turning bulb power off and on in order to insert and remove replicate samples for exposures. The flux at the sample exposure area was measured with a GigaHertz Optik X1-1 Optometer with a UV-3718-4 UVC detector calibrated at the factory for 253.7-nm radiation. The calibration was confirmed by comparing the detector output with an absolute irradiance measurement with an Ocean Optics USB2000 spectrometer, calibrated at the factory for wavelength and irradiance. The flux contour at the exposure plane was mapped using the UVC detector and varied less than 0.5 W across the exposed filter surface. Bulb power was applied an hour prior to exposures so that during the exposures, the flux at the sample was typically 0.94 W and varied less than 0.2% over the duration of the set of replicate exposures, typically less than 1 hour.

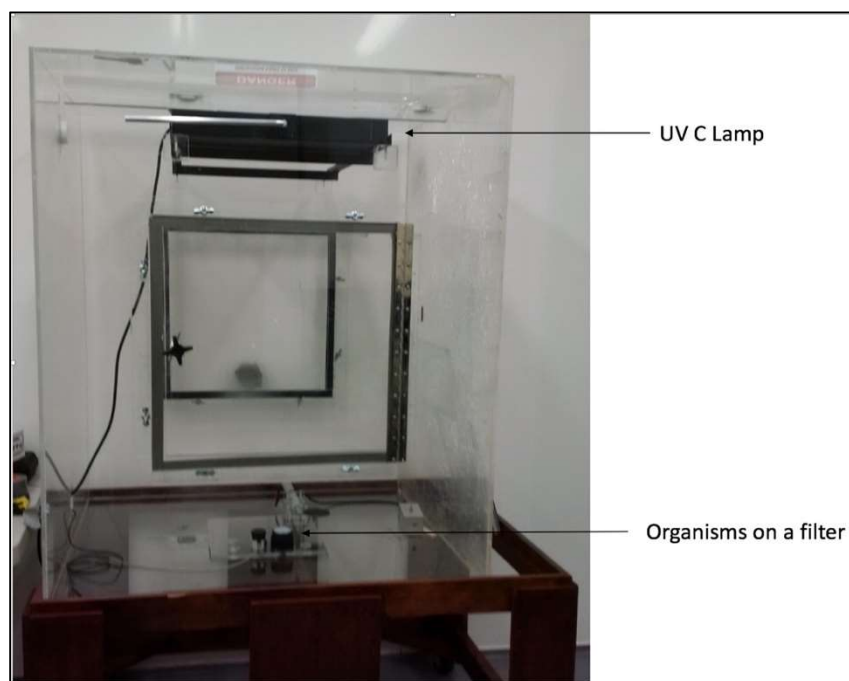


Fig. 16—UV-C exposure setup for single spores and clusters on surfaces

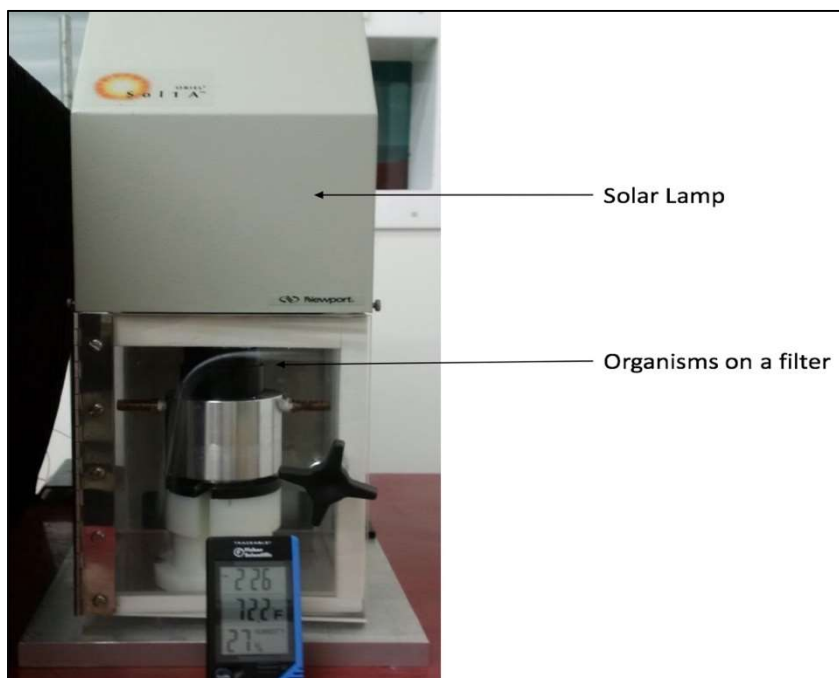


Fig. 17—Solar simulator exposure setup for single spores and clusters on surfaces

Initially, we observed that the temperature of the sample increased with time during exposure to solar simulator radiation, which caused inconsistent results. Therefore, the base of the exposure stand was modified to allow temperature-controlled water to pass through the stand to maintain a constant surface temperature of the sample during the exposure.

For each experiment, six control (unexposed) samples ($n = 6$) were prepared along with test samples ($n = 6$). The spore-loaded filters were placed into the appropriate chamber for irradiation for specific time intervals to provide various doses. The experiments were conducted with two different sizes of spore clusters (measured at nominally 2.5 and 4 μm aerodynamic diameter) and also nonclustered single spores.

After test samples were exposed, recovery of the spores from both the controls and the test surfaces was accomplished by placing each filter into a centrifuge tube containing 20 mL of phosphate buffer saline mixed with 0.01% Tween-80 (PBST) and vortexing for 10 minutes. After extracting the spores into liquid, samples were plated, incubated, and enumerated to quantify the culturable spores in the sample. The extent of kill due to irradiation was determined by comparing exposed samples with control samples. Solar exposures ran from 30 min to 3 h and UV-C exposures varied from 30 s to 5 min.

3.2 Surface Exposures Under Various Temperature and Relative Humidity Conditions

Initial tests did not control the temperature of the samples during simulated solar radiation exposure. Later, in order to check for any impact of temperature on biological decay apart from radiation-induced effects, the survival of spore samples was plotted as a function of the average sample temperature for a fixed simulated solar exposure time. This analysis included the presence or absence of the surfactant Tween 80 as a second variable. Figure 18 shows the survival fraction of 4 μm *B. anthracis* Sterne clusters and single spores on membrane filters exposed to simulated solar radiation for 30 min with and without temperature control and Tween-80. (Section 5.1 discusses the survival fraction (SF) parameter, which is used to characterize biological decay, in more detail.) The results indicate that no difference in culturable fraction was observed for 4 μm

clusters with and without Tween-80 under constant temperature. However, there is a significant difference in survival fraction for single spores and 4 μm clusters with and without temperature control: lower temperatures produced higher survival fractions for the spores.

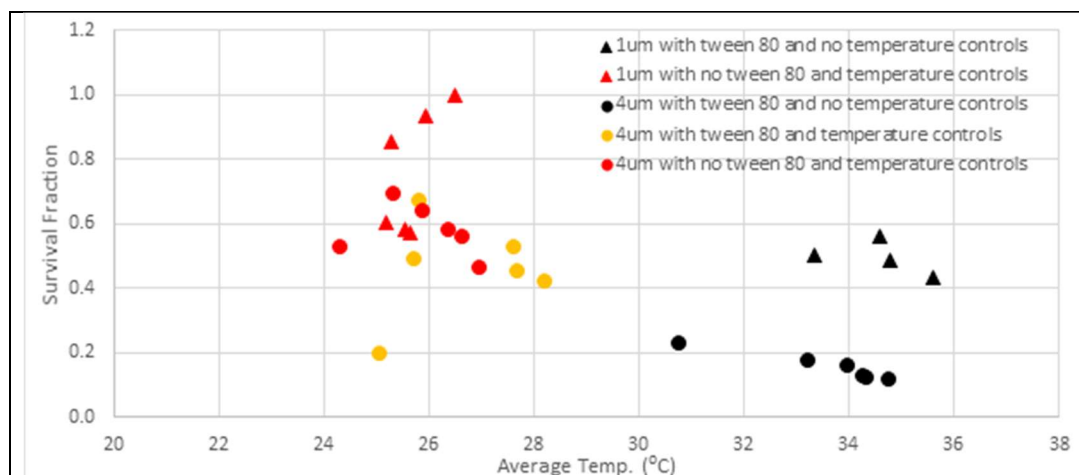


Fig. 18—The effect of temperature on the survival fraction of single spores (triangle) and 4 μm clusters (circles) exposed to solar simulator light for 30 min. Temperatures ranged from 24 to 29 °C and from 30 to 36 °C for temperature-controlled and temperature-uncontrolled experiments, respectively. The results indicate a clear trend, with lower survival fraction at the higher, uncontrolled temperature for single spores and 4 μm clusters. The added Tween-80 did not affect the survival fraction.

In addition, the combined effects of controlled temperature and relative humidity on the survival of spores pipetted onto membrane filters was determined for constant solar exposures. Temperatures of 50 °F and 70 °F and relative humidity (RH) values of 20% and 80% were selected. Single spores were exposed to solar simulator radiation for 1 h under the four temperature and RH combinations. Figure 19 shows the effect of temperature and relative humidity on survival fractions of single-spore *Ba Sterne* when exposed to simulated solar radiation. The results indicate a decrease in the survival fraction as the temperature increases and the humidity decreased. These results indicate that the temperature and relative humidity conditions should be controlled or at least documented for solar exposure experiments.

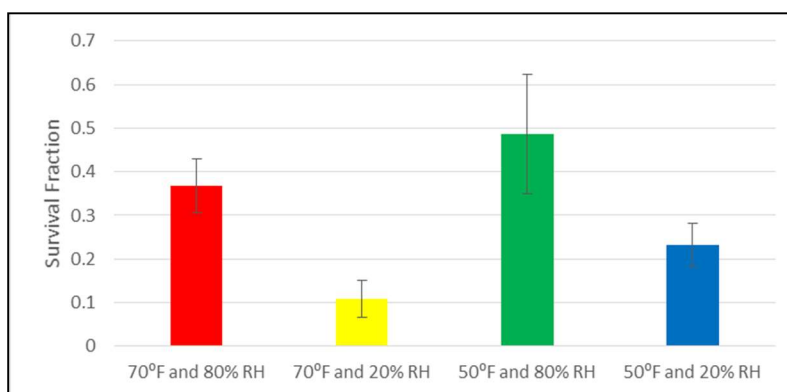


Fig. 19—The effect of temperature and humidity on the survival fraction of single-spore *Ba Sterne* exposed to simulated solar radiation for 1 hour. The results indicate a decrease in survival fraction as humidity decreased and as the temperature increased.

4. AEROSOL EXPOSURE EXPERIMENTAL APPROACH

The experimental approach for aerosol sample suspension, confinement, exposure and collection, is based on prior development and experience with electrodynamic quadrupole trapping of charged aerosol particles. There are good reasons for this. We know from previous measurements of solar radiation exposure of *Bacillus* spores on surfaces that their biological decay could require hours of exposure time. Maintaining a suspended aerosol for such long periods is nearly impossible using conventional approaches. A recent review paper³³ clearly indicates that traditional experimental approaches (static or flowing chambers, rotating drums, etc.) would be inadequate to address the challenge of maintaining a suspended aerosol size distribution and concentration for such long periods.

There are several specific electrodynamic trap designs as well as variations in nomenclature traps that are described in published literature going back at least as far as 1953.³⁴ Over that time, the technique has seen periods of greater and lesser interest, but until now, the method was mostly applicable to the study of individual particles, or just a few particles, at a time. One of the more significant developments from the first year of this program was the exploitation and refinement of handling large numbers of confined particles (meaning a few hundred) at a time. This capability is a direct consequence of our choice of electrode geometry, referred to as a linear electrodynamic quadrupole (LEQ) trap. While we had significant prior experience with the implementation of LEQ devices, the proposed approach of specifically trapping a population of bioaerosols for exposure and degradation studies had not been previously developed or even attempted. The basic principles of operation for this specific design are described in Appendix F in greater detail, and some of this work also has been previously published.^{35, 36} As part of this program, development of this capability represents a significant milestone in experimental aerosol research, and it is reasonable to say that the results obtained in this program could not be achieved in any other way with the same degree of control over particle size and composition.

Our original idea was to maintain multiple particles in an LEQ as a linear array of particles along the trap's vertical axis, where the AC field created by the trap electrodes forces charged particles to an equilibrium position. Since particles injected into the trap will carry the same charge, they will repel each other and maintain a uniform spacing between them along the center line. However, the total number of particles that could be confined in this manner was estimated to be limited to less than 2 or 3 per mm of length along the axis of the trap. To confine hundreds of particles in this mode would require extending the length of the trap to tens of centimeters. Fortunately, we soon discovered (rather rediscovered) particles could be stably trapped away from the center axis. The general principle of particle-particle repulsion remains true even when they are pushed away from the equilibrium axis. This allows higher densities of charged particles per unit length of the trap, so particles occupy a greater volume of the trap (where "trap" refers to the volume between the vertical electrodes).

Much of our effort during the first year and a half of the PAVES program was invested in development of the basic LEQ chamber through iterative designs. There were normal development issues and hurdles such as codifying a procedure for reliable generation of microdroplets with repeatable sizes, accurate measurement of initial microdroplet sizes, and allowing time in their trajectory for microdroplets to evaporate, and other minor problems. Resolving these issues took more time and effort than initially anticipated. However, in Q4 of year 2, when the aerosol chamber system was installed in our BSL-2 bioaerosol lab to begin use with *Bacillus anthracis* Sterne strain spores, a major unexpected problem occurred with particle extraction and collection from the LEQ chamber. Prior to that point, system development work had been performed with either polystyrene latex (PSL) microbeads or BSL-1 (*B. thuringiensis*) spore samples. Using PSLs of different sizes, a reliable particle-collection method and apparatus had been developed and demonstrated that was described in some detail in the quarterly and annual program reports. However, after starting work with *B. anthracis* Sterne spores in Q4 Y2, difficulties were immediately encountered in the form of inconsistent results

with spore particle collection from the LEQ chamber. This was essentially the status of our PAVES aerosol effort at the beginning of the third project year (Feb. 2019).

4.1 Development and Refinement of the LEQ Aerosol System

Consequently, as of the beginning of this report period (Feb. 2019), we were in the midst of a very intense effort to isolate and mitigate the source of inconsistent results for Ba Sterne spore particle collection. A historical summary of technical details regarding these problems has been extracted from the EOY2 and Q1-Y3 PAVES periodic reports and is included in this report as Appendix G. Fortunately, a solution to Ba spore collection was discovered toward the end of February and by mid-March 2019, consistent results were being obtained using an all-metal collection system. Briefly, the one variable that seemed to allow consistent particle collection out of the LEQ was the use of a metal (conductive) filter substrate. Initially, our desire was to employ the same filter materials used in the surface deposition studies being conducted at CBC (Edgewood, Maryland) in keeping with our over-arching goal of parallel methods and processes to facilitate data comparison between the two efforts. However, for reasons that are still not completely clear, the Ba Sterne spore aerosol samples frequently did not work with polycarbonate filters. That is, few, and in many cases zero, spores were recovered (see Appendix G). However, by mid-March, we had been able to validate a new particle-collection method using metal frits instead of membrane filters and to verify a frit collection efficiency of roughly 75%. The stainless-steel frit material³⁷ was specified with a porosity of less than 0.5 μm . The frits can be autoclaved for potential re-use; however, in this project, sterile frits were used once and discarded. In contrast to the track-etched polycarbonate filters, frit filtration is achieved through a “torturous path,” so some degree of deeper penetration with possibly reduced sample recovery may be expected. Figure 20 shows a photograph of the sample collection apparatus that was used throughout the remainder of the program.



Fig. 20—Metal collection frit (Restek # 25072), mounted on the end of a stainless-steel tube, which is inserted into the lower LEQ chamber to collect particle samples

As a brief overview, Fig. 21 depicts the experimental arrangement as a composite of two images. The schematic drawing on the left side illustrates three main apparatus components (from top to bottom): (1) a piezoelectric transducer (PZT) droplet generator, (2) an upper aerosol chamber with windows for observation and radiation exposure, and (3) a lower aerosol chamber for particle counting. Particles exiting the lower chamber immediately enter a filter-based particle collection assembly (not shown). An image of the final system on the right side shows a close one-to-one correspondence with the elements in the schematic. The assembled system is sealed and purged with a dry nitrogen gas flow, but it can release any chamber overpressure through a HEPA filter.

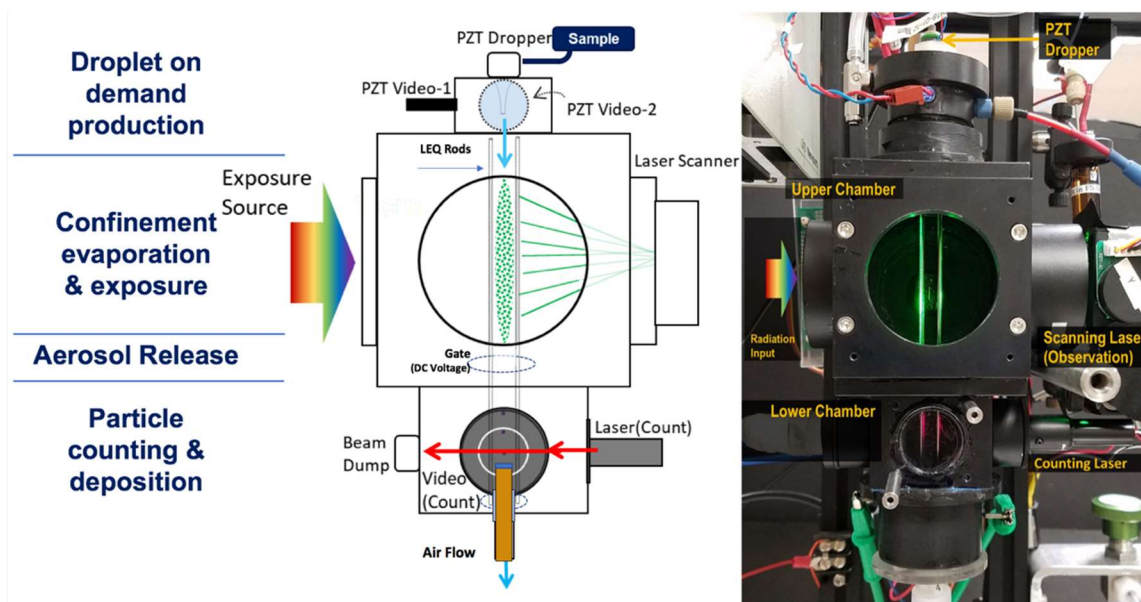


Fig. 21—The LEQ aerosol system appears both as a schematic drawing (left) and as a photograph (right). It's configured with three main sections arranged vertically as labelled: On top is a droplet generator comprising a PZT-driven 70- μm ID capillary with video monitoring feedback that places microdroplets directly into the LEQ. In the middle, the LEQ confines and holds aerosol particles, where they can be observed and exposed to radiation through large windows. On the bottom, individual aerosol particles are counted en route to the frit collection assembly (not shown).

For the aerosol exposure experiments, it was necessary to generate and hold a population of spore aerosol particles to determine the effects of ultraviolet (UV) radiation exposure on their culturability. Aerosol particles were charged and generated by sample solution droplet evaporation and then were held in the upper chamber for set periods of time to be exposed to radiation sources outside the chamber. A ring electrode functions as a “gate” that separates the upper and lower LEQ chambers by either blocking or allowing charged particles to flow through it, depending on its voltage (that matches the polarity of the charge on the particles). At higher voltage, charged particles are blocked, but as this voltage is gradually lowered, particles are able to flow down into the lower chamber in single file. After a timed exposure, the group of suspended particles confined to the upper chamber was carefully released to the lower chamber, where each particle was individually recorded by video and counted as it was collected onto the metal frit. An “aerosol dwell time,” defined as the period between the introduction of the last droplet into the upper LEQ chamber and the departure of the last particle from the lower chamber, was recorded for each sample. The aerosol dwell time provides a metric to check for any dependence of viability on the overall time that particles remain suspended as an aerosol, apart from radiation exposure effects.

For each series of experiments, the sample solution spore concentration was determined by titer. In each experiment, defined as the challenge of a single group of particles in the trap, the initial size of the microdroplets was directly determined from video camera images acquired as they were generated. Therefore, an accurate average number of spores per particle can be calculated from the initial droplet volume times the liquid sample spore concentration for each experiment. Consequently, the total number of spores in the collected aerosol sample can be accurately estimated from the counted particle number times the mean number of spores per particle. Filters were removed for subsequent sample recovery into a resuspension fluid, followed by plate inoculation, overnight incubation, and next-day culture counting. Samples were obtained in sequential experiments for the same conditions with and without radiation exposure to determine survival fractions due to radiation damage.

4.2 Spore Aerosol Irradiation Experiments

Exposures with the 254 nm Hg lamp were initially scoped and then systematically were filled in for both the large and small particle (cluster) sizes. Overall exposure times were short due to the efficient inactivation of 254 nm radiation. Exposure times from 5 to 120 s were attempted for the small size clusters (comprising primarily single, double, and triple spore particles). Exposures up to 300 s were attempted for the larger clusters (nominally 55 spores per particle), which showed increased survivability because inner spores of the cluster were shielded to some extent by spores on the outside (See Section 7). Experiments with the solar simulator required much longer exposures because the effective radiation wavelengths are longer (nominally between 290 and 300 nm) with much less intensity and therefore are less effective in causing biological damage. Consequently, a series of experiments exposed large and small clusters, containing approximately 58 and 2.1 spores per particle, respectively, to simulated solar radiation for up to 181.5 and 91.0 min, respectively.

As discussed in Section 2.5.3, distribution of the actual number of spores generated in the individual aerosol clusters are expected to be described by a Poisson probability based on the fixed droplet size and the sample fluid concentration. This would be true for any method of spore cluster generation based on microdroplet generations and subsequent drying (MicroFab Technology, IJAG and CAD). For the low-concentration samples generated using the MicroFab PZT microdroplet generator, the production of nominally 2.2 spores per cluster should be distributed as roughly 20% single spores, 25% two spores, 22% three spores, 14% four spores, and the balance as ever-decreasing fractions of increasing numbers of spores. The clusters are not locked precisely in space by the LEQ trap, but are observed to exhibit small oscillations and nutations about their loci, and are also presumably free to rotate; we expect their radiation exposure to be isotropic from all directions. Additionally, the optical depth for spore material is estimated to be a few microns even at 254 nm wavelength. Therefore, we expect shielding effects for these small clusters of two to five spores should not be significant, and their survival fraction degradations due to irradiation should be equivalent to those of single spores.

Over the course of this study, the number of spore aerosol particles that could typically be suspended, exposed, and collected varied from about 200 to 500. Mean values of the number of individual particles for exposures to 254 nm and simulated solar radiation were approximately 350 and 260, respectively. It can be immediately appreciated that for larger clusters, the total number of spores in a given sample (e.g., 260 particles x 58 spores per particle = 15,080 spores) is about 27 times more than for the small clusters (e.g., 260 x 2.1 spores per particle = 546 spores). To determine the rate of viability loss, having fractions over two logs of decay, or roughly 1% survival fraction, was the minimum desired criterion. However, clearly, 0.01 survival out of a population of 600 would be only 6, and the random (Poisson) error for numbers that small become large. While our standard deviations from the number of replicates (a goal of 6) were generally good, the amount of total execution time for this labor-intensive effort became an issue. Rather than collect many more replicates to smooth out the small number random error, we chose an alternate approach in which a single frit was used to collect multiple *fills* of equally exposed particles. In this way, the expected number of viable particles was co-added into a single sample. This process, referred to as *multifill*, is a method to obtain valid sample plate counts from otherwise scarcely viable samples. Depending on radiation source and exposure time, two, four, five, seven, or 12 fills were collected for a given multifill sample. To account for any possible effects from reinserting the frit, multifill exposure samples were referenced to multifill controls (no exposure) to obtain survival fractions (vide infra). One can further appreciate that the lowest survival numbers occur for the longest exposures, meaning that for, say, using 8 fills for a 1.5-hour exposure time equates to a 12-hour-long experiment, not counting set-up time, time for particle generation and collection, and post-experiment cleanup time.

4.3 Spore Recovery and Viability Assay Procedures

Once all particles are collected onto the frit, the frit holder is withdrawn from the bottom of the LEQ chamber and placed into a sterile container inside the secondary enclosure. The closed container is transferred to an adjacent Class II/A2 biological safety cabinet (BSC) for aseptic recovery of spores. Using universal precautions, a sterile, empty, 1.5 ml snap cap microcentrifuge vial is inverted over the frit and the frit holder. The assembly is flipped over and the vial is set into a rack with the frit and holder now inverted inside the vial. A sterile stainless-steel rod inserted through the hollow holder and pressed against the back of the frit transfers the frit directly into the recovery vial. As the holder is withdrawn from the vial, the snap cap is closed. Rod and holder are placed into containers of absolute and 70% ethanol, respectively, leaving hands free to transfer 1,000 μ l of sterile phosphate buffer saline containing 0.1% (v/v) Tween 80 (PBSTw) into the recovery vial. Note that each frit is immersed in PBSTw recovery fluid within 5 min of removal from the LEQ.

The spore particles are extracted from the frit into the PBSTw recovery solution by vortexing the recovery vial. This process also promotes breakup of the clusters. Two manual vortexers were equipped with accessory holders for hands-free, continuous operation. However, these accessories limited vortex speed to 50% of its maximum. Therefore, a prescribed vortex schedule was followed for all samples in order to include some time with individual vials at 100% speed and to standardize the vortex process for all samples. Immediately after PBSTw addition, an individual vial was vortexed at 100% speed for 1 minute and again for another minute. Then the vial was set aside to allow time for flame sterilization of the stainless-steel rod and decontamination of the frit holder and transfer box, followed by recovery of the next sample. After the initial 2 x 1-min vortexing of the second sample, both samples were vortexed at 100% speed for 2 min and were returned to the rack. Once all samples had reached this same point of recovery, each vial was vortexed individually again for 1 min at 100% speed. Then all samples were vortexed simultaneously for 30 min at 50% speed, followed by individual vortexing for 2 min at 100% speed, and finally 1 min at 100% speed just before serial dilution or plating.

Because each sample recovery volume comprises only 1,000 μ l, dilution factors, plating volumes, and number of plates per sample had to be estimated carefully and varied among samples with different exposures in order to obtain valid plate counts from the assays for those samples. Fortunately, in the LEQ system, droplet volumes and numbers of collected particles are accurately known. The bulk titer, in units of colony-forming units (cfu) per ml, of the spore suspension that's used to generate microdroplets in the chamber, together with the measured droplet size and the enumeration of the number of particles collected, permits confident calculation of the maximum number of viable spores that could be in any given sample:

$$\# \text{ of spores} = \text{titer (cfu/ml)} \times \text{vol./drop (ml/drop)} \times 1 \text{ drop/particle} \times \# \text{ of collected particles} \quad (10)$$

Ideal plate counts are 100 ± 50 cfu/plate, but counts between 25 and 300 were accepted as valid. For controls, estimating the necessary dilution factor and plating volume so as to achieve that range of counts is straightforward. With irradiated samples, the spore count is reduced by some number from the maximum possible, and the object is to determine the degree of reduction for the given radiation source. For the aerosol experiments, this initially requires a certain amount of trial and error in dilution factor and plate volume for each exposure time and particle size. Once the dose break point is found, then the end point of lowest survivability that still yields measurable counts can be iteratively approached with reasonable predictability. For samples reported here, dilution factors ranged from 1 (no dilution) to 20 (typical values) and up to 100 for some large cluster controls. Plating volumes varied from 100 to 200 μ l (typical) up to 900 μ l for samples exposed to highest doses. The objective of this procedure is to precisely measure the dose at which nothing is left to measure.

Whenever possible, a given dilution was plated on at least three tryptic soy agar (TSA) plates. However, high plating volumes necessarily limit the number of plates possible, so as we approached the target end-point dose, the data necessarily becomes more uncertain. Plates were incubated overnight in a humidified oven at

37 °C for 14.5 ± 0.5 h before counting. (Note: Enumeration at 14 h rather than 16 h in preliminary tests showed no difference in counts; however, because the boundaries between adjacent colonies were more visible at the shorter time, 14 h was adopted as the standard target incubation time to facilitate plate counting for the aerosol studies.) Plate counts less than 25 cfu or greater than 300 cfu were recorded but were excluded from calculations of biological decay rates due the excess error that those counts incur. Blanks prepared from PBSTw recovery and serial dilution fluids consistently showed no colony growth.

4.3.1 Colony Counting Conventions: Damaged Colonies

Assessments of viability rested entirely on the recovery of culturable spores in plating assays, so the conventions followed for enumeration of plating assays are critical, particularly in this case, with collaborators from multiple institutions. As described in Section 2, we took steps to align our procedures between the surface exposure group at CBC and the aerosol exposure group at NRL as closely as possible. One aspect that was perhaps inadequately addressed at the beginning of the program was how to handle “damaged” colonies that appear small in size and sometimes irregular in shape compared to other colonies observed after the normal incubation time. Although damaged, these colonies remain viable and continue to grow. The lag in colony growth compared to undamaged counterparts arises from the additional time needed for repair from radiation damage.

Figure 22 illustrates the difference between a plate from a liquid suspension titer (no stress from aerosolization, drying, or radiation exposure) to one from a sample exposed to simulated solar radiation for 90 min. In general, post-exposure plates yield reduced viability, and the colonies appear smaller and with greater variation in size. Additionally, other fine, irregularly shaped material appears as some sort of nonviable debris proportionate in quantity to exposure dose. (Although present, the debris is not visible in photographs.) Colonies below a certain size were classified as “too small to count” (TSC) by NRL. The plate counts at 14-h incubation appear in the lower left corner of each image in Fig. 22. Post-exposure colonies that were TSC at 14 h are circled in red and the TSC count is shown in the lower right corner. In this case, the TSC count represents 25% of the total number of colonies.

Early in the program, it was established between the two groups that these damaged colonies were to be excluded from plate counts of viability for the sake of consistency with the recent Degrade program sponsored by DTRA Reachback. However, the criterion for distinguishing “damaged” colonies from countable ones is subjective, and therefore, it was difficult to maintain self-consistency over the long period (greater than 18 months) that plate analysis continued. As a hedge against this uncertainty, NRL maintained a record of the number of TSC colonies on each sample plate for future reference. TSC colonies are rare among stock titers and controls, so they’re primarily a product of environmental stress. Small colonies were reported³⁸ in a study involving gamma irradiation of spores. However, as exposure dose increases, the number of TSC colonies increases, passing through a maximum at intermediate exposure times. Beyond the maximum, the TSC count declines as more lethal doses of radiation kill more spores, finally removing any opportunity for cell-repair mechanisms to operate.

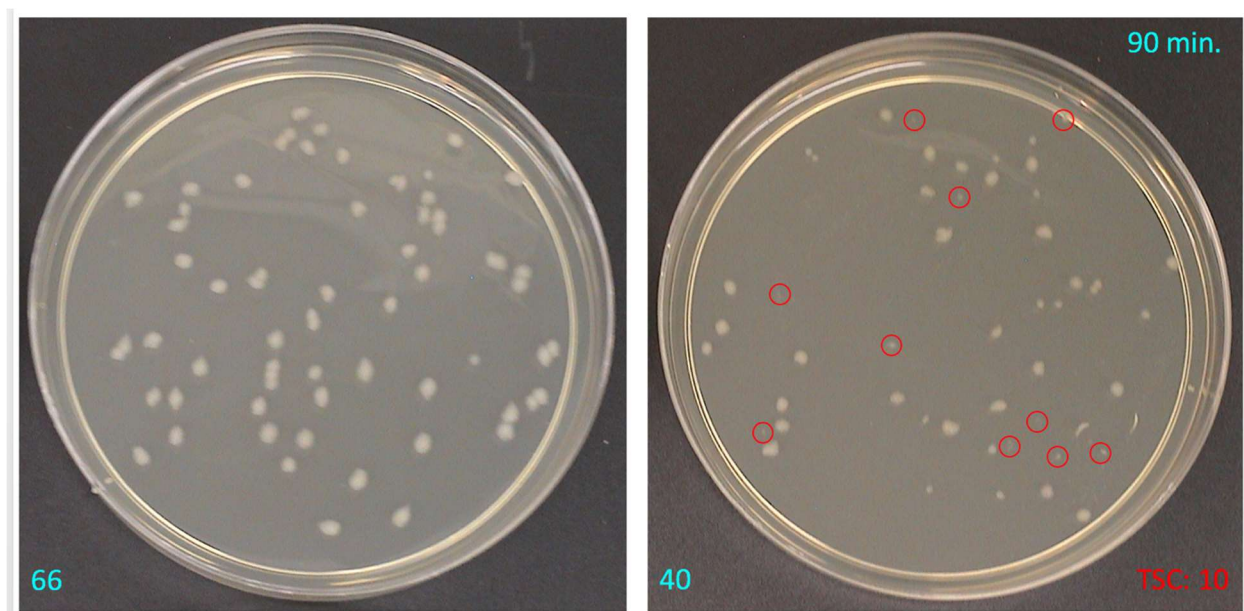


Fig. 22—Photos of *B. anthracis* colony morphology: aerosol particle recovery and incubation from (left) a liquid suspension titer compared to (right) an aerosolized sample exposed for 90 minutes to simulated solar radiation

Figure 23 and Fig. 24 demonstrate this trend for small and large particles exposed to both 254 nm and simulated solar radiation, respectively. The percent increase in plating assay was calculated from the difference in total plate count with and without the TSC colonies, divided by the total count without TSC colonies. (Analogous plots of the percent change in recovery fraction or survival fraction show similar trends at slightly lower y-axis magnitudes.) Figure 23 displays clear maxima for both small and large particles irradiated at 254 nm. However, the maxima differ not just in fluence values (dose) but also in the degree of change due to TSC colonies because the outer spores shield inner spores from damage in the large clusters. The maximum proportion of TSC colonies relative to the standard 14 h count in large clusters at 401 J/m^2 (90 s exposure) is 35% of that in small clusters at 134 J/m^2 (30 s exposure). Likewise, Fig. 24 demonstrates a size-dependent shielding effect for particles exposed to the solar simulator illumination, but here, the large particle maximum at 376 kJ/m^2 (120-minute exposure) is only 19% of that in small particles at 266 kJ/m^2 (85-minute exposure). Note that the final data point in the small particle dependence on fluence is exaggerated because the small particle plate count at 285 kJ/m^2 (91-minute exposure) was too few to count (TFC). Therefore, the TFC result is statistically unreliable as a starting point for the calculation of percent increase with TSC colonies.

It's tempting to propose that the maxima in Fig. 23 and Fig. 24 approximate the highest doses at which maximum repair is still possible and that these maxima point to fluence values beyond which repair becomes impossible for more and more spores. However, additional exposure-time measurements are needed to define the position of the maximum change, the slopes of the approach to and the descent from the maxima, and their reproducibility.

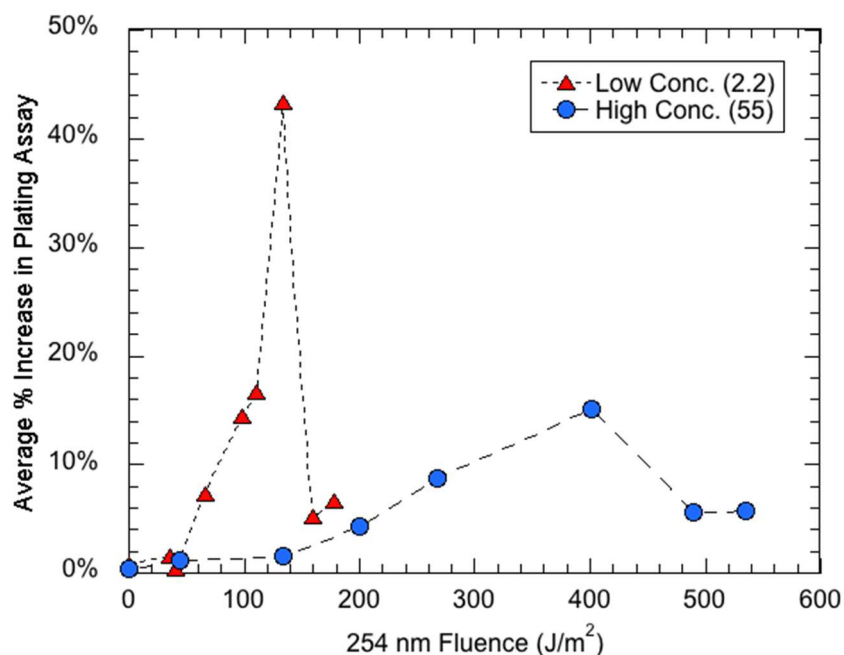


Fig. 23—Dependence of the average percent increase in plate counts, due to inclusion of “too small to count” (TSC) colonies in the assay, on exposure dose and particle size. Small (triangles) and large (circles) particles were irradiated at 254 nm. Both sizes show that the percent change in plate counts goes through a maximum before decreasing at more lethal doses.

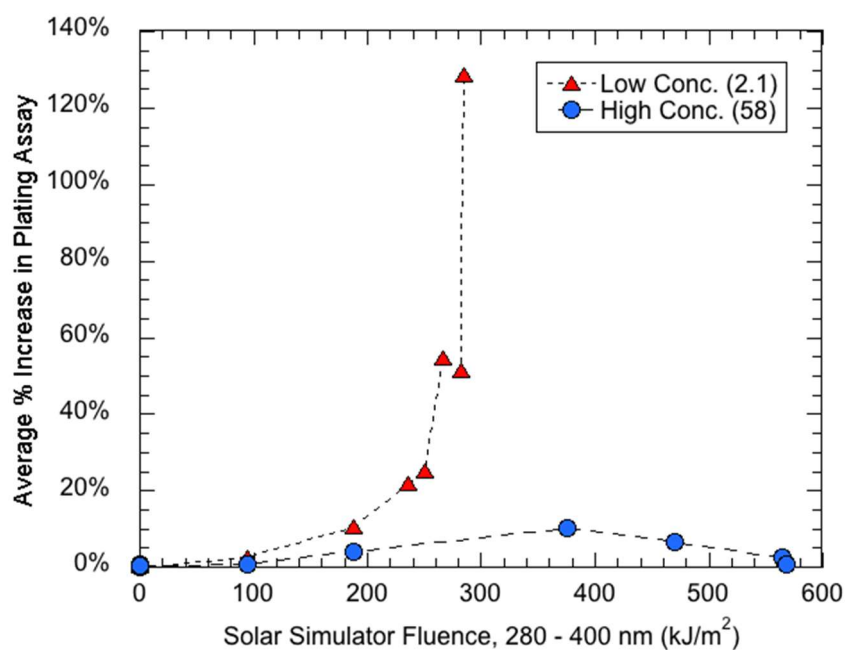


Fig. 24—Dependence of the average percent increase in plate counts, due to inclusion of “too small to count” (TSC) colonies in the assay, on exposure dose and particle size. Small (triangles) and large (circles) particles were irradiated by the solar simulator (SS). The final data point for small particles at 285 kJ/m² is skewed by a statistically unreliable TFC plating result before the addition of TSC colonies. Both sizes show that the percent change in plate counts goes through a maximum before decreasing at more lethal doses. However, the small particle results are limited by the paucity of valid data beyond the maximum.

When normal colonies dominate, a few TSC counts will not affect SF values. However, at high radiation exposures, when most colonies present show variable sizes and irregularity in shape, categorizing some as TSC and others “normal” becomes more difficult. In these cases, the TSC counts may represent a large proportion of the total plate count and their impact on survival fractions is significant. Add to this the observation that the calculated biological decay rate relies on accurate measurement of the linear decay region. The slope of that line is sensitive to its end points, the samples at the intermediate and longest exposures. Under these circumstances, exclusion of TSC colonies from the plating assays becomes more concerning. It is possible that the most important aspect of colony count data collection is consistency, and that an alternate method of colony counting, inclusive of damaged organisms that recover, would result in similar first-order decay rates to when TSC colonies are excluded. However, even if that turned out to be true, the initial slow-growth shoulder region would certainly be extended by the additional TSC counts in the intermediate fluence regions.

As a graphic example of the potential impact of TSC colonies on plate counts and viability assays, Fig. 25 images colonies counted vs. TSC colonies at our standard incubation time of 14.5 h. It also follows the progression in TSC growth and appearance of previously unobservable colonies as incubation time extends to 18.7 and 41.7 h. The plate was inoculated and enumerated using aseptic techniques within a Class II/A2 biological safety cabinet. Media blanks handled concurrently showed no growth. This plate represents the recovery of a multifill sample under simulated solar radiation of small clusters for which 900 μl of the 1,000 μl undiluted recovery suspension had to be plated. Thus, this plate was the only chance of a result for this trial at 91 min of exposure time. Note that because the initial plate count of 21 did not meet the threshold for a valid count, this plate was rejected as “too few to count” (TFC). However, if the TSC colonies at the same 14.5-h incubation time had been included, the plate count would have increased to 47, a valid count, and more than double the initial number. After 4 h more of incubation, another 84 colonies appeared that weren’t visible at 14.5 h, taking the plate count to 131. Finally, by 41.7 h, 42 more colonies appeared, for a total of 173 colonies.



Fig. 25—*B. anthracis* Sterne strain morphology of damaged colonies that continue to grow with extended incubation. Key: Counted colonies at 14.5 h: basis for assay result = 21 (red, solid dots), damaged colonies barely visible at 14.5 h = 26 (thick, red, open circles), damaged colonies visible at 18.7 h = 84 (thin, blue, open circles), damaged colonies visible at 41.7 h = 42 (black, solid dots). Note that the criterion for minimum valid plate counts (25) would reject this plate as too few to count (TFC).

Counts at 18.7 and 41.7 h are not meant to be quantitative, since colony overlap begins to obscure boundaries. The point is that TSC colonies survive the radiation, and they can recover under suitable growth conditions. Excluding TSC colonies from the assay result may underestimate bacterial survival. Furthermore, including TSC colonies in plate counts would reduce some of the subjectivity in assays since all viable colonies at an agreed-upon incubation time would be counted. Whether or not to include TSC colonies in plating assays of bacterial survival clearly needs to receive greater consideration for any future project planning.

4.3.2 Recovery Assessment

Observed recoveries are a function of physical and biological aerosol decay. The reproducibility of these losses depends on the experimental uncertainties associated with handling procedures. Controls provide a means to estimate the magnitude and precision of these uncertainties apart from biological degradation due to irradiation. Thus, normalization of irradiated sample recoveries by controls reduces the contribution of non-radiative effects from the calculation of biological decay. The plating assays described above (recorded as cfu/ml in the 1-ml recovery volume) provide the number of culturable spores recovered from each frit. Division of the plating assay by the maximum number of spores that *could* be viable on that frit (vide supra) yields the “*recovery fraction*” for each sample, including individual controls and exposed replicates. Because extraction efficiencies from the frits might be more variable than from polycarbonate filters due to the tortuous porosity of the frits, it seemed advisable to decouple the extraction efficiency variability from irradiation effects on plating assays. Therefore, for a given set of exposures, the corresponding *recovery fractions* of individual controls were averaged together. Section 5.1 describes in more detail the use of these averaged control recovery fractions to normalize individual exposure replicates and to obtain the *survival fraction* of each sample.

Table 4 summarizes *average control recovery fractions*, their standard deviations (SD), and the ranges of sample exposures in which they were used for normalization. Rows 1, 3, and 4 report controls used for samples exposed to 254 nm radiation for such short periods that aerosol dwell times necessarily exceed exposure times. Rows 2, 5, and 6 show control data for samples exposed to simulated solar radiation. Under the constrained project schedule, only four *multifill* controls could be executed. Three of these were averaged in order to normalize the small clusters exposed to 254 nm radiation (Row 4). All four were averaged to normalize the small clusters irradiated by simulated solar light (Row 6). Rows 1, 2, 3, and 5 reflect independent sets of controls.

Table 4—Summary of Control Recoveries for BaS Spore Aerosols

Row No.	Bulk Titer (x10 ⁸ cfu/ml)	No. of Fills	Aerosol Dwell Time (min)	Corresponding Exp. Time (min) ^a	Average Recovery (%)	% Recovery SD	No. of Controls
1	3.4	1	13 – 18	0.17 – 2.0	66	9	8
2	3.4	1	36 – 161	30 – 181.5	78	17	17 ^b
3	0.14	1	14 - 23	0.13 – 0.50	104	28	18
4	0.14	4	12 – 31	0.60 – 0.67	103	33	3 ^c
5	0.14	1	34 – 90	30 – 75	90	9	3
6	0.13	2, 4	12 - 31	80 – 90	96	30	4 ^c

^a. Corresponding exposure times of irradiated samples that were normalized by each average control.

^b. This set was subsequently subdivided by aerosol dwell time (vide infra).

^c. The three multifill controls averaged together in Row 4 are also included in Row 6.

The average of all four multifill control recoveries produced a 30% standard deviation (SD), higher than the more typical single-fill controls. Although this might be expected, given the increased handling of the sample frit, additional multifill control replicates might have improved the statistics for the two cases shown in Table 4. However, low replicate numbers alone don't account for the higher SD in Row 6. Consider that there are 18 single-fill controls included in Row 3, and yet there, the SD is still 28%. That particular single-fill data set was acquired immediately after implementation of LEQ operation adjustments to optimize small particle confinement and collection. It's possible that operator familiarity with the new procedures contributed to subsequent reductions in single-fill control SDs in Rows 1 and 5, down to 9% for both large and small clusters. So, these data suggest a nonrandom factor also contributing to recovery fraction variability for the cases where SDs are near 30%. For the long dwell times in Row 2, the 17% SD for large cluster controls is discussed next.

