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Inactivation of *Bacillus anthracis* and *Bacillus atrophaeus* spores on different surfaces with ultraviolet light produced with a low-pressure mercury vapor lamp or light emitting diodes

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Abstract

Aims: To obtain quantitative efficacy data of two ultraviolet light (UVC) technologies for surface inactivation of *Bacillus anthracis* Ames and *Bacillus atrophaeus* spores.

Methods and Results: Spores were deposited onto test coupons and controls of four different materials, via liquid suspension or aerosol deposition. The test coupons were then exposed to UVC light from either a low-pressure mercury vapor lamp or a system comprised of light emitting diodes, with a range of dosages. Positive controls were held at ambient conditions and not exposed to UVC light. Following exposure to UVC, spores were recovered from the coupons and efficacy was quantified in terms of $\log_{10}$ reduction (LR) in the number of viable spores compared to that from positive controls.

Conclusions: Decontamination efficacy varied by material and UVC dosage (efficacy up to 5·7 LR was demonstrated). There was no statistical difference in efficacy between the two species or

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Author contributions

All authors have contributed to the (i) conception and design or the acquisition and analysis of data, (ii) drafting or critically revising the manuscript, and (iii) approval of the final submitted version.

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Conflict of Interest

The authors declare there is no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

between inoculation methods. Efficacy improved for the LED lamp at lower relative humidity, but this effect was not observed with the mercury vapor lamp.

Significance and Impact of the Study: This study will be useful in determining whether UVC could be used for the inactivation of *B. anthracis* spores on different surface types.

Introduction

There is a need to manage the risk of biological incidents, which can be naturally occurring, accidental, or deliberate in origin (U.S. Department of Defense et al. 2018). One goal of a national biodefense strategy is to ensure preparedness for recovery from such incidents, which can be accomplished (among other measures) via the development and verification of decontamination techniques for various types of infrastructure, equipment, and environments. Consistent with such a strategy, the research presented here evaluated the use of ultraviolet light (UVC) for the inactivation of *Bacillus anthracis* spores, a Category A bioterrorism agent (U.S. Centers for Disease Control and Prevention 2018) and the bacterium causing anthrax disease.

Exposing contaminated surfaces to UVC in the C range (UVC; 190–290 nm wavelength; Coohill and Sagripanti 2008) is a decontamination technique that may be considered for use in the event of a wide area release of a biological agent such as *B. anthracis* spores. The majority of research on the microbicidal effects of UVC have focused on UV with a wavelength of 254 nm, which is produced via low-pressure mercury vapor lamps (Coohill and Sagripanti 2008) and is a commercially available UVC generating technology. Research on the use of UV light (vis-à-vis sunlight and its spectral components) to inactivate spores of *B. anthracis* began over 125 years ago (Ward 1893).

While UVC is a fairly common technique for disinfection (inactivation of vegetative bacteria and viruses) of water (Masschelein and Rice 2016) and air (Kesavan and Sagripanti 2014), e.g., aerosols, and air ducts (Kowalski 2009), its use on surfaces for the inactivation of bacterial spores (e.g., those of *B. anthracis*) is not widely used commercially. Most commercial UV germicidal equipment for surface disinfection is used for building and ventilation system surfaces or for dental and medical equipment. UVC inactivation rates for microbes in air are typically much higher than for surfaces (Kowalski 2009). Spores of bacteria are generally 5–10 times more resistant to UVC than their corresponding vegetative cells (Coohill and Sagripanti 2008). The benefit to using UVC as a surface decontaminant is that some of the issues associated with liquid or gaseous chemical sporicides can be avoided, such as health effects from exposure, higher costs, time requirements, and incompatibility with materials (Owens et al. 2005). UVC may be a useful decontamination method for sensitive materials such as electronics, artwork, historical artifacts, or other valuable materials or equipment that cannot be readily discarded or may be damaged by use of strong reactive harsh chemicals.

One of the objectives of the present study was to improve inactivation efficacy for bacterial spores that could be achieved on building interior surfaces, by increasing the UVC dosage and varying other parameters. In our review of the literature for UVC inactivation of bacterial spores on surfaces (see for example, Blatchley et al. 2005; Umezawa et al. 2012),

we were unable to locate any data demonstrating efficacy greater than approximately a 4 $\log_{10}$ reduction (LR). For this reason it has been suggested that UVC may achieve complete kill at lower spore loadings (Owens et al. 2005). This lower efficacy of UVC is in contrast to chemical decontaminants such as acidified bleach, peracetic acid, hydrogen peroxide vapor, or chlorine dioxide gas that can typically achieve greater than 6 LR on many types of materials (Wood and Adrion 2019). A LR level ≥6·0 is considered "effective" for chemistries used against spores of *B. anthracis* (U.S. Environmental Protection Agency 2018). The relatively lower decontamination efficacy achieved by UVC may be due to issues with shading and shielding of live spores underneath a layer of inactivated spores (Coohill and Sagripanti 2008). Because of this potential shielding and clumping effect, we tested two methods for inoculating spores onto materials (suspension inoculation and aerosol deposition), hypothesizing that the aerosol deposition method may result in less clumping of spores on materials, and in turn increase efficacy. We note that many UVC and other decontamination studies of bacterial spores on surfaces typically deposit the spores via a suspension inoculation.

