

# Ultraviolet germicidal irradiation inactivation of airborne fungal spores and bacteria in upper-room air and HVAC in-duct configurations

Elmira Kujundzic, Mark Hernandez, and Shelly L. Miller

**Abstract:** The efficacy of ultraviolet germicidal irradiation (UVGI) for inactivating airborne fungal spores and bacterial vegetative cells was evaluated under three configurations — intrinsic, upper-room air, and in-duct. Correspondingly, experiments were performed in (1) a pilot-scale chamber (0.8 m<sup>3</sup>), fitted with four corner UV lamps that irradiated the entire chamber (average UV fluence rate  $10.6 \pm 0.8 \mu\text{J s}^{-1}\text{cm}^{-2}$ ); (2) a full-scale room (87 m<sup>3</sup>), fitted with a UVGI system that irradiated the top 30 cm of the room (5 fixtures, 216 W total lamp power, average upper-zone UV fluence rate  $26 \pm 1 \mu\text{J s}^{-1}\text{cm}^{-2}$ ); and (3) the supply air duct of a heating ventilation and air-conditioning (HVAC) system. Fungal spores of *Aspergillus versicolor* and vegetative cells of bacterium *Mycobacterium parafortuitum* were aerosolized continuously such that their numbers and physiologic state were comparable both with and without the UVGI lamps operating. The Z value (UVGI inactivation rate normalized to UVGI fluence rate) was estimated to be  $1.2 \pm 0.4 \times 10^{-4} \text{ cm}^2 \mu\text{J}^{-1}$  for aerosolized *A. versicolor*. Upper-room air UVGI inactivated culturable airborne fungal spores with a first-order rate constant of  $0.4 \pm 0.2 \text{ h}^{-1}$ . Ultraviolet lamps enclosed in ventilation system ductwork inactivated fungal spores and vegetative bacterial cells at single-pass efficiencies of 75% and 87%, respectively, at an air stream velocity of  $2.2 \text{ m s}^{-1}$ . There was no detected inactivation of fungal spores and vegetative bacterial cells at an air stream velocity of  $5.1 \text{ m s}^{-1}$ .

**Key words:** bioaerosols, UVGI, air disinfection.

**Résumé :** L'efficacité de l'irradiation germicide par UV (UVGI) pour inactiver les spores fongiques et les cellules bactériennes végétatives dans l'atmosphère a été évaluée dans trois configurations – intrinsèques, dans l'air de la partie supérieure des pièces et dans les conduits de ventilation. Les expériences ont été réalisées respectivement dans (1) une chambre à l'échelle pilote (0,8 m<sup>3</sup>) munie de lampes UV aux quatre coins qui irradiaient la chambre en entier (taux moyen de fluence UV de  $10,6 \pm 0,8 \mu\text{J s}^{-1}\text{cm}^{-2}$ ); (2) une salle à pleine échelle (87 m<sup>3</sup>), munie d'un système UVGI qui irradiait les 30 cm supérieurs de la salle (5 luminaires, puissance totale de 216 W, taux moyen de fluence UV dans la partie supérieure de  $26 \pm 1 \mu\text{J s}^{-1}\text{cm}^{-2}$ ); et (3) la conduite d'amenée d'air d'un système chauffage, ventilation et climatisation (CVC). Les spores fongiques d'*Aspergillus versicolor* et des cellules végétatives de bactéries *Mycobacterium parafortuitum* ont été projetées en aérosol de manière continue de manière à ce que leur nombre et l'état physiologique étaient similaires que les lampes UVGI fonctionnaient ou non. La valeur de Z (taux d'inactivation UVGI normalisé au taux de fluence UVGI) était estimée à  $1,2 \pm 0,4 \times 10^{-4} \text{ cm}^2 \mu\text{J}^{-1}$  pour *A. versicolor* pulvérisée. L'UVGI dans l'air de la partie supérieure de la pièce a inactivé les spores fongiques pouvant être cultivées dans l'atmosphère avec une constante de vitesse des réactions du premier ordre de  $0,4 \pm 0,2 \text{ h}^{-1}$ . Les lampes UV encastrées dans les conduits du système de ventilation ont inactivé les spores fongiques et les cellules bactériennes végétatives avec des efficacités monopasses de 75 et de 87 % respectivement à une vitesse d'écoulement d'air de  $2,2 \text{ m s}^{-1}$ . Aucune inactivation des spores fongiques et des cellules bactériennes végétatives n'a été détectée à une vitesse d'écoulement d'air de  $5,1 \text{ m s}^{-1}$ .