4.3.3 Effect of Aerosol Dwell Time on Control Recoveries

Particles remained suspended as aerosols for various times in order to accommodate radiation exposure times that ranged from 8 s to 3 h. The aerosol dwell time for each sample is the sum of the exposure time and the times required to both fill and empty the chamber. Consequently, the dwell times for all samples extended from 12 to 192 min. Although controls had no radiation exposure time, matching their total dwell times to that of exposed samples as closely as possible essentially removed aerosol dwell time as a variable from calculated recovery and survival fractions. This clarified the contribution to biological decay from irradiation alone vs. time as aerosol.

Examination of the control data also presents an opportunity to expose any effect that aerosol dwell time alone might have on the culturability of spores. Such an effect would impact decisions over the number and range of controls needed to normalize the exposure data. Figures 26–29 plot the individual control recoveries, represented by the averaged values in Table 4, against aerosol dwell time.

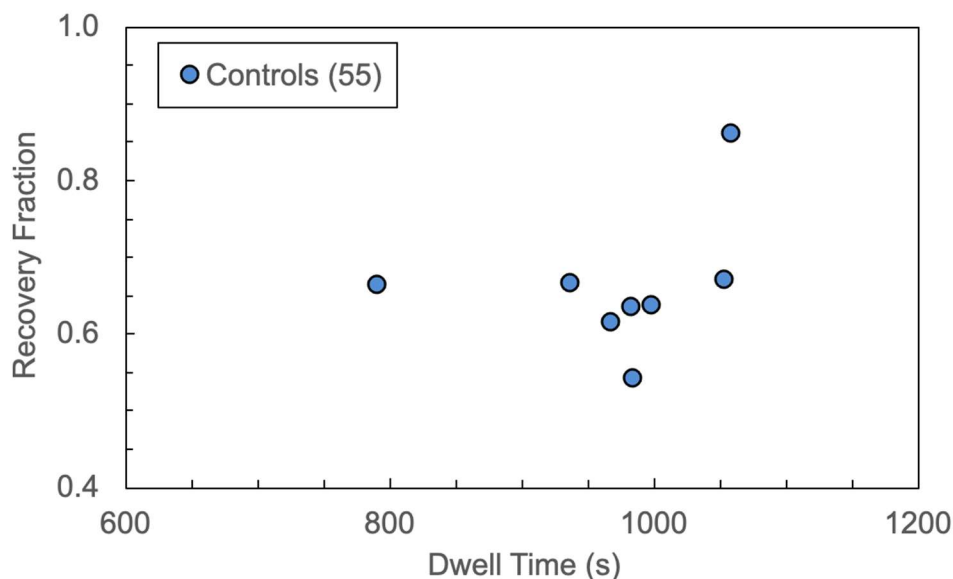


Fig. 26—Recovery fractions of large (55 spores per particle) *Bacillus anthracis* spore clusters vs. total time suspended as an aerosol in the LEQ chamber for less than 20 min. Row 1 of Table 4 summarizes these controls.

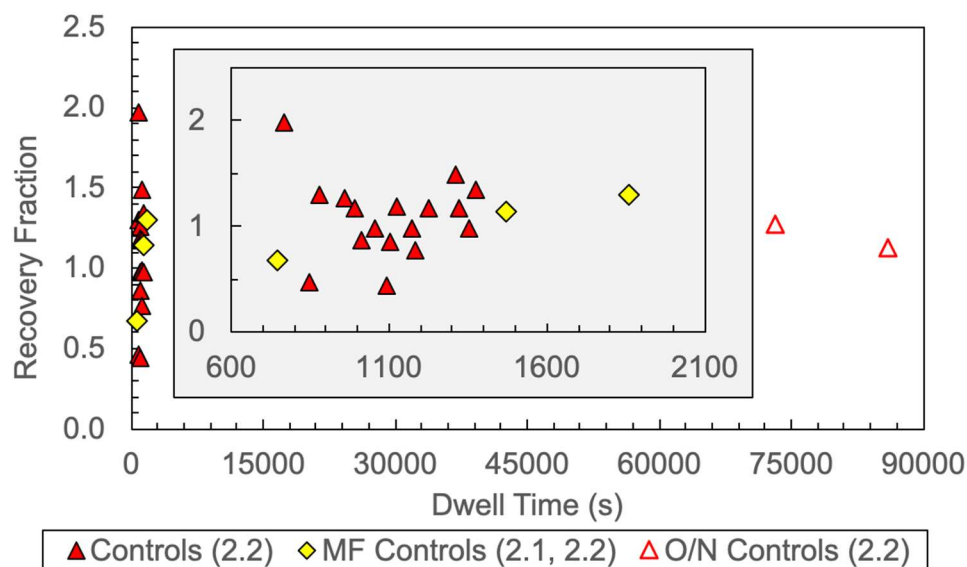


Fig. 27—Recovery fractions of small (2.2 spores per particle) *Bacillus anthracis* spore clusters vs. total time suspended as an aerosol in the LEQ chamber. Overnight (O/N) controls remained suspended for 20 and 24 hours. The inset expands the dwell time region between 10 and 35 min. The same three multifill (MF) controls, used to normalize multifill samples, also appear in Fig. 28. Rows 3 and 4 of Table 4 summarize these controls.

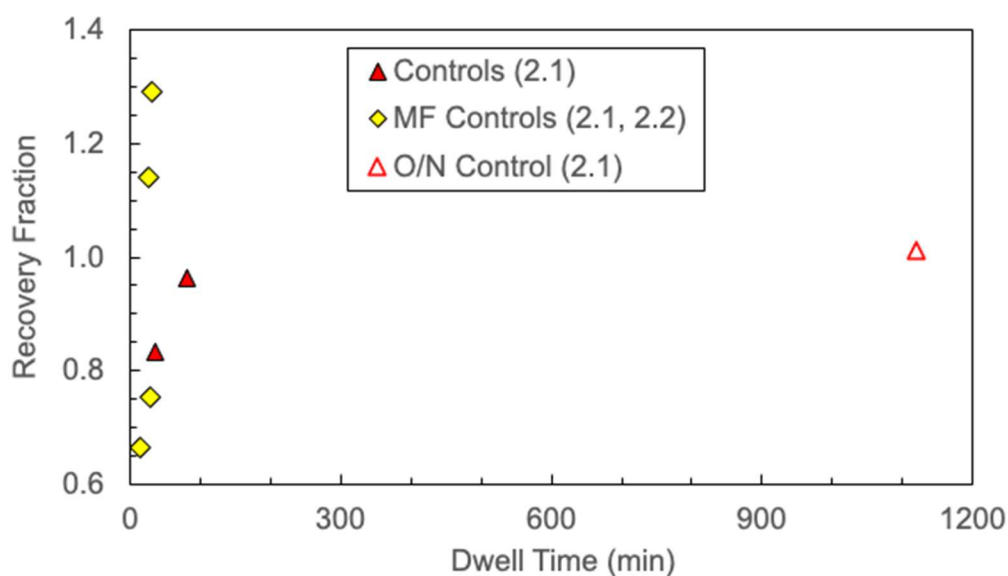


Fig. 28—Recovery fractions of small (2 spores per particle) *Bacillus anthracis* spore clusters vs. total time suspended as an aerosol in the LEQ chamber. The overnight (O/N) control remained suspended for 18.6 hours. Multifill (MF) controls were used to normalize multifill samples. Three of the four multifill (MF) controls also appear in Fig. 27. Rows 5 and 6 of Table 4 summarize these controls.

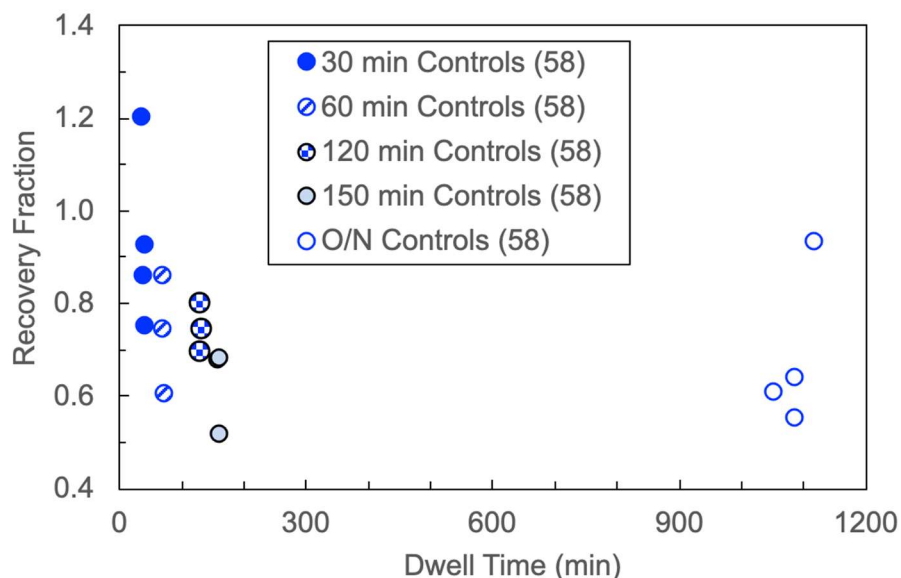


Fig. 29—Dependence of large cluster control recoveries on total time suspended as an aerosol in the LEQ chamber for more than 30 min. The legend refers to the corresponding exposure time for which those controls were acquired, and to overnight (O/N) dwell times. Row 2 of Table 4 summarizes these controls.

When possible, a few controls were held in the LEQ chamber overnight (O/N) to ascertain endpoints. Each set of controls demonstrates a random distribution of recoveries with respect to the aerosol dwell time, with one exception. As shown in Fig. 29, the controls for large clusters under long dwell times (Row 2 in Table 4) show a declining trend in recovery with increased aerosol dwell time that levels off after about 160 min. The overnight (O/N) controls show about the same average recovery as the 160-min point. This suggests that the 17% standard deviation shown in Table 4, obtained from the average of all large-cluster, long-dwell time controls, is due in part to an underlying dependence on dwell time. This set of controls formed the basis for normalizing large clusters exposed to simulated solar radiation.

Although the radiation source has no direct impact on control recoveries, use of the solar simulator necessitates that controls remain suspended longer than in experiments under 254 nm illumination. Comparing Fig. 26 and Fig. 29, it may be that the large cluster controls acquired in the former experiments weren't suspended long enough to observe the same effect. (No O/N controls were recorded for large cluster controls during the 254 nm exposure series.) Small cluster controls all showed no apparent dependence on aerosol dwell time, including O/N periods; however, the data isn't conclusive because replicates with dwell times greater than 30 min were too limited in number. Therefore, while the effect of aerosol dwell time on recovery is intriguing, it bears further study.

Notwithstanding, given these results, recoveries of large clusters irradiated by simulated solar light were normalized by the average recoveries of their time-correlated controls. That is, sets of replicates of controls having similar aerosol dwell times as sets of exposed samples were averaged together, and each time-correlated average control was used to normalize samples having the corresponding exposure time. Thus, the controls summarized in Row 2 of Table 4 are broken out into the time-correlated controls that appear in Table 5. If, among the controls represented by Row 2a, a single outlier is excluded, the average control recovery changes from $(94 \pm 19)\%$ over 4 replicates to $(85 \pm 9)\%$ with 3 replicates.

Table 5—Time-Correlated Control Recoveries for Large Cluster BaS Spore Aerosols

Row No.	Bulk Titer (x10 ⁸ cfu/ml)	No. of Fills	Aerosol Dwell Time (min)	Corresponding Exp. Time (min) ^a	% Av. Recovery	% Recovery SD	No. of Controls
2a	3.4	1	36 – 41	30	94	19	4
2b	3.4	1	68 – 70	60	74	13	3
2c	3.4	1	129 – 131	120	75	5	3
2d	3.4	1	157 – 161	150 – 182	63	9	3
2e	3.4	1	1050 – 1115	Not Used	69	17	4

^a. Exposure times of large cluster, solar-irradiated samples that were normalized by average controls correlated to aerosol dwell times of the exposed samples.

5. SURVIVAL FRACTION RESULTS AND DECAY RATE ANALYSIS

5.1 Introduction

The survival fraction (SF) parameter represents the number of organisms that survive experimental conditions as a fraction of the total number of viable organisms that were exposed to those conditions. Since individual organism survival is inherently probabilistic, and since each organism's viability can only be determined once, SF is a conceptual construct that is approached by measurements of organism populations. Consequently, results are subject to both random experimental uncertainties and possible systematic factors inherent in the measurement process. Repetition of measurements under a given set of conditions provides an assessment of random error. Referencing results from experimental samples to those from controls, which differ by a single condition of interest, normalizes for the effects of unknown or systematic variables on sample viability.

In this work, possible systematic contributions to biological decay include temperature, humidity, physical particle loss, droplet drying temperature, aerosol dwell time, and extraction efficiency. Although system designs incorporated standardization of these factors wherever possible, residual systematic effects can't be completely ruled out. In order to isolate the effects of radiation exposure on spore viability from other possible factors, control particles were aerosolized, collected, and assayed the same way as experimental samples except controls were never irradiated. SF calculations used the plating assays of recovered spore controls to "normalize" the recoveries of irradiated samples and account for any systematic uncertainties.

In execution, the SF determinations for each sample of a given particle size at each exposure time for each of the two radiation sources were carried out as separate experiments. Each experiment was replicated a number of times (2 to 6, depending on the level of difficulty) and included controls that were not exposed to radiation. Moreover, in the sample plating and enumeration step, multiple plates were prepared from each recovered sample in order to reduce random error. Ultimately, the individual SF determinations were compiled to provide SF versus fluence (or dose, in J/m²) dependency data.

As an introduction to this kind of data and its analysis, Fig. 30 reproduces characteristic results for bacterial degradation due to radiation exposure previously published in (left) a textbook on this topic³⁹ and (right) a recent journal article⁴⁰ from the same group. These data exhibit slow initial loss of viability known as the "shoulder" region as the exposure dose, or radiation fluence, increases from zero. However, at higher fluence, the SF decreases more rapidly with an apparent exponential decay, represented as a straight line on a logarithmic/linear plot of SF vs. fluence. A mathematical expression commonly applied to these types of measurements is given by³⁹

$$SF = 1 - [1 - \exp(-k F)]^n, \quad (11)$$

where SF is the measured survival fraction, F is the radiation fluence and n and k are parameters that are used to minimize the mean error between the curve fit and the data points. This expression is frequently referred to as the "multihit" model. The parameter k represents the exponential, or first-order, decay rate obtained from the slope of the linear portion of the data fit at higher fluences, as illustrated by the dashed, red lines in Fig. 30. The variable n refers to the recovery factor (or, notionally, the number of DNA radiation damage sites) needed to cause a loss of viability. Graphically, the value of n is equivalent to the y-intercept of the first order decay line extended to a fluence of zero as illustrated in Fig. 30 (left). This formula is intended for exposure to radiation at a specific wavelength, or a narrow band of wavelengths, and there is some rationale to expect an exponential decay rate based on fundamentals of radiation interaction with materials. In some cases, no shoulder is observed, leaving a simple exponential decay (i.e., the value of n = 1). Mathematically, the value of n controls the extent of the shoulder region, or how large the fluence must be for the exponential dependence to start to dominate. Notionally, the value of n is intended to be an integer that indicates the number of times

a particular cell, or its DNA, could absorb a bond-breaking, or photoreactive, photon before the cumulative damage exceeds the organism's repair capability. In practice, it has come to be regarded as a continuous parameter used to optimize the fit of this equation to experimental data. However, it has been well established that for a given organism, both k and n are typically strongly dependent on the radiation wavelength. Consequently, it is difficult to generalize this analysis approach with a functional dependence of k and n on wavelength, or to describe irradiation damage from a source with a broad range of wavelengths, such as a solar spectrum. A recent article¹⁶ by a coauthor of this report presents a new analytical approach to this topic that may offer another option for analysis of PAVES data, particularly for the solar simulator data.

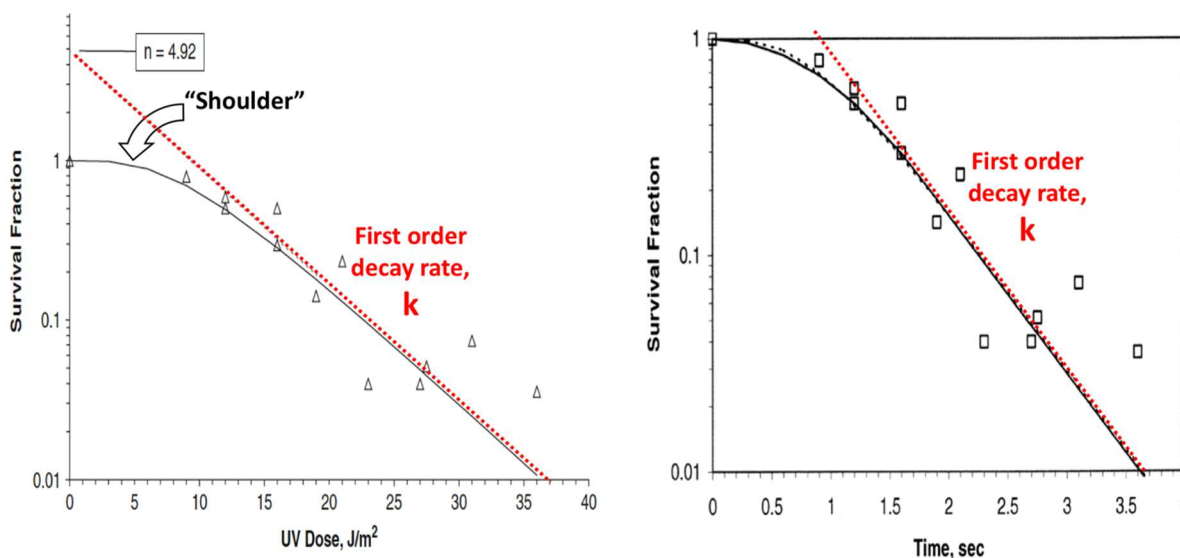


Fig. 30—Two figures are reproduced from references 39 (left) and 40 (right) that illustrate typical SF vs. radiation fluence behavior for bacterial samples. Initially, there is a slow loss of viability, often referred to as a “shoulder” region (left) that is followed by a more rapidly decreasing survival at higher fluences, which is typically modeled as a first-order exponential decay.

As a final example of expected radiation damage measurement, Fig. 31 reproduces a plot of SF versus radiation damage from an earlier landmark study⁴¹ that's particularly relevant to the biological samples of interest here, *Bacillus* spores. This plot of SF vs. fluence that was conducted on different species of *Bacillus* spores when exposed to a narrow band of radiation centered at 255 nm (essentially equivalent to measurements using a germicidal Hg lamp with a 253.7 nm atomic emission line). In Fig. 31, the sample designated UVR is a wild-type *Bacillus subtilis* strain, #HA101 (*Bacillus* Genetic Stock Center BGSCID 1A476⁴²) that represents normal, repair-capable *B. subtilis* spores. A blue box in Fig. 31 has been added to outline the data labeled UVR.

The other samples in this particular plot were *B. subtilis* spores that had certain genes associated with DNA repair removed or compromised, making them more susceptible to radiation damage, as indicated by the steeper slopes of their decay curves (higher decay rate (k) values), and most importantly, the absence of a shoulder effect at low fluences. Since their repair capabilities have been compromised, these other spore samples begin to degrade with exposure to even the lowest level of radiation. One can see that for the wild-type stain data (designated UVR in the original publication) there is a well-defined linear fit for higher fluences indicative of an exponential decay rate. Furthermore, this exponential decay line does not intersect the Y-axis at a fluence value of 0.0, but is offset, giving a positive Y-intercept value. This is characteristic of a shoulder, or a slower initial decay rate in the spectral region of low fluences (between 0.0 and 0.2 on the fluence axis of

Fig. 31). This independent measurement provides an example of a *Bacillus* spore species with typical radiation decay behavior described by the multihit formulation.

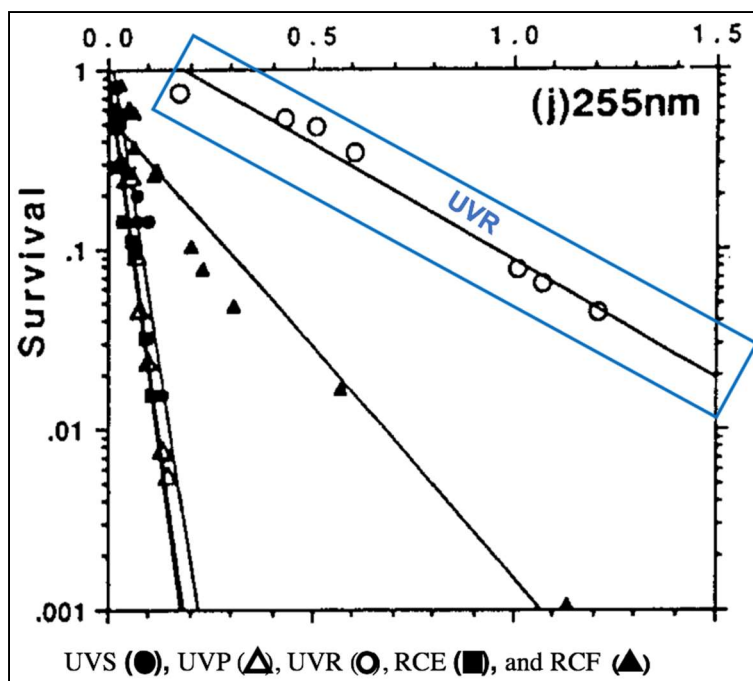


Fig. 31—A reproduction of Figure 1j from reference ⁴¹ is shown here as example of SF vs fluence at 255 nm for *Bacillus* spores. Several different species are shown in this plot, and of interest is the wild strain, which has been highlighted with a blue box and labeled UVR.

5.2 Survival Fraction (SF) Results from Irradiated *B. anthracis* Sterne Strain Spores

Both aerosol- and surface-illumination experiments report the effect of radiation on spore viability in terms of SF versus radiation fluence. This section summarizes those results for all individual replicates recorded at the NRL and CBC facilities with corresponding experimental conditions. Section 2.4 explains in detail the steps taken to enable direct comparison of the outputs from sources used at the two laboratories. As described above, the SF parameter has the same meaning for both aerosol and surface sample results, but there are some differences in the details regarding the use of controls and the SF computation method.

Aerosol sample “survival fractions” are calculated from the *sample recovery fraction* divided by the corresponding *average control recovery fraction*. (See the discussion of aerosol sample recovery fractions in Section 4.3.2.) Thus, by definition, *average survival fractions* for aerosol controls equal unity at zero radiation fluence, and random variations in individual frit-to-frit extraction efficiencies are mitigated through averaging of replicates. *Individual control recovery fractions* are also normalized by the corresponding *average control recovery fraction* such that the resulting range in *individual control survival fractions* provides an empirical measure of the experimental uncertainty in the absence of radiation effects.

Surface-exposed sample plating assays (*recoveries*) were normalized to individual control recoveries to obtain *surface-irradiated survival fractions*. Controls had been aerosolized and deposited on surfaces for the same amounts of time and under the same conditions as irradiated samples in order to provide the reference for the number of pre-irradiated spores for each exposure time. Therefore, the survival for every *individual* surface control is defined as 100%, and these controls typically don’t appear on surface data plots.

5.2.1 254 nm UV Irradiation of Spores as Aerosols and on Surfaces

Figure 32 depicts the individual sample SF values obtained for both large and small clusters, nominally consisting of 55 and 2.2 spores/cluster, respectively, after exposure to 254 nm radiation while suspended as aerosols. Particles were aerosolized from titrated suspensions of *B. anthracis* Sterne strain spores at low and high concentrations using a Microdrop PZT generator and were held suspended in the LEQ chamber for drying and subsequent radiation exposure as described in Section 4. (Average survival fractions, used to extract biological decay rates, appear in Section 5.3.1.) In Fig. 32, a shielding effect of larger spore clusters (55 spores average size) is clearly evident from their slower decay rates (higher SF values) compared to small clusters (2.2 spores average size). Individual replicates show reasonable consistency. In order to measure low survival fractions for small particles under high exposure doses, chamber fills had to be co-added in the multifill process described previously (Section 4.2). In this way, valid plate counts were achieved for small cluster survival as low as about 1% under 254 nm illumination.

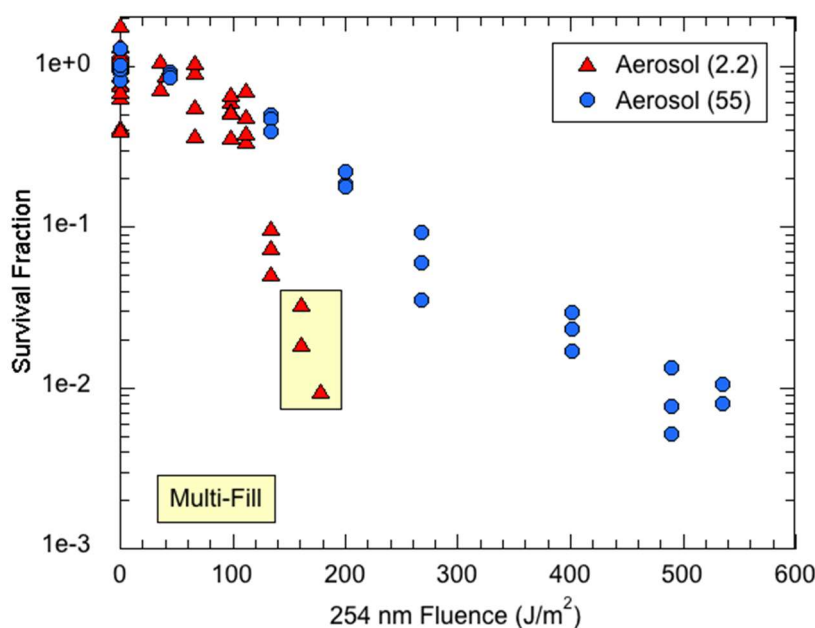


Fig. 32—Aerosol-irradiated spore SF versus 254 nm radiation fluence (J/m^2). Mean cluster sizes containing 2.2 and 55 spores per particle were obtained by LEQ Microdrop aerosol generation from low- and high-concentration spore suspensions, respectively. Each data point represents a single experiment. The multi-fill (MF) procedure extended the measurable range for small particles to 1% survival.

Figure 33 shows survival fraction results of *B. anthracis* Sterne strain spores as small clusters (nominally single spores) and as clusters with aerodynamic diameter mode distributions of 2.5 and 4 μm . These were generated as aerosols, collected onto polycarbonate membrane filters, and subsequently exposed to narrow-band UV (254 nm). The aerosols were generated as microdroplets from three concentrations of spore suspensions and were dispersed into a drying tube. An Ink Jet Aerosol Generator (IJAG) was used for the medium and high concentrations. Unfortunately, the IJAG system could not generate sufficient quantities of particles from low spore concentrations. This necessitated changing the dispersal device to a modified Sontotek aerosol generator (CAD device, see Fig. 15, Section 3.1) for low-concentration samples. For comparison, dilute spore suspensions were also deposited directly onto filters using a metered pipet, then the filters were dried by flowing clean air through them. Knowing the spore concentration and the deposited volume permits the estimation that each residue particle consists of a single spore. This process notionally

creates a non-aerosol deposit of “single” (nonclustered) spores. Consequently, there are a total of four types of surface exposure samples. The two that notionally provide small to no clusters (pipetted samples and clusters generated by the CAD device) are shown in a separate plot (top) for clarity, while the two samples of larger clusters generated by the IJAG device are also together (bottom). In all of the surface-deposition-of-aerosol experiments, a TSI Aerodynamic Particle Sizer (APS) was used to determine the mean aerodynamic diameter of the (dried) spore clusters, and that feedback was used to make small changes in concentration so that a specific cluster’s aerodynamic diameter was consistently maintained as the surface samples were produced. These nominal aerodynamic diameters were standardized as 2.5 and 4 μm clusters from the medium and high concentrations produced by the IJAG generator, respectively. APS data indicated that 1- to 2- μm -aerodynamic-diameter clusters were produced by the CAD generator that has an inherently broader initial droplet size distribution compared to the IJAG.

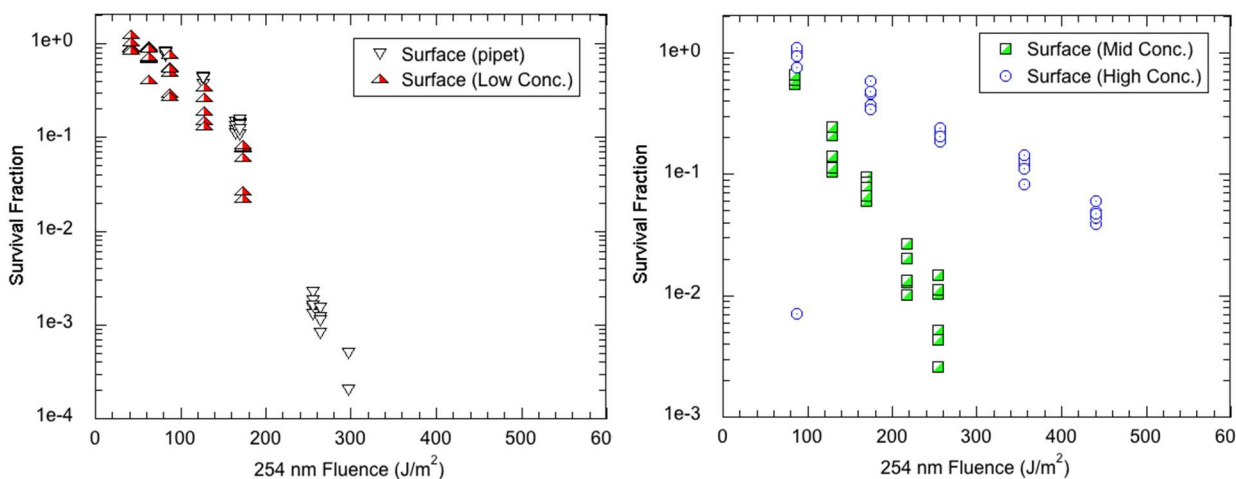


Fig. 33—Surface-irradiated spore SF versus 254 nm radiation fluence. Particles were generated and deposited onto polycarbonate filters prior to illumination by (top) pipet and a modified Sonotek aerosol generator (CAD) and (bottom) an Ink Jet Aerosol Generator (IJAG). Particle diameters are approximately (top) 1 μm (pipet) and 1 to 2 μm (CAD) depositions from low concentration suspensions; and (bottom) approximately 2.5 and 4 μm for IJAG-generated clusters from medium and high concentrations, respectively.

In looking at the data plots in Fig. 32 and Fig. 33, one can immediately see a general trend in which the survival fraction of spores decreases with increasing fluence across all the samples as expected. Moreover, in many of the plots, it is apparent that the rate of decrease in survival fraction is lower at low fluences compared with higher fluences. Additionally, results for both the aerosol- and the surface-exposed samples (Fig. 32 and Fig. 33) indicate larger clusters decay more slowly than smaller clusters. This behavior is expected due to a shielding effect of spores on the surface of the cluster, which reduces the effective fluence experienced by spores in the cluster interior. This shielding effect has been observed in previously published reports.^{43, 44} Finally, for both the aerosol and surface deposition data, the individual data points at a given fluence show reasonably good agreement/consistency with each other. A more detailed and quantitative discussion of these results is provided in Section 5.3.1, where curve fitting is applied to the averages of the replicate values. With the exception of a single outlier among the 4.5- μm -surface-deposited clusters at a fluence of $\approx 85 \text{ J/m}^2$ that was omitted in subsequent analyses, the individual data point replicates are reasonably self-consistent and their variation at each fluence value is taken as an indication of experimental error.

5.2.2 Solar Simulator Irradiation of Spores as Aerosols and on Surfaces

Figure 34 depicts the individual sample SF obtained for both large and small clusters exposed to simulated solar radiation while suspended as aerosols. Particles were dispersed from titered suspensions of *B. anthracis* Sterne strain spores at low and high concentrations in the same way that samples for 254-nm exposures were handled (see Fig. 32). Once again, the shielding effect of larger spore clusters is evident from their slower decay compared to that of smaller clusters. One immediate difference that should be pointed out in comparing Fig. 32 and Fig. 34 is the fluence axis scale factor. As described in Section 2.4.3 regarding the simulated solar radiation, the wavelengths for solar exposure are significantly longer than for the 254 nm germicidal Hg lamp, and most of the effective radiation for spore degradation is located between 305 and 315 nm. Consequently, due to both weaker absorption cross sections and lower quantum efficiencies for photochemical reactions at these longer wavelengths, significantly higher fluences are required to achieve similar reductions in survival fraction. Therefore, the axis units for the fluence plots in Fig. 34 and Fig. 35 differ by a factor of approximately 1,000 from Fig. 32 and Fig. 33 and are listed in kJ/m² instead of J/m². As discussed in Section 2.4.2, these fluence scales were calibrated to reflect the total continuum radiation fluence from the solar simulator over the spectral region from 400 to 280 nm. Otherwise, similar general behavior is observed. Small-particle survival drops off rapidly after a fluence of about 250 kJ/m² (about 80 min exposure time), while larger clusters required about twice as much fluence to achieve similar survival fractions.

Individual data point values for a given fluence level also remained reasonably consistent, although their spread at specific fluence levels is generally increased compared to the 254 nm series data. In order to measure low SF for small spore clusters as aerosols at high exposure doses, chamber sample fills had to be co-added in the multifill process described previously. Using this approach, valid plate counts were obtained for small cluster survival down to 6.3%. For the highest fluence data points, each exposure time lasted 90 min under simulated solar illumination. Moreover, due to project schedule constraints, it was not feasible to expand collection of multiple fills for small clusters beyond that point. For the same reason, the total number of small aerosol replicates was limited. Given the lack of prior data on solar exposure decay rates for either single spores or small spore clusters as aerosols, this type of limitation was difficult to anticipate. In retrospect, to achieve these measurements in an economical manner (both time and cost), it would seem more reasonable to first invest in the design and development of an expanded LEQ apparatus capable of confining and holding a significantly larger number of spore clusters in a single exposure. Given our current state of LEQ development, we believe such a capability would be straightforward to develop, and once an expanded capability was validated, then acquisition of experimental data could proceed much more efficiently.

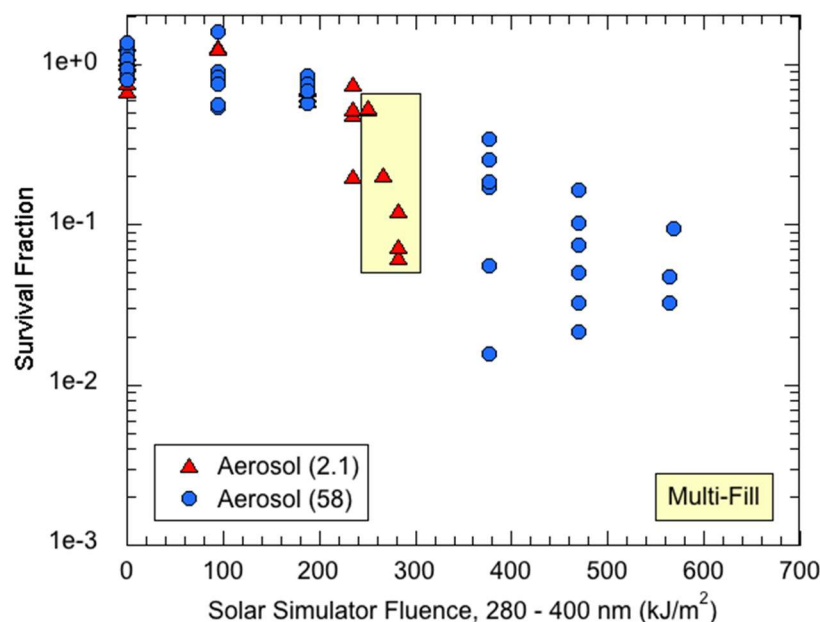


Fig. 34—Aerosol-irradiated SF versus the integrated fluence (kJ/m^2) of the solar simulator illumination from 280 to 400 nm. Mean cluster sizes containing 2.1 and 58 spores per particle were obtained by LEQ Microdrop aerosol generation from low- and high-concentration spore suspensions, respectively. Each data point represents a single experiment. The multifill (MF) method extended the measurable range for small particles down to 6% survival.

Figure 35 displays corresponding data for similar spore samples captured on polycarbonate filter substrates, where again, for the sake of clarity, the data for four different experimental sample preparations have been broken out into two separate plots. The first (upper) plot shows the results from pipetted spore sample solutions and low-concentration CAD-generated spore cluster aerosol collected onto filter substrates. The second (lower) plot show results for two “sizes” of spore cluster aerosols (2.5- and 4- μm aerodynamic diameter) generated using an IJAG droplet generator with a drying tube to promote droplet evaporation prior to collection onto the filter substrate. The plots indicate that as the exposure increases, the SFs decrease. In general, the same qualitative trends regarding SF decay versus fluence and cluster size, previously noted in both the aerosol results (Fig. 32 and Fig. 34) and the 254-nm exposure results for spore clusters on filters (Fig. 33) are evident in Fig. 35 as well.

Spore sample solutions deposited onto surfaces using a pipette and exposed to solar radiation showed slightly slower decay compared to low-concentration spore samples that were aerosolized first using the CAD system and then were deposited onto filters. These results suggest that the single-spore SF is affected by the deposition method. The pipette deposition method may be a less stressful method for single spores compared to the aerosol deposition method. However, neither of these two samples was able to obtain sufficiently low survival fractions to provide a high-confidence evaluation of the decay rate. The low-concentration CAD aerosol has a small range of SF values. Although the pipetted sample approaches 3 logs of decay, the lack of intermediate data points between the 520 and 630 kJ/m^2 fluence values leaves some ambiguity with the correct decay rate. What is reasonably clear is that the break point for the low-concentration aerosol seems sharper and possibly has a shorter shoulder than the pipetted sample. For the intermediate- and large-size clusters deposited onto filters in the lower plot of Fig. 35, the most striking feature is that both sizes appear to have very similar decay curves.

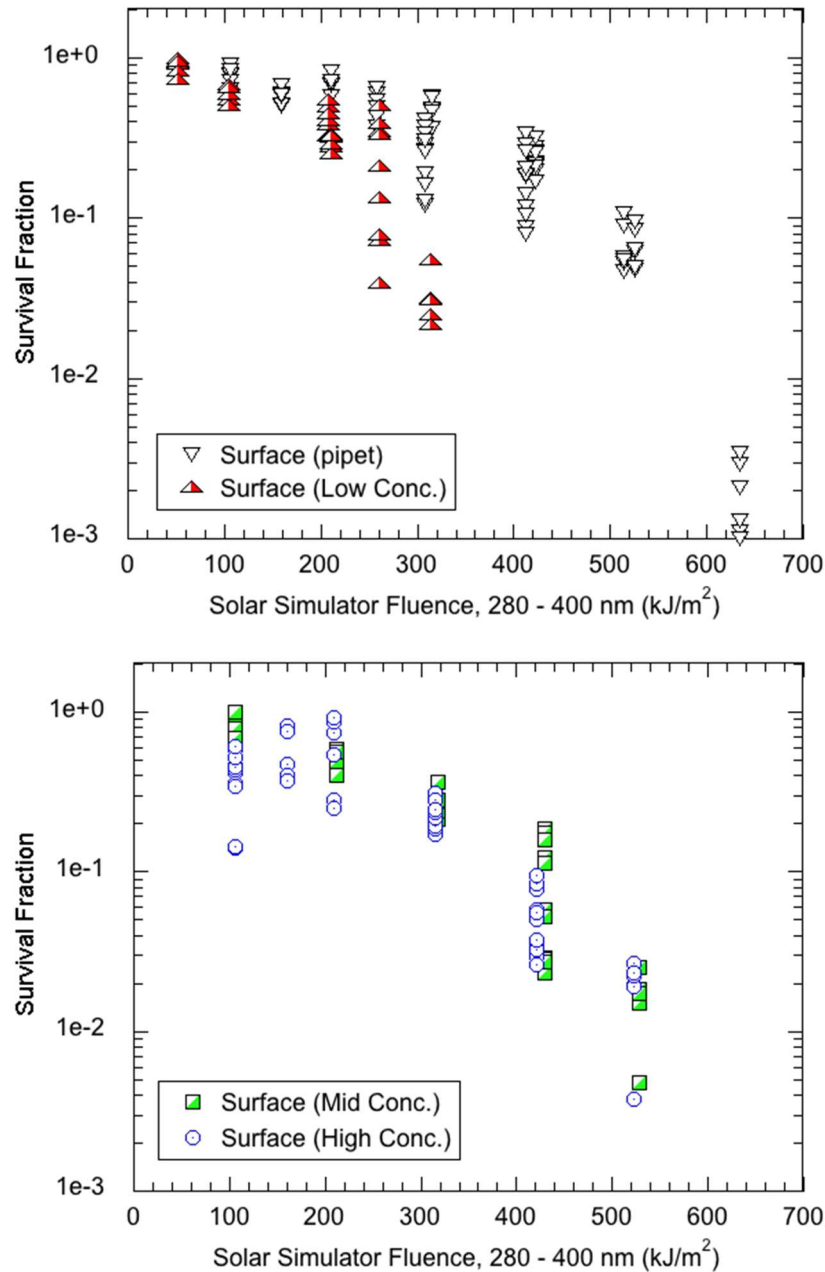


Fig. 35—Survival fraction of (top) single spores pipetted and 1–2- μm particles aerosol-deposited and (bottom) 2.5- μm and 4- μm clusters of Ba Sterne spores on membrane filters exposed to simulated solar radiation (ASTM 177)

5.3 Biological Decay Rate Analysis of Irradiated Spores: Curve Fits and Parameter Extraction

In this section, the survival fraction vs. fluence data shown in the previous section is used to evaluate curve fits of the various spore sample experiments in terms of the two-parameter multihit equation discussed in Section 5.1 (Eq. 11). From an empirical point of view, there are two significant features regarding the decay of the survival fraction (SF) as a function of radiation fluence. First is the breaking point, or fluence value, demarking the end of the shoulder region of the curve and the beginning of the rapid-decay portion of the curve. Second is the rate of the rapid-decay portion. These two curve features are directly reflected by the

values of the two parameters used in the multihit formula. The recovery factor, n , controls the extent of the shoulder portion of the curve, while the decay rate, k , precisely describes the linear rate of decay on a log-linear plot. However, these two factors are somewhat codependent in the sense that the transition from the shoulder region to the exponential-decay region has greater or less curvature depending on both values. From an experimental point of view, accurate determination of k should be reasonably straightforward to extract, and once a good estimate of k is obtained, then an evaluation of n should easily follow.

However, in practice, the most significant data values for the determination of k come at a point where the measured signal (culture counts) effectively decreases to zero. The experimental issue is how low a value of SF is it feasible to measure. For classical microbiological experiments with samples in petri dishes, starting with initial populations greater than 10^7 or even 10^9 are not uncommon, and consequently, measurement of multiple logs of population decline are readily achievable. But in our specific case, where populations of aerosol particles are being suspended in a LEQ trap, such large numbers of organisms are not feasible. Not only for the aerosol exposure measurements, but also for many of the surface exposure measurements, it was a struggle to determine decays of less than 1% (more than 2 logs decay). Therefore, it was not possible to optimize a fit to the multihit formula with a completely automated process.

Instead, we utilized a data analysis and plotting program, KaleidaGraph™, that includes a capability for least-squares-method fitting of the data to a specified formula with initial guesses for the fitting parameters. This automated approach was performed on the data twice, first in its raw form, and again using its logarithm values. This procedure was adopted because analyzing the SF values directly with regard to Eq. (1) meant that contribution from the most significant values for k was minimized. This can be compensated for by analyzing the logarithms of the SF data and fitting it to the appropriate equation (taking the log of both sides of Eq. (1)). Of course, analyzing the log of the SF meant minimizing the data at low fluence values critical for the determination of n (i.e., in the region where survival fractions are close to unity). The values for n and k extracted from each of these two processes were then used in an iterative manner to achieve a final version of a curve fit that visually appeared optimal. Appendix H provides some examples and describes the way this approach was initially developed. The following section shows final results of this analysis procedure for the samples irradiated by 254 nm, and Section 5.3.2 presents results from analysis of the solar simulator radiation exposures. For the sake of visual simplification in all of the remaining plots, the optimized fit curve is plotted as a continuous line together with the corresponding averaged values of all the replicate data at each wavelength. The continuous curve fit and the data points are color coordinated.

While it is understood that the formulation of the multihit model is intended to be strictly applicable only to individual organisms, data for exposed clusters of organisms were also included in this analytical curve-fitting approach simply from the perspective that it would offer a quantitative means for comparing similarly sized clusters of the surface-exposure and aerosol-exposure experiments.

5.3.1 Analysis of Decay under 254 nm UV Irradiation

Figures 36 and Fig. 37 summarize *B. anthracis* Sterne strain spore cluster particle exposure to 254-nm radiation for the aerosol and surface studies, respectively. Each of these two figures shows data for the smallest particles representing least-shielded spores as well as the larger-spore-cluster particles. For these figures, individual SF data shown in Section 5.2.1 was used to arrive at a best fit (described above) to the two-parameter multihit equation that has been computed and displayed for each data set as a solid line. Values of the data for each experiment have been averaged at each wavelength and plotted together with the fit curve. Table 6 provides a summary of the values of the first-order decay parameter (k) and the recovery factor (n) extracted according to the curve fit.