A second purpose of this study was to obtain efficacy data for a virulent strain of *B. anthracis.* While there are some data reported in the literature for UVC inactivation of *B. anthracis* Sterne (vaccine strain) spores on materials (Owens et al. 2005; Menetrez et al. 2006), we were unable to locate any data for a virulent strain such as the Ames strain. Knudson reported on the UVC inactivation of several strains of *B. anthracis* (Knudson 1986), although only the avirulent Sterne strain was evaluated in spore form. Thus, the present study includes side-by-side tests with spores of both virulent *B. anthracis* and *Bacillus atrophaeus*, to compare efficacy in an assessment of the latter's suitability as a surrogate. *Bacillus atrophaeus* and its phylogenetic neighbor *Bacillus subtilis* have been widely used in tests to evaluate UVC (Xue and Nicholson 1996; Gardner and Shama 1998; Coohill and Sagripanti 2008; Raguse et al. 2016).

While low-pressure mercury bulbs are widely used as a UVC source, their disadvantages include the potential leakage and disposal of a product containing mercury (a known neurotoxin), and the bulbs' short lifetime and significant energy usage (Würtele et al. 2011, Tran et al. 2014; Shin et al. 2016). Additionally, when the Minamata Convention on Mercury takes effect in 2020, low-pressure mercury lamps will be prohibited (Kim and Kang 2018). The use of light emitting diodes (LEDs) to produce UV light for germicidal purposes avoids these mercury lamp issues. In addition, LEDs can be tuned to the optimum wavelength to enhance spore inactivation (Shin et al. 2016). For example, it was found that *Bacillus* spores were more sensitive to UVC at a wavelength of 265 nm than to the 254 nm wavelength produced by low-pressure mercury lamps (Mamane-Gravetz et al. 2005). For these reasons, we included UVC produced by LEDs in our evaluation. Nearly all the research and development for LED use as a germicidal technique has been for water or surface disinfection, with minimal data reported in the literature for the inactivation efficacy of bacterial spores on surfaces.

Lastly, the present study included the use of bacterial spores deposited on four types of interior building materials. Decontamination efficacy is strongly dependent on the material with which the microorganisms are associated with, and most if not all UVC studies

reported in the literature typically use only laboratory substrates such as glass or filters, rather than relevant realistic materials. Our study also examined the effect of relative humidity (RH) on UVC inactivation efficacy, which is another data gap (Kowalski 2009).

Materials and methods

Spore preparation

While *B. anthracis* (strain Ames) spores were prepared at the U.S. Army Chemical Biological Center's BSL-3 facility, spores of *B. atrophaeus* (strain ATCC 9372) were prepared in a BSL1/2 laboratory at the same location. The *B. anthracis* spores were prepared from a pathogenic strain, obtained from stock strain pool and characterized by genetic testing. Cells of *B. anthracis* and *B. atrophaeus* were grown on Lab-Lemko sporulation medium (500 ml of sporulation media poured in eight large Petri plates, for each species) as described by (Rastogi et al. 2009), until 90–95% sporulation occurred. Approximately 10^9 spores per plate were generated for each species. The spores were enriched through three centrifugation washes at 8000×g (Beckman Coulter, Allegra 25R, Atlanta, GA) with sterile Milli-Q water (EMD Millipore, Darmstadt, Germany). The enriched *B. anthracis* spores were suspended at a titer of 2×10^8/ml, in sterile distilled water containing 0·01% Tween-80 (Acros Organics, CAS 900565-6; to minimize clumping) and stored at 4°C. The spore suspension quality was evaluated by the titer enumeration following heat shock treatment at 65°C for 30 min in addition to microscopic analysis of spores (to ensure that the vegetative cells component was <5 to 10%). From the microscopic analysis of the spore preparations, no cellular debris was observed, the bulk of the spores were as singles and not agglomerated, and the spores were of uniform size for each species. Before use, the spore preparation was vortexed vigorously to ensure no spore clumping, which is also augmented by the presence of the surfactant.

Coupon materials, replicates, and controls

The four materials (two porous and two non-porous) selected for this study were industrial-grade low pile carpet (Shaw Carpeting, Viking style), pine wood (Spruce Pine Fir Lumber, Lowes, Bel Air, MD), glass (R.G. Collins Glass Co, Inc., Baltimore, MD) and laminate (Formica, Fantasy Marble Scovato, Lowes, Bel Air, MD). Comparable sizes of coupons were used in the study. For the suspension-deposited spores, coupons were 1·5 cm × 1·5 cm, while the aerosol-deposited coupons were somewhat larger (2 cm × 4 cm, with the glass coupons deviating to 2 cm × 5cm), to allow for them to be sampled (discussed later) rather than extracted. The coupons were sterilized prior to testing by autoclaving in Petri plates. Sterilization was used to eliminate contamination by non-target microorganisms. Autoclaving was conducted at a minimum of 103 kPa and 121°C for 30 min. Sterility checks were conducted prior to inoculation by incubating a set number of sterile coupons in tryptic soy broth overnight to ensure no growth was observed.

Test coupons were inoculated with spores and exposed to UVC; four replicates of each test coupon material were used for each time point per experiment. Positive control coupons were inoculated with spores but not exposed to UVC; typically, one positive control coupon for each material was used for each time point in each experiment. Thus, in an experiment

with four time points, four positive control replicates were used for each material. Negative control coupons were not inoculated and not exposed to UVC; typically, one control coupon was used for each time point in each experiment.

Suspension inoculation of spores

For both species, *B. anthracis* and *B. atrophaeus,* the spores were suspended (separately) in 70% ethanol at a target titer of ~5×10^7 colony forming units (CFU) per ml when used for suspension inoculation. An aliquot of 100-μl (using 10 droplets, each 10 μl) was placed onto each coupon via pipette and allowed to dry for at least 4 h prior to testing. The actual titer of the suspension was determined for each day that suspension inoculations occurred and these ranged from 2×10^7 to 9×10^7 CFU per ml, resulting in an actual inoculum range of 2×10^6 to 9×10^6 spores per coupon.