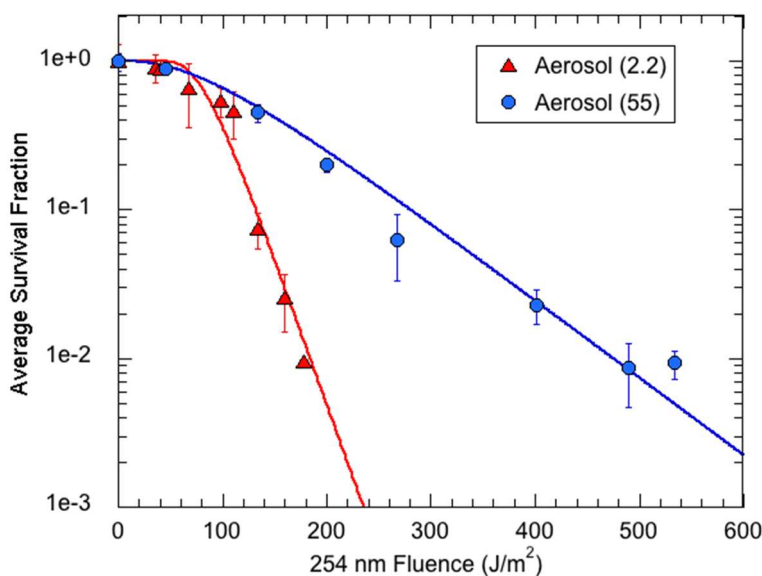


Fig. 36—Average SF vs. fluence at 254 nm for *Bacillus anthracis* Sterne strain spores suspended as aerosols in the LEQ chamber. The two sample types represent small- (red) and large- (blue) cluster particle size distributions with mean sizes of 2.2 and 55 spores per particle, respectively. The multifill procedure enabled measurement of small cluster survival as low as 0.96%. Curve fitting to a multihit model over the linear regions provides the decay rate constants n and k listed in Table 6.

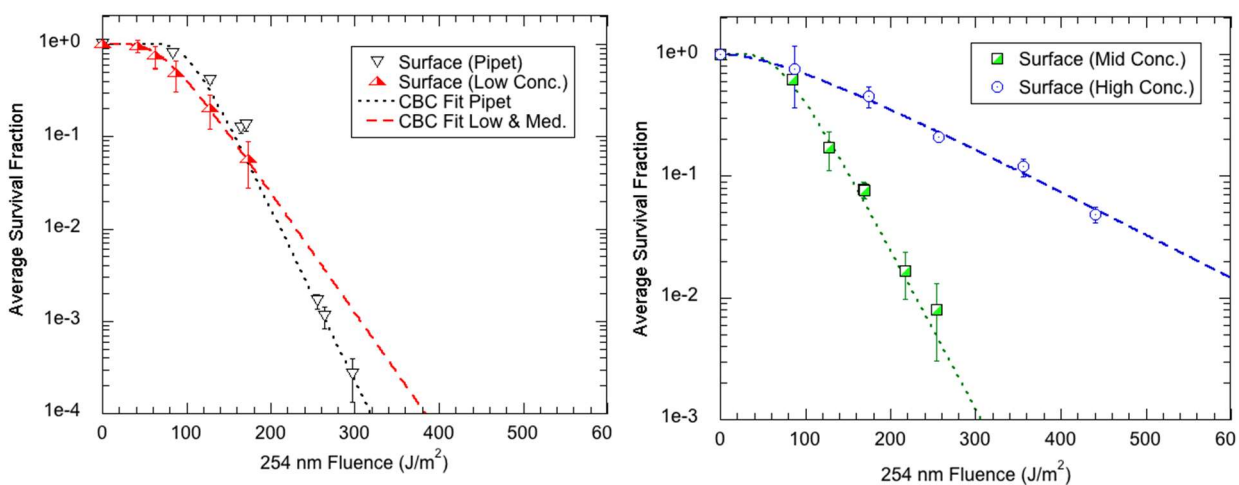


Fig. 37—Average SF vs. fluence at 254 nm for different particle size distributions of *Bacillus anthracis* Sterne strain spores irradiated at 254 nm after deposition onto polycarbonate filter surfaces. The four sample types include (top): single spores pipetted onto the membrane (black), 1–2 μm particles generated by the CAD (red), and (bottom): mid-sized ($\approx 2.5 \mu\text{m}$, green) and large ($\approx 4 \mu\text{m}$, blue) clusters generated by an IJAG. Curve fitting to a multihit model over the linear regions provides the decay rate constants n and k listed in Table 6.

Table 6—Estimated Values of First-Order Decay Rates for Different Particle Sizes^a of *Bacillus* Spores Irradiated at 254 nm

Row #	Exposure to 254-255 nm	Decay Rate (k)	Recovery Factor (n)
1	Aerosol (low conc.)	-0.045	40
2	Surface (pipet)	-0.043	90
3	Munakata et. al. 1991 ⁴¹	-0.038	NA
4	Surface (low conc.)	-0.030	10
5	Surface (mid conc.)	-0.030	10
6	Aerosol (high conc.)	-0.012	3
7	Surface (high conc.)	-0.008	2

^a. Sample concentration determines particle size.

For “single” spores (pipetted samples) and small clusters, the individual log-linear plots in each figure clearly show behavior similar to the examples described in Section 5.1, with well-defined initial shoulder regions followed by steeper linear decays with increasing fluence. In both the aerosol and surface data, smaller, less shielded spore cluster particles decay at a significantly faster rate than the largest, most shielded spore cluster particles, which is consistent with previous studies.^{43,44} This effect is attributed to radiation shielding of spores on the interior of the cluster particle by those in the outer layers of the cluster particle. It should be made clear there is no *a priori* expectation that the multihit formulation should be applicable to spore clusters if they are large enough to exhibit radiation shielding for spores on the interior of the cluster. However, use of the multihit equation to reduce all the data provides a convenient means for quantitative comparison of similar samples with regard to aerosol or surface exposures.

A more detailed discussion and comparison of the quantitative analysis between surface and aerosol exposures is provided in another section below. However, visually, the smallest spore clusters in Fig. 36 and Fig. 37 and the pipetted samples in Fig. 37 exhibit the expected behavior of a defined shoulder region followed by a well-defined first-order exponential decay. Values of the linear decay rate (k) and the recovery factor (n) used for the optimized curve fit are summarized below in Table 6 for each of the sample plots shown in Fig. 36 and Fig. 37. The UVR decay rate shown in Fig. 31 was also included as an independent reference point. It is worth noting that the data shown in Fig. 31⁴¹ was measured by depositing spore samples onto transparent slides for radiation exposure, similarly to the method used to deposit the PAVES pipetted surface samples onto polycarbonate filters. The first four rows in Table 6 contain samples that are supposed to represent unshielded spore exposure decay rates, and while they show fairly close agreement in their k values (from -0.30 to -0.45), their range in n values is more pronounced. Another striking immediate result is that the low concentration and intermediate concentration curve fit parameters of surface exposure (rows 4 and 5) are essentially identical. The samples in last two rows (6 and 7) are the largest spore clusters. These two samples show significantly lower decay rates than those in the first three rows. This result is expected, as previously discussed, due to radiation shielding effects in larger spore clusters. While it is difficult to speculate as to the meaning (if any) of the derived large cluster k and n values, it is nevertheless gratifying to see that they are also reasonably close to each other.

5.3.2 Analysis of Decay under Solar Simulator Irradiation

In a completely parallel fashion, the aerosol exposure and surface exposure data using solar simulator sources is shown in Fig. 38 and Fig. 39. For these figures, individual survival fraction data shown in Section 5.2.2 for both the aerosol and surface studies was used to arrive at a best fit to the two-parameter multihit equation, displayed for each data set as a solid line together with data values for each experiment averaged at each wavelength and plotted together with the fit curve.

For the aerosol exposures in Fig. 38, significantly slower decays are seen for large clusters compared to small clusters, which is consistent with all the previous data. Likewise, for surface exposure results shown in the two plots in Fig. 39, low concentration and pipetted samples decay more rapidly than large clusters, in keeping with an expectation that sufficiently large clusters will shield the inner spores to some extent. Table 7 provides a summary of the parametric values for the optimal curve fits for all the experimental conditions. Note that the k values shown in Table 7 are in terms of the fluence axis scale of kJ/m^2 , so that for comparison of the k values in Table 6 (J/m^2), these would have to be divided by a factor of 1,000 to make them in the same units. Likewise, the recovery factor (n) values for the various experiments were uniformly larger than their counterparts for the 254-nm exposure. Additional discussion and comparisons will be deferred to Section 6, where comparative plots of the surface exposure and aerosol exposure data will be presented.

Figure 39 results indicate that no significant differences in decay were observed for 2.5 μm and 4 μm spore clusters; however, the exposure of single spores after aerosol deposition resulted in a faster decay than 2.5 μm and 4 μm spore clusters. Pipetted single spores showed a slower decay.

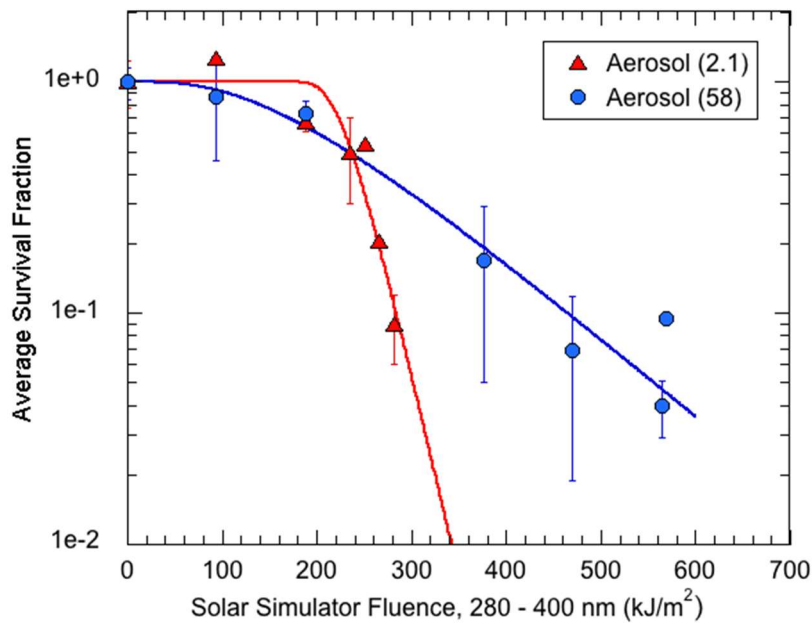


Fig. 38—Average SF of aerosol particles of similar sizes as in Fig. 36 after exposure to simulated solar radiation (ASTM G177) in the LEQ aerosol chamber. Sufficient multiple fills for small cluster exposure times beyond 90 min were infeasible under the project schedule. Curve fitting to a multihit model over linear regions provides the decay rate constants n and k listed in Table 7.

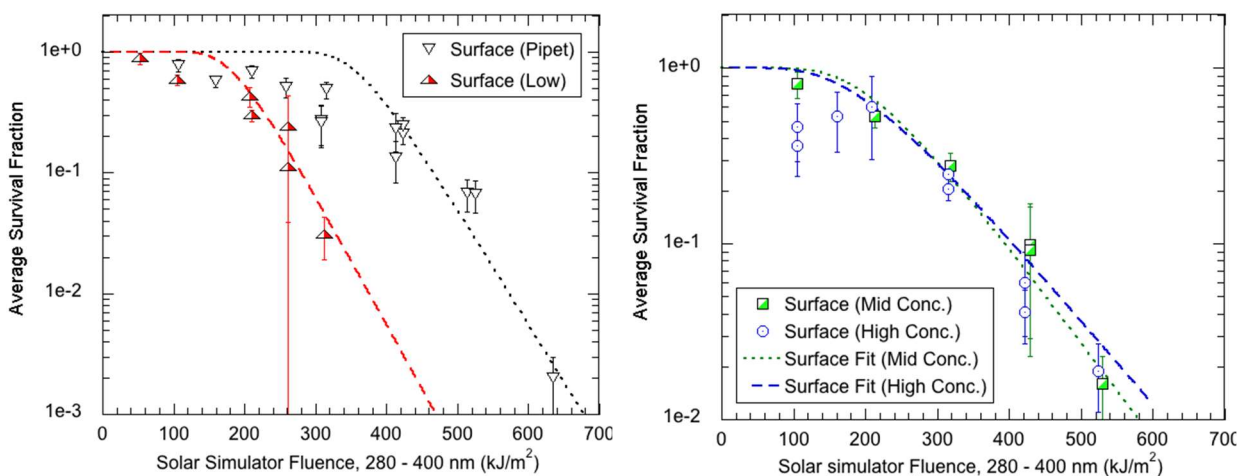


Fig. 39—Average SF vs. fluence of particles of similar sizes as in Fig. 37 exposed to solar radiation (ASTM G177) after deposition onto polycarbonate filter membrane surfaces. The four sample types include (top) single spores pipetted onto the membrane, 1–2 μm particles generated by the CAD, and (bottom) mid-sized and large clusters generated by an IJAG. Curve fitting to a multfit model over the linear regions provides the decay rate constants n and k listed in Table 7.

Table 7—Estimated Values of First-Order Decay Rates for Different Particle Sizes^a of *Bacillus* Spores Irradiated by a Solar Simulator

Exposure to 280 - 400 nm ^b	Decay Rate (k)	Hit Factor (n)
Aerosol (low conc.)	-0.040	9,000
Surface (pipet)	-0.022	3,000
Surface (low conc.)	-0.245	100
Surface (mid conc.)	-0.0126	15
Aerosol (high conc.)	-0.0078	3.9
Surface (high conc.)	-0.011	9

^a. Sample concentration determines particle size.

^b. Wavelength range over which integrated fluence causes biological decay.

6. COMPARISON OF SURFACE AND AEROSOL DEGRADATION AND DISCUSSION

This section presents four figures comparing exposures of *B. anthracis* spores irradiated while suspended as aerosols in the LEQ chamber with similar spore samples deposited and exposed on polycarbonate filter membranes. These two methods of sample exposure are described in detail in Sections 4 and 3, respectively. The plots below show the same average survival fraction (SF) values versus radiation fluence together with corresponding multihit model curve fits presented in Section 5.3, but they are rearranged here to permit direct comparison of decay rates for similar surface-deposited and aerosol samples. The plots are presented in two successive sequences. First, by illumination source: germicidal Hg lamp (254 nm) (Section 6.1) and solar simulator (Section 6.2), and then within each illumination source section by cluster size: (a) small clusters (notionally single spores) and (b) larger clusters.

6.1 Surface vs. Aerosol Decay under 254-nm Radiation

Figure 40 provides a plot showing three sets of data and their corresponding curve fits representing the smallest spore sample clusters: (1) exposure as aerosol with an average of ≈ 2.2 spores per cluster (red, solid curve and red triangles), (2) exposure of a spore sample solution pipetted directly onto filters (open triangles and black, dotted curve) and (3) exposure of low-spore-concentration samples aerosolized by the CAD apparatus, allowed to dry while suspended in air and collected onto filters (red/white triangles and a red, dashed curve). One can immediately see that all of these samples exhibit a well-defined shoulder region initially, followed by a rapid linear decay with increasing fluence (on a logarithmic Y-axis and linear X-axis). The decay rates given by the slopes of the linear part of the decay curves are summarized in Table 6. One can readily see the pipetted filter sample and the aerosol sample exhibit essentially the same decay rate: $k = 0.044 \pm 0.002 \text{ (J/m}^2\text{)}^{-1}$ (within experimental error). For these two data sets, this feature implies that neither the aerosol sample nor the pipetted sample includes any significant effects of shielding that would decrease the decay rate from a single spore value (or, at least, they both coincidentally provide equivalent amounts of shielding).

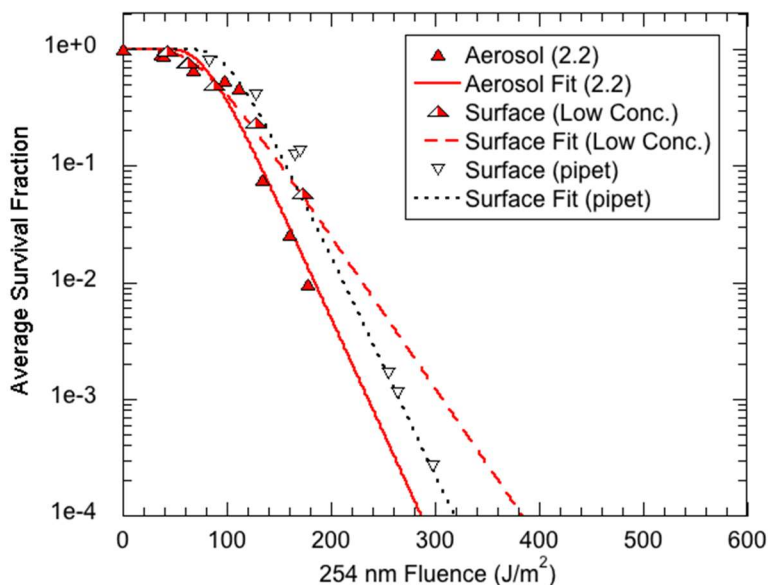


Fig. 40—Effect of 254-nm radiation on the survival fraction of *B. anthracis* spores in small ($\approx 1\text{--}2 \mu\text{m}$) particles when exposed as LEQ aerosols (solid red) or after deposition by pipet (open black) or by CAD (open red) on polycarbonate filter surfaces

Among the three sets of data, the pipetted samples provide the greatest range of survival fraction due to the large number of spores (up to 10^6) that could be repeatedly loaded onto a single filter with this method. Consequently, it was possible to determine a decrease of more than 3 orders of magnitude by direct culture plating and colony counting. In general, the dynamic range of the survival fractions for samples involving aerosols (including those collected onto filters for exposure) was typically closer to 2 orders of magnitude due to the decreased total amount of clusters (spores) that could be feasibly collected. For the low concentration spore cluster samples exposed as aerosols, two orders of magnitude decrease were obtained using the multifill sampling method previously described. In the case of low concentration sample clusters collected and exposed on a filter surface, the linear decay rate is critically determined by the lowest SF value of 0.06 and the next lowest data point at 0.22. Consequently, uncertainty in the linear decay rate associated with this data set is somewhat larger compared to the other two sets of data.

Returning to the pipetted and aerosol-exposure sample decay curves, the main difference between them is that the shoulder region extends to a slightly higher fluence for the pipetted sample than for the aerosol sample. The appearance of these two plots is that one of the decay curves has been horizontally translated with respect to the other by a differential fluence amount of about 30 J/m^2 . At first, this fact raised the possibility that perhaps some systematic error had been made between the two UV intensity calibration instruments used by aerosol and surface exposure experimental groups. However, a careful review showed that that was not a viable possibility. In January 2020, both NRL and CBC met at the National Institute for Standards and Technology (NIST) in Gaithersburg, Maryland, to conduct a calibration experiment with each of their UV radiometers in comparison with a third radiometer maintained by NIST at the Synchrotron Ultraviolet Radiation Facility III (SURF III).⁴⁵ From the calibration measurements taken there, a correction factor was obtained for each of the UV radiometers to normalize them to the NIST standard radiometer reading. From these data, the relative variation between the corrected NRL and CBC UV radiometers is within $\pm 2.5\%$, which is too small to account for the discrepancy between the aerosol and pipette sample decay curves shown in Fig. 40. Taken at face value, the implication of these two sets of data is that the pipetted sample was somewhat more repair capable than the aerosol sample, which is reflected by the difference in their respective recovery factors of 90 and 40 from the curve-fitting process as shown in Table 6. The fact that the decay rates for the two samples are essentially the same eliminates any alternative explanation that perhaps the pipetted sample contained layers of spores that could afford some radiation shielding for those samples.

The remaining plot shown in Fig. 40 is data from the CAD-generated low-concentration aerosol subsequently collected on filters for exposure. Comparing it to the other two data sets, we see that the decay rate appears to be lower (less slope). However, the recovery factor is also lower. Empirically, these features are consistent with results from the remaining data sets for larger clusters. Consequently, discussion of the low-concentration filter-exposed data is deferred to the discussion of Fig. 41.

Figure 41 also shows plotted curves of three data sets, similarly to Fig. 40. Here, the data sets are: high-concentration spore sample suspension clusters exposed as aerosols (solid, blue circles and solid, blue curve), high-concentration spore sample suspension clusters collected and then exposed on filter surfaces (open circles and dashed, blue curve) and intermediate-concentration spore suspension clusters collected and then exposed on filter surfaces (white/green squares and dotted, black curve). In general, all of these data sets display a shoulder region at low fluences but increasing slopes that become linear at high fluences. While this is descriptive, there is no underlying mechanism for expecting these curve shapes as there is for samples of individual spores (e.g., provided by the multihit formula) since the received radiation of each spore in a given cluster could vary significantly if the cluster is large enough. Nevertheless, the data shows that the two samples of largest clusters (highest-concentration sample materials) show the slowest rates of decay consistent with our expectations regarding the radiation shielding mechanism afforded by a large cluster for organisms near the center of the cluster. Data for medium-concentration suspension droplets (producing mid-sized clusters) exhibits a distinctly faster decay rate compared to either of the large cluster samples, consistent with an expectation that a smaller cluster would afford less shielding. Nevertheless, the surface-exposed large clusters

also have a linear decay rate that is slower than the large clusters exposed as aerosols. This difference is greater than can be accounted for by experimental error. Table 6 summarizes the decay rates for each of these samples from largest to smallest as: -0.030 , -0.012 and -0.008 $(\text{J/m}^2)^{-1}$, with recovery factors of 10, 3, and 2, respectively. Again, the concept of a recovery factor in a strict sense is lost for large clusters. For our purposes here, it is simply a quantitative parameter from curve-fitting. Nevertheless, it seems that the two large-cluster data sets are significantly distinct from the intermediate-cluster data set in terms of both curve-fit parameters. It is unfortunate that the program schedule did not permit exploration of an intermediate cluster size samples exposed as aerosol for comparison to the intermediate cluster size samples shown here for surface exposures. It is also clear that reduced decay rates (due to shielding in large clusters) are tied to reduced recovery factor values using the multihit formula as a generic, two-parameter curve-fit procedure. Returning to the data in Fig. 40 from the CAD-generated low concentration samples, it is unexpected that the curve-fit parameters for this sample are essentially identical to those of the intermediate concentration samples generated by the IJAG. For both of these samples, the independently determined decay rate value is 0.03 $(\text{J/m}^2)^{-1}$ and the recovery factor value is 10. This result is unexpected given the differences in the sample suspension concentrations and the different methods by which the sample droplets were generated. Nevertheless, it does additionally indicate that the low-concentration, CAD-generated clusters are not representative of single spores, as originally intended.

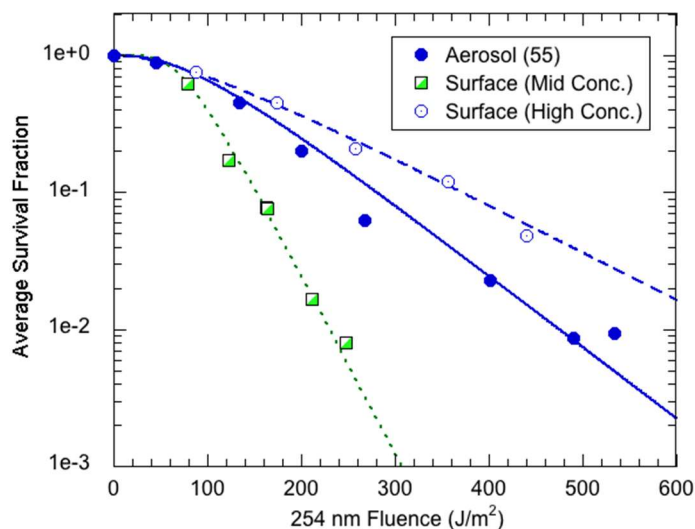


Fig. 41—Effect of 254-nm radiation on the survival fraction of *B. anthracis* spores in large (≈ 4 μm , blue) and mid-sized (≈ 2.5 μm , green) clusters when exposed as LEQ aerosols (solid) or after deposition (open) by IJAG onto polycarbonate filter surfaces

6.2 Surface vs. Aerosol Decay Under Solar Simulator Radiation

This section provides the same type of comparative data plots of similar types of samples with solar simulator (SS) irradiation instead of the 254-nm Hg vapor lamp source in the previous section. The convention adopted to calibrate SS irradiation was based on the ASTM G177 solar spectral standard described in Section 2.4.2. Normalizing SS spectra over the 280–400-nm band provides *Bacillus* spore decay parameters (see Table 7) that can be applied to modeling of environments for a wide range of insolation conditions. With this normalization, plots of the SS decay curves shown previously in Section 5.3.2 have a fluence scale of kJ/m^2 , which implies that the effectiveness of the SS radiation for promoting photochemical degradation reactions in the spore DNA is substantially reduced due the longer wavelength of the SS output relative to the 254-nm source. However, it is not as simple as just comparing the scales and concluding that there is a 1,000x difference in effectiveness between 254-nm radiation and solar radiation. Actinic dose refers to the radiation

absorbed that produces a specified effect, in this case, the photochemical reactions that damage the spores' DNA. As pointed out in Section 2.4.3, most of the wavelengths included in the solar normalization will not have any contribution to these reactions. Referring to Fig. 9, a rough estimate of the effective SS spectral band for photochemical reaction would be between 297 and 327 nm. Integrating the intensity values over this 30-nm band yields a value of 7.07 W/m^2 or roughly 11% of the total SS radiant energy from 280 to 400 nm. Therefore, conversion of decay rates given in Table 7 to actinic values more comparable to the decay rates for 254 nm shown in Table 6 should divide them by a factor of 110 rather than 1,000 to obtain decay constants in J/m^2 .

Recognizing the spectral band responsible for photochemical degradation of the spores is confined between roughly 297 and 327 nm may have a collateral benefit as well. The formulation of the multihit model is, strictly speaking, applicable to radiation at a specific wavelength in the action spectrum. It is difficult to generalize this formulation for a broad, continuous spectrum such as SS irradiation. A relatively recent publication¹⁶ has tackled the problem of computing degradation due to continuous spectra by formulating the problem in terms of photon interactions rather than classical radiation energy. However, the fact that the actinic spectrum in our particular case is relatively narrow may lend some validity to the multihit model approach taken here.

Figure 42 provides a plot showing two sets of data and their corresponding curve fits representing the smallest spore sample clusters (notionally single spores): (1) exposure as aerosol generated with the low-concentration spore suspension that results in smaller clusters, nominally with an average of ≈ 2.2 spores per cluster (red solid curve and red triangles), and (2) exposure of a spore sample solution pipetted directly onto filters (open triangles and black, dotted curve). Based on the 254-nm exposure data results discussed above (Fig. 40), low-concentration, CAD-generated aerosol data results have been combined with the larger spore cluster samples (see Fig. 43) rather than with these samples that notionally represent single spores. In general, differences between the surface-exposed pipetted sample data and the aerosol exposed small cluster sample data are similar to the 254 nm results in that the pipetted sample data shows a significantly extended shoulder region relative to the aerosol exposed sample data. In Table 7 the normalized SS decay rates are given as -0.000040 and $-0.000022 \text{ (J/m}^2\text{)}^{-1}$ with corresponding recovery factors of 9,000 and 3,000 respectively. Unlike the situation with 254-nm radiation, these two samples show somewhat different decay rates given by the slopes of the linear regions of their decay curves. Despite this difference, a similar conclusion is drawn that the relative repair capability of the pipetted sample is greater than that of the aerosol exposed sample. An apparent conflict with regard to the inverted order of the recovery factor parameters is understandable in terms of the difference in decay rates. Had the two sets of data shown similar decay rates (regardless of what their absolute values were) while retaining the difference in the "breaking point," or extent of the shoulder region, then the recovery factor parameters would have also reflected the fact that the pipetted sample shoulder region was greater than the that of the aerosol sample data. A higher slope of the aerosol sample data puts the value of the recovery factor higher despite the fact that the shoulder region is substantially shorter. That being said, the remaining question is why do the decay rates differ by almost a factor of 2 for the SS exposure experiments when there was such good agreement for the 254-nm exposure data?

As discussed at the beginning of this section, actinic decay rates are about two orders of magnitude lower for the SS radiation dose compared to that for the 254-nm radiation dose, and experimentally, this translates to much longer exposure times. It also means that other environmental factors affecting spore viability will have a relatively greater contribution to the total rate of degradation. From prior studies,^{46, 47} we know that environmental parameters such as relative humidity (RH) and temperature can also influence spore state and viability. Initially, our intent was to directly explore dependence on these two environmental factors as part of this program. Indeed, the CBC research team conducted experimental investigation of these factors as presented in Section 3.2 by establishing active control over those two factors. As shown in Fig. 19 under conditions of SS radiation with longer exposure times, differences in survival fractions with factors of 2 to 3 were observed for modest changes in temperature and a variation between 20% and 80% RH. The NRL

aerosol measurement effort took so much time to develop the LEQ chamber hardware and to resolve sample collection procedures that active control of temperature and RH had to be reduced to measurement and monitoring to ensure consistent run-to-run results. Therefore, it is possible that the observed differences in spore decay rates could be the result of slight differences in these other environmental factors over the long exposure times of these samples combined with the relatively weak effect of the SS radiation. We note that even with the multifill method for increasing total spore number in the collected sample that the aerosol exposure was only able to achieve an ultimate survival fraction of 0.08. At the greatest fluence, the exposure times were more than 90 minutes, and three fills (4.5 hours of total exposure) the collection of a single data point was taking more than 9–10 hours to achieve. While it would have been desirable to perform more measurements to explore more parameters, the program schedule could not accommodate them.

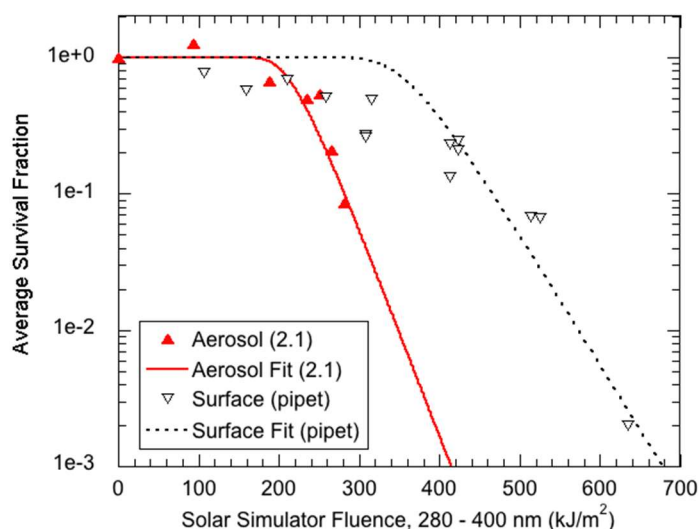


Fig. 42—Effect of simulated solar radiation on the survival fraction of *B. anthracis* spores in small ($\approx 1\text{--}2\ \mu\text{m}$) particles when exposed as aerosols (solid red) or after deposition by pipet (open black) or CAD (open red) on polycarbonate membrane surfaces

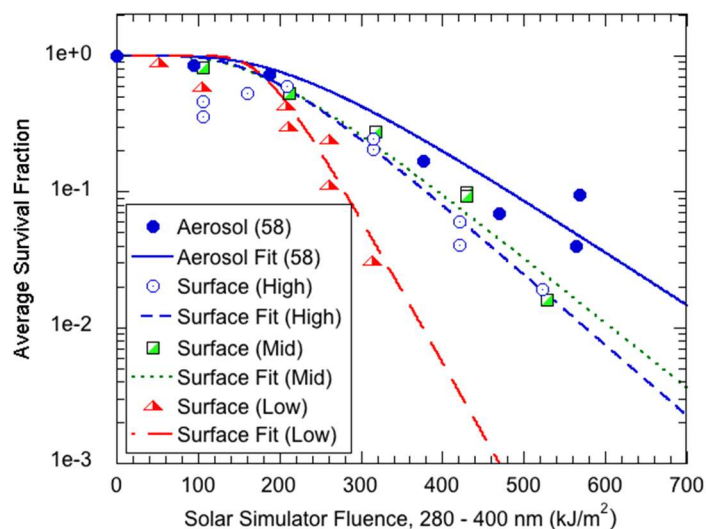


Fig. 43—Effect of simulated solar radiation on the survival fraction of *B. anthracis* spores in large ($\approx 4\ \mu\text{m}$, blue) and midsized ($\approx 2.5\ \mu\text{m}$, green) clusters when exposed as aerosols (solid) or after deposition by IJAG (open) on polycarbonate filter surfaces

In a similar fashion, Fig. 43 shows plotted curves of sets of data: high-concentration spore sample suspension clusters exposed as aerosols (solid, blue circles and solid, blue curve), high-concentration spore sample suspension clusters collected and then exposed on filter surfaces (open circles and dashed, blue curve), intermediate-concentration spore suspension clusters collected and then exposed on filter surfaces (white/green squares and dotted, black curve), and exposure of low-spore-concentration samples aerosolized by the CAD apparatus, allowed to dry while suspended in air, and collected onto filters (red/white triangles and a dashed, red curve). Unlike the previous situation with 254-nm radiation, the low-concentration CAD generated data separates from the IJAG generated mid-concentration results. The slope of the linear portion of the low-concentration CAD-generated cluster data curve provides a decay rate value of only $-0.000025 \text{ (J/m}^2\text{)}^{-1}$ which is within experimental error of the decay rate of the pipetted sample data. Nevertheless, the decay curve exhibits a short, rounded shoulder that has come to be associated as a feature of spore clusters with a recovery factor value of 100, roughly more than an order of magnitude lower than for pipetted and aerosol samples. Data from intermediate- and high-concentration spore sample clusters generated by the IJAG exhibit decay curves that appear the same within experimental error, with slopes of -0.000013 and -0.000011 , and recovery factors of 15 and 9.0, respectively. This result is unexpected and, at least at this point, unexplained. Apparently, influences of other environmental factors such as temperature and RH are also mitigated to a great extent by the protection afforded by large clusters. For high-concentration spore samples generated and exposed as aerosols, the data yields the slowest decay of all samples, with a decay rate of -0.000008 and a recovery factor value of 3.9. This result is also unexpected because aerosol exposure of large clusters at 254 nm yielded a slightly faster decay than the corresponding samples exposed on surfaces. This is significant because there was an expectation that perhaps the surface exposed samples for large clusters might offer greater shielding than the aerosol clusters of similar size. This expectation comes from the fact that surface clusters would have fixed orientation with respect to the incident radiation, whereas aerosol cluster exposures would be isotropic (roasted equally on all sides) because they are free to rotate their orientation with respect to the incident radiation.

One final observation applies to the plots in Sections 6.1 and 6.2. A feature that appears to be consistent throughout all of the different sample exposure data sets presented, is the data for cluster sizes greater than the “single spore” samples appear modeled and fit to the MH formula as providing rounded transitions from the shoulder region to the steeper linear decay region of the decay curves. Generally, for all intermediate and larger clusters regardless of radiation source, the decay curves begin to depart from the initial, flat region *before* the curves for single spores do. This seems counterintuitive, since clusters of spores should not have any ability to *enhance* radiation exposure to a particular spore within the cluster. All of the MSTM model predictions for individual cluster absorption rates are below the value of a single spore’s absorption rate. If that is the case, then how could clusters of spores begin to show decay before single spores? This feature could perhaps be an artifact of the use of the MH formula for fitting the SF vs. fluence data, but the consistency with which it appears in the plots. Of course, in these experiments, the viability of individual clusters is not being determined. Once the clusters (exposed either as aerosols or on filters) are eluted for measurement by plating and culture colony counter, the samples are then subjected to intense agitation (vortexing) so that the collected clusters are completely disintegrated into individual spores. Consequently, the determination of sample decay (loss of viability) is basically an ensemble average over all individual spores contained in all collected clusters. However, this fact alone still does not account for this observed decay curve feature.

7. COMPUTATIONAL MODEL DEVELOPMENT AND ANALYSIS OF OPTICAL PROPERTIES FOR RANDOM CLUSTERS OF PRIMARY PARTICLES

Modeling the UV intensities within the spores of clusters of spores on surfaces is a new component of the overall modeling effort. (Refer to Appendix G for background on the spore cluster model development.) In this approach, individual spores are modeled as homogeneous spheres, and the internal electromagnetic field intensities of clusters of a fixed number of homogeneous spheres (touching but randomly placed) is rigorously calculated from first principles in order to investigate the distributions of incident light radiation inside individual “spores.” The computational method for this is referred to as the multi-sphere T-Matrix (MSTM) model.^{48,49} This approach allows an unambiguous visualization of the relative radiation intensities to which each spore in the cluster is subjected. Although the presentation here strongly emphasizes spores, most of the analytical method described could readily be applied to vegetative bacterial samples as well, or, for that matter, any agglomeration of individual units that can justifiably be approximated as spheres. In this method, the absolute amount of radiation absorbed in the individual units of the cluster are determined by their optical properties (meaning complex refractive index). One of the greatest uncertainties of inputs to this model are the values of complex refractive index as a function of the incident light wavelength. We anticipate that results from an independent experimental investigation of these values may be available in the near future. Each cluster of spheres (representing spores or other organisms) may be encased in a spherical homogeneous matrix material of known composition, or may be clean, with only air in the spaces between the organisms. When the spore cluster was encased within a matrix material, the encasing sphere was modeled as one more sphere (a large one) in the MSTM. (See the description of the homogeneous sphere model in Appendix G) During the performance period of this report, the main activity was to extend the freely suspended cluster model to include clusters sitting on flat surfaces.

7.1 Multi-Sphere T-Matrix (MSTM) Cluster Model Approach (Year 1)

To provide a context in this section for the new results, Fig. 44 illustrates UV intensities in clusters of 100 individual spheres (also termed “spores”) enclosed within a spherical matrix. The incident intensities within each of the spores of the cluster were calculated using the MSTM method. The complex refractive indexes (m) chosen as a hypothetical case for the individual spheres are $1.5 + i\ 0.001$. The large sphere that encloses the 100 smaller spheres has a size parameter of 72.466, which corresponds to $d = 6.921\ \mu\text{m}$ at the incident wavelength (λ) of 300 nm.

The m of the enclosing sphere matrix has values of $1.0 + i\ 0.0$, that is, the cluster is only spores in air with no other matrix. The yellow arrow indicates the direction of propagation of the illuminating incident (plane wave) UV radiation. The amount of radiation absorbed by each sphere is indicated by their color, according the color scale on the right of each figure. Because, for the clusters with 100 spores, only a fraction of the spheres in each cluster are visible from any viewing direction, red or white spheres with the highest intensity in that cluster may not be visible in the illustration.

Since the material between the spores in Fig. 44 is air, the refractive index difference at the spore-air interface is relatively large. Therefore, the light scattered at these many surfaces throughout the cluster is relatively large. The black and purple spheres on the side opposite the incoming wave (opposite the yellow arrow) illustrate that these spores are partially shielded by the other spores in the cluster. Although both the scattering and the absorption by the upstream spheres contribute to the shielding, the small value of m_i , 0.001, suggests that scattering is the dominant contribution to this shielding. The variation of the UV intensity absorbed by the spores with the distance across the large sphere is more clearly illustrated in Fig. 45, in which the internal intensity of radiation in each of the 100 individual spheres is plotted on the vertical axis against their distance along the diameter of the cluster parallel to the incident light beams (yellow arrow in Fig. 44).

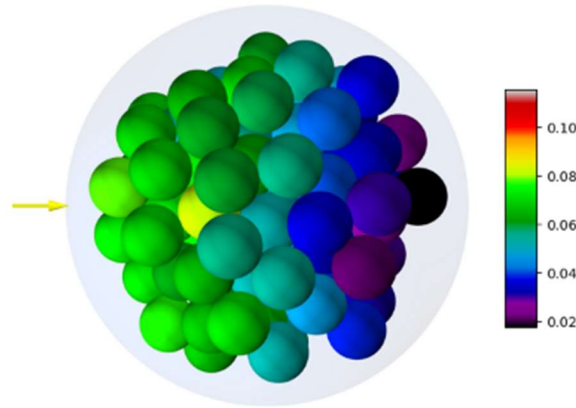


Fig. 44—False color illustration of UV intensities absorbed by individual model spores of a cluster of 100 spores. Each spore is modeled as a homogeneous sphere with complex refractive index $m = 1.5 + i 0.001$. The enclosing sphere has $m = 1.0 + i 0.0$, as does the surrounding air. The illuminating UV plane wave ($\lambda = 300$ nm) propagates in the direction of the yellow arrow. The color scale on the right indicates the UV intensity absorbed by each sphere.

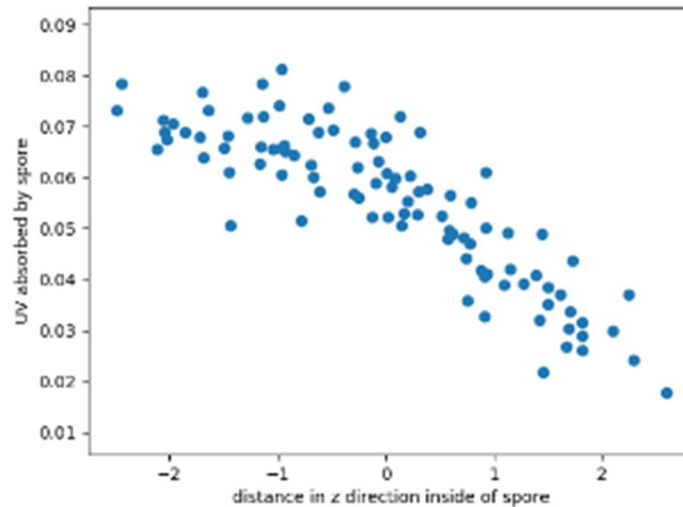


Fig. 45—UV energy absorbed for each spore in a cluster of 100 vs. radial position of the spore in the direction of propagation of the incident wave (from negative z to positive z). The individual spheres have complex refractive index $m = 1.5 + i 0.001$ and the enclosing sphere has $m = 1.0 + i 0.0$ (as in Fig. 44).

The value of the location of each sphere center in a plane perpendicular to this diameter is shown on a scale ranging from -3 to $+3$, where the unit of distance is the diameter of a single sphere (spore, notionally $1 \mu\text{m}$). (A cluster of 100 $1\text{-}\mu\text{m}$ spheres notionally would be approximated by equivalent volume to a diameter of $(100)^{1/3} \mu\text{m}$, or roughly $4.6 \mu\text{m}$ (i.e., $\pm 2.3 \mu\text{m}$ from center) if the packing density could be 1.0. For a more reasonable packing density of 0.6, the diameter could be closer to $(100/0.6)^{1/3} \mu\text{m}$, or roughly $7.7 \mu\text{m}$ (i.e., $\pm 3.87 \mu\text{m}$ from center). Note that from the angle illustrated in Fig. 44 none of the spheres appears to be in contact with the surface of the outer sphere. The range of absorbed energy among the 100 spores is about a factor of 4.5 in this case. (See Fig. 45.)

7.2 UV Intensity in Models of Spore Clusters on Planar Surfaces: Development and Results (Year 2)

In Year 2, the absorption efficiencies of clusters of spores on surfaces were calculated. To provide a context for the new model development and results, Fig. 46 illustrates UV intensities in clusters of 54 spheres (also termed “spores”) enclosed within a spherical matrix that is in contact with a planar surface.

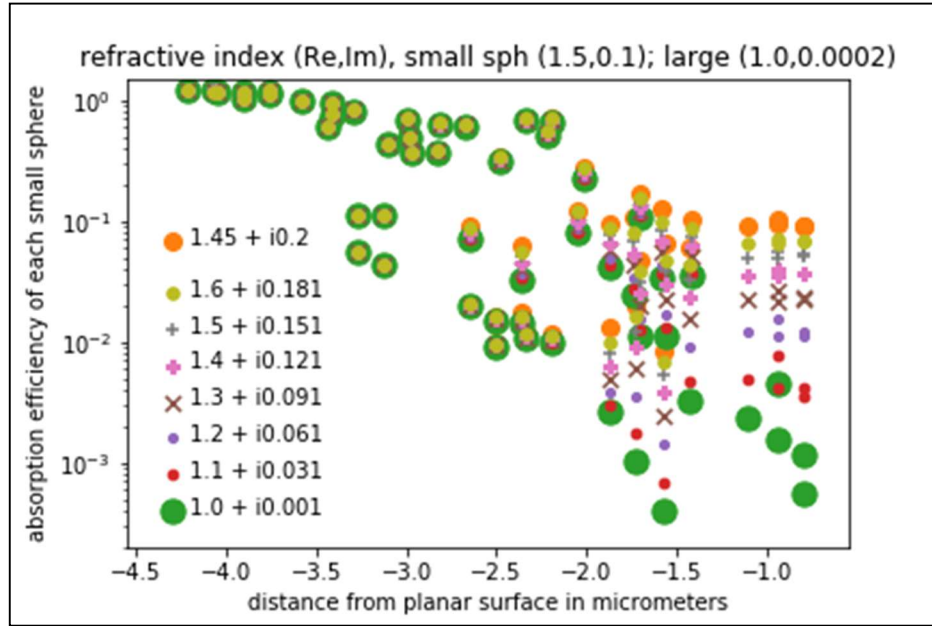


Fig. 46—Absorption efficiency at 300nm (vertical axis) is plotted for each spore in a cluster vs. the distance of each sphere from the surface. The surface is perpendicular to the direction of propagation of the incident wave (from negative z to positive z). The individual spheres have complex refractive index $m = 1.5 + i 0.1$. The sphere matrix enclosing the cluster has $m = 1.0 + i 0.0002$ (for air). The complex refractive index of the surface in each of the eight cases is noted in the legend by the marker for that surface. Individual small spheres are 1 μm in diameter, and each cluster has 54 individual spheres.

The incident intensities within each of the spores of the cluster were calculated using the MSTM method as extended to include a planar surface. In Fig. 46, the complex refractive index (m) chosen as a hypothetical case for the individual spheres is $1.5 + i 0.1$. The large sphere that encloses the 54 smaller spheres has $m = 1.0 + i 0.0002$ to approximate air. The 0.0002 for the imaginary component of m is higher than that of actual air but is used to avoid computational issues in dealing with the interaction of the cluster of spheres with the planar surface. The diameter of the sphere enclosing the smaller spheres is 5 μm . The wavelength $\lambda = 300 \text{ nm}$. The absorption efficiencies are illustrated for eight different planar surfaces, where the refractive indexes are given in the legend. The sizes and shapes of the markers are chosen so that it is possible to tell when a marker is being covered. The large, green dot is for a surface with the refractive index of air, but with a relatively large imaginary component (0.001) to avoid computational problems. Although 0.001 is much larger than the imaginary component of m for actual air, any differences for these calculations is very likely to be so small as to be unnoticeable. In Fig. 46, the spheres closest to the surface, i.e., the spheres on the far right, are most strongly affected by the surface. Some differences are large. The absorption efficiencies for some of the spheres increase by more than 200-fold as the surface changes from one approximating air (green dots) to one with $m = 1.45 + i 0.2$ (orange dots). In comparing the spheres near the surface for two different refractive index values ($m = 1.45 + i 0.2$ vs. $1.6 + i 0.181$), it appears that the imaginary part of refractive index (m_i) is more important in increasing the intensity in the spheres near the surface than is the m_r (real part of m).

7.3 UV Intensities in Models of Nonspherical Spores (Year 3)

One key objective of the year 3 modeling effort is to develop accurate computational methods of predicting the distribution of ultraviolet electromagnetic intensity within realistically represented spores. The models developed in years 1–2 was based on idealized representations of biological particles. Specifically, they were assumed to be spherical and homogeneous in composition. The advantage of this approach is that it leads to an exact-yet-efficient computational scheme and makes feasible the relatively fast calculation of intensity distributions within clusters of spherical spore particles over a range of UV wavelengths. However, the spores in this study are much more accurately represented as prolate ellipsoids. In addition, experimental components of the project were performed with spores in contact with a surface, and the presence of the surface can alter the incident radiation conditions from those associated with isolated, airborne particles.

The computational model used to predict the distribution of EM intensity within the nonspherical particles is based on a volume integral solution to the time-harmonic Maxwell's equations.⁵⁰ The formulation involves a discretization, into small volume elements, of the refractive index distribution within the particle, and as such has no restriction on the shape, composition, and homogeneity of the particles. The original motivation for this formulation was to predict the light-reflection characteristics of discretely inhomogeneous layers of particles, such as those occurring in pigment films, planetary regolith, snow packs, and the like. The formulation, however, can be used for the purposes of this project, i.e., to predict the spatial UV intensity distributions within model particles, either individually or in clusters, representing spores and, potentially, other particles of future interest, such as vegetative cells.

The volume integral computational method has been used to predict the intensity distributions within individual particles having the shape of a prolate spheroid. The initial computations involved particles with an equivalent sphere radius (the radius of the sphere having the same volume) of $0.65\ \mu\text{m}$; this corresponds to a dimensionless particle size parameter of about 10 at 400 nm wavelength. An example of the discretized spheroid having an aspect ratio (major/minor axis length) of 2 is shown in Fig. 47, in which about 5×10^5 discrete volume elements are used to model the particle.

The refractive index of the particle is taken to be $m = 1.54 + 0.001i$, which corresponds to the value used in previous modeling of spore material. Figure 48 shows the predicted absorption efficiency factor of the particle as a function of the particle aspect ratio; for the prolate spheroid, an aspect ratio of 1 corresponds to a sphere. The two curves correspond to incidence directed perpendicular (broadside) and parallel (axial) to the spheroid symmetry axis. The efficiency factor Q_{abs} is defined so that the absorption cross section of the particle is $\pi a_V^2 Q_{abs}$, with a_V the volume-based radius. Since a_V is constant for these calculations, the curves describe the relative change in total particle absorption for a fixed particle volume due to elongation of the particle. The analytical Mie theory prediction is $Q_{abs} = 0.051$, and the volume integral results overpredict the sphere absorption by about 15%; the error is primarily due to the scale of the volume discretization and can be made smaller at the expense of considerably larger computational memory and time requirements.

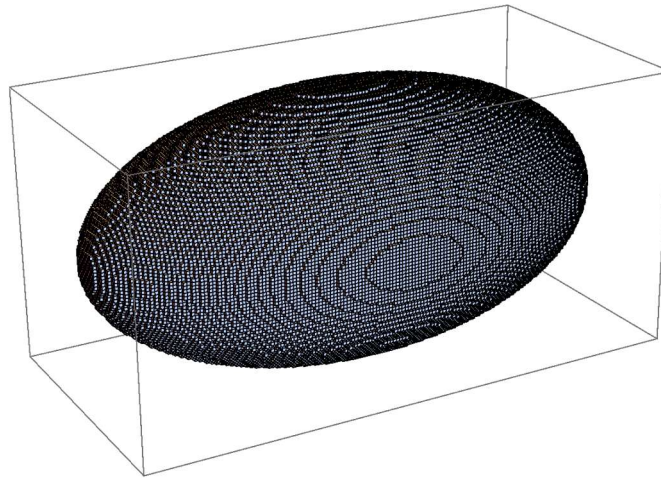


Fig. 47—Discretized particle

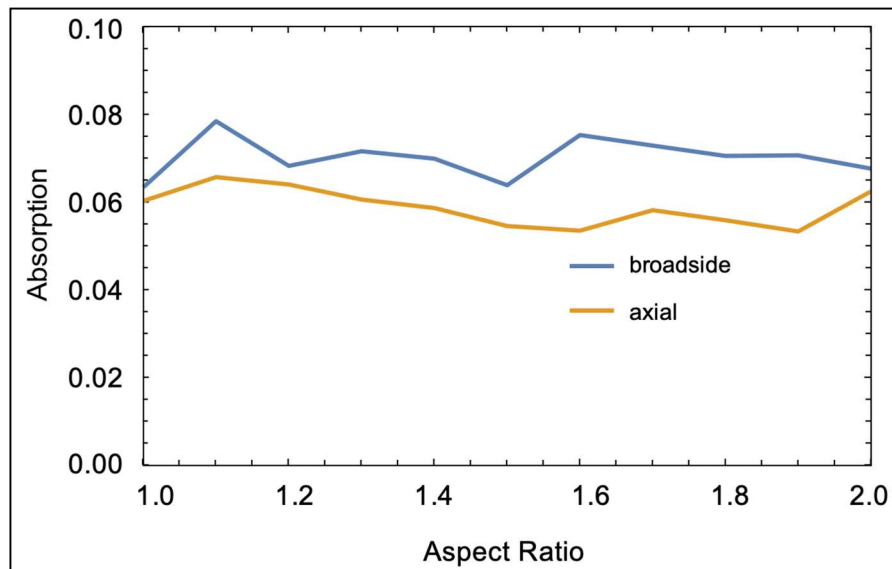


Fig. 48—Absorption efficiency vs. elongation

The calculations indicate that elongation of the fixed particle volume has no apparent overall trend on particle absorption generally, irrespective of the orientation of the particle with respect to the incident beam. This behavior is known to be the case for particles that are either small relative to the incident wavelength, or that have a refractive index magnitude close to unity (i.e., “soft” particles), neither of which is the case here. However, the so-called skin depth, defined as $\lambda/\text{Im}(m)$ and which represents a characteristic length of radiation absorption, is significantly larger than the particle radius for all conditions examined. Under such conditions, the attenuation, due to absorption, of radiation along a line of sight within a particle would be minimal, and the amount of radiation absorbed by the particle would be governed primarily by refraction effects at the particle surface. This behavior is born out in the intensity distribution plots described in the following paragraph.

The intensity distribution within the particle is illustrated in Fig. 49 to Fig. 51. These figures correspond to different geometries of a sphere, a prolate spheroid with an aspect ratio 2 with broadside incidence, and the same prolate spheroid with axial incidence, respectively. The volume-based radius, wavelength, and refractive index are the same as the previous plot, and the contours are normalized with respect to the intensity of the incident beam. All three cases show similar features in that the incident radiation is focused, by the convex shape of the particle, into regions near the side opposite that illuminated. For the sphere and the axial-incidence spheroid, the intensity distribution varies by roughly two orders of magnitude within the particle volume, and a somewhat lower range occurs for the broadside incidence spheroid. This variation in intensity, however, is very much an artifact of the monochromatic plane wave incident beam used in the calculations. As wavelength changes, the specific locations of minima and maxima in the distributions would shift. In this respect, the intensity distributions for natural (continuum) illumination conditions corresponding to a range of wavelengths would be considerably more uniform than those shown in Fig. 49 to Fig. 51.

The work so far has focused on the absorption of EM radiation by individual nonspherical spore particles, and the basic conclusion for the model nonspherical particle examined (a 2:1 prolate spheroid) is that the overall magnitude of the absorption and the distribution of intensity within the particle are not radically different than those for a spherical particle having the same volume and refractive index. A much more important factor affecting the amount of radiation exposure by a single particle is whether the particle is part of an aggregated cluster; this essentially creates a significantly larger overall particle with dimensions that become comparable to the skin depth. For such conditions, computational results using spherical particles have shown that the intensity exposure of spores located in the regions opposite to illumination can be several decades lower than those directly illuminated. It would be expected that similar behavior would occur for nonspherical spores; indeed, nonspherical spores offer a greater packing density than spheres, and in this regard, the characteristic length over which radiation is absorbed by a set amount would likely be smaller for a cluster of packed spheroids than for packed spheres.

A precise modeling of this effect, however, is computationally difficult. Basically, the key advantage of the sphere model — when applied to the calculation of EM scattering and absorption by a cluster of particles — is that the overall degrees of freedom of the model scale with the net surface area of the system. In the traditional volume-integral model (such as the DDA, for discrete dipole approximation), the DOF scale with the overall particle volume, and, for the conditions examined here, the latter is considerably larger than the former. As a specific example, the single particle calculations performed here required about the same computational time and memory requirements as those required for the sphere model applied to a 100-sphere cluster having the same monomer.

Nevertheless, the specific volume-integral method used in the investigation can, with some computational feasibility, be applied to a layer of nonspherical particles having an overall depth of two to three particle radii; this is due to the fact that the formulation is based on periodic conditions in the lateral plane, and for this situation, a system of two or more particles side by side does not significantly increase the overall DOF compared to that for a single particle. However, the DOF will scale linearly with the depth of the particle layer. A possible approach would be to perform calculations on dense deposits of spheroid-shaped particles with the objective of identifying a characteristic radiation absorption coefficient (basically, an absorption mean-free-path) for the layer.

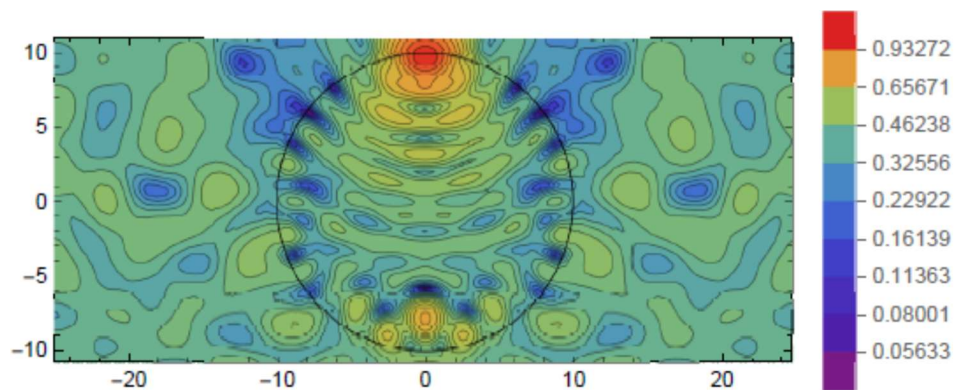


Fig. 49—Internal incident field intensity distribution in the sphere

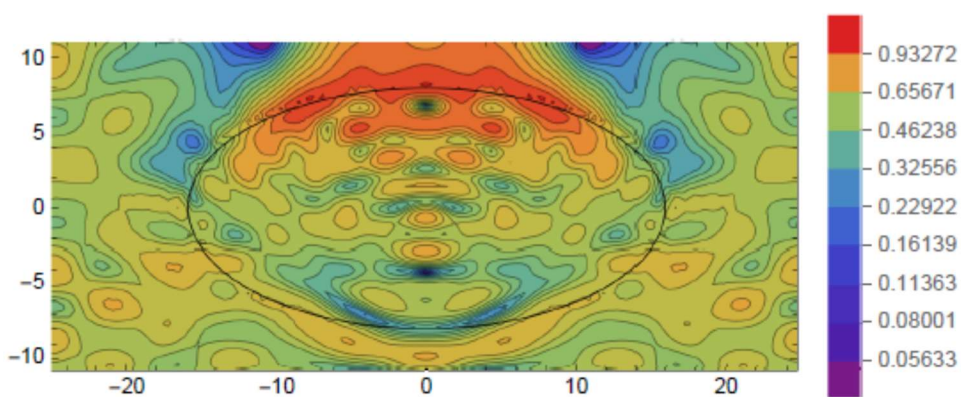


Fig. 50—Internal incident field intensity distribution in the spheroid, broadside incidence

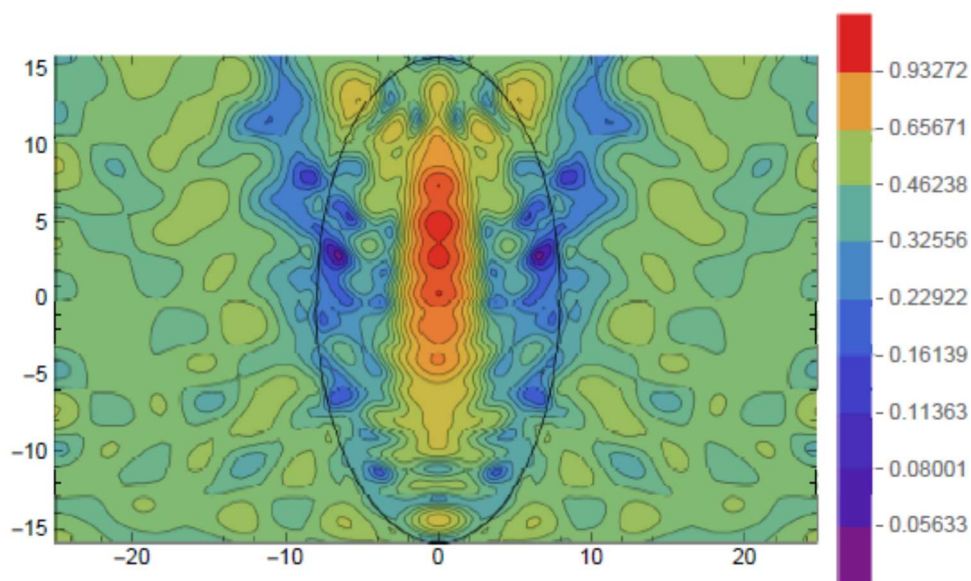


Fig. 51—Internal incident field intensity distribution in the spheroid, axial incidence

7.4 UV Intensities in Models of Spores and Clusters of Spores on Planar Surfaces: Results for Various Refractive Indexes, Cluster Sizes and Incident Angles (Year 3)

The year 2 results used refractive indexes for spores that were less than ideal. In the following calculations, the refractive indexes are consistent with approximations that are more likely to be similar to those of actual spores (see Table 8). Refractive index values used for *Bacillus* spores in this report were taken from published articles^{51, 52, 53} when available. Not all values are used in the calculations, but the values not used specifically are still useful for understanding the relevance of the calculations of the UV intensities at different wavelengths. The rows in Table 8 are grouped according to three ranges of penetration depth: less than 3 μm , 4–8 μm , and greater than 33 μm .

Table 8—Complex Refractive Index Values ($m_r + i m_i$)^{51, 52, 53} and Penetration Depths (δ) for *Bacillus* Cells and Spores at Various Wavelengths

Wave Length (nm)	<i>Bacillus</i> Spores (98% Solids)			<i>Bacillus</i> Veg. (98% Solids)			<i>Bacillus</i> Spores (60% Solids)		
	m_r	m_i	δ (μm)	m_r	m_i	δ (μm)	m_r	m_i	δ (μm)
260	1.548	0.0175	1.18	1.588	0.0350	0.591	1.467	0.00968	2.14
266	1.546	0.0192	1.11	1.585	0.0346	0.61	1.467	0.0106	2.0
273	1.543	0.0189	1.15	1.580	0.0319	0.681	1.466	0.0105	2.08
280	1.54	0.0161	1.38	1.575	0.0261	0.854	1.464	0.0089	2.50
290	1.538	0.00543	4.25	1.536	0.0061	3.75	1.463	0.0030	7.69
302	1.536	0.00071	33.7	1.534	0.00028	85.5	1.462	0.00039	61.0
317	1.534	0.0004	63.4	1.532	0.00006	445	1.461	0.00022	115
325	1.532	0.00041	62.7	1.530	0.00008	336	1.46	0.00023	113
339	1.530	0.00023	117	1.548	0.00009	308	1.459	0.00013	212
355	1.526	0.00025	113	1.544	0.00009	303	1.456	0.00014	203
450	1.518	0.00008	448	1.527	0.00008	434	1.449	0.00004	810

7.4.1 UV Absorption in Spore Clusters with Air Between the Spores

Figures 52 and Fig. 53 illustrate the effects of a surface on the intensities within the spores in a cluster. The UV absorption by a sphere is proportional to the product of the average UV intensity within that sphere and the imaginary part of m_{small} for that sphere. So, for any given figure in Section 7 of this report, where the m_{small} is the same for every spore in the cluster, the UV intensity absorbed by the sphere is proportional to the UV intensity within the sphere. There are 54 spheres (spores) in the clusters. In Fig. 52, the spores are weakly absorbing, with $m = 1.536 + i0.0007$. The cluster is enclosed in a spherical matrix with $m = 1.0003$ to approximate air. (The number in the figure is rounded for brevity.) In Fig. 52, the individual spores absorb very little UV light. The spores in the cluster are highly scattering because air fills the spaces between the spores and so there are large refractive index differences at the air-spore interfaces. The net effect is that the most-absorbing spheres only absorb approximately 60% more light than the least-absorbing spheres. The reflection of light by the surface increases the absorption by the spheres, but not more than approximately 2.5%. In contrast, in Fig. 53, because of the increased absorption by the spores at 266 nm, the most-absorbing spores absorb about 10 times as much light as do the least-absorbing spores, and the effect of the surface is to increase the absorption by spores by approximately 1%.

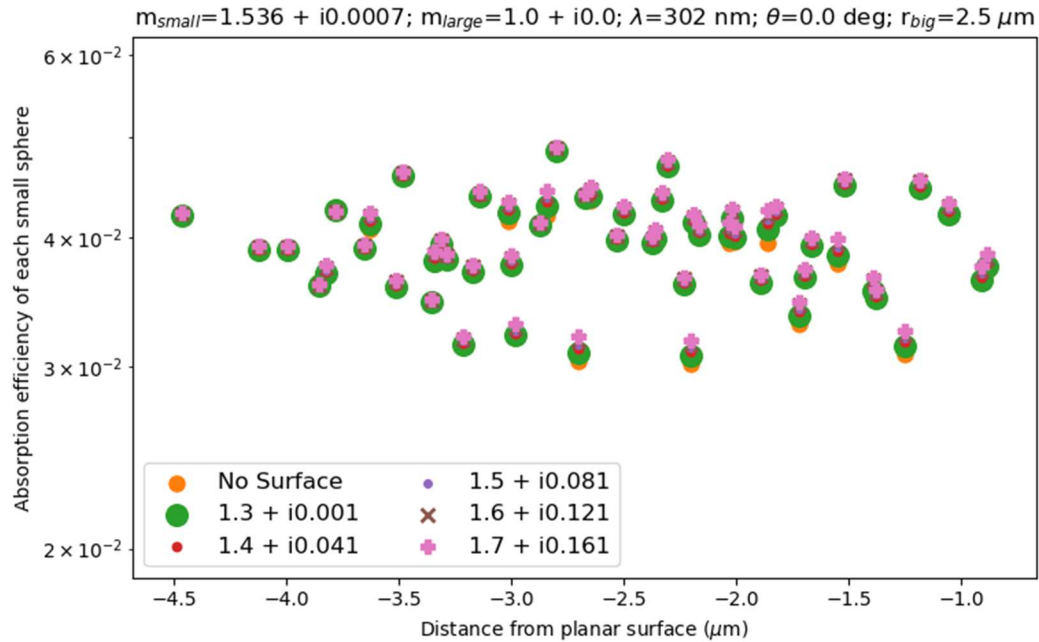


Fig. 52—UV absorption efficiency for each spore in a cluster (vertical axis) vs. distance of each sphere from the surface. The surface is perpendicular to the direction of propagation of the incident wave (from negative z to positive z). The individual spheres have complex refractive index, m_{small} , and the enclosing sphere has the $m = m_{large}$ given at the top of the figure. The complex refractive index of the surface in each of the six cases is noted in the legend by the marker for that surface. Individual small spheres are 1 μ m in diameter. Each cluster has 54 individual spheres.

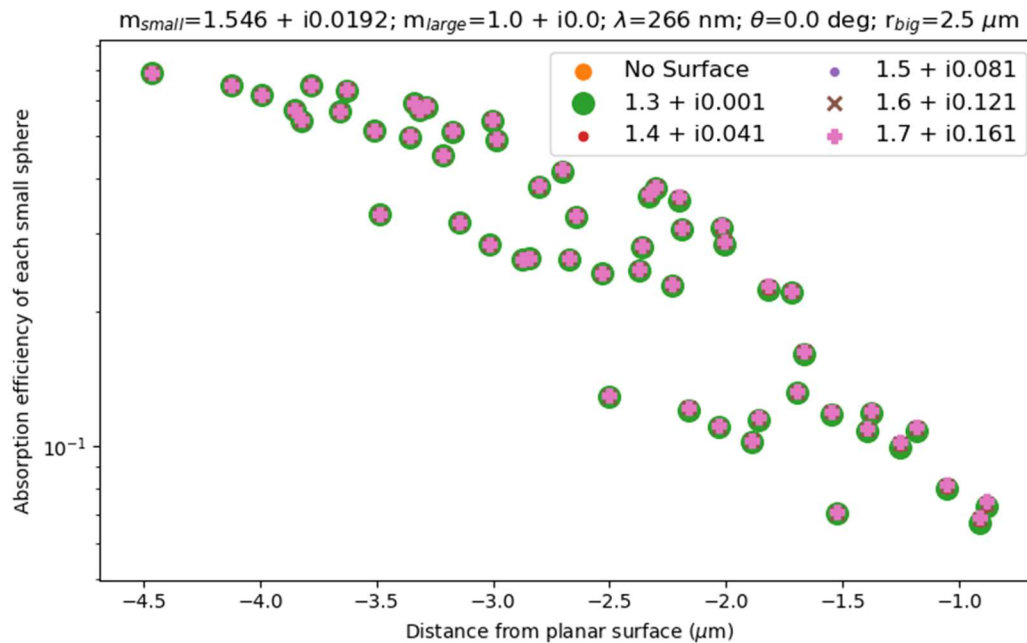


Fig. 53—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

7.4.2 UV Absorption in Spores within a Weakly Absorbing Encompassing Sphere

In Fig. 54 to Fig. 59, the effects of a surface on the intensities within the spores in a cluster are illustrated as in Section 7.4.1, except the encompassing sphere is not air, but a weakly absorbing material, with $m = 1.4 + i0.002$. Because the change in refractive index at the interface between the spores and the encompassing sphere is only approximately one-fifth as large as it is in Fig. 52, the scattering is less, and the UV light is better focused in the forward direction, as evidenced by the highest intensities within the cluster occurring in spheres nearest the surface.

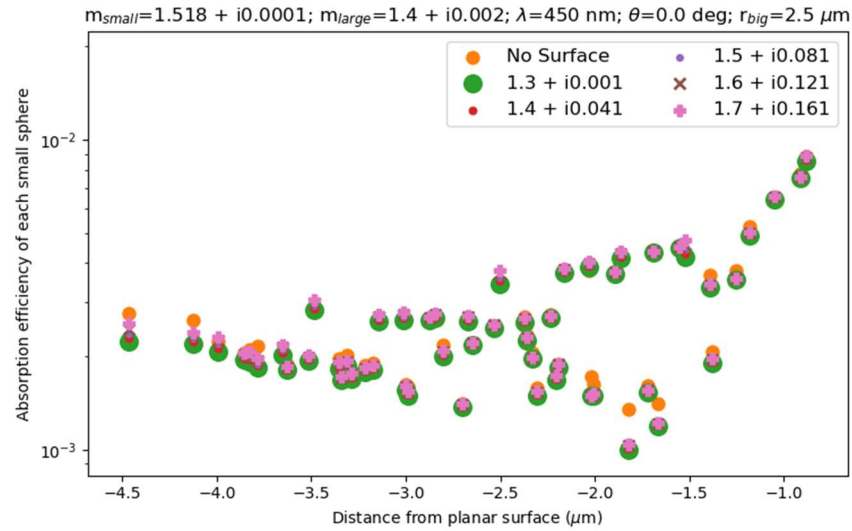


Fig. 54—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

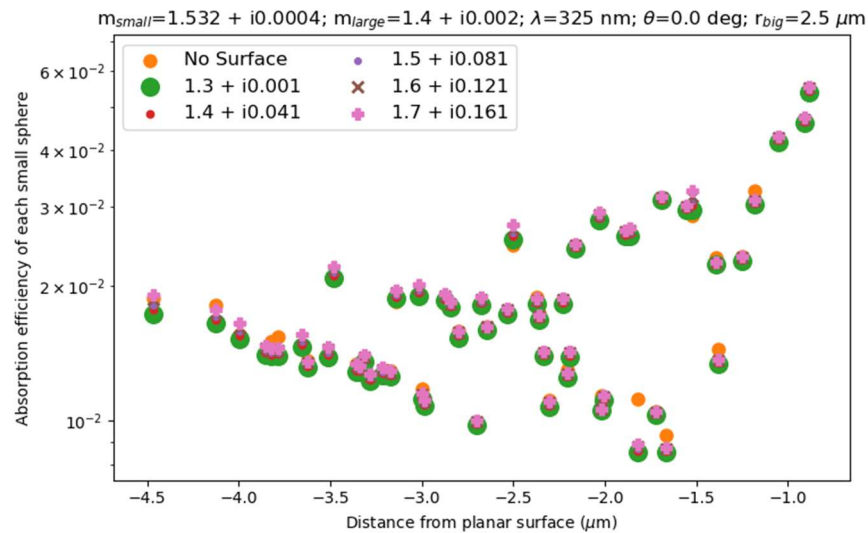


Fig. 55—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

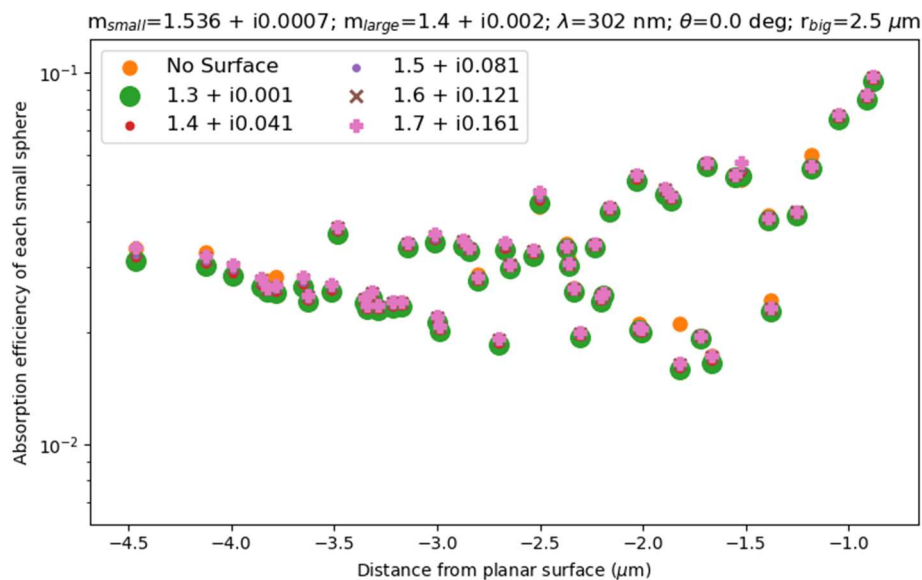


Fig. 56—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

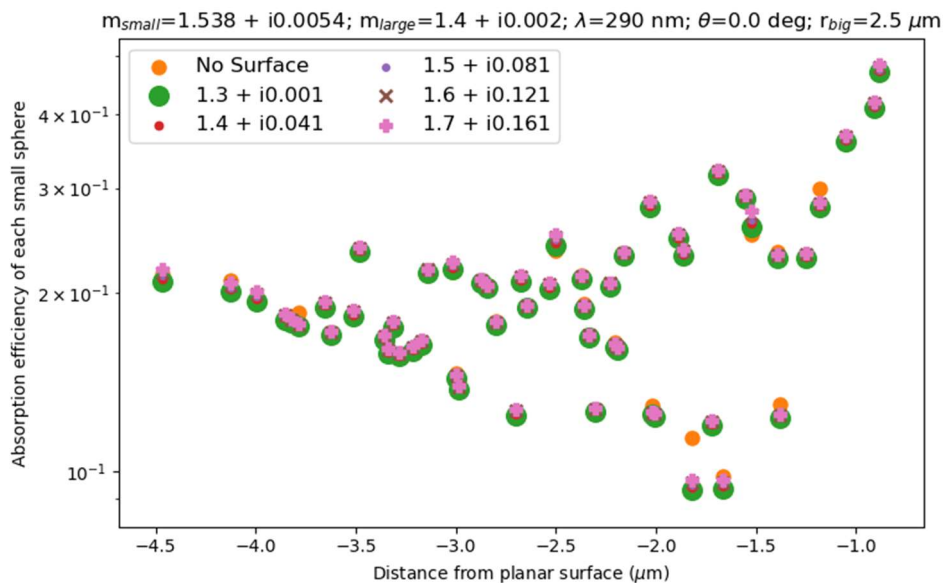


Fig. 57—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

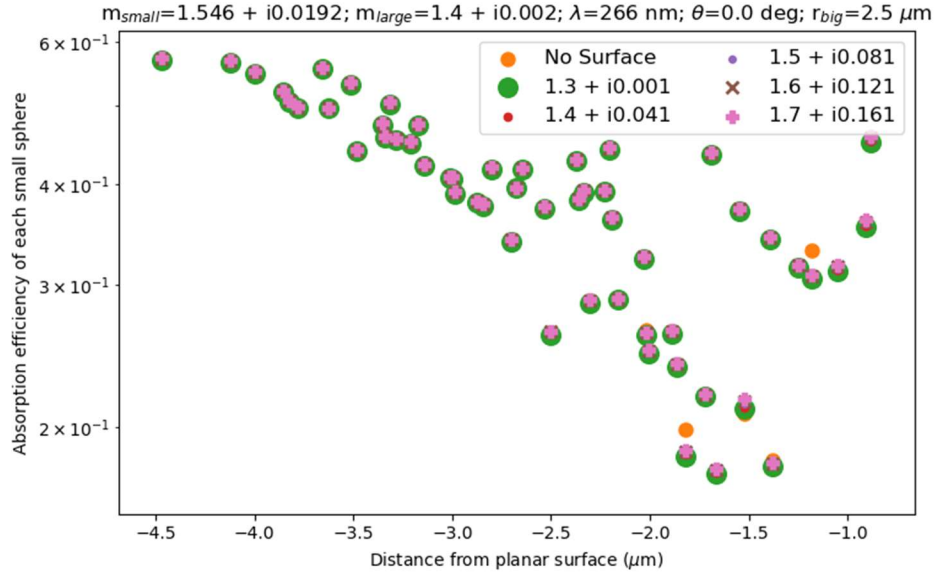


Fig. 58—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

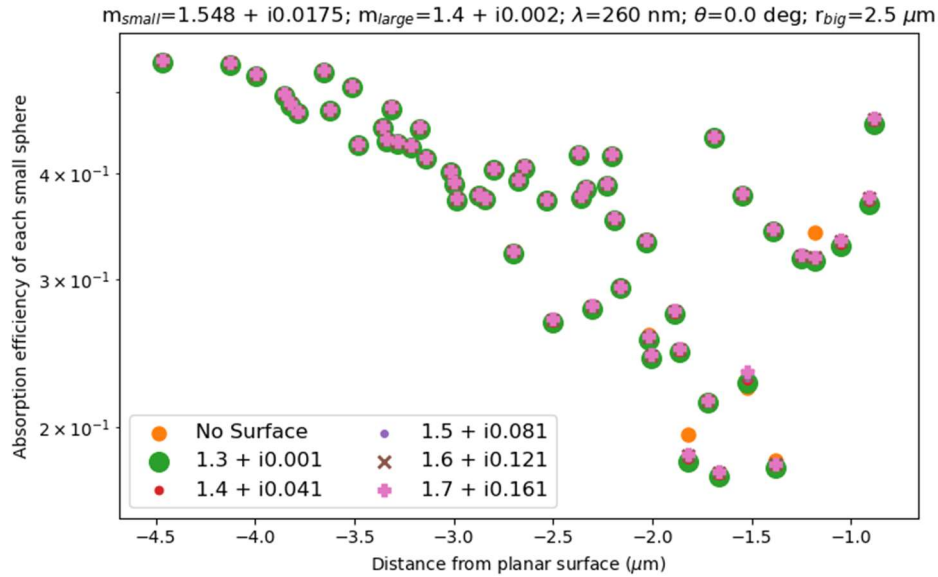


Fig. 59—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

This focusing of light inside the encompassing sphere in the forward direction is so pronounced in these clusters with a weakly absorbing encompassing sphere that even in Fig. 59, where the $m_i = 0.0175$, this focusing is still very apparent, even though it is not sufficient to make these spheres (near the focus point) the most absorbing ones in the cluster. In Fig. 54 to Fig. 59, the ratio of the most highly to most weakly absorbing individual sphere is as large as approximately 8 in Fig. 54 and as small as approximately 3.5 in Fig. 58 and Fig. 59.

7.4.3 UV Absorption in Spores within a Highly Absorbing Encompassing Sphere

In Fig. 60 and Fig. 61, the spores are within a highly absorbing encompassing sphere, with $m_{\text{ilarge}} = 0.03$, far larger than the relatively small $m_{\text{ilarge}} = 0.002$ used in Section 7.4.2.

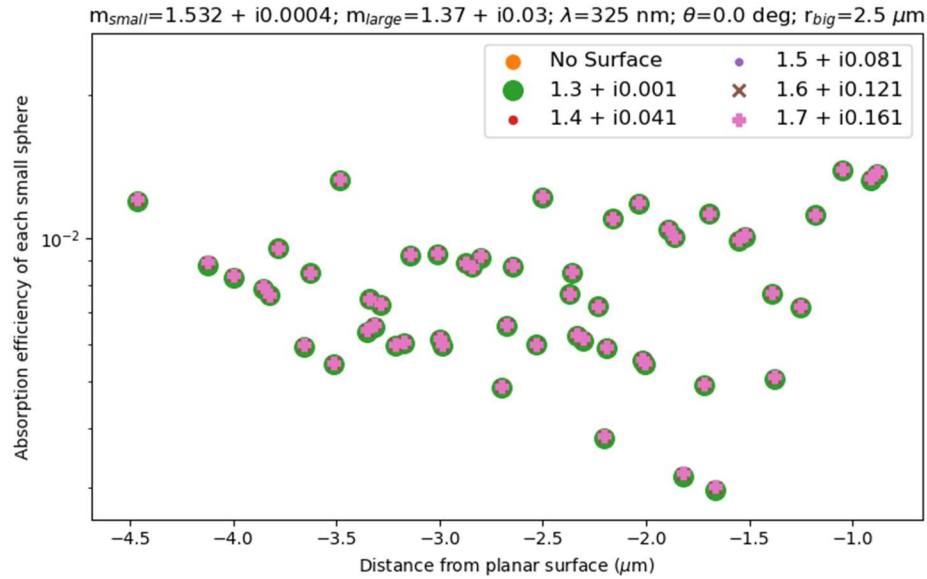


Fig. 60—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

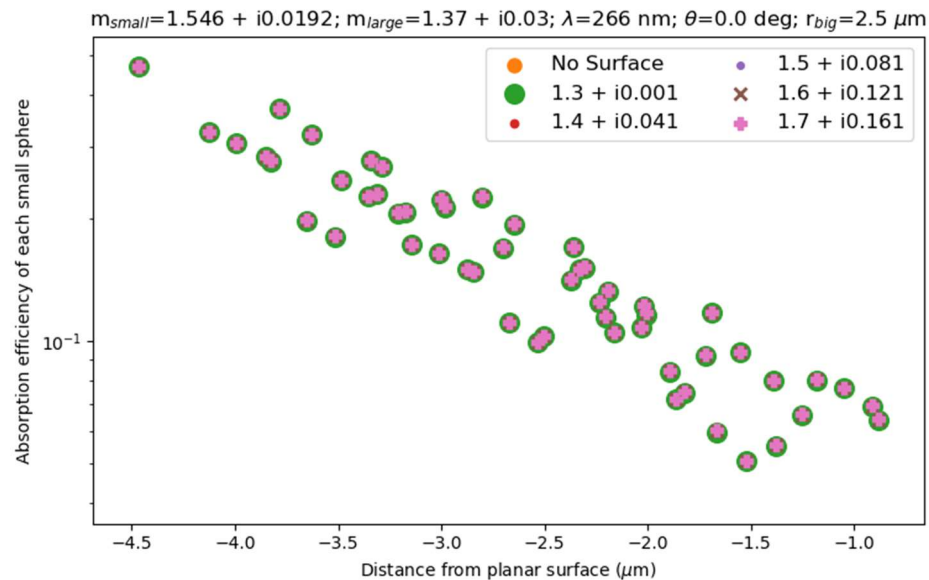


Fig. 61—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

7.4.4 Clusters with a 10- μm Diameter Weakly Absorbing Encompassing Sphere

In Fig. 59 and Fig. 63, the effects of a surface on the intensities within the spores in a 10- μm diameter cluster containing 54 1- μm diameter spheres are investigated. These single spheres are far more separated than they are in Sections 7.4.1 through 7.4.3. Because such clusters could exist in nature, the UV intensities in individual spheres in these large particles are illustrated. The individual spheres have the complex index of refraction consistent with 325 nm and 266 nm in Fig. 62 and Fig. 63, respectively. The enclosing sphere has a low absorption in Fig. 62 and Fig. 63 with $m_i = 0.002$. This focusing of light inside the encompassing sphere in the forward direction is so pronounced in these clusters with a weakly absorbing encompassing sphere that even in Fig. 63, where the $m_i = 0.0192$, this focusing is still very apparent. The spheres near the focus are the most absorbing ones in the cluster.

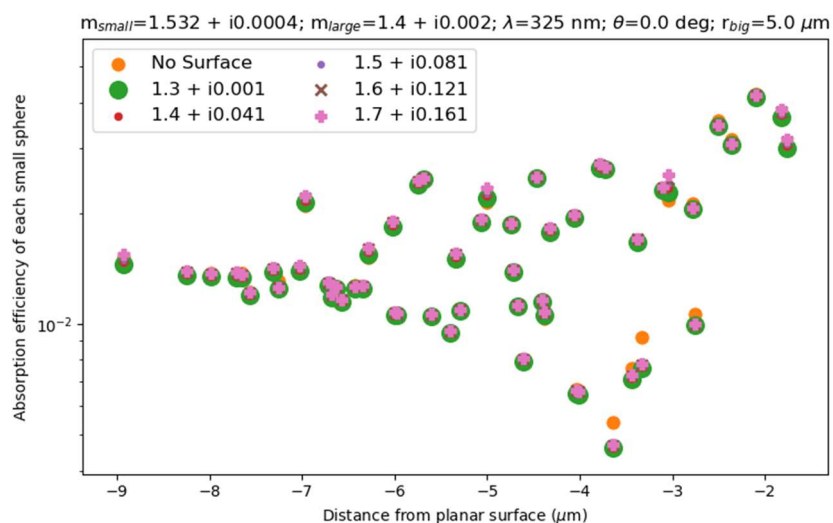


Fig. 62—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

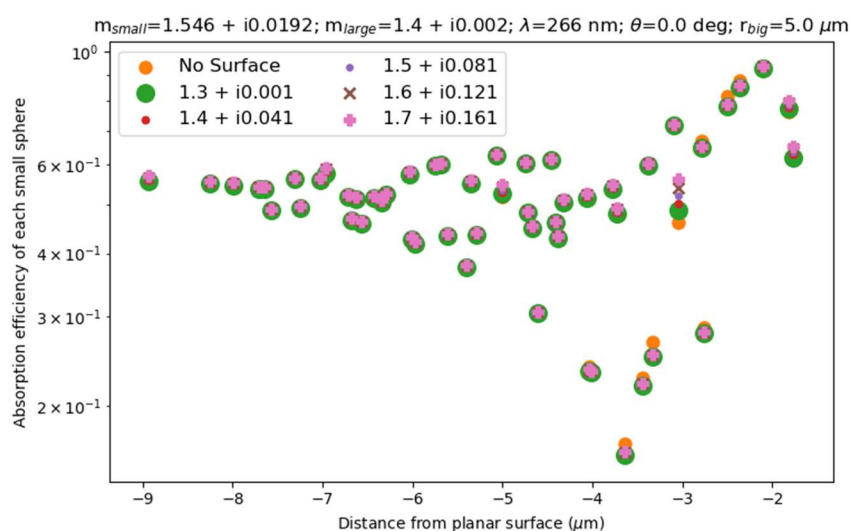


Fig. 63—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

7.4.5 Clusters with a 10- μm Diameter Strongly Absorbing Encompassing Sphere

In Fig. 64 to Fig. 66, the m_i values of the large spheres are 0.02, 0.05, and 0.05, respectively. The ratio of most- to least-absorbing spore is approximately 100 in Fig. 64, 2,000 in Fig. 65, and 60,000 in Fig. 66. The material of the large sphere is so absorbing, and the diameter of the large sphere is so large (10 μm), that the UV intensity reaching the side of the sphere opposite the incident wave is very small. Consequently, the light reaching the planar surface is very small and the effects of the surface are so small as to be not be detectable. Also, the effects of focusing within the large sphere of these figures are much less apparent than in Section 7.4.4. Here, this focusing in the forward direction appears in Fig. 64 as a bump on an otherwise downward-trending log (absorption efficiency) as the UV light moves closer to the surface. In Fig. 64, the penetration depth of the material of the spores is 1.11 μm and not very different in the material of the larger sphere. In Fig. 65 and Fig. 66, with $m_{\text{large}} = 0.05$, the penetrations depths are even smaller, only approximately 40% as large as in Fig. 64.

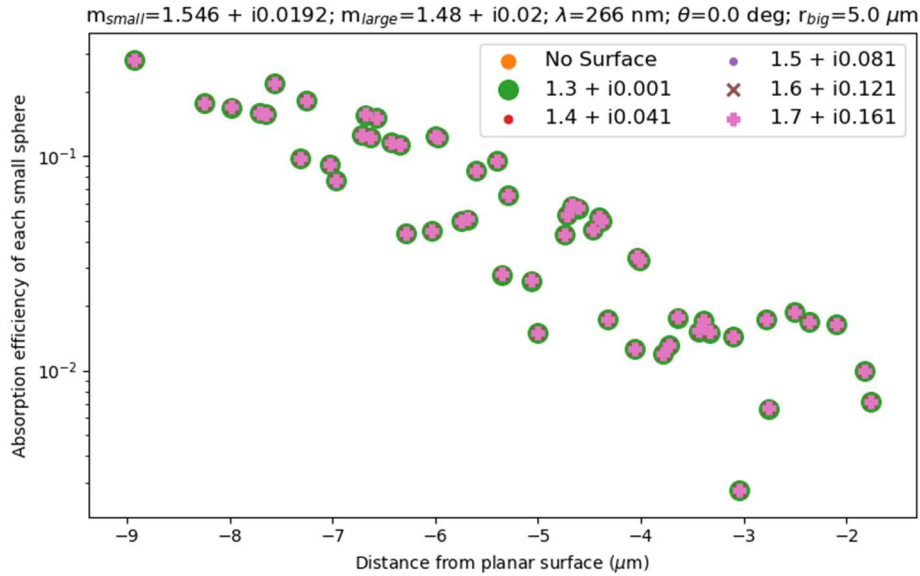


Fig. 64—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

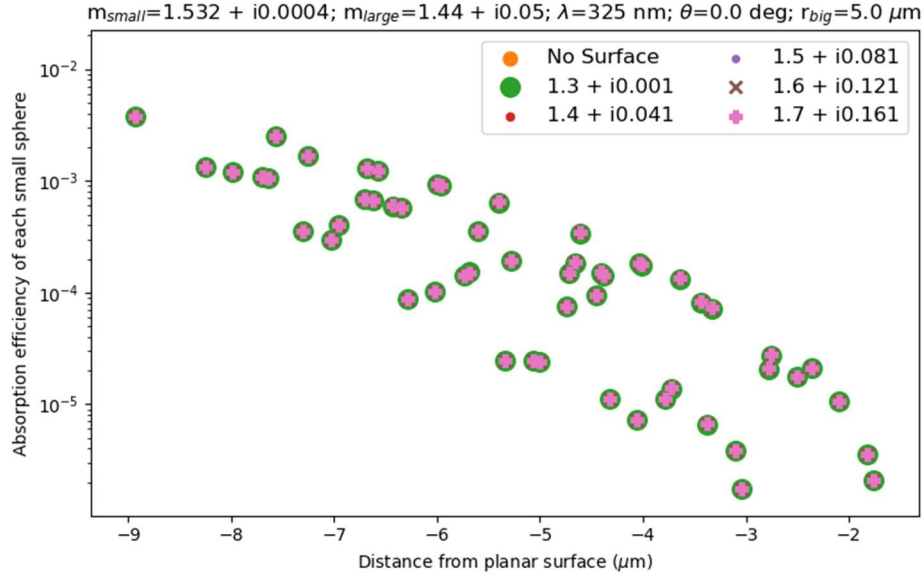


Fig. 65—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

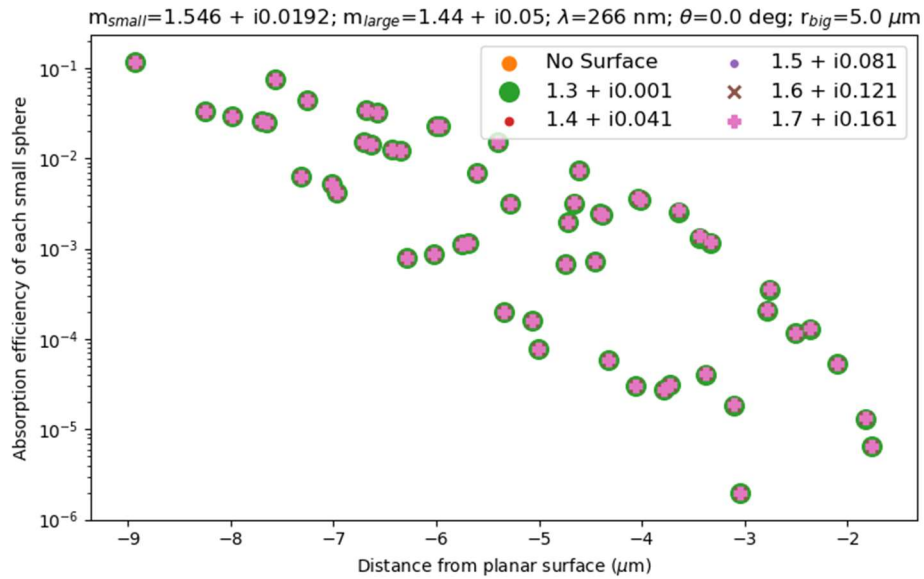


Fig. 66—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

7.4.6 Variation in UV Intensities with Angle of Incidence

The spores and surfaces in Fig. 67 to Fig. 70 all have the same compositions. However, the zenith angle of the incident UV plane wave (i.e., the angle from the normal to the surface) varies from 0 (normal incidence) in Fig. 67 to 40°, 80°, and 89° in Fig. 68, Fig. 69, and Fig. 70, respectively. In Fig. 67 to Fig. 70, in the case with no planar surface, absorption efficiencies of the spores vary from a minimum of approximately 0.17–0.24 to a maximum of approximately 0.55–0.57. The variations in the ranges occur because the spores are

positioned at random locations, and so, e.g., for each different zenith angle, spores are at different random distances from the illuminated side of the large sphere. In Fig. 70, where the incident angle is 89° , the absorption by each spore is, in most cases, higher than that of the no-surface case. It is larger for almost all particles farther than about 2.2 to 3.0 μm from the surface, no matter which surface is present (other than air). Spores within approximately 1.3 μm of the surface have lower absorption efficiencies than when the surface is not present.