Aerosol deposition of spores

Due to safety concerns, only *B. atrophaeus* spores were used in aerosol deposition tests. Since aerosol deposition was performed over an 1855-sq-cm surface area and 84773-cu-cm space within a deposition chamber, high spore titers were required to get a comparable deposition over small coupon surface (8–10 sq.-cm). As a result, *B. atrophaeus* spores were suspended in 70% ethanol at a titer of ~10^9 CFU per ml. An aliquot of 10-ml spore suspension was aerosolized into the spore deposition chamber using an aerosol generator producing 18 micron droplets (SonoTek Corp., Model # 8700-120, Milton, NY). Target spore deposition was between 6 and 6·5-log CFU per coupon. The 10-ml spore suspension was pumped through the sonic nozzle of the aerosol generator at a flow rate of 1-ml per minute after being thoroughly vortexed. The aerosol deposition chamber (45·7 cm × 40·6 cm × 45·7 cm) was fabricated with Lexan Plexiglas (0·95 cm thick), with a door on one side to allow for placement and removal of coupons. The inside of the door was lined with sponge neoprene stripping with adhesive to ensure a hermetic seal, which was reinforced with pressure clamps. Each Petri plate contained coupons of multiple material types to ensure equal and random distribution of spores onto each material. Negative control coupons were placed in the Bio-safety cabinets. A small fan (Orion, Knight Electronics, Inc.) placed in the top corner of the deposition chamber (opposite of the sonic nozzle) was operating during spore aerosolization, to assist with mixing and uniform deposition. After aerosolization, the fan was turned off and the spores were allowed to gravitationally settle overnight (typically >16–18 h) before UVC exposure.

Low-pressure mercury vapor lamp

A 15W mercury vapor bulb (Sankyo Denki Co., Japan) was used in a ballast/lamp fixture apparatus (ClorDiSys, Inc., Lebanon, NJ). The UVC lamp housing (Model XX-15S, UVP LLC, Upland, CA) was suspended from the ceiling of the chamber.

LED UVC source

Since there was limited commercial availability of UVC LED lamps providing the intensity we contemplated for surface decontamination, a custom LED lamp system was fabricated. The system consisted of 48 UVC LEDs (Crystal IS, Inc., Green Island, NY; Klaran model

LED), with each LED producing UVC with a nominal peak wavelength of 265 nm. The LED system was mounted on a 44·5 cm × 44·5 cm stainless steel plate via four strips of 12 LEDs. The strips were mounted on the plate 11·4 cm apart, and in each strip the LEDs were spaced 3·5 cm apart. The LED system plate was then mounted on top of the LED UVC exposure chamber. On the opposite side of the plate, a 250 W power supply and fan for cooling the plate were attached.

Mercury lamp UVC exposure chamber

A chamber was fabricated for each UV-C source/lamp type due to differences in lamp and mounting characteristics. The dimensions of the mercury lamp chamber were 48 × 43 × 43 cm and was constructed of 0·95 cm thick Plexiglas. A transparent polypropylene plastic sheet of 0·32 cm thickness was placed between the mercury bulb and inoculated coupons to reduce the UVC intensity to be more consistent with the lower LED lamp UVC intensity. The inoculated coupons were placed in four Petri plates which were then placed on a battery-operated turntable (Winter Lane, model no. 546-035) at the bottom of the chamber. The 26·7 cm diameter turntable rotated at 1·3 rev min^{-1} to allow for uniform exposure to the UVC produced from the narrow bulb. The distance between the UVC bulb and the top of the coupons was ~28 cm. At each prescribed exposure time, a Petri plate of coupons was removed from the UVC chamber. Refer to Fig. S1 for a photograph of coupons inside the mercury lamp chamber.

LED UVC exposure chamber

The construction of the LED UVC exposure chamber was similar to the mercury lamp chamber, with somewhat different dimensions of 46 × 46 × 46 cm. A small scissor jack was used in the chamber to adjust the height of coupons in relation to LED lamp plate. The inoculated coupons were placed in four Petri plates with the top of the coupons approximately 11 cm from the LED lamp. Measurements of UVC intensity were made below the LED lamp to ensure uniformity of intensity and to confirm no rotation of the coupons was necessary. See Fig. S2 for a photograph of the LED lamp inside the exposure chamber.

UVC measurement

The UVC intensity was measured using a spectral radiometer (UVpadE Model #670027, Opsytec Dr. Groebel, Ettlingen, Germany), which was calibrated at the factory prior to shipment. This spectroradiometer provided an intensity value at each wavelength for the whole UV range (200–440 nm), and then integrated the individual intensities to determine a total intensity for the UVA, UVB, and UVC regions. We report here the total intensity integrated over the UVC band. The UV sensor was placed at the same height as the top of the coupons would be and UVC measurements were taken before and after exposing the coupons. For the mercury lamp chamber, five UVC measurements were taken on the turntable (one in each corner and one in the center). Twelve UVC measurements were taken for the LED lamp, equally distributed in the area where coupons would be placed. For the relatively higher intensity of the low-pressure mercury lamp, the high sensitivity setting on the spectroradiometer was used, with a sampling rate of 1500 ms. For the LED lamp, the fixed (resolution 0·01) setting was used, resulting in a sampling rate of 10 000 ms. The

spectroradiometer reported UVC intensity in units of mW cm^{-2}, and UVC dosage (units of mJ cm^{-2}) was calculated as the intensity multiplied by the time of exposure in seconds. Typical spectra for the mercury and LED lamps are presented in Figs S3 and S4.