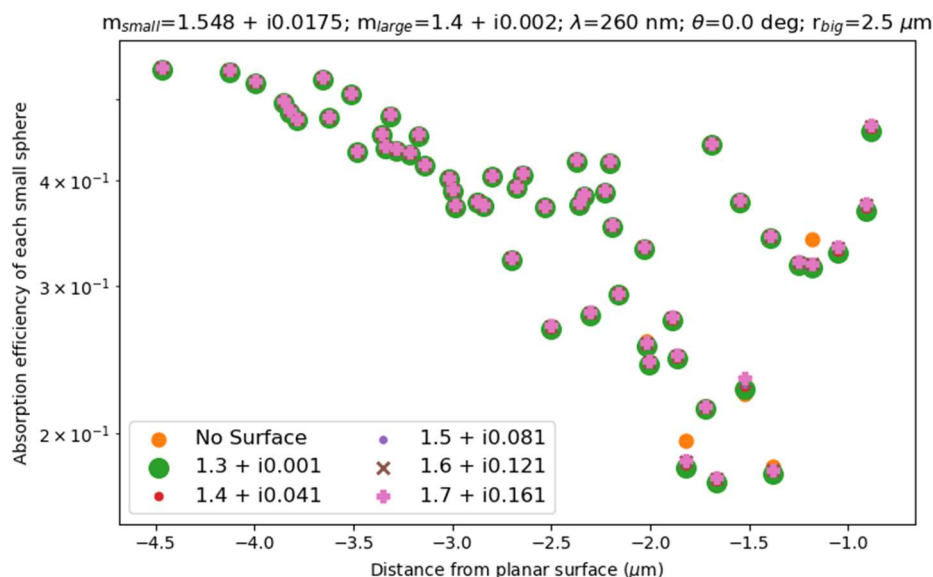


Fig. 67—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure

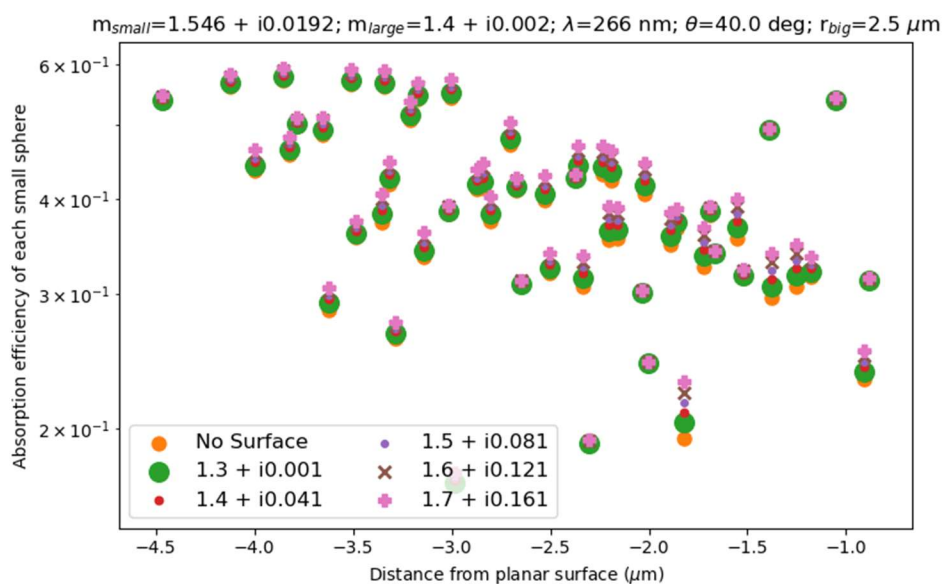


Fig. 68—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure, and with the UV incident 40° from the vertical

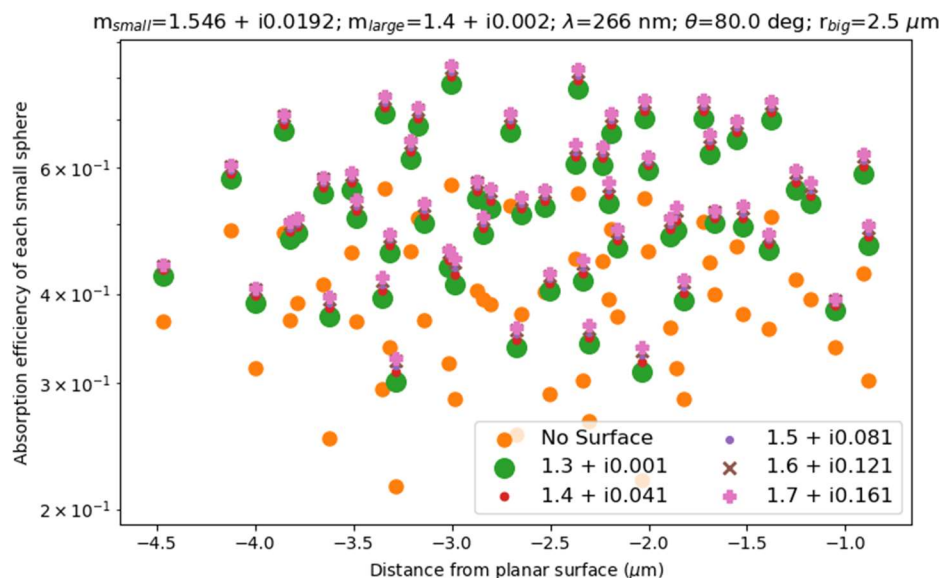


Fig. 69—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure, and with the UV incident 80° from the vertical

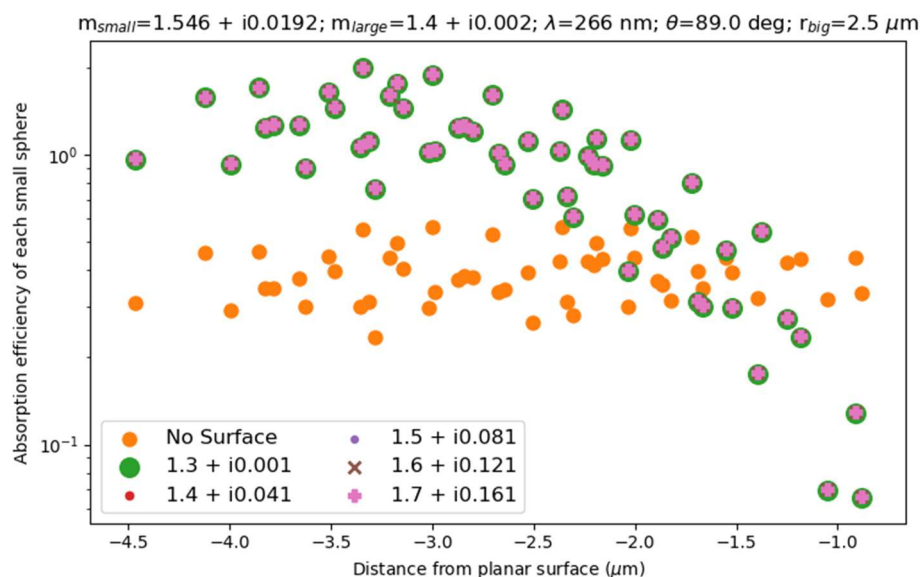


Fig. 70—UV absorption efficiency for each spore vs. distance from the surface as in Fig. 52, except that the m_{small} , and m_{large} are as indicated at the top of the figure, and with the UV incident 89° from the vertical

Table 9 summarizes the parameters used for the model clusters studied here using the MSTM. For each scenario, the absorption efficiencies of spores in the clusters were calculated and illustrated in the figures listed. The wavelength-dependent optical properties of the bacterial spores are the same as in Table 8.

Table 9—Refractive Indices of Bacteria⁵⁴, Encompassing Sphere, and Surfaces Used in the Calculations

Figure	Refractive Index of Spores		Refractive Index and Diameter (μm) of Encompassing Sphere			Wave-length (nm)	Refractive Index of Surface		Incident Angle (from Normal)
	m_r	m_i	m_r	m_i	d		m_r	m_i	
52	1.536	0.0007	1.0	1e-12	5	302	1.0-1.7	1e-12 - 0.161	0
53	1.546	0.0192	1.0	1e-12	5	266	1.0-1.7	1e-12 - 0.161	0
54	1.518	0.0001	1.4	0.002	5	450	1.0-1.7	1e-12 - 0.161	0
55	1.532	0.0004	1.4	0.002	5	325	1.0-1.7	1e-12 - 0.161	0
56	1.536	0.0007	1.4	0.002	5	302	1.0-1.7	1e-12 - 0.161	0
57	1.538	0.0054	1.4	0.002	5	290	1.0-1.7	1e-12 - 0.161	0
58	1.548	0.0175	1.4	0.002	5	260	1.0-1.7	1e-12 - 0.161	0
59	1.546	0.0192	1.4	0.002	5	266	1.0-1.7	1e-12 - 0.161	0
60	1.532	0.0004	1.37	0.03	5	325	1.0-1.7	1e-12 - 0.161	0
61	1.546	0.0192	1.37	0.03	5	266	1.0-1.7	1e-12 - 0.161	0
62	1.532	0.0004	1.4	0.002	10	325	1.0-1.7	1e-12 - 0.161	0
63	1.546	0.0192	1.4	0.002	10	266	1.0-1.7	1e-12 - 0.161	0
64	1.546	0.0192	1.48	0.02	10	266	1.0-1.7	1e-12 - 0.161	0
65	1.532	0.0004	1.44	0.05	10	325	1.0-1.7	1e-12 - 0.161	0
66	1.546	0.0192	1.44	0.05	10	266	1.0-1.7	1e-12 - 0.161	0
67	1.546	0.0192	1.4	0.002	5	266	1.0-1.7	1e-12 - 0.161	0
68	1.546	0.0192	1.4	0.002	5	266	1.0-1.7	1e-12 - 0.161	40
69	1.546	0.0192	1.4	0.002	5	266	1.0-1.7	1e-12 - 0.161	80
70	1.546	0.0192	1.4	0.002	5	266	1.0-1.7	1e-12 - 0.161	89

7.5 Implications of the Modeling of UV Intensities in Clusters of Spores

Implications of the above calculations for clusters, made with the MSTM, including the MSTM for clusters on a surface, and made with homogeneous sphere models, are as follows:

1. Absorptive shielding

- Absorptive shielding does not appear significant when the only absorbers are primary metabolites at wavelengths longer than 290 nm. It can protect some spores in large clusters if illuminated at 280 nm or less, or may protect at longer wavelengths for sufficiently pigmented spores (see Fig. 65).
- For the unpigmented bacteria approximated here, at wavelengths of 290 nm and larger, the effect of absorption of UV on the lowest UV intensities within relatively homogeneous clusters is relatively small. It is especially small for clusters rotating randomly in air (for example, see Fig. G5 in Appendix G).
- For pigmented (brownish) bacterial spores of the type studied by Tuminello et al.,⁵⁴ if the m given by the authors is accurate, then the UV intensities within rotating or not-rotating clusters are likely to be substantially decreased at all relevant wavelengths, even for wavelengths greater than 450 nm. Such shielding from UV becomes more prominent as cluster size increases, at least up to sizes of 6 to 8 μm .

2. Shielding by scattering

- a. Shielding by multiple scattering appears to be most significant in nonrotating clusters with only air between the spores. Such clusters are highly inhomogeneous. If the spheres are also absorbing, longer paths resulting from multiple scattering could increase the absorption of UV light.
- b. Shielding by scattering is small in an approximately index-matched cluster of spheres, clusters that can be approximated with homogeneous sphere models, and where refractive shielding can be significant.
- c. For highly inhomogeneous clusters, in which the material between the clusters is air, the extinction efficiency for the individual spheres (not in a cluster) oscillates around a value of 2.0 but is not less than 1.2 for the UV at wavelengths that occur in the atmosphere and that are most harmful to spores. Also, the scattering efficiency of these spore-approximating individual spheres exceeds the absorption efficiency.

3. Refractive shielding

- a. The ratios of lowest intensity to highest intensity within a cluster are largest for the approximately index-matched cases, i.e., where the spores and the material between the spores have a quite similar refractive index. These large ratios are the result of refraction at the surface of the large sphere, along with relatively low refraction and scattering within the overall cluster.
- b. Refractive shielding in nonrotating clusters can be significant even when there is no absorptive shielding, so long as the cluster is at least somewhat homogeneous.

4. Diminished shielding in rotating spheres

- a. Spores in rotating clusters illuminated at wavelengths longer than 290 nm are protected very little, if at all, if the only materials in the clusters are primary metabolites. The spores are unprotected from the UV light whether or not the cluster is homogeneous.
- b. Even at the center of a 4- μm cluster of spores within an index-matched sphere (i.e., the homogeneous sphere case), and where the m is that of very dry (98% solids) spores, for the rotating cluster case, the intensity is still approximately 20% of that in an individual sphere. A 20% decrease is somewhat small compared to the decreases in the cases of nonrotating clusters and in light of all the other uncertainties and approximations involved.

5. Effects of a planar surface

- a. For an *individual* spherical spore in air illuminated with UV light, the effect of moving the spore onto a surface at *near normal* incidence can be to either increase or decrease the UV absorbed by the sphere. The effect appears to be relatively small, especially when compared to the effect on the UV intensity within some spores in a cluster on a surface. In the simple approximation of a spore as a homogeneous perfect sphere, the effect of surface can be dominated by its impact on the optical resonances of the sphere, especially when the absorption by the sphere is small.
- b. For an *individual* spherical spore in air illuminated with UV light at incident angle 89° from the normal to the surface, the effect of the surface can be to approximately double the absorption efficiency.
- c. For a cluster of spores in a sphere resting on a surface and illuminated by a UV plane wave propagating in a direction normal to the surface, the surface has relatively small effects on the absorption efficiencies of the spores. These absorption efficiencies tend to be slightly higher than without a surface, although some individual spores had lower absorption efficiencies than when no surface was present.

- d. For a cluster of spheres resting on a surface and illuminated by a UV plane wave propagating in a direction 89° from the normal to the surface, the surface can increase the absorption efficiencies of the spores not too close to the surface. However, for spores near the surface, for example, within $1.2\ \mu\text{m}$ for a $5\text{-}\mu\text{m}$ particle, the absorption efficiencies were smaller than when no surface was present (in the range of five times smaller).
6. Effects of uncertainties in the complex refractive index
- a. The uncertainties in the complex refractive index are substantial because different spores and vegetative cells may have different compositions because of different ratios of protein, nucleic acids, peptidoglycan, CaDPA, and pigments such as melanins, carotenoids, and scytonemin, and other materials.
 - b. Uncertainties in the complex refractive index are substantial in cases of homogeneous $8\text{-}\mu\text{m}$ -diameter clusters rotating in air, especially at the UV wavelengths of $280\ \text{nm}$ and shorter, which do not occur in the atmosphere but are important in germicidal UV of clusters or aggregates containing bacteria, viruses, or other microbes.

8. SUMMARY CONCLUSIONS AND RECOMMENDATIONS

8.1 Program Report Overview

This report presents cumulative results of the PAVES research program conducted between February 2017 and February 2020, with the caveat that data analysis and report writing continued intermittently afterward through February 2022. Additionally, further experimental work continued at CCDC-CBC into 2020 under a no-cost extension of the original program funds. This final segment of experimental work covered collection of exposure data of the same *B. anthracis* Sterne strain samples on alternate surface materials including quartz slides and synthetic “spiderweb” fibers, as well as exposure of *B. anthracis* Ames strain spores pipetted directly on polycarbonate filters. The experimental work performed after February 2021 is not included in this report. No further data collection of spores exposed as aerosol took place beyond what is included in this report.

In summary, this report provides measurements of small spore clusters (including single spores) exposed to two UV radiation sources as confined, suspended aerosols and, in a parallel, coordinated effort, measurements of similar spore samples deposited onto polycarbonate filters prior to exposure with the same radiation sources. It also provides measurements made on larger spore clusters both as aerosol and as surface deposition with exposure to both radiation sources. The two radiation sources used by both efforts were a germicidal Hg discharge lamp with emission predominantly at 254 nm and a commercial solar simulator lamp customized to provide a UV spectral output closely corresponding to the ASTM G177 standard (enhanced UV) solar spectrum representative of the continental U.S. The utility of data collected using the solar simulator is to provide basic decay rate data that could be used as input to modeling simulations for estimating *B. anthracis* viability in different outdoor environmental scenarios (with differing insolation). The purpose of 254 nm source exposure data was to provide a reference point for aerosol and surface sample measurements, permitting their comparison to prior 254 nm measurements in published scientific literature and thus establishing confidence in the solar simulator exposure results. It also provides a direct measure of the efficacy of solar exposure relative to 254 nm exposure.

The overarching technical/scientific goals of this program were (a) to measure, for the first time as well-characterized aerosols, *Bacillus anthracis* Sterne strain survival fractions (SF) as a function of UV radiation exposure, (b) to concurrently measure survival fractions using the same materials and similar aerosols deposited on surfaces and exposed to the same UV radiation sources, (c) to analyze the SF data to determine decay rates of the spore samples exposed to UV radiation, (d) to compare the decay curves as a function of UV fluence (dose) of aerosol exposure samples to those deposited and exposed on surfaces (for similar sample preparation, and for the same UV source), (d) to develop and demonstrate, for the first time, a first-principles computational model for the absorbed radiation of spore clusters in terms of individual spore irradiation within the cluster, and (e) to evaluate the relationship between surface particle exposure samples and aerosol particle exposure samples in terms of their decay rates and behavior. The motivation for this undertaking was to see if there is a practical means by which spore aerosol decay rates could be inferred from decay measurements of the same spore materials on surfaces or substrates.

In addition, dispersions of spore materials as aerosol typically will include aerosol particles consisting of multiple spores. While it is possible, using special preparation methods, to disperse predominantly single spores,¹⁷ it requires a much higher level of effort to achieve, especially for generating high concentrations and/or large total quantities. In general, dispersion methods of *Bacillus anthracis* spore aerosols would be expected to have most of their particulate mass consist of multiple-spore clusters. Consequently, this study also included an objective to measure survival of characterized spore clusters exposed to UV radiation both as aerosols and also as residing on surfaces.

Survival fraction data for all the samples was analyzed in term of a traditional multihit (MH) model formula that has been extensively used in prior studies and published literature on radiation exposure decay of microorganisms. The two main features of this traditional model for microorganism population radiation degradation are (1) a shoulder region at low fluences/doses during which little or no organism death is observed, followed by (2) a steeper rate of decay, characterized as a linear decrease on a logarithmic survival fraction axis with increasing fluence/dose. Consequently, the model contains two parameters that govern the shape of the decay curve: (a) k , the decay rate given by the slope of the linear decrease in SF, and (b) n , a recovery parameter that controls the extent of the shoulder region along the fluence axis.

From a purely experimental perspective, it is critical to determine two key features of the survival fraction data as a function of fluence. They are: first, a “breaking point,” or fluence value where survival fractions start to increase rapidly in contrast to little or no decay up to that point, and second, the slope of the rapid decay as fluence increases beyond that point. The linear slope is critically determined by accurately determining the breaking point and then determining survival fraction at the highest fluence measurable, i.e., the point where the signal (surviving colonies) effectively goes to zero.

Additionally, there are two caveats regarding the application of the MH model to the data obtained by the PAVES program. First, the model description is intended for a disperse population of individual organisms, each capable of forming a new colony of organisms under appropriate conditions. It is not intended to describe groups of organisms that may derive some collective benefit by their physical formation. Second, this model is formulated in terms of a single wavelength of radiation. It is not well-suited to describe a more complex circumstance in which organisms are subjected to a broad, continuous band of wavelengths and the probability of causing damage to a given organism may change significantly for different wavelengths.

With regard to the first caveat, we acknowledge that the mechanistic meaning of the two parameters will be lost for data on samples that consist of large (roughly greater than 10) numbers of spores. However, the equation for the MH formulation does provides a convenient means for quantitative characterization of decay response in the measured data. This is somewhat analogous to the use of Gaussian or normal distribution as a two-parameter curve fit for experimental data when the underlying probability distribution is not known a priori. For our purposes here, no claims are made that the interpretation of the recovery parameter is meaningful in terms of organism damage sites, or that the fitted curves necessarily describe decay response beyond the measured fluence range.

With regard to the second caveat, it should be pointed out that although the SS radiation is a very broad-continuum spectrum, only a very small fraction of it is active in promoting organism viability loss due to the wavelength dependence of the *Bacillus* spore action spectrum. The SS continuum contains *no* UV-C and a very small percentage of UV-B radiation (see Section 2.4.3 for detail), so the actinic radiation dose is estimated to be confined to a relatively narrow band centered approximately at 312 ± 15 nm.

8.2 Conclusions

Given the qualifications and guidelines above, the PAVES measurement results are summarized in Table 10. Of greatest importance are the values obtained for single-spore sample measurements. As explained previously, the “single spore” aerosol in fact consists of a combination of mostly single spores, doublets, and triplets (2-spore and 3-spore clusters) with decreasing percentages of spore cluster increments. In terms of optical exposure, there should be very little, if any, consequence to a distribution of such small clusters in comparison to an aerosol of only single spores. Likewise, the pipetted samples were never aerosolized, but simply were deposited directly onto filter substrates, and by random chance could leave spores in contact with each other as the liquid evaporated. However, the remarkably close results of their measurements, especially for the 254-nm exposures, indicates that these samples are primarily representative of single spores.

Table 10—PAVES Survival Fraction Data Results

Cluster “Size”	Radiation Source	Sample Exposure Type	Spore Sample Conc.	Sample Generation Method	Decay Rate (k) (J/m ²) ⁻¹	Recovery Factor (n)
Single Spore	254 nm	Aerosol	Low	PZT	-0.045	40
Single Spore	254 nm	Surface	—	Pipet	-0.043	90
Single Spore	SS	Aerosol	Low	PZT	-0.000040	9000
Single Spore	SS	Surface	—	Pipet	-0.000022	3000
Small	254 nm	Surface	Low	CAD	-0.030	10
Intermediate	254 nm	Surface	Mid	IJAG	-0.030	10
Small	SS	Surface	Low	CAD	-0.000025	100
Intermediate	SS	Surface	Mid	IJAG	-0.000013	15
Large	254 nm	Aerosol	High	PZT	-0.012	3.0
Large	254 nm	Surface	High	IJAG	-0.008	2.0
Large	SS	Aerosol	High	PZT	-0.000008	3.9
Large	SS	Surface	High	IJAG	-0.000011	9.0

In Table 10, data has been presented first (column 1) in terms of effective cluster “size,” meaning numbers of spores in clusters for the different samples. Single spore values are highlighted in yellow and refer to cluster sizes between 1 and 5 spores. The size terms “small” and “intermediate” mainly signify a difference in generation method. CAD-generated cluster sizes are not as well characterized. Intermediate size clusters are expected to have a mean spore count of about 13. Large cluster size samples are highlighted in blue and have a mean spore count of about 54. Significant program summary results are as follows:

- A detailed physics computational model for exact numerical solution to the radiation exposure of each spore within a cluster of a specified number of randomly arranged spores has been developed and validated as part of this program. Known as the multiple-sphere T-matrix (MSTM) model, it assumes incident plane-wave illumination at a single, fixed wavelength and that the individual spores can be approximated as volume-equivalent spheres with an effective uniform refractive index. The MSTM model can provide results for a freely suspended spore cluster (aerosol particle), or a cluster in close proximity to a plane surface of known refractive index. (See Section 7.5 for a summary of model implications/results.)
- Single spore decay rates due to 254-nm radiation were determined to be the same for aerosol and surface exposures (Table 10).
- Single spores exposed to 254 nm on surface samples appear to be slightly more repair-capable than as aerosols, indicated by the difference in recovery factors (90 versus 40, Table 10).
- For 254-nm radiation exposure, single spore decay parameters measured for both aerosol and surfaces are generally close to prior published values for naturally occurring *Bacillus* spores (Table 6).
- Single spore decay rates due to SS radiation between aerosol and surface exposures are close (meaning less than a factor of 2 difference in decay rate and a factor of 3 difference in recovery factor).

- Similar to 254-nm radiation exposure, SS exposure of surface single spore samples appear to possess somewhat greater repair capability than the corresponding aerosol exposure sample.
- Values for SS exposures in Table 10 are normalized to the integrated total fluence of the ASTM G177 standard integrated over its complete range from 280 to 400 nm.
- In Table 10, the decay rates for surface and aerosol exposures differ by a factor of $\approx 1400\times$ between SS and 254-nm radiation sources. However, a more meaningful comparison would account for *actinic* SS spectral normalization (based on the integrated SS intensity of a spectral band from 297 to 327 nm). This normalization provides a factor of $9.09\times$ higher than the full spectrum values in Table 10, yielding $0.00036 \text{ (J/m}^2\text{)}^{-1}$ and $0.00020 \text{ (J/m}^2\text{)}^{-1}$ for aerosol and surface SS exposure, respectively.
- Using the *actinic* spectral SS responses (see previous bullet) the SS decay rates are $\approx 156\times$ lower than those for 254-nm exposure. Based on spore refractive index estimates, the difference in radiation absorption between 254-nm and 314-nm radiation wavelengths is roughly $18\times$. Therefore, the remaining factor ($\approx 8.7\times$) can be ascribed to the difference in photochemical reaction probability between these two wavelengths.
- For the large cluster sizes, both 254-nm and SS exposures yield decay curves for which the aerosol exposure and surface exposure results are in reasonably good agreement (meaning the measured decay rates for each radiation source are within 20% of their average value and the recovery factors differ by less than $3\times$). However, the order of the decay curves for aerosol and surface samples is inverted. Aerosol exposure decay was somewhat faster than surface exposure for 254-nm radiation, but vice versa for SS radiation.
- For 254-nm exposures, the small and intermediate cluster sizes were characterized by the same exact decay curve. Their collective decay curve was well placed as intermediate values between the single spore decay curves and the large cluster decay curves.
- For SS exposures, the small cluster size sample decay curve was well placed between the single spore decay curves and the large cluster decay curves. However, the intermediate cluster size sample decay curve was almost the same as the large cluster surface exposure sample. This is an unexpected result.

8.3 Recommendations

1. The detailed physics computational model should be used to provide computational predictions of decay curve behavior for clusters of nominal cluster sizes of about 13 and 54 spores. These results could be compared to the experimental decay curves obtained for the 254-nm-radiation exposure data.
2. A future opportunity exists in the assessment of the growth of “damaged” cell colonies compared to unexposed colonies after the “damaged” colonies have begun to grow again as described in detail in Section 4.3. Our expectation is that the “repair” process that occurs for radiation-damaged spores is only needed for spore-to-vegetative-cell germination, and that subsequent cell division is not as permanently affected as a genetically based casualty of light irradiation. Had the project schedule allowed, we could have specifically collected samples of damaged cell colonies that took longer to appear in the collected sample culture plates and propagated them as a separate *B. anthracis* Sterne cell line for characterization and comparison to the stock cell line. If future experiments on radiation damage are carried out, this topic should be specifically addressed.
3. An investment should be made to develop a higher-capacity LEQ aerosol particle trap. The version developed for this program was innovative and offers a unique capability to supplement, or more

likely, to *replace* Goldberg drum devices. This work establishes an LEQ as an experimental tool for obtaining data on samples as aerosols that require long suspension times. However, this first version was too small to be labor and cost effective. For a modest investment, a straightforward extension of this design could be implemented that would increase the trap volume by an order of magnitude, which would be sufficient to make this really valuable tool for aerobiological research.

4. The BW defense community at large is in great need of a standard model organism for a microbial aerosol that is *not* a *Bacillus* (or other) spore. So much work over the past 30 years has been done targeting *B. anthracis* simply because of the physical ease of use for spores. However, there is only one pathogen on the CDC class A list that has a spore form and only one pathogen on the CDC A, B and C lists that is a *Bacillus* spore. It is time to move forward with development of methods and capabilities to directly study microbiological model organisms (vegetative bacterial and viral) that can be directly studied as aerosols.

APPENDIX A: *BURKHOLDERIA THAILANDENSIS* VIABILITY TESTS

A.1 Vegetative Cell Preparation and Aerosolization Meeting with NBACC

In October 2017, PAVES hosted the Vegetative Cell Workshop at CCDC-CBC to hear presentations from invited subject matter experts on their experience with survival of various bacterial vegetative cells as aerosols. One outcome of that workshop was the selection of *Burkholderia thailandensis* for further study as a PAVES candidate simulant for *B. mallei* and *B. pseudomallei*. Another was a continuing dialog about *B. thailandensis* with the National Biothreat Assessment and Counterproliferation Center (NBACC). In a follow-up meeting on November 19, 2018, members of the PAVES team met with a group of researchers at NBACC at Fort Detrick, Maryland, to discuss methods for preparation and the composition of the dissemination fluid. In particular, the team sought to identify the additives needed to maintain vegetative cell viability throughout aerosol dissemination. The following summary describes some salient points from that meeting.

Overnight broth cultures, grown to log phase at 37 °C, are enumerated and diluted to prepare bacterial suspensions. Plating assays after 24 to 48 h of incubation at 37 °C provide reference points for the viability of the suspensions used to generate aerosol particles. The culturable fraction of collected aerosols is determined by comparing recovered sample assays to those reference results. In an effort to remedy periodic sample loss, the vortex extraction time was increased to 10 or 20 minutes for the aerosol sample of *Burkholderia* vegetative cells as aerosols. Tests and experiments run at NBACC typically are concerned with assessing the results and consequences of potential bioterrorist activities. For that reason, NBACC investigations emphasize “low-tech” and unsophisticated methods and preparations. Essentially all of their experience in aerosolizing *Burkholderia* species involves the use of spent media in various amounts. Their microbiologists acknowledged that spent media was, in fact, detrimental to the health of vegetative bacterial cells to the point of being toxic. This well-known property is nominally the reason the exponential, or log, phase of bacterial growth transitions to the stationary phase. Although the NBACC team didn't have direct experience for guiding a more optimal choice of composition to maintain viability, they did suggest some earlier classified documents on that topic for which they agreed to request a transfer, but as of this writing, we haven't received them.

However, another important aspect clarified during this meeting was that just because *B. thailandensis* cells can be maintained as culturable in pure water does not imply that they remain in the same biological or metabolic state as they age in storage. Therefore, their susceptibility to loss of viability under stress is known to increase with storage time in pure water. This realization had a significant impact on the remaining PAVES program. It essentially voids the strategy of storing the vegetative cell samples at 4° C and using them as needed over extended times in the same way as the *Bacillus anthracis* spore samples. Knowing that the robustness of the vegetative cells depends on how long they have been stored dictates that our approach for *Burkholderia thailandensis* cell data collection should involve a fresh sample growth each day before experiments are run. Because *B. thailandensis* requires 18-h broth cultures with subsequent concentration and dilution, and because plating assays need 24–48 h of incubation, this significantly increased the projected labor overhead for this activity.

A.2 Characterization of *B. thailandensis* Vegetative Cell Viability

Experiments were conducted at CBC to determine the storage survival of *B. thailandensis* and any dependence on suspension composition. *B. thailandensis* was grown by inoculation of 20 ml tryptic soy broth with three colonies and culturing in an orbital shaker at 175 rpm and 37 °C for 18 h. The suspension was concentrated tenfold by centrifuging at 5,000 RPM for 20 min and removing 90% of the supernatant to achieve between 10^{10} and 10^{11} cells per ml. Three solutions were prepared by dilution for this evaluation:

A. Organisms in water with 0.001% Tween

B. Organisms in Butterfield's Buffer

C. Organisms in Butterfield's Buffer with 0.1% spent media

Organism decay was determined by periodic incubation of an aliquot sample on culture plates for two days. Survival of the organisms was observed over a 28-day period. The results in Fig. A1 show that by the end of that period, survival decreased to 80.4%, 93.5%, and 73.2% for suspensions A, B, and C, respectively. Organisms in Butterfield's Buffer had slightly higher survival compared to the other two solutions, but overall, the culturable fraction for all three compositions remained acceptably high, consistent with previous literature findings.^{3, 4, 5}

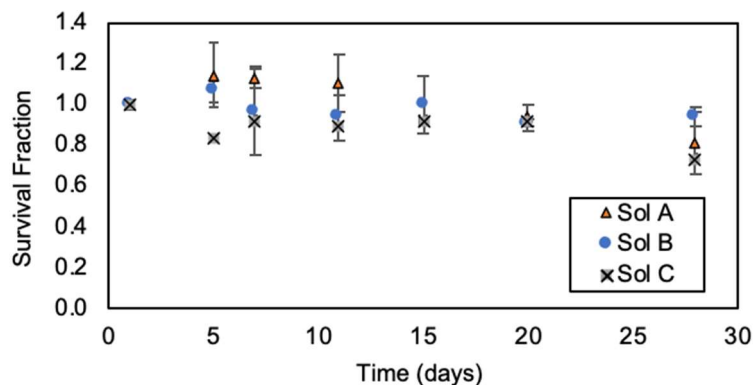


Fig. A1—Survival Fraction of *Burkholderia thailandensis* in solutions A, B, and C over a period of 28 days

In the next test, sample degradation due to drying of organisms on filters was determined over a 28-day period. Samples were pipetted onto filters. Half the filters were immediately placed into the recovery solution (PBS with 0.01% Tween-80) and the other half were placed into the recovery solution after the same total time period as the exposed samples (approximately 65 minutes). The results indicate that less than 7% of the organisms survived as a result of drying on the filter in tests where the filters were placed immediately into recovery solution. Figure A2 shows that a lower number (1.6 % or less) of cells survived when the organisms stayed on the filters for a longer time before placement in the recovery solution.

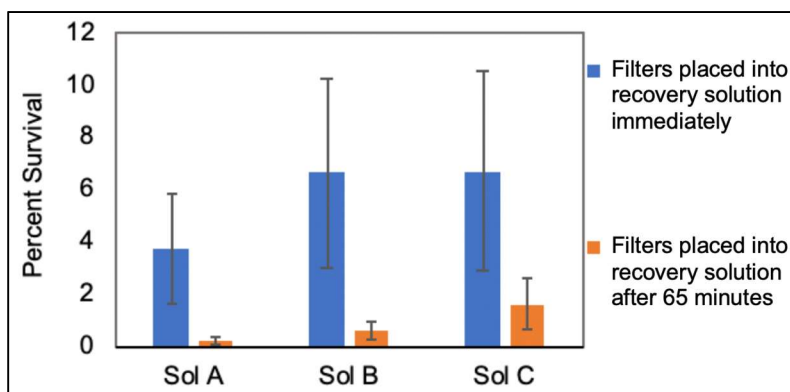


Fig. A2—Percent survival of organisms in solutions A, B, and C pipetted onto filters and placed into recovery solution immediately (blue) or 65 min (orange) after deposition. Percent survival is relative to the titer of an equivalent volume of stock.

Lastly, the effect of solution type (solutions A, B, and C) on the solar kill was determined by exposing the organisms on filters to solar radiation for 30 seconds and comparing them to control filters that were not exposed. These experiments were conducted over a 28-day period. A 30-second solar exposure kills 2 logs of organisms compared to control filters as shown in Fig. A3. There is no statistically significant difference in percent survival due to the solution composition.

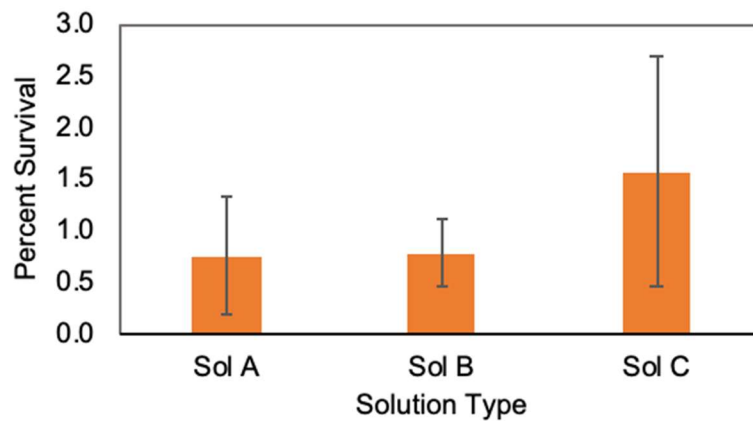


Fig. A3—Survival percent after 30 seconds of solar simulator exposure compared to unexposed filters (controls)

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APPENDIX B: *BACILLUS* SPORE PREPARATION PROTOCOL

All procedures were performed at U.S. Army Combat Capabilities Development Command (CCDC) Chemical and Biological Center (CBC) in Aberdeen, Maryland. Dr. Vipin Rastogi prepared two batches of *B. anthracis* Sterne strain spores for the PAVES program. Each batch was divided for use in surface and aerosol exposure experiments at CBC and NRL, respectively. Aliquots were placed into 15- or 50-ml sterile tubes before distribution. This protocol was written for the first of the two batches, prepared July 14, 2017.

Day 1

Struck a plate of *Bacillus anthracis Sterne* from frozen culture collection (Lot# 032607). Plates left at RT for 48 hours due to holiday.

Day 3

Growth observed, single colonies present. Plates stored at 4 °C in Lab 307 fridge. Started two 20 mL TSB cultures of *Ba Sterne* prior to storage.

Day 5

Aliquoted 400 µl of the 0/N/G broth culture and spread on to 4 Lab Lemko plates (P062617-02). (Microscope service pending, so, did not use all available sporulation media: 10 plates remain of same prep.)

Day 11

Checked spores from sporulation set #1 to assess number of vegetative cells vs. spores (borrowed microscope to analyze wet mounts). Culture seeded was at ~90%. Spores will be harvested tomorrow.

Day 12

Harvested spores off the 4 plates from sporulation set #1.

Washed 2 plates each with 15 ml of sterile water. Rewashed 4 plates total with 15 ml of sterile water before washing all with a third 10 ml of sterile water. Therefore, to harvest all the plates, 55 ml sterile water was used.

Harvested spores from the plates were filtered through an autoclaved paper towel.

Centrifuged the harvested suspension for 2 hours.

Aspirated the clear supernatant and washed the pellet with 40 ml of sterile water.

Centrifuged the suspension again for 1 hour.

Aspirated the clear supernatant and washed the pellet with 40 mL 0.1% Tween 80 (P021617-03).

Centrifuged the suspension again and aspirated the supernatant.

Resuspended the pellet in 30 ml 0.1% Tween 80.

Stored the suspension at 4 °C for the weekend prior to being heat shocked due to water bath not being at the appropriate level.

Day 16

Heat shocked Sporulation Set #1 suspension at 65 °C for 30 minutes to eliminate any remaining vegetative cells.

Sonicated stock for 5 min and vortexed for 2 min to break up any clumps of spores, enumerated the stock culture, then returned to storage at 4 °C.

Spores analyzed under the microscope looked >95% with no vegetative cell burden.

Day 17

Counted enumeration plates: the stock titer averaged $\sim 3.6 \times 10^9$ cfu/mL.

Prepared a working stock by adding 5 ml of 3.6×10^9 parent stock to 95 ml of 0.01% Tween 80; then divided the working stock between two tubes into each of which 2.5 mL of parent stock was added to 47.5 mL Tween-80.

Enumerated the working stock separately by two researchers.

Day 18

Counted enumeration plates: the average titer = 1.25×10^8 cfu/mL.

Labeled this working stock as 1.25E8_P07142017.

Second batch addendum:

The second batch was prepared similarly on October 23, 2017: average titer = 7.5×10^8 cfu/ml.

APPENDIX C: SOLAR SIMULATOR CHARACTERIZATION (YEAR ONE)

UV light sources may include narrowband sources used in previous studies, a low-pressure UV-C bulb⁸ and narrowband UV LEDs⁵⁵ at various wavelengths between 255 nm and 315 nm, as well as a solar simulator⁹ tested by NBACC and Battelle for prior studies. To perform solar exposure measurements under controlled laboratory conditions, special custom filters were procured to mimic the ASTM G173 and G177 standard spectra. The chief challenge to accurate measurements is the rapid drop in optical intensity (almost 9 logs) in the UV-B range (290–300 nm). Although the flux of shorter-wavelength photons is orders of magnitude smaller, the impact of these shorter-wavelength photons is orders of magnitude greater as well. As a result, it is critical to get accurate measurements of intensity at these wavelengths to properly characterize UV kill. The requirement for dynamic range in optical sensitivity is nontrivial. The conventional solid-state CCD detector within our calibrated Ocean Optics Spectrometer only has approximately three logs of dynamic range. As shown in Fig. C1, the noise becomes intolerable precisely in the wavelength range that is critical. We also performed measurements using a double monochromator with a photomultiplier tube (PMT) detector to improve the dynamic range for detection. The double monochromator is essential to eliminate stray light that limits low-level sensitivity. The single-channel PMT also inherently has more dynamic range than any pixel in a CCD array. The double monochromator and the PMT were part of a commercial Fluorolog 3 fluoro spectrophotometer that we adapted for our measurement. However, this experimental setup was still limited in dynamic range, giving us about six logs. For many measurements, this was insufficient.

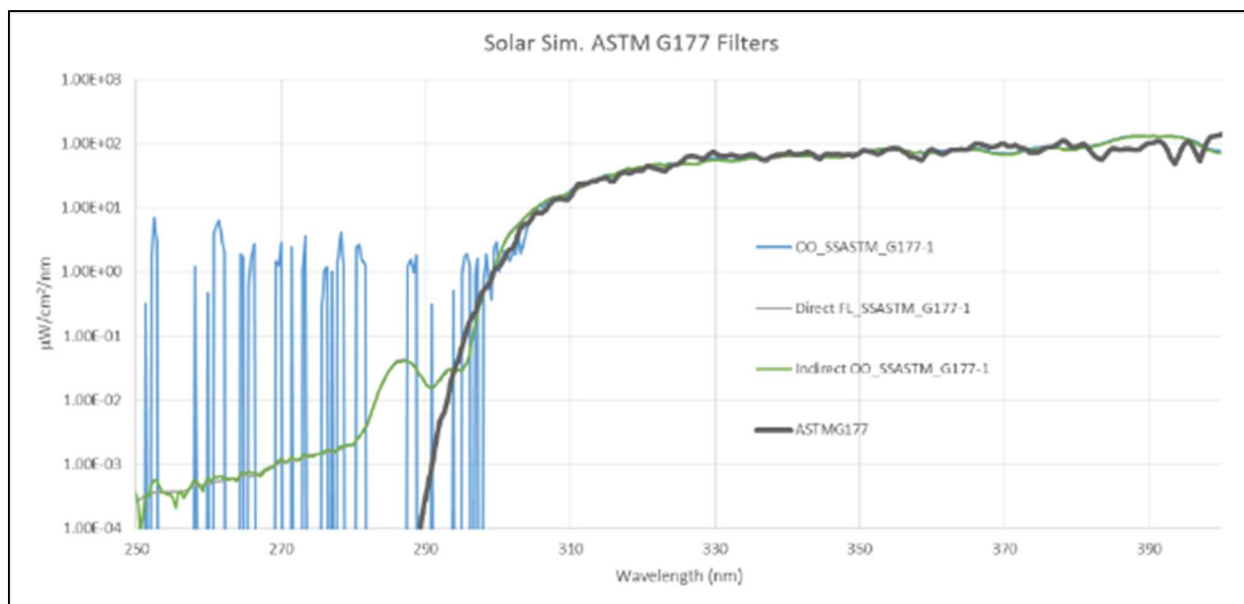


Fig. C1—Measurements of solar simulator intensity (optical flux/unit area/unit wavelength) vs. flux after transmission through a custom filter designed to give the ASTM G177 standard solar spectrum. The ASTM G177 standard (black) is presented as well. The OO_SSASTM_G177-1 labeled trace (blue) utilizes a calibrated Ocean Optics spectrometer. Due to dynamic range, noise obscures the measurement at wavelengths shorter than 300 nm. A direct measurement using a Fluorolog 3 dual monochromator to limit stray light interference (Direct FL_SSASTM_G177-1, gray) compares well to an indirect measurement using the unfiltered solar simulator fluence and the G177 filter transmittance (green).

To simplify measurement (the double monochromator measurements were technically cumbersome), we combined measurements of the transmittance of the custom G177 and G173 filters with measurements of the unfiltered solar simulator. When unfiltered, the output of the solar simulator is comfortably within the dynamic range of the calibrated Ocean Optics spectrometer. Our spectrophotometer that measured the transmittance of

the filters had about five logs of dynamic range. Multiplying the transmittance by a measurement of the unfiltered solar simulator yielded an indirect measurement of the solar simulator output. Agreement between the direct double monochromator measurements and the indirect measurements obtained from the unfiltered solar simulator and filter transmittance was excellent until the double monochromator measurement became engulfed with noise. The same measurement was performed for the G173 filters.

APPENDIX D: MINIMUM REQUIRED TWEEN 80 STUDY

As noted in Section 2.2, stock suspensions of *B. anthracis* Sterne strain spores contain 0.01% (v/v) Tween 80 (Polysorbate 80) to prevent clumping and surface adhesion during storage. However, the minimum amount of Tween 80 needed to achieve the same result is unknown. In the interest of reducing the fraction of Tween 80 in the final spore-containing particulate aerosol as much as possible, for reasons described in Section 2.5.1, we assessed the effect of Tween 80 concentration on spore plating results in a limited screening study. Working under the hypothesis that agglomeration of spores or adherence of spores to their containers would manifest as reduced colony counts in serial dilution and plating assays, we prepared suspensions of BA Sterne spores with three concentrations of Tween 80 for analysis.

Tween 80 solutions were stored at 4 °C in amber bottles and were warmed to ambient temperature just before use. Dilutions of a stock solution of 0.1015% (v/v) Tween 80 (derived from Fisher Scientific #BP-338) with reverse-osmosis-purified water afforded solutions at 0.010%, 0.0010%, and 0.00010% (v/v) strength. Each dilution was sterile-filtered through 0.1 µm polyether sulfone membranes just before loading each set of sterile serial dilution vials with 900 µl of the appropriate Tween 80 solution. From a single batch of *B. anthracis* Sterne stock containing 0.01% (v/v) Tween 80, 1,000 µl was transferred aseptically to each of four empty vials designated as Samples 1–4. Samples 1–3 were co-centrifuged 10 minutes at a relative centrifugal force (RCF) of 16,000 to pellet the spores. From each vial, removal of 900 µl of supernatant without evidence of disturbing the pellet enabled replacement by the same volume of 0.010%, 0.0010%, or 0.00010% Tween 80. Pellets were resuspended by vortexing at high speed for 3 minutes to complete the wash. Centrifugation and washing with the corresponding Tween 80 concentration were repeated two more times, except that the centrifuge time was increased to 20 min. After the third wash, the pellets were resuspended in 900 µl of the corresponding Tween 80 solution. (During this procedure, intermittent noise from the microcentrifuge could be heard, not unlike the sound of a bearing going bad. Each centrifugation cycle produced well-compacted spore pellets in all three samples; however, due to uncertainty about the uniformity of centrifugal force applied throughout the process, the three supernatants from each washed sample were retained and combined to a final volume of 2,700 µl each for subsequent analysis.)

Calculations showed three washes would be sufficient to reduce the Tween 80 concentration in Samples 2 and 3 from 0.01% to 0.0010% and 0.00011%, respectively. Assuming no spore loss in the supernatants, Samples 1–3 should contain the same number of spores as Sample 4 and should produce the same titer unless reduced Tween 80 causes clumping or surface adhesion. Sample 1, washed in 0.010% Tween 80, served as the control for comparing the influence of Tween 80 concentration. As a check on the effect of centrifugal washing alone, Sample 4 was used as is in 0.01% Tween 80 without washing. All four vials were serially diluted with the corresponding sterile-filtered Tween 80 solution. The 10^4 -, 10^5 -, and 10^6 -fold dilutions were plated in triplicate on pre-poured tryptic soy agar (TSA) plates (Hardy Diagnostics #G62). Additionally, single TSA plates were inoculated with the three supernatants and blanks for each sterile-filtered Tween 80 solution. Plates were incubated in a humidified oven at 37 °C for 14–16 h before counting. The four sample vials and three supernatants were sealed and stored at 4 °C between assays. Serial dilution and plating from the stored samples was repeated after 6 and 13 days storage, except that (1) dilutions proving to be too few or too numerous to count were omitted, and (2) on day 13, the 10^5 dilutions in 0.0010 and 0.00011% Tween 80 were plated in quadruplicate rather than in triplicate. Plate counts used to calculate viable concentrations among all four samples ranged from 31 to 203. Resulting titers and sample standard deviations appear in Table D1. Day 6 results on Sample 1 were lost to contamination.

Table D1—Dependence of *B. anthracis* Spore Titer on Tween 80 Concentration and Storage Time

Sample #	Tween 80 Conc. ^a (% v/v)	Washed?	Day 0 (10 ⁸ cfu/ml)	Day 6 (10 ⁸ cfu/ml)	Day 13 (10 ⁸ cfu/ml)
4	0.01	No	1.8 ± 0.2	1.66 ± 0.09	1.8 ± 0.2
1 (Control)	0.010	Yes	1.4 ± 0.1	no data ^b	1.4 ± 0.2
2	0.0010	Yes	1.1 ± 0.2	1.2 ± 0.1	1.30 ± 0.08
3	0.00011	Yes	0.5 ± 0.1	0.5 ± 0.2	0.38 ± 0.04

^a. Calculations showed the volume attributable to spores had a negligible effect on final Tween 80 concentrations.

^b. Two of the three plates for the control at day 6 were significantly contaminated by a nonsterile spreader. The third plate was countable but contained enough contamination to question the validity of the result. No other plates showed any contamination at any time.

The three supernatants, comprising three combined washes each for Samples 1–3, each contained more than 400,000 spores. Counts obtained from the control supernatant in a single plate on Day 13 translates to a spore concentration of 1.5×10^5 cfu/ml and 4.05×10^5 total spores recovered from the three washes. This constitutes 0.3% of the total spores that remained in the control pellet throughout the three wash cycles, but only 1% of the difference in mean total spores between the unwashed sample and the control. Since both samples employ the same concentration of Tween 80, differences due to surface adhesion or spore agglomeration aren't expected. Sample 1 was subjected to potential stress from the centrifugal force and vortexing that Sample 4 did not encounter. However, the reputed hardiness of *Bacillus* spores argues against decreased viability caused by these stresses. Additional replicates are needed to clarify this discrepancy. However, it's clear that the centrifugal washing process left measurable spores in the supernatant, contributing to loss in the washed samples. The possibly malfunctioning centrifuge is being replaced to ensure consistent pelleting. Meanwhile, we note that the mean titers of Samples 1 and 4 are within one or two standard deviations of each other on days 13 and 0, respectively. The low percentage of spores in the control supernatant may simply indicate that the mean titers of these samples are within experimental uncertainty of each other and not as many spores are lost by the washing process as the current results suggest. Lastly, titers of supernatants from the remaining two samples exceeded that of the control supernatant. Plates from Samples 2 and 3 were too numerous to count, although a rough estimate of 420 colonies obtained from the latter suggests a titer of 4×10^5 cfu/ml with the same order of magnitude as the supernatant from Sample 1.

With caution about overinterpreting data from this single screening study, some observations follow from Table D1:

1. During 13 days' storage at 4 °C, the titers for a single sample remain constant within one standard deviation.
2. For a given assay day, mean titers decrease with use of centrifugal washing and with decreasing Tween 80 concentration.
3. Comparing the unwashed sample to the control suggests some loss of spores during centrifugation and washing, although both titers lie within one or two standard deviations of each other on day 13 and day 0, respectively. At least 78% of the stock spores were retained through three wash cycles, although only 1% were accounted for based on a single supernatant plate (vide supra).
4. Sample 2 assays to within one standard deviation of the control on day 0 and day 13.
5. Although Sample 2 titers to within two standard deviations of the spores in Sample 3 on day 0, that agreement diverges on days 6 and 13 to three and eight standard deviations, respectively.

6. The control titer exceeds that of Sample 3 by more than four standard deviations on both day 0 and Day 13.

These results suggest that the Tween 80 concentration in spores suspended for environmental exposure analysis could be reduced by a factor of 10, to 0.0010% (v/v), without substantial losses to spore titer. On the basis of observations 5 and 6 above, further reduction of Tween 80 concentration seems unwise. Samples could be washed just before experiments or perhaps up to one week before. Any variability introduced from samples being washed separately at CBC and NRL would be countered through the planned use of exposure controls. However, the preceding assessment doesn't take into account potential clumping or wall losses for longer periods of time (e.g., in transfer tubing between exposure replicates or storage). This scenario may be more likely with long exposures during the LEQ aerosol experiments than the surface studies, since the former entails substantially smaller volumes of solution per replicate. In that case, subsequent particle sizing may have sufficient resolution to reveal clumped particles, but direct comparison of exposure results with surface exposures from the same nominal concentration of spores would not be reliable.

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APPENDIX E: EXAMPLE APS DATA FOR THREE STANDARD CLUSTER AERODYNAMIC DIAMETERS: 1.0, 2.4 AND 4.0 MICRON

E.1 Mass Analysis

Sample #	4 um samples (Mass)			2.5 um samples (Mass)			1 um Samples (Mass)		
	46	47	50	93	95	97	43	44	45
Start Time	23:05:00	23:05:30	23:07:05	00:27:47	00:28:47	00:30:21	00:37:21	00:37:51	00:38:21
Aerodyn	dM/dlogDp	dM/dlogDp	dM/dlogDp	dM/dlogDp	dM/dlogDp	dM/dlogDp	dM/dlogDp	dM/dlogDp	dM/dlogDp
<0.523	7.92E-07	6.79E-07	5.65E-07	2.04E-06	3.62E-06	3.28E-06	0.000925	0.000888	0.000863
0.542	0	0	0	0	0	0	0.001161	0.001027	0.001065
0.583	0	0	0	0	0	0	0.001832	0.001912	0.001713
0.626	0	0	0	0	0	0	0.002817	0.002867	0.002982
0.673	0	0	0	0	0	0	0.004549	0.004692	0.004672
0.723	0	0	0	0	0	0	0.007548	0.00808	0.007941
0.777	0	0	0	0	0	0	0.014026	0.013081	0.013365
0.835	0	0	0	0	0	0	0.025121	0.024984	0.024613
0.898	0	0	0	0	0	0	0.047924	0.046978	0.043924
0.965	0	0	0	0	0	0	0.075865	0.075534	0.076797
1.037	0	0	0	0	0	0	0.118109	0.122327	0.115272
1.114	0	0	0	0	0	0	0.183068	0.178713	0.169773
1.197	0	0	0	0	0	5.75E-05	0.287649	0.274025	0.267472
1.286	0	0	0	0	7.13E-05	7.13E-05	0.499693	0.46531	0.45197
1.382	0	0	0	8.85E-05	8.85E-05	8.85E-05	0.898131	0.846258	0.80067
1.486	0	0	0	0	0.00022	0.00011	1.57907	1.50921	1.44539
1.596	0	0	0	0.000545	0.000545	0.000682	2.47017	2.41455	2.32581
1.715	0	0	0	0.001184	0.002199	0.001522	3.56282	3.48586	3.4483
1.843	0	0	0.00021	0.003988	0.005038	0.004408	4.84968	4.89565	4.88327
1.981	0	0	0.00026	0.005991	0.008075	0.008336	5.5774	5.82643	5.85821
2.129	0.000647	0.000647	0.000647	0.015516	0.022951	0.019719	5.78367	6.08171	6.12406
2.288	0.000802	0.00361	0.000802	0.023667	0.033696	0.04212	5.82253	6.03714	6.01588
2.458	0.005973	0.003982	0.00448	0.03833	0.050774	0.05177	5.63248	5.52844	5.35322
2.642	0.011119	0.00803	0.008648	0.057448	0.053742	0.06795	5.29266	5.05052	4.87261
2.839	0.017631	0.028363	0.022997	0.032962	0.040628	0.060558	4.44757	4.11335	3.86192
3.051	0.039953	0.046611	0.042806	0.023781	0.031391	0.03044	3.33128	3.17813	2.99929
3.278	0.040135	0.060203	0.056661	0.011804	0.007083	0.018887	2.44942	2.26763	2.10237
3.523	0.071778	0.095216	0.067383	0.007324	0.007324	0.008789	1.67579	1.63624	1.52784
3.786	0.09089	0.081801	0.081801	0.003636	0.003636	0.005453	1.00706	0.934347	1.02524
4.068	0.063162	0.096998	0.069929	0.002256	0.002256	0.004512	0.665452	0.57071	0.51206
4.371	0.061584	0.07838	0.069982	0.008398	0	0	0.394697	0.310719	0.341511
4.698	0.041685	0.062527	0.052106	0	0	0	0.166739	0.208423	0.184107
5.048	0.008621	0.038796	0.047417	0	0.004311	0.004311	0.12932	0.090524	0.081903
5.425	0.032096	0.026746	0.026746	0.005349	0	0	0.037445	0.053493	0.048144
5.829	0.019914	0.053105	0.026553	0	0	0	0.053105	0.046467	0.026553
6.264	0.041187	0.024713	0.016475	0	0	0	0.016475	0.008237	0.008237
6.732	0.020444	0	0.010222	0	0	0	0.020444	0.010222	0.010222
7.234	0.038055	0	0	0	0	0	0.012685	0.012685	0
7.774	0	0.031483	0	0	0	0	0	0	0
8.354	0	0	0	0	0	0	0	0	0
8.977	0	0	0	0	0	0	0	0	0
9.647	0	0	0	0	0	0	0	0.030081	0
10.37	0	0	0	0	0	0	0	0	0
11.14	0	0	0	0	0	0	0	0	0
11.97	0	0	0	0	0	0	0	0	0
12.86	0	0	0	0	0	0	0	0	0
13.82	0	0	0	0	0	0	0	0	0
14.86	0	0	0	0	0	0	0.109849	0	0
15.96	0	0	0	0	0	0	0	0	0
17.15	0	0	0	0	0	0	0	0	0
18.43	0	0	0	0	0	0	0	0	0
19.81	0	0	0	0	0	0	0	0	0
Event 1	7	6	5	18	31	29	7521	7215	7020
Event 3	10	13	10	1	0	1	4799	4599	4304
Event 4	0	0	0	0	0	1	0	0	0
Dead Time	2	2	1	2	2	3	963	947	929
Inlet Press.	986.7	985.5	986.4	985.2	985.2	985.5	997.2	997.5	997.8
Total Flow	4.97	4.96	4.96	4.97	4.97	4.96	4.97	4.97	4.98
Sheath Flow	4	3.98	3.98	3.97	3.99	3.99	3.98	3.99	4
Analog Inpi	0	0	0	0	0	0	0	0	0
Analog Inpi	0	0	0	0	0	0	0	0	0
Digital Inpi	0	0	0	0	0	0	0	0	0
Digital Inpi	0	0	0	0	0	0	0	0	0
Digital Inpi	0	0	0	0	0	0	0	0	0
Laser Powe	75	75	75	75	75	75	75	75	75
Laser Curre	53.5	53.5	53.5	53.5	53.5	53.5	53.5	53.5	53.5
Sheath Pun	2.818	2.818	2.809	2.814	2.812	2.821	2.837	2.84	2.848
Total Pump	2.871	2.873	2.87	2.872	2.871	2.87	2.86	2.86	2.86
Box Tempe	30.6	30.7	30.2	30	30	30	31.9	31.9	31.8
Avalanch P	27.5	27.5	27.5	27.5	27.5	27.5	29	29	29
Avalanch P	204	204	204	204	204	204	204	204	204
Status Flag	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Median(nnr	4.03764	4.05401	3.9953	2.65533	2.59723	2.65076	2.28278	2.26012	2.24792
Mean(nm)	4.41538	4.2994	4.14605	2.78483	2.65666	2.70564	2.4005	2.36307	2.35006
Geo. Mean	4.25068	4.16064	4.04552	2.7224	2.61382	2.66417	2.29613	2.27598	2.26706
Mode(nm)	3.78552	4.06794	3.78552	2.64165	2.64165	2.64165	2.28757	2.12875	2.12875
Geo. Std. D	1.31203	1.28546	1.24676	1.2279	1.19273	1.1882	1.33205	1.31251	1.30653
Total Conc.	0.018928	0.023163	0.018942	0.007571	0.008564	0.010307	1.78946	1.76168	1.72017

E.2 Number Count Analysis

	4 um samples (number)			2.5 um samples (number)			1 um samples (number)		
Sample#	46	47	50	93	95	97	43	44	45
Start Time	23:05:00	23:05:30	23:07:05	00:27:47	00:28:47	00:30:21	00:37:21	00:37:51	00:38:21
Aerodynamic	dN/dlogDp	dN/dlogDp	dN/dlogDp	dN/dlogDp	dN/dlogDp	dN/dlogDp	dN/dlogDp	dN/dlogDp	dN/dlogDp
<0.523	0.055999	0.047999	0.039999	0.143997	0.255995	0.231995	65.3987	62.7827	61.0708
0.542	0	0	0	0	0	0	13.8877	12.2878	12.7357
0.583	0	0	0	0	0	0	17.6636	18.4316	16.5117
0.626	0	0	0	0	0	0	21.8876	22.2716	23.1675
0.673	0	0	0	0	0	0	28.4794	29.3754	29.2474
0.723	0	0	0	0	0	0	38.0792	40.7672	40.0632
0.777	0	0	0	0	0	0	57.0229	53.1829	54.3349
0.835	0	0	0	0	0	0	82.3024	81.8544	80.6384
0.898	0	0	0	0	0	0	126.525	124.03	115.966
0.965	0	0	0	0	0	0	161.405	160.701	163.389
1.037	0	0	0	0	0	0	202.492	209.724	197.628
1.114	0	0	0	0	0	0	252.923	246.907	234.555
1.197	0	0	0	0	0	0.063999	320.25	305.082	297.786
1.286	0	0	0	0	0.063999	0.063999	448.311	417.464	405.496
1.382	0	0	0	0.063999	0.063999	0.063999	649.331	611.828	578.868
1.486	0	0	0	0	0.127997	0.063999	919.982	879.278	842.095
1.596	0	0	0	0.255995	0.255995	0.319994	1159.72	1133.61	1091.95
1.715	0	0	0	0.447991	0.831983	0.575988	1347.94	1318.82	1304.61
1.843	0	0	0.063999	1.21598	1.53597	1.34397	1478.56	1492.58	1488.8
1.981	0	0	0.063999	1.47197	1.98396	2.04796	1370.28	1431.46	1439.27
2.129	0.127997	0.127997	0.127997	3.07194	4.54391	3.90392	1145.07	1204.07	1212.46
2.288	0.127997	0.575988	0.127997	3.77592	5.37589	6.71987	928.941	963.181	959.789
2.458	0.767985	0.51199	0.575988	4.9279	6.52787	6.65587	724.146	710.77	688.242
2.642	1.15198	0.831983	0.895982	5.95188	5.56789	7.03986	548.341	523.254	504.822
2.839	1.47197	2.36795	1.91996	2.75194	3.39193	5.0559	371.321	343.417	322.426
3.051	2.68795	3.13594	2.87994	1.59997	2.11196	2.04796	224.124	213.82	201.788
3.278	2.17596	3.26393	3.07194	0.639987	0.383992	1.02398	132.797	122.942	113.982
3.523	3.13594	4.15992	2.94394	0.319994	0.319994	0.383992	73.2145	71.4866	66.7507
3.786	3.19994	2.87994	2.87994	0.127997	0.127997	0.191996	35.4553	32.8953	36.0953
4.068	1.79196	2.75194	1.98396	0.063999	0.063999	0.127997	18.8796	16.1917	14.5277
4.371	1.40797	1.79196	1.59997	0.191996	0	0	9.02382	7.10386	7.80784
4.698	0.767985	1.15198	0.959981	0	0	0	3.07194	3.83992	3.39193
5.048	0.127997	0.575988	0.703986	0	0.063999	0.063999	1.91996	1.34397	1.21598
5.425	0.383992	0.319994	0.319994	0.063999	0	0	0.447991	0.639987	0.575988
5.829	0.191996	0.51199	0.255995	0	0	0	0.51199	0.447991	0.255995
6.264	0.319994	0.191996	0.127997	0	0	0	0.127997	0.063999	0.063999
6.732	0.127997	0	0.063999	0	0	0	0.127997	0.063999	0.063999
7.234	0.191996	0	0	0	0	0	0.063999	0.063999	0
7.774	0	0.127997	0	0	0	0	0	0	0
8.354	0	0	0	0	0	0	0	0	0
8.977	0	0	0	0	0	0	0	0	0
9.647	0	0	0	0	0	0	0	0.063999	0
10.37	0	0	0	0	0	0	0	0	0
11.14	0	0	0	0	0	0	0	0	0
11.97	0	0	0	0	0	0	0	0	0
12.86	0	0	0	0	0	0	0	0	0
13.82	0	0	0	0	0	0	0	0	0
14.86	0	0	0	0	0	0	0.063999	0	0
15.96	0	0	0	0	0	0	0	0	0
17.15	0	0	0	0	0	0	0	0	0
18.43	0	0	0	0	0	0	0	0	0
19.81	0	0	0	0	0	0	0	0	0
Event 1	7	6	5	18	31	29	7521	7215	7020
Event 3	10	13	10	1	0	1	4799	4599	4304
Event 4	0	0	0	0	0	1	0	0	0
Dead Time	2	2	1	2	2	3	963	947	929
Inlet Press	986.7	985.5	986.4	985.2	985.2	985.5	997.2	997.5	997.8
Total Flow	4.97	4.96	4.96	4.97	4.97	4.96	4.97	4.97	4.98
Sheath Flow	4	3.98	3.98	3.97	3.99	3.99	3.98	3.99	4
Analog Inpt	0	0	0	0	0	0	0	0	0
Analog Inpt	0	0	0	0	0	0	0	0	0
Digital Inpt	0	0	0	0	0	0	0	0	0
Digital Inpt	0	0	0	0	0	0	0	0	0
Digital Inpt	0	0	0	0	0	0	0	0	0
Laser Powe	75	75	75	75	75	75	75	75	75
Laser Curre	53.5	53.5	53.5	53.5	53.5	53.5	53.5	53.5	53.5
Sheath Pun	2.818	2.818	2.809	2.814	2.812	2.821	2.837	2.84	2.848
Total Pump	2.871	2.873	2.87	2.872	2.871	2.87	2.86	2.86	2.86
Box Tempe	30.6	30.7	30.2	30	30	30	31.9	31.9	31.8
Avalanch P	27.5	27.5	27.5	27.5	27.5	27.5	29	29	29
Avalanch P	204	204	204	204	204	204	204	204	204
Status Flag	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Median(nm)	3.52726	3.51164	3.49141	2.48672	2.42411	2.47164	1.83412	1.84499	1.84958
Mean(nm)	3.63715	3.63362	3.60543	2.49731	2.43556	2.48672	1.88047	1.88162	1.88204
Geo. Mean	3.54692	3.55104	3.52872	2.46077	2.40286	2.45345	1.80015	1.80302	1.80448
Mode(nm)	3.78552	3.52269	3.27812	2.64165	2.45824	2.64165	1.84342	1.84342	1.84342
Geo. Std. D	1.24452	1.23478	1.22847	1.18548	1.17901	1.17924	1.35258	1.34896	1.34666
Total Conc.	0.643987	0.801984	0.683986	0.877982	1.10598	1.23798	419.934	415.862	407.498

E.3 Raw Count Analysis

Sample#	4um samples (raw counts)			2.5 um samples (raw counts)			1 um samples (raw counts)		
	46	47	50	93	95	97	43	44	45
Aerodynarr	Raw Counts	Raw Count	Raw Counts	Raw Count	Raw Count	Raw Counts	Raw Count	Raw Count	Raw Counts
<0.523	7	6	5	18	32	29	8175	7848	7634
0.542	0	0	0	0	0	0	217	192	199
0.583	0	0	0	0	0	0	276	288	258
0.626	0	0	0	0	0	0	342	348	362
0.673	0	0	0	0	0	0	445	459	457
0.723	0	0	0	0	0	0	595	637	626
0.777	0	0	0	0	0	0	891	831	849
0.835	0	0	0	0	0	0	1286	1279	1260
0.898	0	0	0	0	0	0	1977	1938	1812
0.965	0	0	0	0	0	0	2522	2511	2553
1.037	0	0	0	0	0	0	3164	3277	3088
1.114	0	0	0	0	0	0	3952	3858	3665
1.197	0	0	0	0	0	1	5004	4767	4653
1.286	0	0	0	0	1	1	7005	6523	6336
1.382	0	0	0	1	1	1	10146	9560	9045
1.486	0	0	0	0	2	1	14375	13739	13158
1.596	0	0	0	4	4	5	18121	17713	17062
1.715	0	0	0	7	13	9	21062	20607	20385
1.843	0	0	1	19	24	21	23103	23322	23263
1.981	0	0	1	23	31	32	21411	22367	22489
2.129	2	2	2	48	71	61	17892	18814	18945
2.288	2	9	2	59	84	105	14515	15050	14997
2.458	12	8	9	77	102	104	11315	11106	10754
2.642	18	13	14	93	87	110	8568	8176	7888
2.839	23	37	30	43	53	79	5802	5366	5038
3.051	42	49	45	25	33	32	3502	3341	3153
3.278	34	51	48	10	6	16	2075	1921	1781
3.523	49	65	46	5	5	6	1144	1117	1043
3.786	50	45	45	2	2	3	554	514	564
4.068	28	43	31	1	1	2	295	253	227
4.371	22	28	25	3	0	0	141	111	122
4.698	12	18	15	0	0	0	48	60	53
5.048	2	9	11	0	1	1	30	21	19
5.425	6	5	5	1	0	0	7	10	9
5.829	3	8	4	0	0	0	8	7	4
6.264	5	3	2	0	0	0	2	1	1
6.732	2	0	1	0	0	0	2	1	1
7.234	3	0	0	0	0	0	1	1	0
7.774	0	2	0	0	0	0	0	0	0
8.354	0	0	0	0	0	0	0	0	0
8.977	0	0	0	0	0	0	0	0	0
9.647	0	0	0	0	0	0	0	1	0
10.37	0	0	0	0	0	0	0	0	0
11.14	0	0	0	0	0	0	0	0	0
11.97	0	0	0	0	0	0	0	0	0
12.86	0	0	0	0	0	0	0	0	0
13.82	0	0	0	0	0	0	0	0	0
14.86	0	0	0	0	0	0	1	0	0
15.96	0	0	0	0	0	0	0	0	0
17.15	0	0	0	0	0	0	0	0	0
18.43	0	0	0	0	0	0	0	0	0
19.81	0	0	0	0	0	0	0	0	0
Event 1	7	6	5	18	31	29	7521	7215	7020
Event 3	10	13	10	1	0	1	4799	4599	4304
Event 4	0	0	0	0	0	1	0	0	0
Dead Time	2	2	1	2	2	3	963	947	929
Inlet Pressu	986.7	985.5	986.4	985.2	985.2	985.5	997.2	997.5	997.8
Total Flow	4.97	4.96	4.96	4.97	4.97	4.96	4.97	4.97	4.98
Sheath Flov	4	3.98	3.98	3.97	3.99	3.99	3.98	3.99	4
Analog Inpi	0	0	0	0	0	0	0	0	0
Analog Inpi	0	0	0	0	0	0	0	0	0
Digital Inpu	0	0	0	0	0	0	0	0	0
Digital Inpu	0	0	0	0	0	0	0	0	0
Digital Inpu	0	0	0	0	0	0	0	0	0
Laser Powe	75	75	75	75	75	75	75	75	75
Laser Curre	53.5	53.5	53.5	53.5	53.5	53.5	53.5	53.5	53.5
Sheath Pun	2.818	2.818	2.809	2.814	2.812	2.821	2.837	2.84	2.848
Total Pump	2.871	2.873	2.87	2.872	2.871	2.87	2.86	2.86	2.86
Box Tempe	30.6	30.7	30.2	30	30	30	31.9	31.9	31.8
Avalanch Pi	27.5	27.5	27.5	27.5	27.5	27.5	29	29	29
Avalanch Pi	204	204	204	204	204	204	204	204	204
Status Flag	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Median(nm)	3.52726	3.51164	3.49141	2.48672	2.42411	2.47164	1.83412	1.84499	1.84958
Mean(nm)	3.63715	3.63362	3.60543	2.49731	2.43556	2.48672	1.88047	1.88162	1.88204
Geo. Mean	3.54692	3.55104	3.52872	2.46077	2.40286	2.45345	1.80015	1.80302	1.80448
Mode(nm)	3.78552	3.52269	3.27812	2.64165	2.45824	2.64165	1.84342	1.84342	1.84342
Geo. Std. D	1.24452	1.23478	1.22847	1.18548	1.17901	1.17924	1.35258	1.34896	1.34666
Total Conc.	0.643987	0.801984	0.683986	0.877982	1.10598	1.23798	419.934	415.862	407.498

E.4 Solution Concentrations

Solution	Concentration (cfu/mL)									
4 μ m Stock	1.50E+08			3.75E+08	2.5x	7.50E+08	5.00E+00	5	1.25E+08	8.33E-01
2.5 μ m Stock	5.40E+07			3.78E+08	7x	7.50E+08	1.39E+01	14	1.25E+08	2.31E+00
1 μ m Stock	7.13E+06			3.78E+08	53x	7.50E+08	1.05E+02	100	1.25E+08	1.75E+01

APPENDIX F: DESIGN OF A LINEAR ELECTRODYNAMIC QUADRUPOLE (LEQ) SYSTEM FOR QUANTITATIVE MEASUREMENT OF BIOLOGICAL DECAY RATES FROM IRRADIATION OF BACTERIAL AEROSOLS

We originally proposed a unique approach for aerosol particle exposure experimental study utilizing a linear electrodynamic quadrupole (LEQ) trap to levitate and confine charged micron-sized aerosol particles. The LEQ apparatus permits study of aerosol particles over extended periods of time under controlled environmental conditions. This section provides a brief summary of the operational principles, applications of this type of experimental apparatus, and a detailed description of a new design specifically for the PAVES program. Although we have previously used LEQ devices^{35,36} primarily for the study of individual suspended particles, this technique is amenable to high-volume, high-throughput data collection, where multiple particles can be generated, captured and maintained in suspension using repeatable, semi-automated procedures.

F.1 Background on LEQ Operation

The LEQ trap utilizes the quadrupole field created by four parallel rods, spaced in a square pattern, with an alternating voltage (AC) applied to pairs of diametrically opposing rods as shown in Fig. 21 and Fig. F1. The AC field constrains charged particles to a point in the plane perpendicular to the rod axes located on the geometric center of the square, resulting in a central axis of stability along the LEQ center as depicted in Fig. F1b. Absent other forces, trapped particles are constrained to be on this axis but can freely move up or down. The LEQ method is exploited in commercial ion mass spectrometers for separation of ionized molecular fragments. However, the same principles can be applied to much larger, micron-sized particles in normal atmosphere.

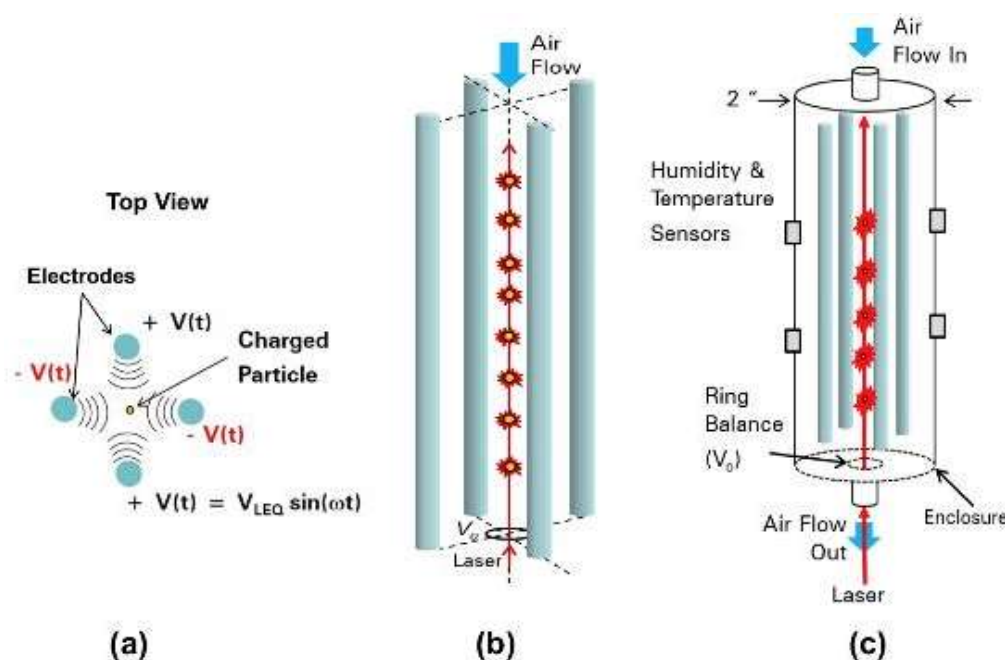


Fig. F1—Schematic illustrations of (a) quadrupole geometry with a particle confined at the center of the poles, (b) multiple particle confinement along the stability axis of the LEQ showing airflow direction, electrostatic balance ring and illumination laser from the bottom, and (c) LEQ system enclosed with temperature and several humidity sensors

Figure F1c represents schematically how the LEQ particle traps are enclosed in order to avoid disturbing or losing trapped particles due to room air currents. With an enclosure, single or multiple particles can be routinely held for periods of minutes to hours, even days. An enclosure also offers a controlled environmental chamber in which surrounding gas composition can be regulated, and temperature (T) and relative humidity (RH) can be monitored. The enclosure is made airtight, and a controlled gas flow enters and exits at ends of the enclosure along the LEQ axis. For observation of trapped particles through enclosure windows, Fig. F1b and Fig. F1c illustrate illumination using a laser beam aligned along the LEQ axis to make the particles visible to the eye.

Positional control of particles along the LEQ axis is achieved by an electrostatic (DC) potential applied to a balance ring at the bottom of the LEQ trap in Fig. 21. (Also see Fig. F1b and Fig. F1c. The DC potential, V_o , has the same polarity as the particle charge and creates an upward repulsion force on trapped particles. As a particle along the central axis approaches the bottom of the trap, the repulsive force of the balance ring increases until it reaches a position along the axis where the repulsive force from the DC ring voltage exactly balances the combined downward forces of aerodynamic drag from the gas flow and gravity. In previous devices, this balance point was exploited using video image acquisition and analysis software to create a feedback loop to the DC voltage, thereby locking the position of a specific particle along the vertical axis. If the particle charge-to-mass ratio, or the gas flow rate, changed, the balance voltage automatically adjusted to maintain the particle position, enabling for data acquisition (such as evaporation rates) over long periods of time.

One of the important advantages of a linear trap geometry is the ease of particle insertion and extraction. For droplet generation, as described in Section 2.5, reliable and repeatable charged microdroplet insertion is achieved by aligning the capillary nozzle with the LEQ central axis so that the initial droplet trajectory is coincident with the LEQ stability line. The magnitude of the droplet's initial velocity quickly equilibrates to the gas flow by drag forces, and more than 99% of the injected droplets are captured along the axis within a few millimeters. Another unique feature is that the balance ring at the LEQ exit can be used as an electric valve. The DC voltage can be momentarily lowered, permitting an individual particle to be released through the trap exit. In designing a new system for bioaerosol containment and exposure, we conceived of an LEQ with longer electrodes that would enable placement of a balance ring in the middle, dividing the LEQ into two (upper and lower) trap chambers. Figure 21 shows an image of the new LEQ design, which consists of four 1.5-mm-diameter electrodes arranged on a square with 8.0-mm sides and a total length of 120 mm.

F.2 LEQ System Design for Bioaerosol Environmental Exposure Experiments

When multiple droplets were injected into an LEQ trap, they would be trapped in a single column along the equilibrium symmetry line as depicted in Fig. F1b and Fig. F1c. Since they were all generated and charged with the same polarity and magnitude, they repelled each other and would be nominally equally spaced along the axis. In our original conception, we planned to create a population of bioaerosol particles as a dense line for evaluating irradiation exposure. One of the concerns with this approach was achieving sufficient numbers of particles to be able to make reliable population decay measurements. Since viability of microorganism populations are typically measured in terms of exponential decay rates, samples on the order of 1,000 particles would be necessary to achieve meaningful results.

Fortunately, there can be many stable configurations for trapping multiple charged particles in this type of electrodynamic field. As the number of injected particles increases, they interact with each other and begin forcing each other away from the central equilibrium axis. The “density” of particles per unit length of the trap therefore increases significantly, effectively creating a small cloud of particles. However, in this cloud, the particles are essentially in fixed positions relative to one another and do not freely move about. We have generated, charged and trapped as many as approximately 1,000 polymer particles, producing a cloud of particles within the chamber. Under trapping conditions with lower-number-density chamber fills, when it's

possible for individual particles to be forced away from the equilibrium axis of the trap, they undergo small oscillatory movements at the same frequency as the trap voltage. These small orbits manifest visibly as an elongated appearance of individual particles due to the relatively slow camera exposure time compared to their oscillation frequency. The apparent shapes of the particles are actually the trajectories of their oscillatory motion.

F.2.1 Prototype Power Supply

One early challenge associated with development of a new trap capability was design and fabrication of a new, high-voltage power supply for the LEQ electrodes. This effort was necessary because analog components that had been previously used (high-voltage transformers for low audio frequencies) were no longer available for purchase due to the lack of commercial market. We conceived a novel circuit design using available high-frequency transformers and fabricated a prototype power supply, shown in Fig. F2 (clear, plastic box on left). The power supply is operated wirelessly via Bluetooth using custom software running on a control tablet display shown in Fig. F2 (right). The required low-frequency, high voltage was achieved by amplitude modulation of a high-frequency carrier voltage. The new unit also incorporates four separate DC supplies for balance rings as well as the high-voltage AC frequency generation, amplitude control, and even a phase control between the two diametrically opposed pairs of LEQ electrodes. We have observed that adjusting the phase shift of the driving voltage between electrode pairs causes the entire cloud of particles to rotate around the central symmetry axis of the LEQ trap at a rate that is proportional to the phase difference. The all-digital unit is much more compact and lightweight than our original high-voltage supply, which consists of several separate, large components.

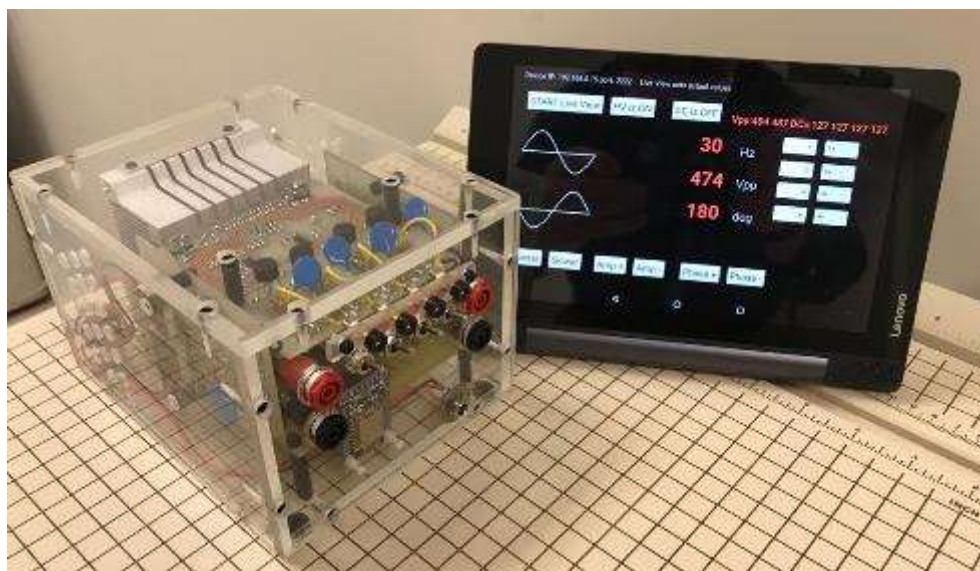


Fig. F2—All-digital LEQ power supply (left) provides both high-voltage AC for the trap electrodes (rods) and up to four low-voltage DC outputs for balance rings. Shown on the tablet display is a custom software panel that wirelessly controls and displays the supply voltages, AC frequency, and the phase of the trap high voltage between the diametrically opposed sets of electrodes.

F.2.2 Droplet Generation Modification

A second element that required improvement was droplet generation, which initially involved just installing a second video camera and a telescope to image the tip of the PZT capillary tube where the droplets are formed. Decent-resolution images were necessary to obtain reasonably accurate measurements of the initial droplet diameters. Direct, precise measurements of droplet size enable estimation of the number of spores in the residue clusters from known sample concentrations. This improvement led to some unanticipated consequences, since it allowed the operator to directly see the quality and consistency of the droplets as well as to measure physical size. As a result, it was determined that the most stable PZT droplet generation occurs in its fundamental mode, in which the droplet diameter is close to the capillary diameter, 70 μm . This mode for PZT produces the most stable and reliable operating point. However, larger droplets mean that more liquid must evaporate before getting to the exposure chamber for confinement, and consequently, the distance between the droplet generator and the LEQ electrodes had to be extended by about 6.5 cm to allow more time for droplet drying. Figure F3 shows a typical photo of *Bacillus* spore particles confined in the LEQ exposure chamber after modification to increase the flow path length.

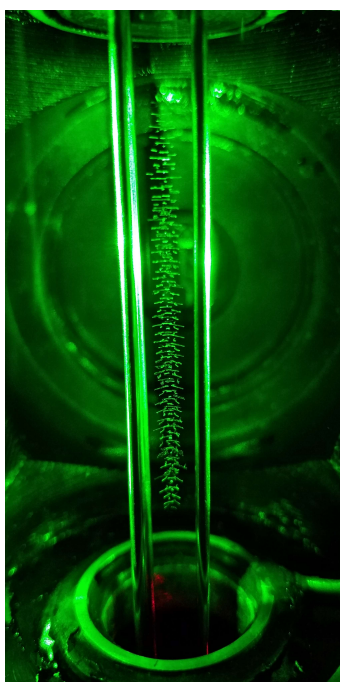


Fig. F3—*Bacillus* spore particles suspended in the LEQ exposure chamber. The elongated particle appearance is an artifact of the trajectory of individual oscillatory motions and the relatively long camera exposure time compared to oscillation frequency.

As shown in Fig. 21, the PZT droplet generator housing fits into the top of a long extension tube, just above the LEQ chamber. (The extension tube can be wrapped with heating tape to promote additional drying, but this capability was not used.) Below that, the exposure chamber is formed by a 3" optical cube that has a large observation window in the front and a large, fused-silica window on the left side to allow radiation from the solar simulator lamp or other radiation sources to expose the particles held there. Directly beneath that, the counting chamber consists of a 2" optical cube. The bottom of the counting chamber is a standard optical tube that accommodates the custom base supporting the LEQ electrodes and also houses the metal exit tube that connects to the collection assembly (see below). The collection exit tube is plumbed to a stainless-steel tee that leads to an in-line HEPA filter and a digital flow meter in the vacuum pump line. Although dry

nitrogen is used as a carrier gas inside the sealed aerosol flow system, the interior pressure is always slightly less than that of the outside lab air, preventing escape of aerosol material. As an added precaution, the entire LEQ system is housed in a 140-ft³ negative pressure enclosure that draws air at 650 cfm up from the floor past the LEQ device and out through a HEPA filter mounted in the ceiling of the enclosure, with an estimated 280 volume changes per hour.

F.2.3 Initial B. anthracis Spore Aerosol Irradiation Experiments

Initial experiments with *B. anthracis* Sterne spore particles with the LEQ aerosol system encountered difficulties right away in the form of inconsistent results. Occasionally, and in apparently random order, samples (even control samples) would show no viable counts. This led to series of increasingly specific experiments in an effort to isolate and understand the cause of these failures to show growth from spore aerosol particles. A detailed, historical account of the results of this effort is captured in Appendix H.

In a nutshell, the immediate hurdle appeared to be the physical presence or absence of the spore particles in collection, not one of spore inactivation. The collection process had been demonstrated to work acceptably well for polystyrene latex (PSL) particles. The spore recovery process was vetted as reliable, and the titer checks on the sample solutions remained consistent. The frustrating aspect was the apparently random nature of the initial results for spore particles, in which sometimes the procedure worked as expected and other times it didn't. Although we did not confirm a plausible reason for why the mechanics of particle collection could be different between spores and PSLs, we did formulate an alternative approach to particle collection that proved to be successful.