Temperature and RH monitoring in UVC chambers

UVC exposures were performed at laboratory ambient temperatures, at either uncontrolled RH, low RH, or high RH. The temperature and RH were measured using a HOBO data logger (Onset Corporation, MX2301) placed at the bottom of the chamber. For high RH test runs (target RH of 75%), the test chamber RH was raised using beakers containing saturated aqueous sodium chloride solutions. For low RH tests (target of approximately 45% RH), Drierite desiccant (Cole-Parmer) was used as needed if the ambient RH was higher than the desired RH.

Spore recovery from suspension-deposited coupons

Each coupon (test or control) was placed in a sterile 50 ml plastic tube containing 10 ml of phosphate buffered saline and 0·01% Tween-80. The coupons were sonicated for 10 min and vortexed for 2 min. The suspensions were diluted (10-fold) as appropriate. Negative control samples were plated undiluted. For each dilution, an aliquot was plated on two tryptic soy agar (TSA) plates. If no CFU were observed at −1 dilution, the entire remaining volume was filtered through 150-ml filters (0·2-μm) Nalgene Analytical filters (Thermo Scientific, P/N 130-4020) and aseptically overlaid on TSA plates. The spread-plated and filtered samples were incubated at 37°C for 16–24 h before counting. The CFU for were enumerated manually for *B. anthracis* and using QCount™ for *B. atrophaeus.* Relative spore recoveries were calculated by number of spores recovered from positive controls relative to the titer control, (number of spores recovered from coupons/number of spores inoculated on the coupons × 100) for the four surfaces, and ranged between 20 and 50% of the inoculum level (based on a range of 2×10^6 to 9×10^6 spores per coupon), and in general, spore numbers were least recovered from wood coupons. The limit of detection (LOD) was 1–3 CFU per coupon, since samples with low viable spores were all filtered and counted after incubation.

Spore recovery from aerosol-deposited coupons

Due to a potential of spores on the side of the coupons not being exposed to UVC, they were not extracted. Rather, the entire top surface of each coupon (test or positive control) was sampled with a sterile foam tipped applicator (Puritan, Model no 25-1607) using an S-shaped pattern. The applicator was then dropped in a sterile 50 ml plastic tube containing 10 ml of PBS with 0·01% Tween-80. The applicators were then extracted and plated as described above for the coupons. The spore recovery from four surface types ranged from 25 to 65%. The LOD for these samples was also 1–3 CFU per coupon, since samples with low viable spores were filtered, and counted after incubation.

Data handling

For spread plates, the CFU counted from the two plates for each coupon was averaged. The CFU per coupon was then multiplied with the plating volume factor (10), dilution factor (1/

dilution read for CFU counting), and extraction buffer volume factor (10). For filter-plated samples, the CFU per filter was the total number of CFU per coupon. The CFU per coupon was converted to its $\log_{10}$ value. Decontamination efficacy in terms of LR was calculated by subtracting the average log CFU of the test samples from the average log CFU of control samples.

Statistical analysis

Statistical analyses were performed using R 3.4.3 (https://www.r-project.org/) to assess the effect of experimental conditions on UVC efficacy. Specifically, the effect of spore deposition technique, spore species, and RH on efficacy was examined for each material. Two methods were used to this end. In the main approach, a *t*-test was performed to assess differences in group means for the pairs (e.g., material, RH) in which normality and variance equality assumptions were found to hold, with the Wilcoxon rank sum test used when they did not. A formal normality check was performed on the pair differences, and two variance homogeneity checks were performed on the original response values, to evaluate the suitability of a *t*-test versus a non-parametric equivalent. The Shapiro–Wilk test was used to assess normality, and *F*-test and Bartlett's test were used to assess homoscedasticity. A secondary approach, used in experiments varying RH, plotted response values in each (material, RH) pair against dose, fit a simple linear regression to the data, and compared slopes like-to-like with respect to material across RH levels. Additionally, an analysis to determine the effect of the material type itself on decontamination efficacy was conducted. A two-tailed t-test was employed for each unique material pair for both spore species. Refer to the Supporting Information (including Figs S10–S14 and Tables S1–S19) for further details on the statistical methods and results.

Test matrix

An overview of the test matrix is presented in Table 1. Due to space limitations within the test chambers, along with multiple test variables and objectives, not all materials were tested in every experiment, and not all experiments utilized the same exposure times. Additionally, typically two or more experiments were conducted to evaluate each test objective. And, most importantly, an adaptive approach to testing was adopted, especially regarding UVC exposures. For example, longer time points were used if previous experiments showed poor efficacy.

Results

Recovery of spores from positive controls

A summary of the average recoveries of *B. atrophaeus* and *B. anthracis* spores from the positive controls, by material and deposition method, is shown in Table 2. Glass was the most used material in the study and had the highest average spore recoveries for both species and ranged from 6·5 to 6·8 log CFU. Spore recoveries averaged above 6 log CFU in all cases except for *B. atrophaeus* on wood (both deposition methods), which had an average recovery of 5·7 log CFU.

Decontamination efficacy results for suspension and aerosol deposited spores

The UVC decontamination efficacy results as a function of the spore deposition method are shown in Table 3 (and Figs S5 and S10). This set of experiments was conducted with *B. atrophaeus* and the mercury lamp. This evaluation was conducted via four separate experiments to allow for side-by-side tests with both spore deposition methods, using multiple materials and exposure times. Average temperatures within the test chamber ranged from 22 to 23°C, and average RH levels ranged from 70 to 71%, except for the second experiment with wood, which had an average RH level of 83%. The UVC intensities ranged from 0·40 to 0·48 mW cm^{-2}. Tests were conducted with exposure times from 15 min to 6 h.

As can be observed in Table 3 (and Figs S5 and S10), there was no obvious pattern in efficacy as a function of the spore deposition method. For the carpet material, the efficacy was higher for the aerosol-deposited spores, while the opposite trend was observed with the laminate material. The LR results were highly variable for the wood and glass with respect to the deposition method. From the statistical analysis (refer to Supporting information for further details) there was no significant difference in the group averages ($P = 0·57$), and thus there is insufficient evidence to posit that the spore deposition method affected LR.

Decontamination efficacy generally increased with increasing UVC dosage, with a few exceptions. The highest mean LR values achieved were 5·12 on glass and 5·27 on laminate, with the suspension inoculation approach, at the highest UVC dosage of 8726 mJ cm^{-2} (corresponding to a 6-hr exposure time). The glass and laminate materials were decontaminated >3·00 LR in every test, whereas efficacy for the carpet and wood materials was generally <3·0 LR. In one test condition (glass, aerosol-deposited spores), an average LR of 5·0 was achieved with a UVC dose as low as 3456 mJ cm^{-2}. As shown in Table 3, the LR values for glass were always greater than for wood, for both inoculation methods; in particular, there were three instances (out of eight with comparable dosages) in which the difference in LR for wood was greater than 2·0 when compared to glass.

Decontamination efficacy results comparing *B. atrophaeus* and *B. anthracis* (Ames)

The results comparing the two *Bacillus* species are shown in Table 4 (and in Figs S6, S7, and S11). This set of experiments was conducted with both *B. atrophaeus* vs. *B. anthracis* Ames in side-by-side tests, suspension-inoculated onto materials, and exposed to the low-pressure mercury lamp. This portion of the study was conducted via two separate experiments to allow for both species to be tested simultaneously, using multiple materials and three exposure times. The first experiment was conducted with all four materials, at an average temperature of 23°C and 59% RH. The second experiment was conducted with only glass and laminate, with the goal of having the RH >70% (though an average of 67% RH was obtained). The average UVC intensity was 0·47 mW cm^{-2} for both experiments, with exposure times of 2, 4, and 8 h. As can been seen in Figs S6 and S7, there was no obvious pattern indicating a difference in sensitivity to UVC for the two species of bacteria, with the exception in the first experiment (Fig. S6), in which decontamination efficacy was higher for *B. anthracis* Ames at all time points for the glass and wood materials.

Out of 18 comparisons for the two species, as shown in Table 4, the LR values for *B. anthracis* were greater than for *B. atrophaeus* in 10 of these. In addition, the statistical analysis (refer to Supporting information for further details, section 2.1 in the Statistical Analysis section, and in particular, Table S6) confirms there was no significant difference in the group means for the two species' pooled data. However, we do acknowledge that the p-value from the paired *t*-test is near to a 0·1 significance level ($P = 0{\cdot}14$).

The efficacy generally increased with increasing dosage, with a few exceptions. The highest LR of 5·7 was achieved against *B. anthracis* on glass using the highest experimental UVC dosage of 13 536 mJ cm^{-2} (an 8-h exposure), in the first test run shown in Table 4. (We do note that the decontamination efficacies achieved against *B. anthracis* on glass in the first test run were on average approximately 1·8 log greater than achieved in the second test run. This difference in efficacy between the first and second test runs was not observed for *B. atrophaeus* on glass, nor for the two species on laminate.) No spores were detected on two of the three glass coupons recovered at this time point, the only instance in the study in which complete kill occurred. From the statistical analysis of the results to compare the effect of species, decontamination efficacy was significantly higher for glass than the three other materials, for both species. Refer to Table S7.

Decontamination efficacy results for the LED UVC lamp at high and low RH

The test conditions and results to evaluate the LED lamp decontamination efficacy at high and low RH are shown in Table 5 (and Figs S8 and S12). This set of experiments was conducted with suspension-inoculated spores of *B. atrophaeus* onto all four materials. The first experiment was conducted at an average temperature of 23°C and 39% RH, while the second experiment was conducted at a similar temperature but higher average RH of 73%. The average UVC intensity of the LED lamp was 0·25 mW cm^{-2} for both experiments, with exposure times up to 6 hr. There is an obvious pattern showing an inverse relationship between RH and efficacy. That is, the lower RH is associated with higher inactivation in every comparison, with an average increased efficacy of 1·2 LR. The statistical analysis (refer to Supporting Information for further details) confirms this observation, i.e., there was significant evidence ($P < 0{\cdot}0001$) that LR changed when the RH was adjusted.

As in the previously discussed experiments, efficacy was generally higher for the glass and laminate materials, for both RH levels. The efficacies for these two materials were nearly all >4 LR at the low RH condition, whereas for the wood and carpet materials, LR values generally ranged from 2 to 4 LR at the low RH. The highest LR of 5·1 was achieved against *B. atrophaeus* on glass using the highest LED lamp UVC dosage of 3600 mJ cm^{-2} (corresponding to a 6-h exposure time). The LR for laminate and glass were both different from wood at the $\alpha = 0{\cdot}01$ significance level; efficacy for laminate and glass was significantly different from carpet at the $\alpha = 0{\cdot}05$–$0{\cdot}1$ significance levels.

Decontamination efficacy results for the low-pressure mercury lamp at high and low RH

The test conditions and results for evaluating the effect of RH when using the mercury UVC lamp are shown in Table 6 (and Figs S9 and S14). This set of experiments was conducted with spores of *B. atrophaeus* that were suspension-inoculated onto the glass and laminate

materials. The first experiment was conducted at an average temperature of 23°C and 39% RH, while the second experiment was conducted at a similar temperature but higher average RH of 73%. The average UVC intensity of the LED lamp was 0·25 mW cm^{-2} for both experiments, with exposure times up to 6 hr. There is an obvious pattern showing an inverse relationship between RH and efficacy. That is, the lower RH is associated with higher inactivation in every comparison, with an average increased efficacy of 1·2 LR. The statistical analysis (refer to Supporting Information for further details) confirms this observation, i.e., there was significant evidence ($P < 0·0001$) that LR changed when the RH was adjusted.