F.2.4 Aerosol Collector and Validation of LEQ for Culture-Based Viability Assay

The aerosol sampling method was redesigned to eliminate any nonconductive parts, including the polycarbonate membrane polycarbonate filter and its nylon filter holder. The new collector substrate is a sintered stainless-steel (316) frit (Restek # 25072) with a specified porosity of 0.5 μm . The frit is 6.35 mm (0.25") in diameter and 1.2 mm (0.047") thick. The frit is mounted into a recessed tip of a 316 stainless-steel tube as photographed in Fig. 20 and shown schematically in Fig. F4. The new holder is inserted into the bottom of the LEQ lower chamber (through the insulated tube used previously to mount the now-obsolete 0.5" polycarbonate filter holder). The chamber's air flow is drawn through the new collection tube. The new filter holder has a diameter that is slightly smaller than the diagonal distance between the LEQ electrodes so that it can be inserted up into the LEQ. A mechanical stop (shoulder) positions the top of the collection tube just below the (red) laser beam in the lower aerosol chamber. Since this laser illuminates particles as they flow to the collection system, each particle may be counted as it is collected. A video camera with a telescopic lens provides both a live view and a recorded file of particles exiting the aerosol chamber. In the new collection system configuration, the imaging camera captures the tip of the filter holder tube so that visual confirmation can be made that each particle is collected onto the frit as it is counted during the collection process. The photograph in the right side of Fig. F4 shows a typical instantaneous particle-collection image.

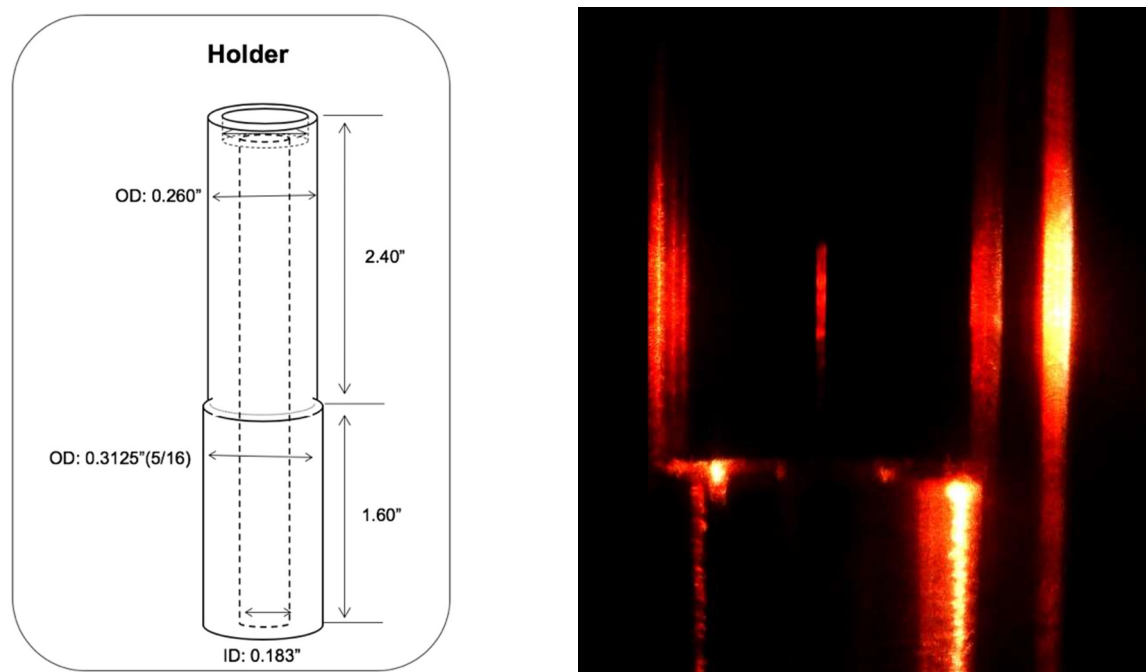


Fig. F4—Redesigned components for collection of bacterial aerosol particles. Left: conductive 316 stainless steel (SS) tube (not to scale) with recess to hold the 316 SS frit particle-collection filter. Right: a particle passing through the red laser beam appears as a streak in the middle of the photo, since the transit time of the particle is shorter than the camera exposure time, and in the lower third of the image, the tip of the tube frit holder can be seen positioned inside three of the LEQ rods (front-left rod is not seen).

With this change to an SS frit collection substrate and holder, and its proximity to the lower ends of the quadrupole rods, plating assays on collected spores became dramatically more reliable and consistent. Recoveries of controls, which include all possible process effects except irradiation, are listed in Table 4 and Table 5 of Section 4 with standard deviations. All data reported here was obtained with this modified collector.

APPENDIX G: MODELS OF UV RADIATION IN SPORES AND SPORE CLUSTERS

Optical phenomena that affect shielding of bacteria in clusters from UV irradiation include the following, most of which are illustrated in Fig. G1:

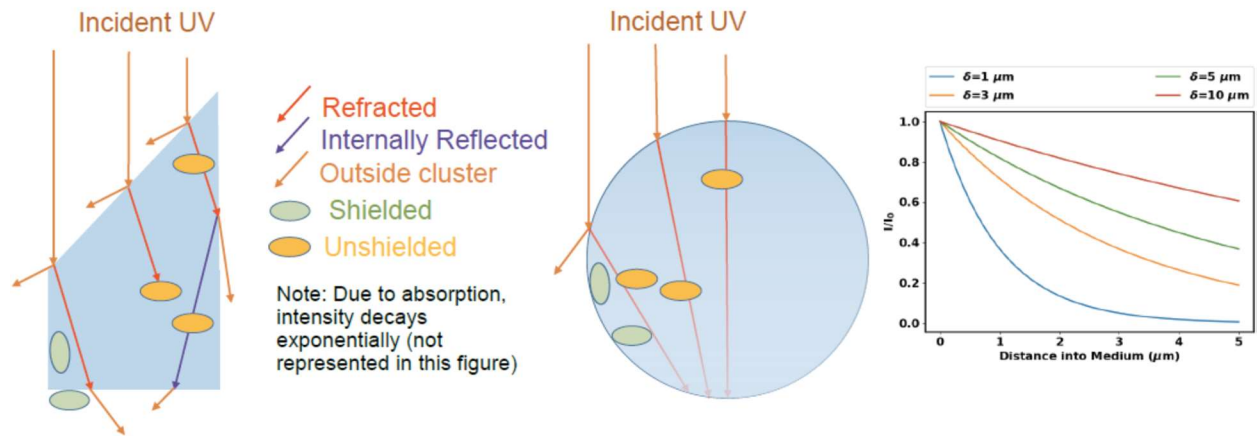


Fig. G1—Illustration of optical features related to shielding of bacteria in agglomerates from UV. The material surrounding the agglomerate is air.

- (i) *Absorption* of UV causes the intensity to decrease exponentially as it travels deeper into the cluster. Absorption thereby reduces the intensity of UV reaching the spore and the DNA in its core. The distance at which the UV has decreased to $1/e$ ($= 0.368$) of the starting intensity is termed the penetration depth, δ , which is the key parameter we use here for understanding shielding by absorption. If the intensity (I) of UV at distance z is $I(z)$, and the intensity at $z=0$ is I_0 , then the fraction of light remaining after traveling a distance z in the homogeneous material is $I(z)/I_0 = 1/e^{z/\delta} = e^{-z/\delta}$. Another parameter used in defining optical properties of materials is m_i , the imaginary part of the complex refractive index, which is $m_i = \lambda/4\pi\delta$, where λ is the free-space wavelength. The penetration depth and m_i depend upon the wavelength of the UV.
- (ii) *Refraction* of UV at a surface can direct light away from a spore underneath the surface. This effect is dependent upon the shape of the object, the angle of the incident UV with respect to a line perpendicular to the relevant surface(s), and on the optical properties of the aggregate and the material outside the aggregate. In the figures in this paper, the material outside the aggregate is air. For aggregates in water, the light refracts (bends) much less at the interfaces, and so the potential for refractive shielding is far less, if not negligible.
- (iii) *Reflection* at the outer surface of a cluster can reduce the UV in the cluster. In inhomogeneous clusters, reflection also occurs at the surfaces separating regions of different refractive index within the cluster. Reflection is typically greater for greater differences in the refractive indexes at the surface. For unpolarized light such as sunlight, reflection is typically greater for larger angles between the direction of the incident UV and a line perpendicular to the surface.
- (iv) *Diffraction* can increase the UV in regions that may otherwise be very dark because of refraction, or refraction plus absorption. It can increase the intensity in the least-well-illuminated bacteria in clusters. Diffraction is typically most important for particles such as spores, which have sizes in the range of the wavelength. It is not negligible for the wavelengths, spores, and clusters treated here.

Two methods are used to calculate the UV intensities. The more general method, needed for most accurate results for almost all spore clusters, the Multi-sphere T-Matrix (MSTM) model⁴⁹, is used to analytically calculate realistic intensities within clusters. The code accommodates an arbitrary number of separated or touching spheres. It provides exact solutions for the UV intensities inside the spheres of the cluster. It is applicable to clusters of bacteria, with or without additional material in the spaces between the bacteria, where the individual spores or bacteria are reasonably approximated as homogeneous spheres. However, because the MSTM is computationally intensive, especially for larger clusters (e.g., greater than 4- μm diameter at UV wavelengths), and for rotating clusters, we also used a homogeneous sphere (HS) model, which is solved by separation of variables, often referred to as “Mie theory.” UV in HS models is computationally simple enough that calculations for spheres with differing refractive index vs. wavelength and for any relevant diameters can be done much more easily and quickly. HS models are applicable to the special case of particles formed by drying liquid droplets containing bacteria in which the droplets include sufficient soluble material (spent growth media, lysed cells, Tween, dextran, phosphate buffered saline, or mixtures of these) to cover all the spores in a cluster, and in which the optical properties of the non-spore material is not too different from that of the spores. Example clusters that may be reasonably approximated by the HS models are particles made by drying drops that comprise vegetative cells or spores, as well as lysed cells, with or without spent culture medium. HS models also become more applicable as the volume fraction of the spores or vegetative cells in a cluster, decreases within a sphere that is otherwise homogeneous. That is, the UV in three spores, randomly positioned within a 5 μm sphere of, for example, ammonium sulfate, should be approximated from the UV intensities calculated for the homogenous sphere.

The MTSM (or a similar code capable of representing the spheres and the material in between the spores) is essential for cases in which the refractive index in the spaces between the spores in a cluster is significantly different from the refractive index of the spores. Such differences are especially important when the cluster is made of clean spores, i.e., with air in the spaces between spores. Calculated intensities, using clusters containing as many as 100 spores, show that the UV exposure of individual spores within the cluster can be far smaller than for isolated spores under the same ambient light conditions. The partial protection of some of the spores from UV, which is due to both absorptive and refractive shielding, is far more significant for particles in a fixed orientation, as when held on a surface. In contrast, such effects are less significant for aerosol particles that are free to rotate in air, and hence in sunlight are exposed to UV from all sides.

We first describe our efforts in approximating the optical properties of spores and spore clusters, then describe the HS models and methods used to approximate the UV intensities in clusters of spores and individual spores. Results from the MTSM model appear in Section 7.

G.1 Estimates of Optical Properties of Bacterial Spores

Table G1 lists the penetration depth (δ) and the real and imaginary parts of the complex refractive index, $m = m_r + i m_i$, for different wavelengths for bacterial spores in the genus *Bacillus*. Of course, bacterial spores are not actually homogenous, and are far less homogenous than are vegetative bacteria growing in water. The problem of inhomogeneous spores must be addressed, but we are looking at the simplest problem first. These values for m were estimated from reported measurements of molar absorption coefficients and concentrations of light-absorbing primary metabolites in bacteria^{51, 52, 53}, also including CaDPA in spores.

Bacteria may also include varying amounts of a large variety of additional light-absorbing molecules, e.g., melanin's and carotenoids. Because the potential variations are so large, we focus here on the case of unpigmented bacteria but describe how the results are relevant to pigmented bacteria.

Some comments on uncertainties in the complex refractive index, and hence penetration depth, must be stated. First, for bacteria lacking pigments, the relative uncertainties in δ at wavelengths between 260 and 290 nm are far smaller than those at longer wavelengths. The absorption at 290 nm and shorter wavelengths is

dominated by nucleic acids, amino acids, and calcium dipicolinate (CaDPA), and uncertainties in these and their molar absorption coefficients tend to be smaller and less variable than those of primary metabolites absorbing at longer wavelengths, although absorption by CaDPA in spores at 290 nm and longer wavelengths is less certain. However, as is illustrated later, for the larger d and smaller δ (at 260 to 290 nm) the UV in the low-intensity regions is relatively sensitive to δ . On the other hand, at the longer wavelengths, the δ values (see Table G1) are so large relative to d that uncertainties have a relatively small effect for clusters with d in the ranges modeled here (1 to 9 μm). Second, for pigmented bacteria, especially at wavelengths longer than 280 nm, δ may be much smaller than those listed in Table G1 and may be so small that the UV distributions at the wavelengths longer than 290 nm may be more similar to the distributions illustrated here for the wavelengths at 290 nm and shorter. For example, *B. subtilis* spores (strain 168, which is pigmented) have a relatively flat m_i when suspended in water, which remains between 0.0135 and 0.018 for wavelengths between 260 to 500 nm, and the only m_i greater than 0.0146 occurred at wavelengths longer than 295 nm.⁵⁵

Table G1—Estimates of the Complex Refractive Index ($m_r + i m_i$) and Penetration Depth (δ) Vs. Wavelength for *Bacillus* Spores and Vegetative Cells used in Our Calculations

Wave-length (nm)	<i>Bacillus</i> Spores (98% Solids)			<i>Bacillus</i> Veg. (98% Solids)			<i>Bacillus</i> Spores (60% Solids)		
	m_r	m_i	δ (μm)	m_r	m_i	δ (μm)	m_r	m_i	δ (μm)
260	1.548	0.0175	1.18	1.588	0.0350	0.591	1.467	0.00968	2.14
266	1.546	0.0192	1.11	1.585	0.0346	0.61	1.467	0.0106	2.0
273	1.543	0.0189	1.15	1.580	0.0319	0.681	1.466	0.0105	2.08
280	1.54	0.0161	1.38	1.575	0.0261	0.854	1.464	0.0089	2.50
290	1.538	0.00543	4.25	1.536	0.0061	3.75	1.463	0.0030	7.69
302	1.536	0.00071	33.7	1.534	0.00028	85.5	1.462	0.00039	61.0
317	1.534	0.0004	63.4	1.532	0.00006	445	1.461	0.00022	115
325	1.532	0.00041	62.7	1.530	0.00008	336	1.46	0.00023	113
339	1.530	0.00023	117	1.548	0.00009	308	1.459	0.00013	212
355	1.526	0.00025	113	1.544	0.00009	303	1.456	0.00014	203
450	1.518	0.00008	448	1.527	0.00008	434	1.449	0.00004	810

Colors indicate three penetration depth groups: $\delta < 3 \mu\text{m}$ (orange), $4 \mu\text{m} < \delta < 8 \mu\text{m}$ (green), and $33 \mu\text{m} < \delta$ (blue).

The relatively flat δ for these “brownish” spores suggests that pigments with absorption of both melanins and carotenoids contribute to the absorption. However, neither melanins nor carotenoids alone have absorption spectra consistent with the measured spectra. Absorption of light by melanins decreases too rapidly with wavelength.⁵⁶

Carotenoids, on the other hand, tend to absorb far lighter in the 400- to 500-nm range than they do in the 290- to 380-nm range. Although including values for pigment molar absorption coefficients and concentrations in bacteria is beyond the scope of this work, calculations of UV for the δ of unpigmented bacteria at 280 nm and shorter wavelengths can be useful for estimating the UV in brownish bacteria at wavelengths from 280 to 400 nm.

The relevant diameters (d) of spherical particles for our work vary from approximately 0.8 to 10 μm . The ratio d/δ can help in understanding the variations in calculated intensities. The intensity of an optical wave traveling a distance z in a medium with penetration depth δ is $I(z)/I_0 = e^{-z/\delta}$. So, if $d/\delta = 5$, then we

may expect some positions inside the sphere to have intensities far smaller than the illuminating (incident) wave, maybe on the order of $e^{-d/\delta} = e^{-5}$ times. On the other hand, if d/δ is less than 0.3, the absorption in the sphere should be small and significant protection from UV would need to arise from refractive effects.

In Table G1, m_i and δ separate roughly into three main categories: δ smaller than an approximately 2.5- μm -diameter spore (at wavelengths 260 to 280 nm), δ greater than 33 μm (at wavelengths of 302 nm and larger), and a transition region with δ between 3.75 and 7.69 μm (at 290 nm). Between 280 and 302 nm, the penetration depth increases by a factor of 24–100, whereas the differences in composition resulting from changes in water content are less than approximately two. For UV germicidal irradiation (UVGI), light at 254 nm is often used. Table G shows that the δ changes relatively little in the 260- and 280-nm range. Thus, the UV intensity distribution in the particles at 280 nm should be similar to those at the shorter wavelengths. Similarly, at 302 nm and above, $d/\delta < 0.3$ for the d of the particles investigated here. Therefore, at wavelengths of 302 nm and above, the absorption is sufficiently low that the internal intensities (illustrated in the subsequent figures) are largely independent of illumination wavelength for clusters of spheres of the sizes calculated. This could change in the case of pigmented particles.

G.2 Homogeneous Sphere (HS) Model UV Intensities in Spheres with Optical Properties of Spores: Fixed Orientation

HS model results are shown to augment the MSTM results, partly because they are simpler to understand and partly because they help in understanding the MSTM findings. On the other hand, the MSTM results help in understanding how similar the homogeneous sphere results are to MSTM predictions when the larger sphere has a refractive index that's not too different from that of the spores (as spheres that model the spores).

Figure G2 illustrates how the internal intensity varies with d for one UV wavelength (280 nm) and for spores with 98% solids. For this case, $\delta = 1.38 \mu\text{m}$. As d increases, the lowest-intensity regions, colored in cyan to purple, have UV intensities that are orders of magnitude smaller than the incoming (incident) intensities and can be termed “shielded” or “dark” regions. In these figures, the shielded regions result from a combination of absorptive and refractive shielding. For spheres larger than approximately 2 or 3 μm , these “dark” regions encompass approximately the same fraction of the volume of the sphere for all diameters because the refractive index does not vary. Because the incident wave is unpolarized and the particle is a sphere, the intensity distribution is independent of the angle around the sphere axis aligned with the direction of wave propagation.

The UV intensity at the transition from cyan to purple on the false color scale decreases from 0.16 for $d = 2 \mu\text{m}$, to 0.05 for $d = 3 \mu\text{m}$, 0.01 for $d = 5 \mu\text{m}$, to 0.003 for $d = 7 \mu\text{m}$, to 0.001 for $d = 9 \mu\text{m}$. These figures illustrate that for the larger spheres, regions as large as the spore core (harboring DNA) or a vegetative cell have intensities that are orders of magnitude smaller than the average intensity over the sphere, or the average intensity of a sphere with $d = 1 \mu\text{m}$, which is approximately that for an individual spore. If these spheres were illuminated by UV from both the right and from the left, the low intensities in the refractively shielded regions would be replaced by the relatively high intensities from the wave moving in the opposite direction. The intensities are similar to previous calculations for visible light in a non-absorbing droplet, but are simpler because the UV is unpolarized.

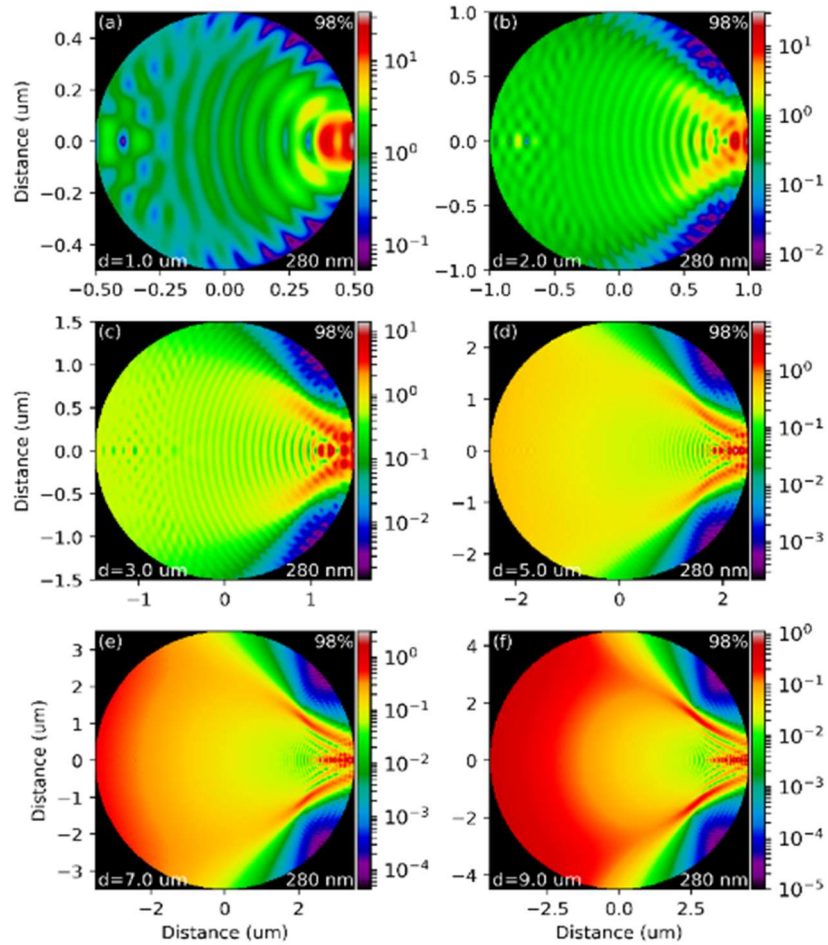


Fig. G2—Logarithm of UV intensities on a cross section inside of homogeneous spheres having a refractive index $(1.54 + i 0.0161)$ approximating that of dry (98% solids) spores at 280 nm. Each is illuminated by an unpolarized UV plane wave propagating from left to right. The logarithm of intensity is shown on the false-color scales. The sphere diameters are 1, 2, 3, 5, 7, 9 μm . The percentage of solids is 98%, and water is 2%.

The peak intensity in the sphere decreases from approximately 33 for the $d=1 \mu\text{m}$ sphere, to 20 at 2 μm , to 6 at 5 μm , to 1 at 9 μm . The peak intensity is in the internal focal region on the right side of the sphere. For smaller δ , e.g., with the vegetative cells in Table G1 at wavelength = 280 nm, the intensity maximum decreases to approximately 0.6 for $d = 5 \mu\text{m}$ and larger (in these cases, the maximum UV is near the sphere surface on the side where the incident wave first enters the particle).

Figure G3 illustrates, for $d = 6 \mu\text{m}$ spheres, the effect of increasing the penetration depth, which increases from $\delta = 1.38 \mu\text{m}$ at 280 nm to $\delta = 63.4 \mu\text{m}$ at 317 nm for these spores with 98% solids. In Fig. G3, as wavelength increases from 280 to 317 nm, the intensities in the diamond/oblong shaped regions colored cyan to black increase from a range of approximately 0.0001 to 0.006 to a range of 0.002 to 0.1. That is, the intensities in the lowest-intensity regions are approximately 15 to 20 times lower at 280 nm than at 317 nm. In each of these cross sections, the regions colored cyan to black, with dimensions roughly 1 and 3 μm , are sufficiently large to encompass the majority a spore roughly 1 μm in diameter, even when δ is many times larger than d . Because the UV is symmetric about the axis of the sphere, these shielded regions form a ring around the axis. Several spores could be shielded within a ring.

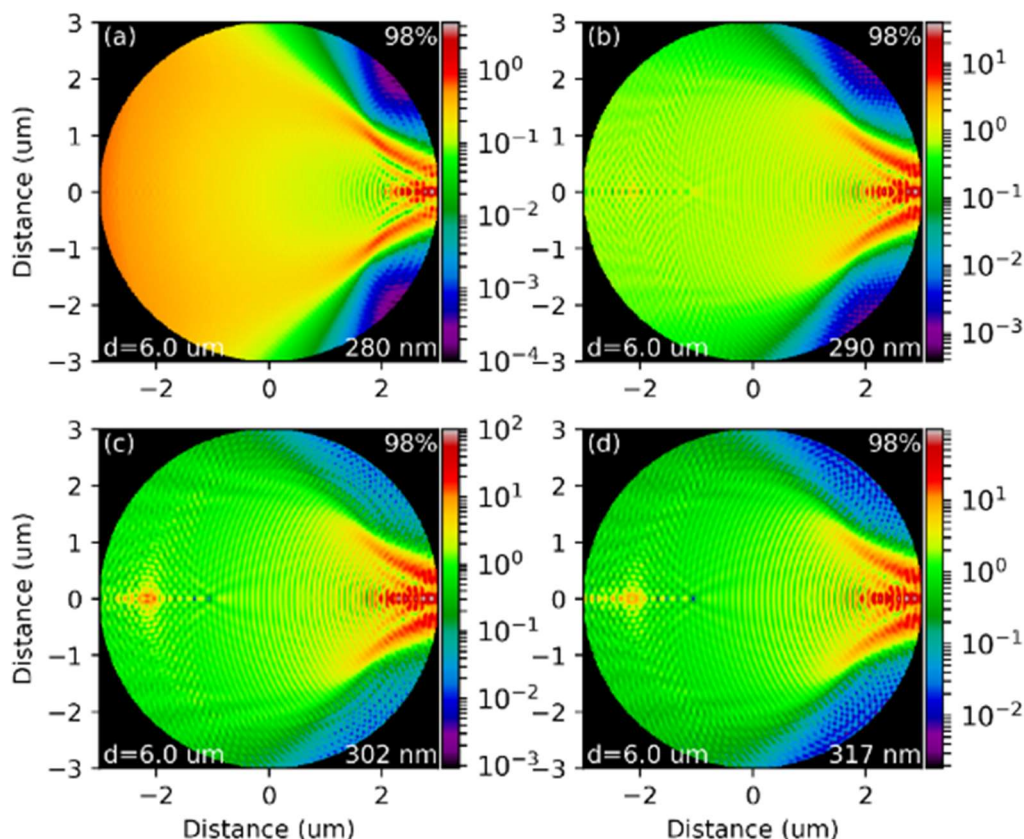


Fig. G3—Logarithm of UV intensity on a cross section inside a homogeneous sphere illuminated by an unpolarized UV plane wave coming from the left side of the page. The sphere diameter is 6 μm in each case. δ is 1.38 at 280 nm, 4.25 at 290 nm, 33.7 at 302 nm, and 63.4 at 317 nm, as specified in Table G along with m .

It is useful to consider how these UV distributions might be different in pigmented bacteria. The measured m_i of “brownish” *B. subtilis* spores in water, throughout the UV-A and UV-B regions, are within 16% of the 0.0161 used for m_i in Figs. G2 and G3a. The m_i measured for spores in glycerol, for wavelengths between 260 and 700 nm, was approximately 50% of the 0.0161 at 280 nm used in Fig. G2. Therefore, for spherical clusters of pigmented spores with the m_i reported by Tuminello et al. (cited above), the UV shown in Fig. G2 and Fig. G3a (for 280 nm) provides better estimates of the UV in the clusters for wavelengths from 280 and 600 nm than do the intensities shown in Fig. G3b and Fig. G3c for wavelengths of 290, 302, and 317 nm.

Figure G4 illustrates the variation in UV intensity with fraction of solids between 45% and 90% for clusters ($d = 5 \mu\text{m}$) illuminated at 280 nm. The variation in UV distributions with the percentages of solids used in Fig. G4 is much less significant than the variations with diameters in Fig. G2 or with wavelength in Fig. G3. This difference between Fig. G3 and Fig. G4 is not surprising. In moving from 90% to 45% solids in Fig. G4, the concentrations of absorbing molecules are approximately cut in half and δ approximately doubles. In Fig. G3, in contrast, the δ at 317 nm is 46 times larger than that at 280 nm. Although in Fig. G4, the size of the refractively shielded region decreases as the percent solids and refractive index decreases, the effect is too small to be easily visible for the range of refractive indexes for these spheres (1.524 to 1.434).

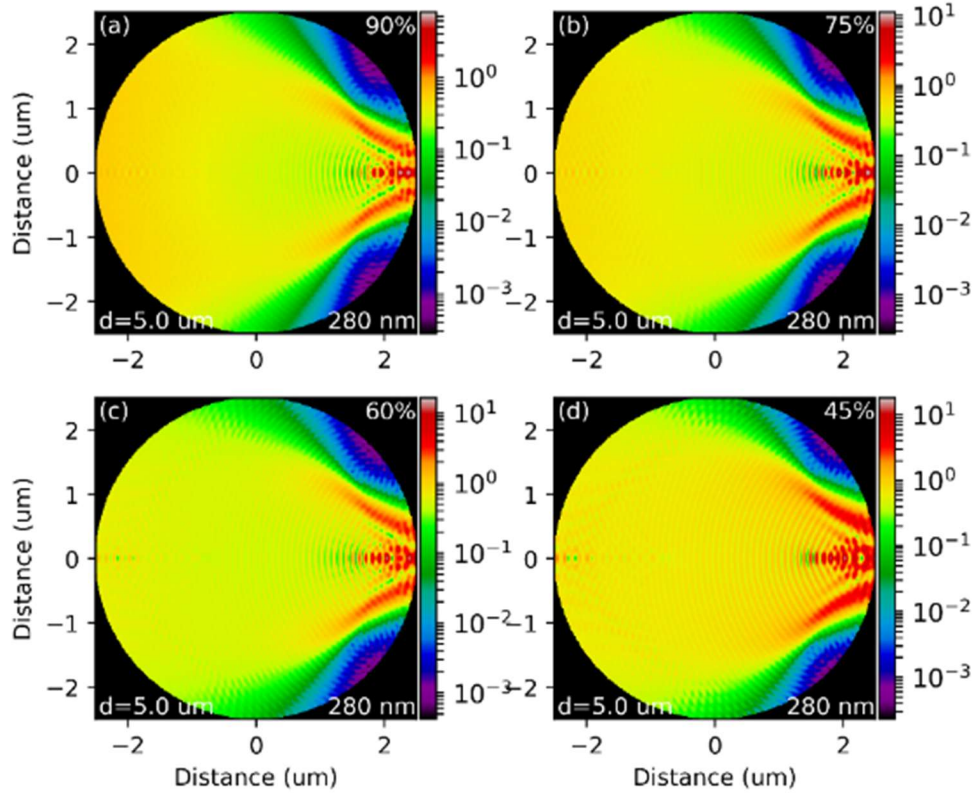


Fig. G4—Logarithm of UV on a cross section of homogeneous spheres approximating clusters of spores, each illuminated by a UV plane wave approaching from the left. The sphere diameter is 5 μm in each case. The δ , m , and percentage of solids in the particle are: 1.54 (1.524+ i 0.0145) at 90%, 1.92 (1.494+ i 0.0116) at 75%, 2.5 (1.464+ i 0.00089) at 60%, and 3.48 (1.434+ i 0.0064) at 45%.

G.3 HS Model UV Intensities in Spheres with Optical Properties of Spores: Random Orientation

In spherical homogeneous particles carried in air, the internal intensity is averaged over all illumination angles as the particles rotate randomly and rapidly relative to illumination times (seconds to hours). Figure G5 illustrates UV intensities in such rotating particles. It shows the angle-averaged intensity for three diameters of clusters of spores (98% solids), and for one diameter of a cluster of cells with a higher m_i than that used for the spores, each at ten wavelengths (Table G1). In Fig. G5, the variation in UV is with the distance from the sphere's center (the radial distance). As suggested in Table G1 and as consistent with previous figures where the wavelength is varied, the results clump into three main categories: 280 nm and below with δ less than 1.38 μm for the spores (less than 0.7 for vegetative cells), 302 nm and above with δ greater than 33.7 μm , and an intermediate category (290 nm) with $\delta = 4.25 \mu\text{m}$ for the spores and 3.75 for the vegetative cells. For 1- μm spheres, the radial variation for the 290-nm case ($d/\delta = 0.056$) is similar to those with wavelengths longer than 290 nm. The peak in the yellow line near radial distance 0.44 in Fig. G5a is an example of the increase in internal intensity near resonances of the sphere, which remained even after we integrated over a band of radius/wavelength to reduce these artifacts.

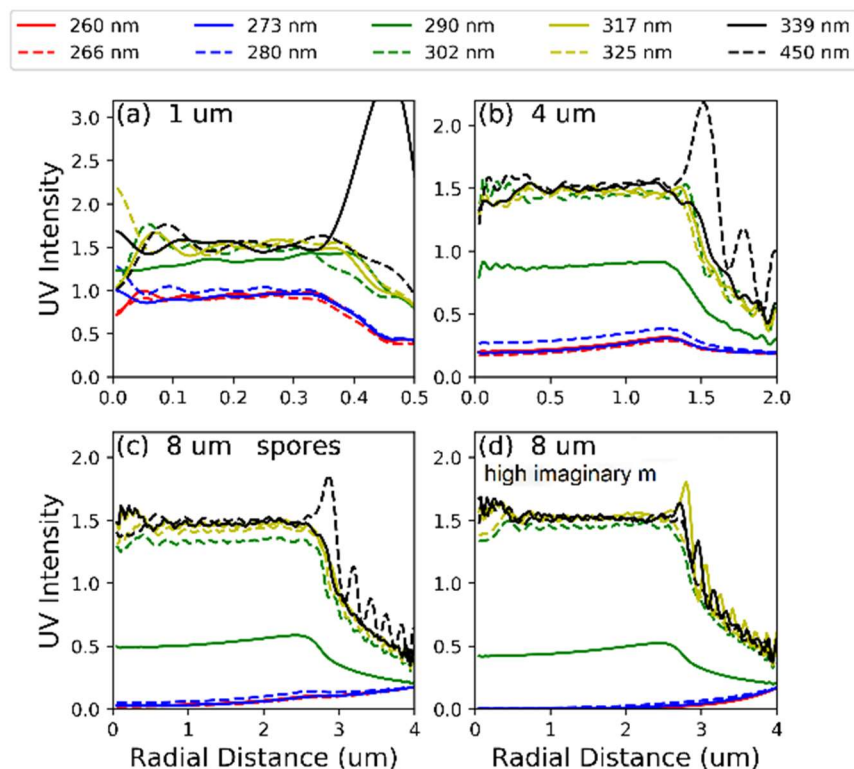


Fig. G5—Variation with distance from the center of the sphere of the angle-averaged UV inside of homogeneous spherical particles of diameters: 1 μm in (a), 4 μm in (b), and 8 μm in (c) and (d). Clusters of spores are modeled in (a), (b), and (c). Cells with a higher m_i are in (d). In all cases, the center of the sphere is at radial distance = 0. In each case, the lowest four curves are at wavelengths 260, 266, 273, and 280 nm, the solid, green curve just above those (except for radial distance greater than 0.33 μm in (a)) is at 290 nm. The upper five curves are at 302, 317, 325, 339, and 450 nm. In (d) the complex m , with increasing wavelength, are: $(1.588+i0.0350)$, $(1.585+i0.0346)$, $(1.580+i0.0319)$, $(1.575+i0.0261)$, $(1.536+i0.0061)$, $(1.534+i0.00028)$, $(1.532+i0.00006)$, $(1.530+i0.00008)$, $(1.548+i0.00009)$, and $(1.527+i0.00008)$.

The differences between the minimal intensities in the rotating and nonrotating spheres are large for the spheres illuminated at 280 nm and below, especially for the 4- and 8- μm spheres. The lowest intensity near the center of the 8- μm rotating sphere in Fig. G5c is 0.08 for UV at 280 nm. However, in Fig. G2, the 7- and 9- μm spheres in fixed position have regions where the large majority of the intensities are less than 0.0001 of the incident wave, and these are large enough to encompass a 1- μm -diameter sphere.

That the 290 nm case is intermediate between the cases of 280 nm and shorter, and of 302 nm and longer, is also seen in the shape of UV vs radial distance. In Fig. G5c, for the 8- μm spheres, the radial variation in UV at 290 nm (with $d/\delta = 0.45$) is shaped more like the 302- and 317-nm cases, with the UV decreasing with radial distances for radial distances greater than 2.2 μm . However, its overall intensity is more similar to cases with wavelengths of 280 nm and shorter. Also, for the 290-nm case in Fig. G5c, at radial distances less than 2.2 μm , the UV decreases in moving toward the center of the sphere. In this way, the 290-nm UV variation for the 8- μm cluster (Fig. G5c) is more similar to the 280-nm results for the 4- μm diameter cluster (Fig. G5b).

Significantly, the results in Fig. G5, when combined with the UV bands of solar radiation contributing most to inactivation of spores, suggest that clusters of unpigmented bacteria rotating in air are not significantly shielded from solar UV at the wavelengths most significant for inactivating spores (300 to 320 nm). This small-to-negligible shielding is not general for this wavelength range, but applies to the nonpigmented bacteria modeled here and to the estimated values used for absorptivity of CaDPA.

APPENDIX H: HISTORICAL DEVELOPMENT OF AEROSOL SUSPENSION AND COLLECTION

H.1 Overview Aerosol Experimental Approach

The experimental approach for aerosol suspension and exposure studies, and data collection, was based on prior development and experience with electrodynamic trapping of charged aerosol particles. There is a range of specific designs as well as nomenclature that can be obtained from published literature going back as far as 1953.³³ The technique has seen periodic use over time, but until recently, the method was mostly applicable to the study of single, levitated particles. One of more significant developments from the first year of this program has been the exploitation and refinement of handling large numbers (hundreds) of confined particles simultaneously. This capability is directly related to our choice of electrode geometry, referred to as a linear electrodynamic quadrupole (LEQ) trap. The basic principles of operation were briefly described elsewhere.³⁴ While we had had significant prior experience with the implementation of these types of devices at the beginning of this program, an apparatus specifically for executing the bioaerosol exposure and degradation studies was just a concept. During the first year of PAVES, an intensive effort went to design, fabrication and initial tests of the bio-aerosol chamber. As context, Fig. H1 is reproduced from the first-year report showing a schematic diagram of the LEQ apparatus with four main elements from top to bottom: (1) the microdroplet generator, (2) the particle exposure chamber, (3) the particle counting exit chamber and (4) the particle collection filter assembly. At the beginning of the second year of the PAVES program, this apparatus had been assembled but was still being tested to explore and optimize its operation. By the beginning of Q1 Y2, there were still significant issues with droplet generation reliability, and particle collection had not been implemented beyond a couple of non-quantitative runs with large (10 μm) PSL particles. Consequently, the PAVES aerosol system continued to be substantially modified over this time. In brief, by the end of Q1 Y2, issues with droplet generation and drying had been resolved for spore samples, and substantial progress had been made with particle collection. However, the reliability and reproducibility of the latter were inadequate at that point. Specific changes are described in the sections below.

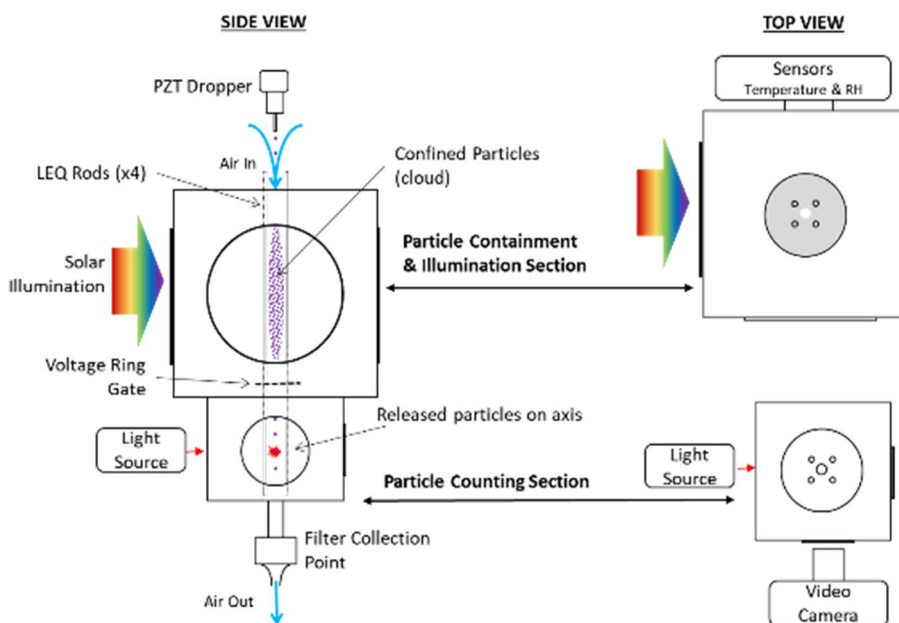


Fig. H1—Current LEQ system showing the top and side views. Particles injected as droplets into the upper section rapidly dry into solid particles. Confined particles are held in this upper chamber for radiation exposure and are then allowed to pass individually into the lower chamber for video counting. As particles exit the lower chamber, a polycarbonate membrane filter is used for collection.

H.2 Continued Development of the LEQ Aerosol System

During the first three quarters of the second performance year, the aerosol apparatus was located in a regular lab space to permit greater access and flexibility during development and initial testing. Experiments with viable BL-2 spores (BA Sterne samples) could not commence until the apparatus was relocated to our BL-2 lab inside a secondary enclosure. It was decided that development work would continue in a regular lab setting until complete functionality was demonstrated. During this period, aerosol sample materials were either commercially available PSL beads or *Bacillus* spores from BL-1 species. Two main issues were addressed: (1) demonstration of particle collection efficiency as acceptable levels, and (2) improved droplet generation reliability and quantification.

H.2.1 Sample Collection Improvement 1

It was quickly determined that the original filter assembly design did not provide consistent, reproducible results. Occasionally, expected samples were obtained, but mostly, no particles were collected on the filter. The absence of particles was attributed to losses in the coupling between the bottom of the lower counting chamber of the LEQ housing, and a new design was developed.

Figure H2 shows a schematic drawing of the base of the aerosol confinement system that supports the four LEQ electrodes running all the way through the aerosol exposure chamber to the droplet generation capillary tip. The main components of the base are machined Lucite plastic (shaded grey in right drawing) which slides into the bottom of the counting chamber. A hermetic seal adequate for small pressure differentials is achieved using O-rings that are omitted in this diagram, as are any electrical connections or feed-throughs. Based on prior experience, we speculated that successful transition of the charged particles from the LEQ confinement region to the filter could be achieved by a conductive metal tube protruding into the LEQ trap region. Except for near the ends of the short tube, the electric field should be nearly zero inside the tube, so charged particles entering along the center line of the tube should not feel any lateral force and would continue due to gas flow toward the filter surface below. The plastic-covered metal tube [ID: 7 mm (0.25"), length $\approx$ 2.5 cm (1")] is press-fit into the plastic base that supports the LEQ electrodes so that it extends into the LEQ region, but also protrudes out the bottom of the base as shown in Fig. H2 (left).

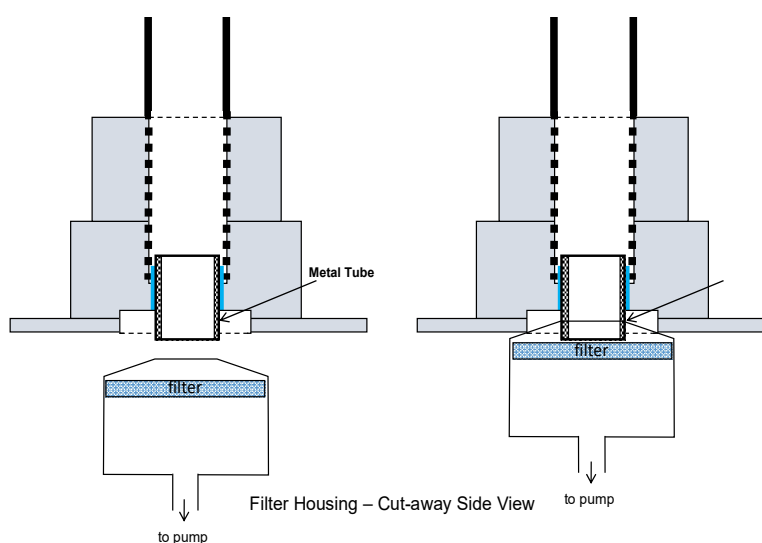


Fig. H2—A schematic of the new LEQ chamber base that now forms the bottom of the aerosol chamber

Figure H3 shows a close-up photo of commercially available 13-mm-diameter filter housings. On the left, the assembled housing outlet has been connected to a standard PVC tubing and SwageLok™ coupling. On the right side, the threaded top cap has been removed (center) and the bottom filter support is oriented vertically so that the filter support surface is visible (far right). The top caps were drilled out to make a press-fit seal to the metal transition tube protruding from the bottom of the lower counting chamber housing. (A penny provides a scale for the photo.) Fig. H2. (right) illustrates how the connection to the transition tube forms a tight seal and positions the filter surface just below the end of the tube. This design positions the filter support immediately beneath ($\approx 1\text{--}2\text{ mm}$) the grounded transition metal tube, presumably eliminating charged surfaces to attract aerosol particles as they move downward to the filter surface. This iteration in the LEQ base support and filter housing was completed in September 2018.



Fig. H3—A close-up photo of the particle collection filter housing. Complete unit at left, with cap removed at right showing filter support. Cap in middle.