As in the previously discussed experiments, efficacy was generally higher for the glass and laminate materials, for both RH levels. The efficacies for these two materials were nearly all >4 LR at the low RH condition, whereas for the wood and carpet materials, LR values generally ranged from 2 to 4 LR at the low RH. The highest LR of 5·1 was achieved against *B. atrophaeus* on glass using the highest LED lamp UVC dosage of 3600 mJ cm^{-2} (corresponding to a 6-h exposure time). The LR for laminate and glass were both different from wood at the $\alpha = 0·01$ significance level; efficacy for laminate and glass was significantly different from carpet at the $\alpha = 0·05–0·1$ significance levels.

Decontamination efficacy results for the low-pressure mercury lamp at high and low RH

The test conditions and results for evaluating the effect of RH when using the mercury UVC lamp are shown in Table 6 (and Figs S9 and S14). This set of experiments was conducted with spores of *B. atrophaeus* that were suspension-inoculated onto the glass and laminate materials. The first experiment was conducted at an average temperature of 23°C and 26% RH, while the second experiment was conducted at the same temperature but higher average RH of 69%. The average UVC intensity of the mercury lamp was 0·42 mW cm^{-2} for the low RH experiment, and a somewhat lower average UVC intensity of $0·36 \pm 0·04$ mW cm^{-2} was measured at the higher RH test condition. In both experiments, coupons were exposed to UVC for up to 3 h.

As can be seen in Figs S9 and S14, there is no obvious trend indicating that the RH affected decontamination efficacy when using the mercury lamp. In fact, there were several instances in which the lower RH and associated higher UVC dosage did not improve efficacy (in contrast to what was observed with the LED tests). Because of the differing UVC dosages associated with the different RH levels, we compared the inactivation rates (increase in LR with respect to change in dose) rather than the LR values for each material and RH condition. From this analysis, we found no significant difference in the inactivation rates, by material, for the two RH levels. Please refer to the Fig. S15 for more details of this analysis.

Discussion

To the best of our knowledge, the present study demonstrates for the first time that bacterial spores deposited on environmental surfaces can be inactivated with UVC at LR levels >4·0. There were five test conditions in which decontamination efficacy was ≥5 LR, which occurred for glass or laminate (non-porous) materials, with both species, and with both UVC techniques. Increased UVC dosages generally resulted in increased efficacy, but with a few

caveats. With the goal of maximizing efficacy and the limited number of time points we could analyze per experiment, we evaluated UVC dosages that were generally higher than what was typically reported in the literature (with exposure times up to 8 h). Thus, more than likely much of the data collected in this study were from the tail end of a biphasic survival curve (Coohill and Sagripanti 2008). Inactivation rates are lower at the tail end, as we show in Fig. S9 and exemplified by (Owens et al. 2005), who showed minimal to no inactivation after an initial rapid decay.

Achieving decontamination efficacy ≥5 LR generally required high UVC dosages ranging from 3456 to nearly 14 000 mJ cm^{-2}. Relative to the literature, a 4 LR of *B. atrophaeus* spores was reported using a dose of 2000 mJ cm^{-2} with a medium pressure mercury lamp (Owens et al. 2005). The highest LR achieved in the study was 5·7, on glass for *B. anthracis* spores at a dose of nearly 14 000 mJ cm^{-2}. While these test results demonstrate that UVC may be more effective on surfaces against spores than previously reported, we were unable to demonstrate ≥6 LR for the technology, a LR level which is considered “effective” for chemistries used against spores of *B. anthracis* (U.S. Environmental Protection Agency 2018).

The generally lower decontamination efficacy achieved by UVC, compared to what may be readily achievable (>6–7 LR) with chemical sporicides such as peracetic acid or hypochlorous acid (Wood et al. 2011), could be due to issues with shading and shielding of the UVC radiation (Coohill and Sagripanti 2008). Because of this potential effect, we evaluated UVC against spores deposited onto materials using both a suspension and aerosol inoculation approach, hypothesizing the latter approach would result in less clumping of spores. However, our results showed there was no significant difference in decontamination efficacy using UVC (mercury lamp) between the two inoculation methods, using spores of *B. atrophaeus.* We acknowledge our dataset for making this comparison is somewhat limited (N = 16 comparisons of material/UVC dosage), and that additional testing to examine this hypothesis would be recommended. Although the spore suspension was thoroughly mixed prior to aerosolization, more than likely some of the spores remained agglomerated during aerosolization, since biological particles typically tend to bind to each other (Kesavan et al. 2014). Additionally, in general, particles within an aerosol collide and coagulate through numerous mechanisms (Friedlander 1977).

The present study also confirms for the first time, based on side-by-side tests, that there was no statistical difference in the inactivation efficacy of UVC for spores of both *B. atrophaeus* and *B. anthracis* Ames strain. We are not aware of any literature reporting the efficacy of UVC to inactivate spores of a virulent *B. anthracis* strain. That *B. atrophaeus* would therefore be an appropriate surrogate for *B. anthracis* Ames when decontaminating with UVC was not totally unexpected, based on data comparing *B. atrophaeus* with the vaccine Sterne strain (Nicholson and Galeano 2003, Owens et al. 2005; Menetrez et al. 2006). This finding is also consistent with a study demonstrating the comparable resistance of *B. subtilis* and *B. anthracis* Ames to simulated sunlight, i.e., spores were exposed to UVA and UVB (Wood et al. 2015).