When this design for the bottom portion of the aerosol chamber was implemented, experiments were carried out to determine particle-capture efficiency onto a filter, compared to direct particle counting as they passed through the lower counting chamber. When particles held in the upper exposure chamber are controlled to slowly exit into and through the lower counting chamber (see Fig. H1), there they intercept a c.w. laser beam and their images are recorded by a video camera as they pass through the beam. For a given sample, the total particle count using this laser illumination can then be compared to the number of particles collected on the filter by examining the filter with a microscope and manually counting the particles present. In September 2018, these design modifications were implemented, and collection efficiency experiments were conducted in October. Figure H4 shows a micrograph of dye-doped PSL cluster particles that were captured on a filter and then were placed into an epifluorescent microscope for observation and particle counting. Not only were the PSL particles reliably collected on the filter, but as this image illustrates, typically all of the collected particles were located in a small area in the middle of the filter. In this case, fluorescent PSL particles are inside a circular area about $500\text{ }\mu\text{m}$ (0.5 mm) in diameter. More than 40 trials were conducted in which more than 6,000 individual dye-doped PSL (mostly $1\text{-}\mu\text{m}$ -diameter) and / spore particles were counted. For both of these particle sample types, concentrations were adjusted to create both small clusters (including single, individual spores or beads) as well as larger ones. For spore samples, a quantity of fluorescein dye was added so the resulting residue particles (from droplet drying) could be observed under the microscope in fluorescence. Overall, the two methods of particle counting for each sample agreed to within 8% on average. These results were adequate to justify moving the LEQ aerosol apparatus to the BL-2 lab.

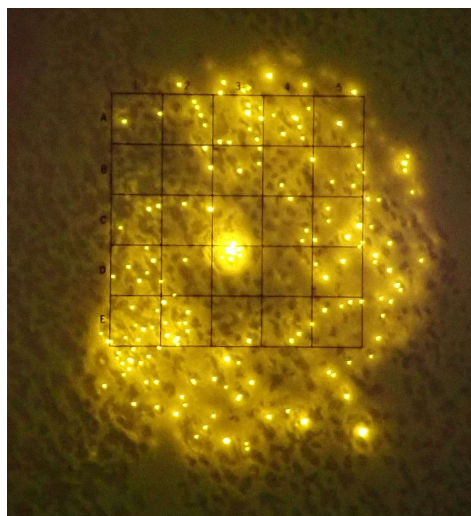


Fig. H4—A microscope image of fluorescent PSL clusters collected on a filter. Viewed through the eyepiece at 10X, the calibrated reticle is 400 x 400 μm .

H.2.2 Droplet Generation Modification

The second element that required improvement was droplet generation, which initially consisted of installing a second video camera and a telescope to image the tip of the PZT capillary tube where the droplets are formed. Image resolution sufficient to obtain reasonably accurate measurements of the initial droplet diameters was necessary for direct measurements of droplet size. Determination of the initial droplet diameter enables estimation of the number of spores in the residue clusters from known sample concentrations. This improvement led to some unanticipated consequences, since it allowed the operator to directly see the quality and consistency of the droplets as well as to measure physical size. As a result, it was determined that the most stable PZT droplet generation occurs in its fundamental mode, in which the droplet diameter is close to the capillary diameter, 70 μm . This mode for PZT operation produces the most stable and reliable droplets. However, larger droplets mean that more liquid must evaporate before getting to the exposure chamber for confinement, and consequently, the LEQ had to be extended by about 6.5 cm to allow more time for droplet drying.

Figure H5 shows a photo of the complete LEQ aerosol system as it appears currently in the BL-2 secondary enclosure. At the top, the PZT droplet generator housing, which fits into a long extension tube that has also been wrapped with heating tape to promote additional drying but is not being used. Below that, the exposure chamber is formed by a 3" optical cube that has a large observation window in the front and a large, fused-silica window on the left side for allowing radiation from a solar simulator lamp or other radiation sources to expose the particles held there. Directly beneath that, the counting chamber consists of a 2" optical cube. The bottom of the counting chamber is a standard optical tube that accommodates the custom base supporting the LEQ electrodes and also houses the metal exit tube that connects to the filter assembly. The white filter housing and connector tube (shown in Fig. H3) can be seen below this base. The collection filter exit tube is connected to a stainless-steel tee leading to an in-line HEPA filter and a digital flow meter (at bottom right, Fig. H5) in the vacuum pump line. Although dry N₂ is used as a carrier gas inside the sealed aerosol flow system, the interior pressure is always slightly less than the outside lab air, preventing escape of aerosol material into the secondary enclosure. Fig. H6 shows a typical photo of spore particles confined in the LEQ exposure chamber taken through the large window seen in Fig. H5.

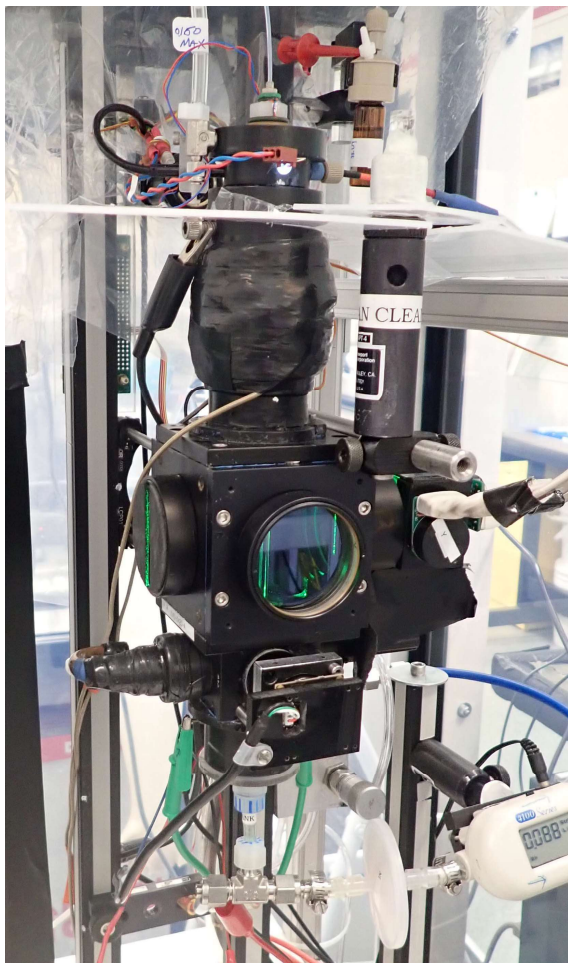


Fig. H5—A current photo of the aerosol generation, exposure and particle collection system in the BL-2 hood

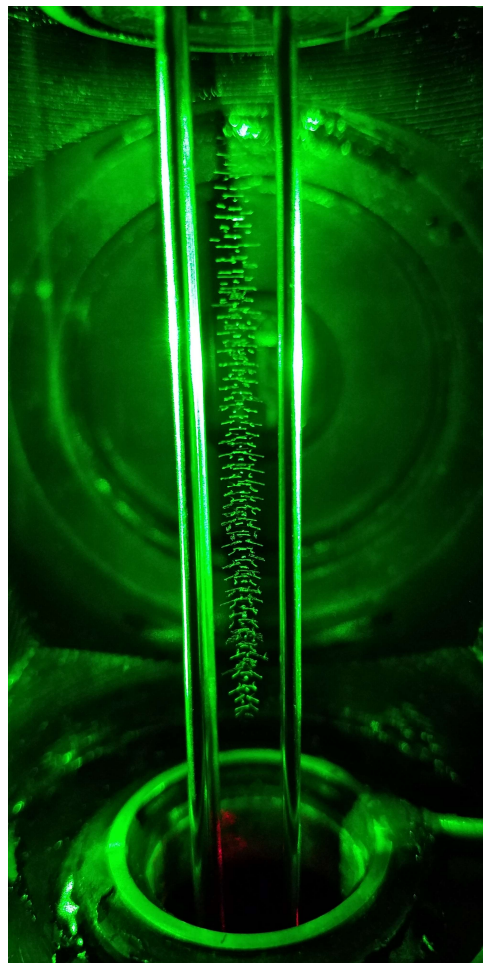


Fig. H6—A current photo of *Bacillus* spore particles suspended in the LEQ exposure chamber

At this point, it appeared that the aerosol particle-counting and filter-collection processes were sufficiently reliable and repeatable to move on to begin Sterne strain spore viability experiments, starting with 254-nm irradiation.

H.3 Initial Spore Aerosol Irradiation Experiments.

In December 2018 (Q4 Y2), experiments with BA Sterne spore particles were begun with the LEQ aerosol system. Difficulties were encountered right away in the form of inconsistent results. Occasionally, and in apparently random order, samples (even control samples) would show no viable counts. This led to series of increasingly specific experiments in an effort to isolate and understand the cause of these failures to show growth from spore aerosol particles. A detailed, historical account of the results of this effort is captured in Appendix F. However, at the time, we had not solved the problem of why our sample collection process onto filters sometimes succeeded but other times failed.

The immediate hurdle appeared to be the physical presence or absence of the spore particles in collection, not one of inactivation. However, the collection process had been demonstrated to work acceptably well for

PSL particles. Additionally, the recovery process had been vetted as reliable, and the titer checks on the sample solutions had been always consistent. The frustrating aspect was the apparently random nature of the results for spore particles, in which the procedure sometimes worked as expected. Our efforts over several weeks during this performance period were fueled by the tantalizing prospect that if we were just able to isolate the hidden variable(s) that controls whether the collection is successful or not, then we could proceed to acquire the needed data. We were unable to come up with a plausible reason for why the mechanics of particle collection could differ between spores and PSLs. However, at that point, it was more important to move forward with the program than try to figure out this puzzle. We formulated an alternative approach to particle collection, which is described in Appendix F, Sections F.2.3 and F.2.4.

APPENDIX I: SURVIVAL FRACTION (SF) ANALYSIS CURVE FITTING METHOD

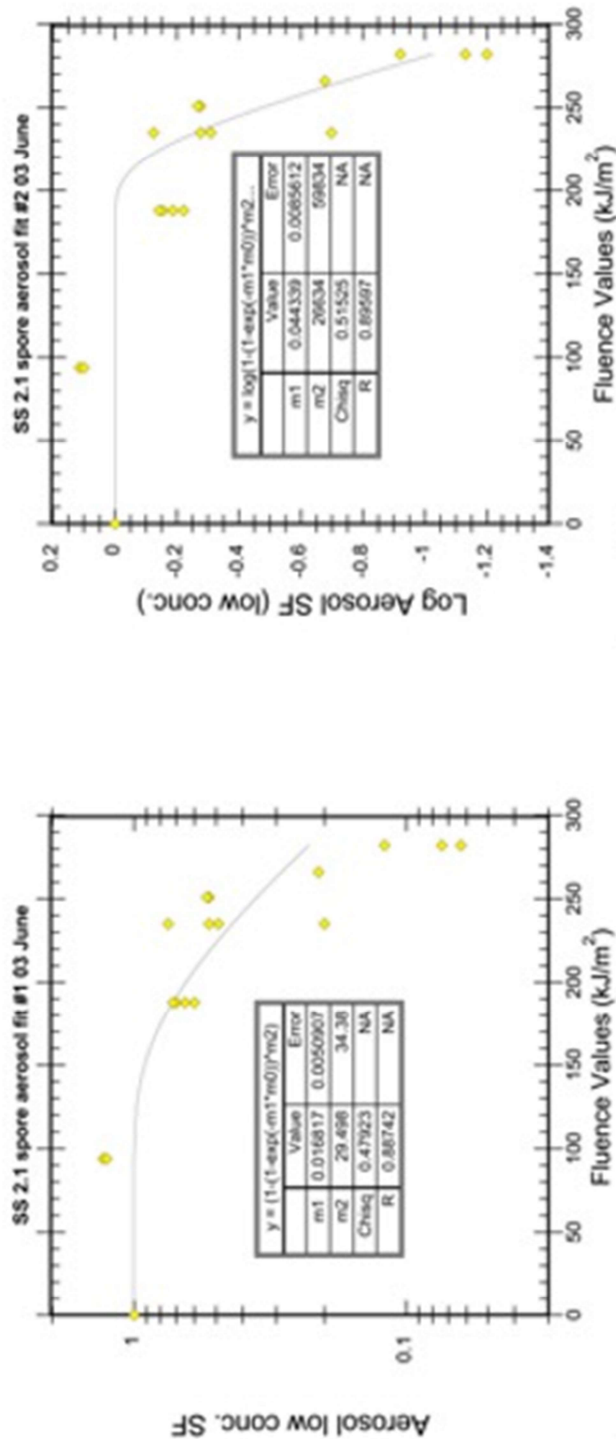
This is a quick overview of the analysis process used to fit the PAVES SF versus Fluence data to the Multi-hit model for comparison within the PAVES program and among other measurements (e.g. DeGrade and prior published data).

I started by using KaleidaGraph to apply curve fits to the data in an automated way, which employs a form of least squares minimization using initial guesses for the two parameters (k & n) of the specified Multi-hit equation. It was immediately obvious that for the data in its original form, this process was inadequate. Since much of the important part of the data were values that were extremely close to zero, a least squares approach de-emphasizes data points at large (highest) fluence values. An approach that I adopted to compensate for that, was to transform the data by taking its logarithm and then using the fitting routine on the transformed data for the log of the Multi-hit equation. However, this may also be sub-optimal, since it inverts the significant of the data, de-emphasizing the data points close to zero ($\log(1)$) at low fluences.

As a final step then I used the Formula Entry function in KaleidaGraph to independently generate a best fit curve which used values for the two parameters that were bounded by the parameter values given by the two automated fitting methods (original data and log-transformed data). I adjusted the values iteratively to achieve a fit to the data that appeared optimal for each sample series.

For the aerosol study, particles were created by generating droplets of a known size ($\approx 67 \mu\text{m}$ diameter) from a suspension of a known concentration of spores in water. The water quickly evaporates from the micro-droplets in air leaving behind a cluster of spores. Two sizes of spore-cluster particles were produced by using suspensions of spores at two different concentrations. For the solar simulator (SS) radiation exposures, these residual spore clusters had an average number of spores/particle ($\bar{N}$) of either 58 spores for the high concentration sample, or 2.1 for the low concentration sample. The curve fitting process is illustrated in the next slide using data from the low concentration sample ($\bar{N} = 2.1 \pm 1.4 \text{ SD}$).

Initial curve fits to 2.1 Spore Aerosol SS Decay



The left-hand plot shows all the 2.1 spore particle SS exposure data as survival fraction (SF) versus calibrated fluence (280 – 400 nm integrated SS intensity – see write-up for details). The least-squares curve fit for the multi-hit equation is shown, where the values of m1 and m2 in the fitting routine respectively represent the first-order decay rate, k (in m²/kJ), and the repair parameter, n. In the first plot, it is clear that the data points between 250 and 300 kJ/m² exposure are not well represented. This is because their values are small. In the right-hand plot, by converting the data to its logarithm, and plotting it on a linear scale, the data values at high fluence become more significant, because those at low fluences are now numerically close to zero. The same curve fitting process now more closely represents the high fluence values which is where we expect the first order decay constant to be determined. The parameter values for the curve fits are bounded by roughly 0.045 and 0.017 for k (m1), and 30 and 27,000 for n (m2).

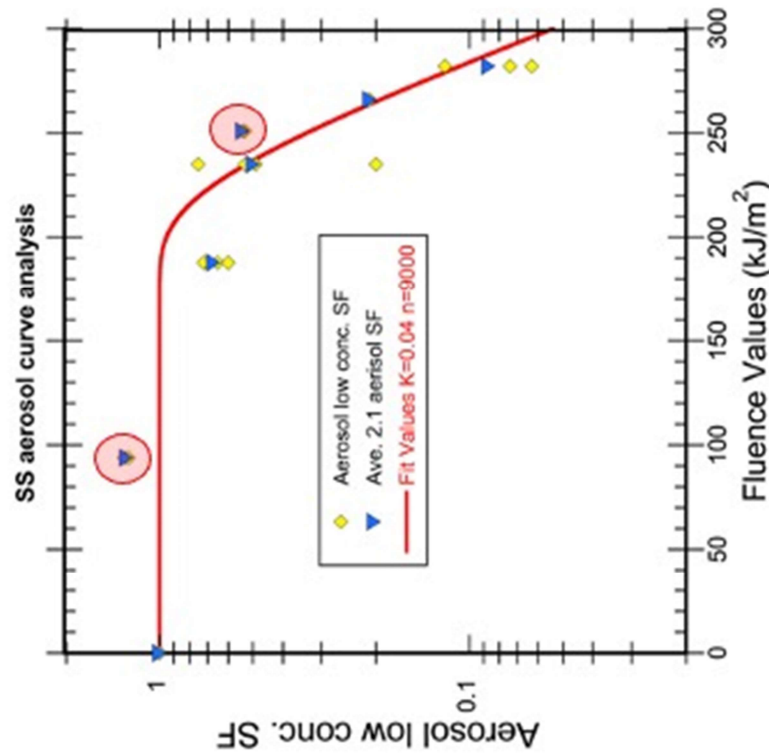
Curve fit to 2.1 Spore Aerosol SS Decay

The plot on the right shows the same data as on the previous slide as the yellow diamonds and now the average values for each exposure level are also shown as blue triangles. The red line is the multi-hit equation using parameter values of $k = 0.04$ and $n = 9000$.

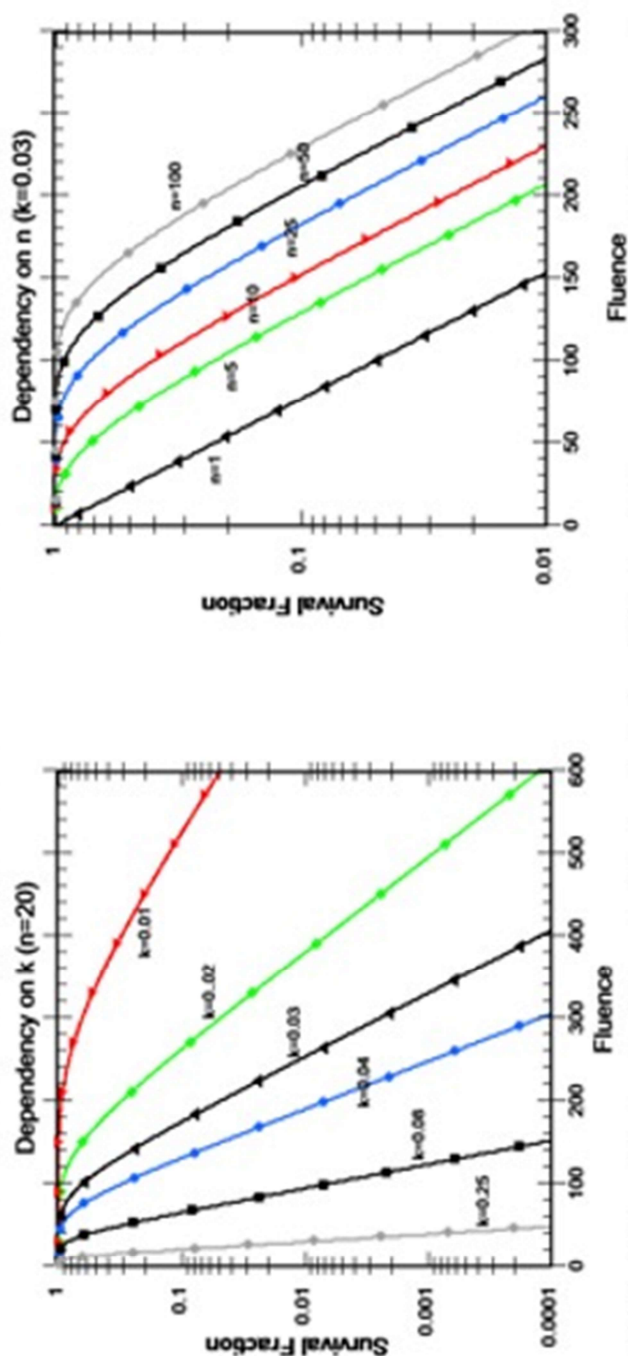
The values for k and n are somewhat inter-dependent as will be illustrated on the next slide.

From the curve fits shown on the previous slide, it was clear that the value of k needed to be close to the 0.044 end of the range. Selecting a value of 0.04 for k allows the value of n to be reduced significantly to 9000 without changing the overall closeness of the fit.

The agreement with three average values data points is extremely good in the high fluence region between 240 and 300 kJ/m^2 . Significant outliers are seen at fluence values of 94 and 251 kJ/m^2 (circled). In the first case the surviving fraction exceeds 1, and in the second case the surviving fraction increases as the fluence increases. Since the viability of the spore samples cannot increase once released, these results are indicative of the level of experimental error.



Parameter interdependency for Multi-hit equation



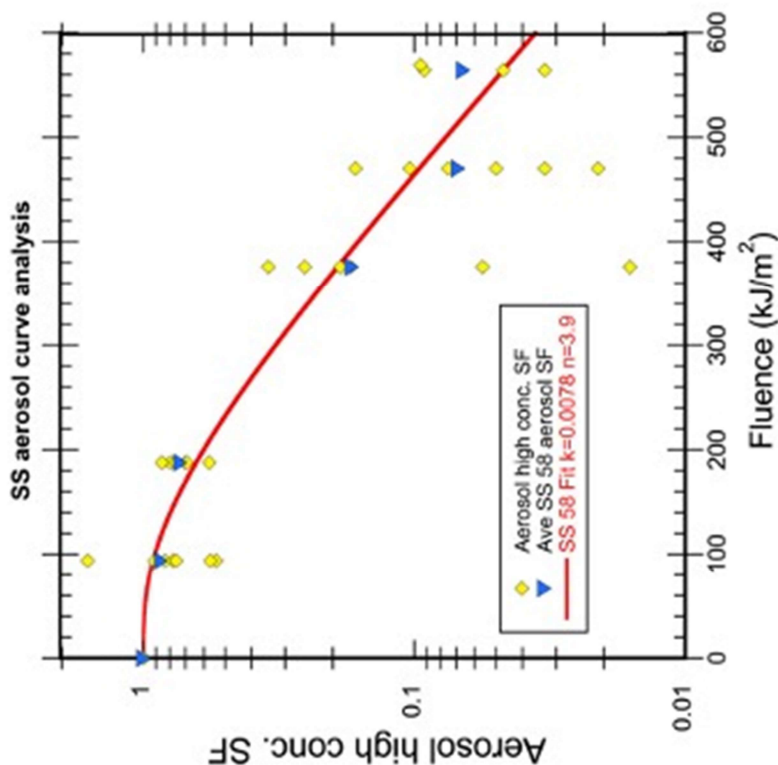
The multi-hit "model" equation for microbial survival fraction due to degradation by radiation exposure is an empirical two-parameter equation (e.g. analogous to the Gaussian or normal distribution equation) that permits matching of observed decay behavior. What this means is that at large fluence values an exponential decay is typically observed. This would be similar to examples of degradation or absorption in materials for which a first-order exponential decay is well known, except that living organisms have a repair capability that compensates for initial damage, and so exponential decay occurs only after the repair mechanisms have been overcome. In the parametric fit, the exponential decay rate is k and the repair capability is represented by n . How long the "shoulder" region extends quantitatively is determined by the combination of n and k . However, procedurally, the value of k can be assessed initially by focusing on the high-fluence region of the data. Once k is estimated, then n can be estimated by the distance to the "breaking-point" in the data.

Curve Fit to Aerosol Large Cluster SS Decay

The plot shows analysis of aerosol particles from the high concentration spore samples. In this sample series, the average number of spores in each particle is 58 with a SD of ± 8 . By mass, or volumetrically, the SD in spore number translates to a diameter change of only $\pm 4.6\%$. Because the outer spores of the cluster particle effectively shield inner spores, measured survival fractions are significantly higher than small clusters for the same fluence.

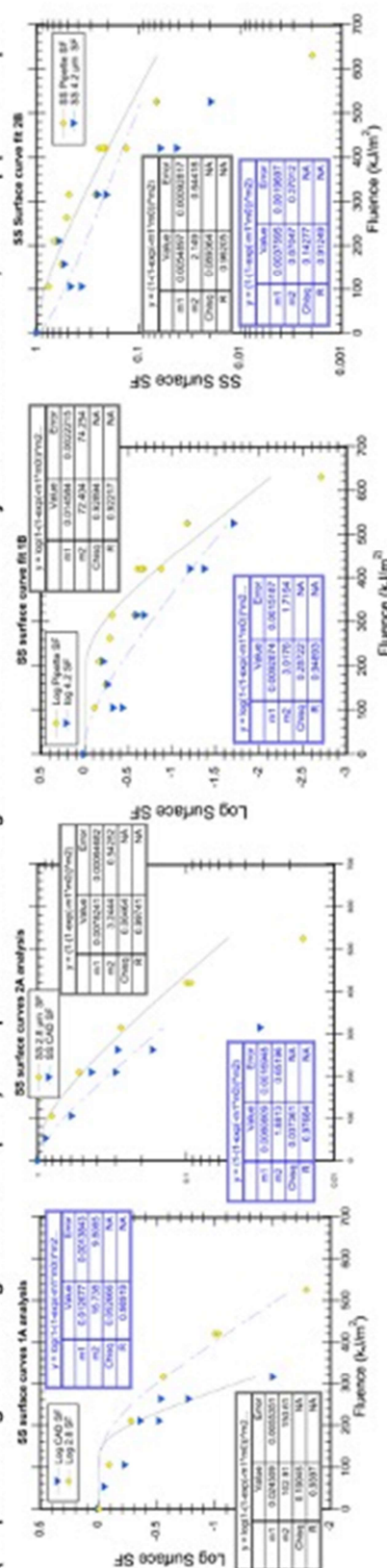
Both the individual measurements (yellow diamonds) and the average values for each exposure level (blue triangles) are shown. The curve fit (red line) shows excellent agreement with the averaged data.

Using the same fitting process shown previously for the particles with an average number of 2.1 spores, values for k and n are ≈ 0.008 and 4, respectively. The first-order decay rate is reduced by about a factor of 5 from the small cluster particles.



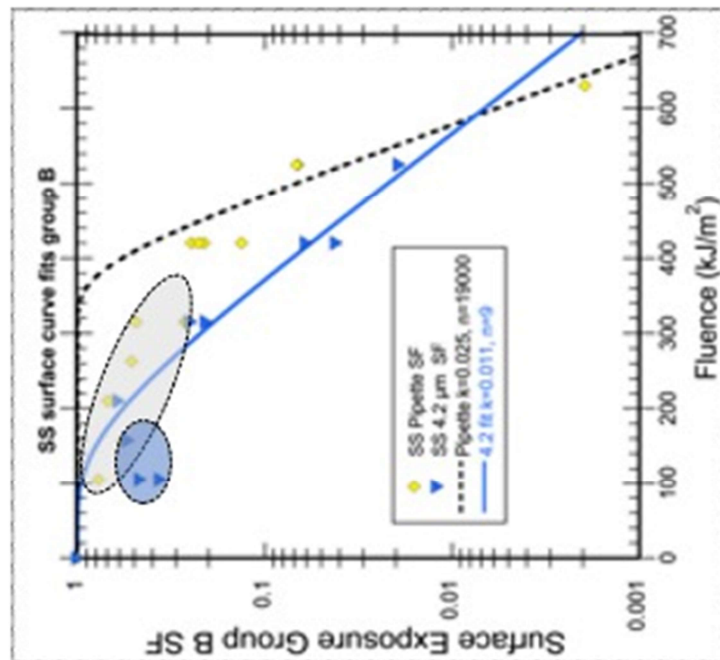
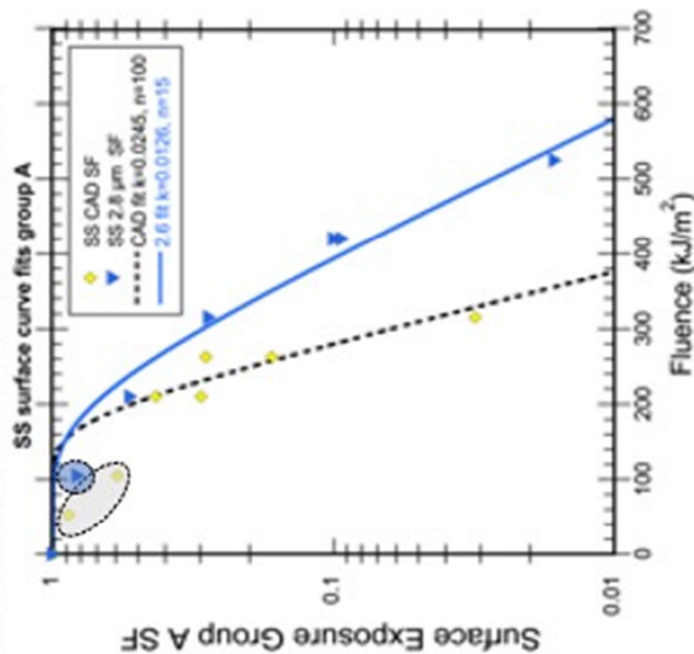
Particle Surface Deposition SS Decay

In the surface deposition particle exposure study three different particle types were investigated, as well as liquid suspensions directly applied to surfaces and then dried. The liquid application method is referred to as "pipette" samples since a metering pipette was used to dispense precision volumes of liquid with a known concentration onto filter membranes. For the three particle samples aerosol samples were generated by the same general method as the aerosol samples, by generating characterized droplets of the spore suspension and allowing the water to evaporate away. The residual spore cluster particles were then collected onto filter membranes by pulling particle-laden air through the filters using a vacuum pump. The four samples are divided into two groups of two for initial assessment, and below plots for the original an log transformed data are shown for each group, and are designated as 2 and 1 respectively. Group A consists of the two smaller particles sizes referred to here as "CAD" that is the smallest cluster particle size with an average aerodynamic diameter of 1.6 μm , and a medium cluster size with an average aerodynamic diameter of 2.6 μm (despite the legend marking of 2.8 in the plots). Group B includes the largest cluster size with an aerodynamic diameter of 4.2, and the pipette sample.



For the two plots showing the original data, labeled "2", the curve fits fail to adequately capture the highest fluence data points in each sample, as expected. In order to capture these points, and provide a better estimate of k , we look to the plots of the log transformed data labeled "1". For the "1" plots, while values of k are improved, one can see that these curve fits show SF values at low-fluences consistently falling well below the curve fit. While the "1B" fit to 4.2 data appears to provide a balanced fit to the data in general, the degree to which the low fluence points drops initially, and then rise, and then drop again is also problematic, and I will address that in the next slide. Previously, I had considered the least squares optimization of the log-transformed data as a sort of upper bound that would be modified downward to accommodate the low-fluence points. For these data, that approach is not appropriate because the low fluence values seem to be generally at odds with expected behavior.

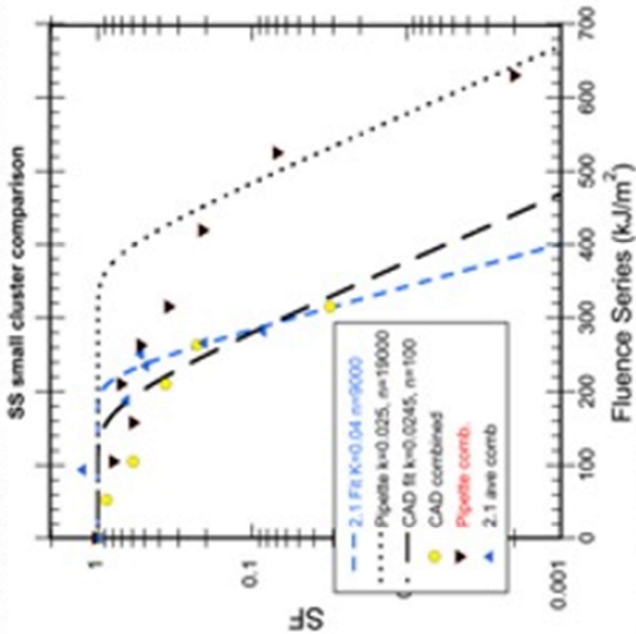
Continued SS Surface Curve Fits



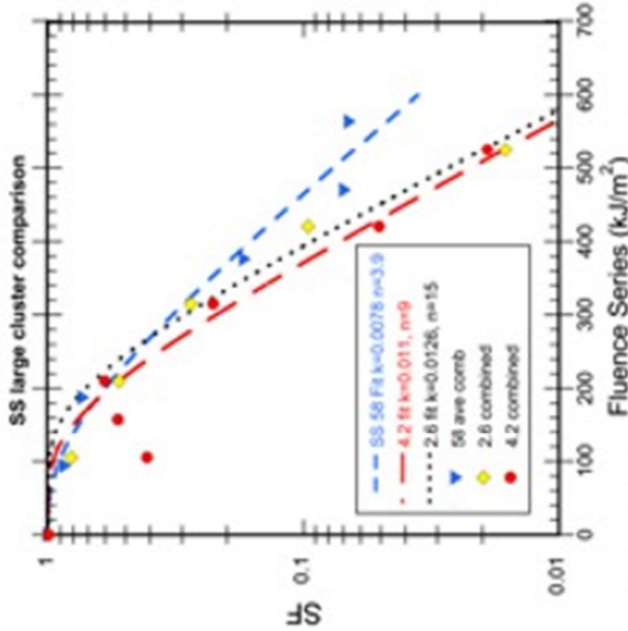
These plots are result from my iterative fiddling with the curve fits to the four sets of data. Overall, they are good quantitative fits. For group A the parameters are essentially the same as those from the "1A" least squares automated process on the previous slide. However, for group B, I essentially had to treat all of the low fluence data points as outliers to apply the multi-hit model. This issue is present in the group A data as well, just less pronounced. The consistency of this issue across all four sets of data is concerning, perhaps Jana and Dan can double check their control normalization procedures.

Summary and comparisons

Using the curve fits on the previous slide for the surface data, here are initial plots comparing the aerosol and surface results.



Extended shoulder regions for all samples
Both surface samples (pipette & CAD) have similar k values but quite different repair parameters (n values) (unexpected)
Aerosol decay rate is clearly a bit faster than surface (expected)
Both surface data samples significantly undershoot shoulder



All larger clusters decay more slowly than small (expected)
Both surface samples (2.6 & 4.2) are almost indistinguishable (unexpected)
Aerosol decay rate is noticeably slower than surface (unexpected)
4.2 surface data sample significantly undershoots shoulder

APPENDIX J: AEROSOL EXPOSURE DATA: 254-NM IRRADIATION

Suspension Conc. (cfu/ml)	Droplet Diameter (µm)	Spores per Particle	Exposure time, (s)	Aerosol Dwell Time (min)	Collected Particle Count	Dose 254 nm (J/m ²)	Chamber Temperature (°C)	Chamber Relative Humidity (%)	Survival Fraction (SF)	No. of Replicates	Average SF	SF Std. Dev.
1.42E+07	65	2.0	0	14.03	347	0	22	20	0.42	22	1.0	0.3
1.42E+07	71	2.7	0	14.58	211	0			1.19			
1.42E+07	63	1.9	0	18.68	295	0			1.08			
1.38E+07	66	2.0	0	12.43	1195	0	22	10	0.64			
1.42E+07	65	2.0	0	12.75	351	0			1.8			
1.42E+07	67	2.2	0	15.90	326	0			1.15			
1.42E+07	63	1.9	0	16.50	261	0			1.07			
1.42E+07	69	2.4	0	16.78	391	0			0.79			
1.42E+07	69	2.4	0	17.50	361	0			0.89			
1.42E+07	73	2.9	0	18.12	329	0			0.40			
1.42E+07	69	2.4	0	18.35	305	0			0.78			
1.42E+07	65	2.0	0	19.50	362	0			0.90			
1.42E+07	69	2.4	0	19.63	361	0			0.70			
1.42E+07	69	2.4	0	20.35	203	0			1.07			
1.42E+07	65	2.0	0	21.83	400	0			1.36			
1.42E+07	66	2.1	0	21.95	392	0			1.07			
1.42E+07	71	2.7	0	22.50	387	0			0.89			
1.42E+07	67	2.2	0	22.87	403	0			1.23			
1.38E+07	68	2.2	0	24.43	1261	0			1.10			
1.38E+07	63	1.8	0	30.96	1069	0			1.25			
1.42E+07	67	2.2	0	1220	335	0			1.17			
1.42E+07	67	2.2	0	1434	404	0			1.03			
1.42E+07	69	2.4	8	19.07	418	36	22	10	0.73	3	0.90	0.17
1.42E+07	65	2.0	8	19.08	371	36			1.08			
1.42E+07	69	2.4	9	18.50	311	40			0.89			
1.42E+07	69	2.4	15	13.50	314	67	22	10	0.37	5	0.7	0.3
1.42E+07	63	1.9	15	13.78	320	67			0.37			
1.42E+07	71	2.7	15	16.00	365	67			0.56			
1.42E+07	69	2.4	15	18.03	465	67			0.92			
1.42E+07	65	2.0	15	20.20	402	67			1.07			
1.42E+07	65	2.0	22	17.50	312	98	22	10	0.61	5	0.54	0.12
1.42E+07	69	2.4	22	19.07	293	98			0.67			
1.42E+07	63	1.9	22	22.47	440	98			0.53			
1.42E+07	67	2.2	22	23.00	457	98			0.53			
1.42E+07	63	1.9	22	31.43	416	98			0.36			

Suspension Conc. (cfu/ml)	Droplet Diameter (µm)	Spores per Particle	Exposure time, (s)	Aerosol Dwell Time (min)	Collected Particle Count	Dose 254 nm (J/m ²)	Chamber Temperature (°C)	Chamber Relative Humidity (%)	Survival Fraction (SF)	No. of Replicates	Average SF	SF Std. Dev.
1.42E+07	65	2.0	25	17.22	336	111	22	10	0.72	5	0.46	0.16
1.42E+07	67	2.2	25	18.52	368	111			0.49			
1.42E+07	63	1.9	25	20.23	364	111			0.34			
1.42E+07	65	2.0	25	21.43	536	111			0.34			
1.42E+07	71	2.7	25	21.75	374	111			0.39			
1.42E+07	65	2.0	30	19.73	379	134	22	10	0.052	3	0.08	0.02
1.42E+07	63	1.9	30	21.35	402	134			0.075			
1.42E+07	61	1.7	30	23.02	382	134			0.10			
1.38E+07	66	2.1	36	14.05	1645	160	22	10	0.019	2	0.03	0.01
1.38E+07	69	2.3	36	14.10	1513	160			0.034			
1.38E+07	66	2.1	40	14.14	4041	178	22	10	0.0096	1	0.010	n/a
3.4E+08	67	54	0	13.18	321	0	22	10	1.00	8	1.00	0.137
3.4E+08	69	59	0	15.60	255	0			1.007			
3.4E+08	71	64	0	16.13	350	0			0.93			
3.4E+08	69	59	0	16.38	330	0			0.96			
3.4E+08	65	49	0	16.40	349	0			0.82			
3.4E+08	65	49	0	16.63	377	0			0.96			
3.4E+08	67	54	0	17.55	380	0			1.01			
3.4E+08	67	54	0	17.65	283	0			1.30			
3.4E+08	71	64	10	12.88	308	44.5	22	10	0.93	3	0.89	0.04
3.4E+08	69	59	10	14.37	349	44.5			0.90			
3.4E+08	67	54	10	20.15	373	44.5			0.86			
3.4E+08	67	54	30	15.85	364	134	22	10	0.50	3	0.45	0.06
3.4E+08	65	49	30	17.78	404	134			0.47			
3.4E+08	69	59	30	19.00	358	134			0.39			
3.4E+08	67	54	45	14.93	312	200	22	10	0.19	3	0.20	0.02
3.4E+08	69	59	45	15.83	309	200			0.18			
3.4E+08	69	59	45	24.68	306	200			0.22			
3.4E+08	67	54	60	15.38	311	267	22	10	0.093	3	0.06	0.03
3.4E+08	67	54	60	16.93	381	267			0.035			
3.4E+08	61	41	60	17.32	339	267			0.060			
3.4E+08	71	64	90	16.25	344	401	22	10	0.029	3	0.023	0.006
3.4E+08	71	64	90	17.55	387	401			0.017			
3.4E+08	67	54	90	19.23	389	401			0.023			
3.4E+08	65	49	110	14.13	328	490	22	10	0.0077	3	0.009	0.004
3.4E+08	69	59	110	14.22	360	490			0.0052			
3.4E+08	67	54	110	16.13	377	490			0.013			
3.4E+08	67	54	120	18.10	280	534	22	10	0.0080	2	0.009	0.002
3.4E+08	61	41	120	19.20	428	534			0.011			

APPENDIX K: AEROSOL EXPOSURE DATA: SIMULATED SOLAR IRRADIATION

Suspension Conc. (cfu/ml)	Droplet Diameter (um)	Spores per Particle	Exposure Time (s)	Aerosol Dwell Time (min)	Collected Particle Count	Dose 280-400 nm (kJ/m ²)	Chamber Temperature (°C)	Chamber Relative Humidity (%)	Survival Fraction (SF)	No. of Replicates	Average SF	SF Std. Dev.
3.4E+08	67	54	0	41.00	298	0	22	10	0.803	17	1.00	0.16
3.4E+08	66	51	0	36.00	211	0			1.285			
3.4E+08	69	59	0	38.00	285	0			0.920			
3.4E+08	65	49	0	39.00	233	0			0.991			
3.4E+08	69	59	0	1085	395	0			0.936			
3.4E+08	72	67	0	70.00	392	0			0.823			
3.4E+08	67	54	0	1050	310	0			0.891			
3.4E+08	65	49	0	68.00	219	0			1.168			
3.4E+08	71	64	0	1085	291	0			0.808			
3.4E+08	73	69	0	69.00	461	0			1.010			
3.4E+08	73	69	0	129.00	297	0			0.933			
3.4E+08	71	64	0	157.00	163	0			1.083			
3.4E+08	71	64	0	159.00	414	0			0.826			
3.4E+08	61	41	0	129.00	195	0			1.071			
3.4E+08	67	54	0	161.00	355	0			1.091			
3.4E+08	69	59	0	131.00	320	0			0.997			
3.4E+08	62	43	0	1115	165	0			1.365			
3.4E+08	67	54	1800	37.00	257	94.0	22	10	0.911	7	0.9	0.4
3.4E+08	67	54	1800	42.00	285	94.0			0.833			
3.4E+08	71	64	1800	40.00	178	94.0			1.606			
3.4E+08	71	64	1800	58.00	161	94.0			0.758			
3.4E+08	73	69	1800	34.00	215	94.0			0.539			
3.4E+08	65	49	1800	40.00	311	94.0			0.778			
3.4E+08	71	64	1800	42.00	327	94.0			0.567			
3.4E+08	67	54	3600	69.00	215	188	22	10	0.797	6	0.73	0.10
3.4E+08	69	59	3600	69.00	291	188			0.856			
3.4E+08	72	67	3600	68.00	168	188			0.701			
3.4E+08	67	54	3600	71.00	261	188			0.692			
3.4E+08	68	56	3600	70.00	257	188			0.763			
3.4E+08	61	41	3600	67.00	251	188			0.572			
3.4E+08	63	45	7200	128.00	241	376	22	10	0.346	6	0.17	0.12
3.4E+08	77	82	7200	126.00	157	376			0.172			
3.4E+08	63	45	7200	130.00	356	376			0.254			
3.4E+08	70	61	7200	127.00	102	376			0.188			
3.4E+08	67	54	7200	128.00	261	376			0.056			
3.4E+08	63	45	7200	127.00	226	376			0.016			

Suspension Conc. (cfu/ml)	Droplet Diameter (µm)	Spores per Particle	Exposure Time (s)	Aerosol Dwell Time (min)	Collected Particle Count	Dose 280-400 nm (kJ/m ²)	Chamber Temperature (°C)	Chamber Relative Humidity (%)	Survival Fraction (SF)	No. of Replicates	Average SF	SF Std. Dev.
3.4E+08	75	75	9000	159.00	292	470	22	10	0.165	7	0.07	0.05
3.4E+08	73	69	9000	162.00	274	470			0.033			
3.4E+08	71	64	9000	163.00	227	470			0.075			
3.4E+08	71	64	9000	167.00	300	470			0.033			
3.4E+08	65	49	9000	160.00	261	470			0.104			
3.4E+08	69	59	9000	165.00	352	470			0.021			
3.4E+08	69	59	9000	160.00	307	470			0.050			
3.4E+08	65	49	10800	188.00	87	564	22	10	0.047	2	0.040	0.011
3.4E+08	69	59	10800	190.00	197	564			0.033			
3.4E+08	69	59	10890	192.00	143	569	22	10	0.095	1	0.095	n/a
1.42E+07	65	2.0	0	12.43	1195	0	22	10	1.029	7	1.00	0.23
1.42E+07	67	2.2	0	24.43	1261	0			1.080			
1.38E+07	63	1.8	0	27.19	584	0			1.342			
1.38E+07	68	2.2	0	30.96	1069	0			1.184			
1.38E+07	67	2.2	0	34.00	291	0			0.890			
1.38E+07	66	2.0	0	80.07	377	0			0.692			
1.21E+07	68	2.0	0	111.9	319	0			0.782			
1.42E+07	69	2.4	1800	45.50	196	94.0	22	10	1.264	2	1.266	0.003
1.38E+07	65	2.0	1800	79.40	152	94.0			1.269			
1.42E+07	65	2.0	3600	74.00	287	188	22	10	0.704	4	0.67	0.06
1.42E+07	71	2.7	3600	78.00	313	188			0.720			
1.38E+07	63	1.8	3600	79.67	225	188			0.650			
1.38E+07	65	2.0	3600	80.45	188	188			0.597			
1.42E+07	65	2.0	4500	91.00	241	235	22	10	0.533	4	0.5	0.2
1.42E+07	65	2.0	4500	92.00	293	235			0.754			
1.38E+07	65	2.0	4500	94.47	224	235			0.491			
1.38E+07	67	2.2	4500	100.55	271	235			0.204			
1.21E+07	68	2.0	4800	93.59	642	251	22	10	0.533	2	0.537	0.006
1.21E+07	69	2.1	4800	98.63	597	251			0.541			
1.21E+07	64	1.7	5100	93.35	554	266	22	10	0.205	1	0.205	n/a
1.38E+07	65	2.0	5400	108.28	119	282	22	10	0.074	3	0.09	0.03
1.38E+07	65	2.0	5400	108.48	1101	282			0.123			
1.38E+07	71	2.6	5400	114.96	984	282			0.063			

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