The low RH (~39%) appeared to increase the efficacy of the LED lamp, but this effect was not evident for the mercury lamp. This lack of consistency may be due to some of the differences in the UVC lamps. The main difference between the two lamps is their radiative output wavelength (the mercury lamp UVC output peaks at 254 nm, but with a narrower band compared to LED lamp, with peak output ~265 nm, but with a broader band; see Supporting information for typical spectra), and perhaps that may have been a factor. The other main difference between the two lamps was their intensity. The average intensity of the LED lamp was 0·25 mW cm^{-2}, measured at 11 cm distance. The intensity of the low-pressure mercury vapor lamp was higher (average of ~0·42 mW cm^{-2}), even when measured at a distance of 28 cm. The UVC dosage, as opposed to the UVC exposure time, was an independent variable in the study. Further research is needed to determine to effect of UVC source on efficacy.

The primary mechanism for inactivation of spores via UVC is through damage to DNA (Setlow 2006; 2016), although it is not clear how the RH level would affect that mechanism. There are few data available in the literature documenting the effect of RH on the inactivation of bacterial spores via UVC, and those data show confounding UVC efficacy results with altering RH, or no effect of RH (Kowalski 2009). In one study examining UVC in air ducts (Van Osdell and Foarde 2002), the increase of RH from 55 to 85% had no effect on the inactivation of *B. subtilis* spores. The general paucity of data in the literature on the effect of RH on UVC efficacy, emphasize the need for more research in this area.

Our study demonstrates that the material with which the spores are associated affects the decontamination efficacy of a UVC system. The nonporous materials glass and laminate were generally decontaminated with greater efficacy when compared to the wood and carpet materials used in the study. This result, that the porous materials were relatively more difficult to decontaminate with UVC compared to nonporous materials, is consistent with nearly all other decontaminants for *B. anthracis* spores (Wood and Adrion 2019). Few studies have documented the effect of material on the efficacy of UVC in the inactivation of spores. However, in one such study, spores dried onto surfaces were more resistant to UVC compared to spores in a suspension, and that there was variation in efficacy depending on the material (Blatchley et al. 2005). Our results are consistent with the authors' hypothesis that the materials' pore structure may shield the UV radiation, and also consistent with the thinking that the microcrevices of some materials will reduce individual spores' exposure to UVC (Coohill and Sagripanti 2008).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Study design/test matrix

Test objective	Spore species	Spore deposition method	UVC lamp	RH	Materials	Time points (min)
Compare deposition method	*Bacillus atrophaeus*	Suspension, aerosol	Mercury	Uncontrolled	Glass, wood	15, 30, 60, 120, 180, 360
					Carpet, laminate	180, 360
Compare species	*Bacillus anthracis* (Ames) and *B. atrophaeus*	Suspension	Mercury	High	Glass, wood, carpet, laminate	120, 240, 480
				Low	Glass, laminate	120, 240, 480
Compare lamp type and RH	*Bacillus atrophaeus*	Suspension	LED	High, low	Carpet, glass, laminate, wood	60, 120, 240, 360
			Mercury	High, low	Glass, laminate	30, 60, 120, 180

Table 2

Average recovery of *Bacillus atrophaeus* and *Bacillus anthracis* from positive controls (log CFU) by material and inoculation method

	Average ± SD recovery of *Bacillus atrophaeus*, aerosol deposition (*N*)	**Average ± SD recovery of *Bacillus atrophaeus*, suspension inoculated (*N*)**	**Average ± SD recovery of *Bacillus anthracis*, suspension inoculated (*N*)**
Glass	6·5 ± 0·0 (2)	6·7 ± 0·2 (8)	6·8 ± 0·0 (2)
Wood	5·7 ± 0·0 (2)	5·7 ± 0·3 (6)	6·3 (1)
Carpet	6·4 (1)	6·1 ± 0·7 (4)	6·7 (1)
Laminate	6·4 (1)	6·6 ± 0·3 (7)	6·5 ± 0·0 (2)

N refers to the number of experiments (inoculation events) occurring with that material/deposition method. Each experiment typically included 4 replicate positive controls for each material.

Table 3

Test conditions and decontamination efficacy results for *Bacillus atrophaeus* spores, suspension versus aerosol deposition, using the low-pressure mercury lamp

Material	Exposure time (min)	Average UVC dose mJ cm^{-2}	Average efficacy (LR) suspension inoculation	Average efficacy (LR) aerosol inoculation	Average *T* (°C)	Average RH (%)	Average UVC intensity (mW cm^{-2})
Glass	15	432	3·23 ± 0·07	3·60 ± 0·30	22 ± 0·9	70 ± 1·7	0·48 ± 0·02
	30	864	3·71 ± 0·15	3·21 ± 1·32			
	60	1728	3·48 ± 0·69	4·70 ± 0·40			
	120	3456	3·28 ± 0·90	5·00 ± 0·39			
Wood	5	141	0·58 ± 0·07	1·14 ± 0·16	22 ± 0·4	83 ± 1·6	0·47 ± 0·02
	30	846	1·63 ± 0·15	1·63 ± 1·63			
	60	1692	1·76 ± 0·18	1·84 ± 1·84			
	120	3384	2·92 ± 0·00	3·10 ± 0·00			
Glass	180	4590	4·94 ± 0·37	3·09 ± 0·38	23 ± 1·0	71 ± 3·0	0·42 ± 0·04
Wood			2·45 ± 0·28	2·00 ± 0·67			
Carpet			1·03 ± 0·18	2·78 ± 0·49			
Laminate			4·93 ± 0·23	3·06 ± 0·90			
Glass	360	8726	5·12 ± 0·57	4·74 ± 0·51	23 ± 0·8	70 ± 2·9	0·40 ± 0·04
Wood			2·38 ± 0·08	3·00 ± 0·61			
Carpet			1·32 ± 0·13	3·47 ± 0·45			
Laminate			5·27 ± 0·29	4·39 ± 0·26			

Table 4

Test conditions and efficacy results for spores of *Bacillus anthracis* and *Bacillus atrophaeus*

Material	Exposure time (min)	Average UVC dose (mJ cm^{-2})	Average efficacy (LR) *B. atrophaeus*	Average efficacy (LR) ames	Average *T* (°C)	Average RH %	Average UVC intensity (mW cm^{-2})
Wood	120	3384	0.04 ± 0.15	0.82 ± 0.02	23 ± 0.9	59 ± 1.7	0.47 ± 0.05
	240	6768	0.42 ± 0.22	0.89 ± 0.23			
	480	13 536	0.74 ± 0.46	2.32 ± 0.13			
Glass	120	3384	2.55 ± 0.29	4.73 ± 0.39			
	240	6768	3.17 ± 0.91	3.93 ± 0.72			
	480	13 536	4.14 ± 0.37	5.73 ± 0.76			
Carpet	120	3384	0.17 ± 0.17	0.58 ± 0.06			
	240	6768	1.2 ± 0.00	1.17 ± 0.03			
	480	13 536	1.19 ± 0.12	2.65 ± 0.09			
Laminate	120	3384	1.53 ± 1.06	1.2 ± 0.05			
	240	6768	1.76 ± 0.92	1.45 ± 0.51			
	480	13 536	2.47 ± 0.59	2.77 ± 1.05			
Glass	120	3384	3.48 ± 1.89	2.55 ± 1.00	22 ± 0.7	67 ± 3.0	0.47 ± 0.05
	240	6,68	3.66 ± 0.41	3.69 ± 0.50			
	480	13 536	3.71 ± 0.04	2.80 ± 0.47			
Laminate	120	3384	1.54 ± 0.73	1.33 ± 1.36			
	240	6768	1.89 ± 1.07	1.42 ± 1.00			
	480	13 536	2.85 ± 0.97	2.64 ± 1.07			

Spores were deposited on materials using a suspension inoculation; UVC source was low-pressure Hg lamp.

Table 5

Test conditions and efficacy results for *Bacillus atrophaeus,* using the LED UVC lamp, high versus low relative humidity

Material	Exposure time (min)	Average UVC dose mJ cm^{-2}	Average Efficacy (LR) LED lamp	Average *T* (°C)	Average RH (%)	Average UVC intensity (mW cm^{-2})
Carpet	60	900	2·94 ± 0·28	23 ± 0·8	39 ± 3·4	0·25 ± 0·03
	120	1800	2·67 ± 0·10			
	240	3600	3·97 ± 0·16			
	360	5400	3·57 ± 0·09			
Glass	60	900	4·33 ± 0·30			
	120	1800	4·95 ± 0·41			
	240	3600	5·06 ± 0·82			
	360	5400	4·02 ± 0·15			
Laminate	60	900	4·31 ± 0·27			
	120	1800	4·8 ± 0·18			
	240	3600	4·79 ± 0·43			
	360	5400	3·95 ± 0·20			
Wood	60	900	2·21 ± 0·29			
	120	1800	2·58 ± 0·66			
	240	3600	3·37 ± 0·54			
	360	5400	3·2 ± 0·10			
Carpet	60	900	1·2 ± 0·79	22 ± 0·8	73 ± 3·6	0·25 ± 0·04
	120	1800	1·61 ± 1·03			
	240	3600	2·29 ± 0·86			
	360	5400	2·08 ± 0·57			
Glass	60	900	2·6 ± 0·80			
	120	1800	2·58 ± 0·93			
	240	3600	3·32 ± 0·63			
	360	5400	2·99 ± 0·64			
Laminate	60	900	3·06 ± 0·85			
	120	1800	3·17 ± 0·93			
	240	3600	3·18 ± 0·46			
	360	5400	3·06 ± 0·55			

Material	Exposure time (min)	Average UVC dose mJ cm^{-2}	Average Efficacy (LR) LED lamp	Average *T* (°C)	Average RH (%)	Average UVC intensity (mW cm^{-2})
Wood	60	900	1·12 ± 0·86			
	120	1800	2·05 ± 0·51			
	240	3600	1·87 ± 1·87			
	360	5400	1·72 ± 0·46			

Spores were deposited onto materials via suspension inoculation.

Table 6

Test conditions and efficacy results for *Bacillus atrophaeus* using the low-pressure mercury lamp, high versus low RH

Material	Exposure time (min)	UVC dose mJ cm^{-2}	Efficacy (LR) mercury lamp	Average *T* (°C)	Average RH (%)	Average UVC intensity (mW cm^{-2})
Glass	30	756	3·31 ± 0·42	23 ± 0·6	26 ± 4·2	0·42 ± 0·05
	60	1512	3·73 ± 0·45			
	120	3024	4·39 ± 0·48			
	180	4536	4·36 ± 0·34			
Laminate	30	756	3·15 ± 0·39			
	60	1512	3·52 ± 0·28			
	120	3024	3·14 ± 0·40			
	180	4536	4·22 ± 0·13			
Glass	30	648	3·18 ± 0·29	23 ± 0·2	69 ± 0·7	0·36 ± 0·04
	60	1296	4·24 ± 0·36			
	120	2592	3·79 ± 0·24			
	180	3888	3·3 ± 0·56			
Laminate	30	648	3·03 ± 0·35			
	60	1296	3·45 ± 0·11			
	120	2592	3·94 ± 0·21			
	180	3888	3·58 ± 0·31			

Spores were deposited onto materials via suspension inoculation.