**Mots clés :** bioaérosol, irradiation germicide par UV, désinfection de l'air.

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## Introduction

Airborne transmission of infectious agents resulting in disease has been well-documented (Hopewell 1986; Behrman and Shofer 1998; Sutton et al. 1998; Li et al. 2005). Efforts to design and optimize appropriate technologies to remove or inactivate causative agents are underway. Engineering controls include direct source control using local exhaust ventilation, maintenance of negative pressure differences between isolation and treatment rooms and adjacent areas, dilution and removal of contaminated air via mechanical ventilation, in-room air filtration, ultraviolet germicidal irradiation (UVGI), and using respiratory protective equipment (CDC 1994). These controls are designed to reduce the concentration of infectious agents within the local environment, to protect those who come into close contact with infectious persons, and to prevent spreading of infectious agents.

One technology that inactivates microorganisms is ultraviolet germicidal air irradiation. Commercially available low-pressure mercury-vapor lamps used for ultraviolet germicidal air irradiation applications emit nonionizing electromagnetic radiation with most of their spectral power density associated with a wavelength of 254 nm (AIHA 1991; Shechmeister 1991). Ultraviolet germicidal irradiation that penetrates to microbial DNA may cause damage sufficient to interrupt cell replication.

Upper-room air UVGI is achieved by suspending lamps from the walls or ceiling so that irradiation is contained within the upper portion of the room (Macher et al. 1992). Studies have been performed evaluating the effectiveness of upper-room air UVGI to inactivate bioaerosols (Macher et al. 1992; Miller and Macher 2000; Green and Scarpino 2002; Ko et al. 2002; Xu et al. 2003, 2005). Upper-room UVGI has been shown to inactivate airborne bacteria at significant rates and it has been shown to be effective in controlling a number of airborne biological contaminants (Miller and Macher 2000; Brickner et al. 2003; Xu et al. 2003).

Installation of UVGI lamps in ductwork of heating, ventilating and air conditioning (HVAC) systems provides another means of inactivating bioaerosols that enter and circulate through the indoor environment. Enclosing UVGI in ventilation system ductwork, where the irradiance levels can be much greater than those used in occupied spaces, showed that inactivation of microorganisms can be significantly enhanced (Menzies et al. 1999, 2003; Levetin et al. 2001; Ryan et al. 2003). While these studies provide much needed, valuable information, data on the effectiveness of ductwork UVGI was missing and no data were provided on design parameters that optimize system performance. For example, in many studies UVGI irradiance levels within the ducts were not measured and ductwork air velocities were not monitored. A recent well-controlled study of two types of commercial short-wave ultraviolet radiation lamps in the "C" band (200 to 280 nm) was conducted in a full-scale recirculating test duct, which provides some information on the physical factors that influence UVGI in-duct applications (VanOsdell and Foarde 2002). Air stream velocities in the duct were in the range from  $1.27 \text{ m s}^{-1}$  to  $1.87 \text{ m s}^{-1}$

( $250 \text{ ft min}^{-1}$  to  $368 \text{ ft min}^{-1}$ ). Results showed that vegetative bacteria were relatively easy to inactivate, while bacterial and fungal spores were very difficult to inactivate.

Our research group at the University of Colorado has conducted previous studies of upper-room air UVGI (Miller and Macher 2000; Xu et al. 2003, 2005). These studies used bacteria and mycobacteria species. Thus there was a need to extend the research to include fungal spores and to investigate in-duct systems. The overall objective of this study was to conduct experiments to quantify the efficacy of ultraviolet germicidal irradiation for inactivating airborne fungal spores in three configurations — intrinsic, upper-room air, and in-duct, and vegetative bacterial cells in the in-duct configuration. Intrinsic refers to experiments conducted in a pilot-scale, uniformly irradiated chamber that allowed for derivation of intrinsic effects of UVGI on microorganisms.

## Materials and methods

### Experimental facilities

#### *Pilot-scale chamber*

A pilot-scale chamber was used to determine intrinsic fungal spore inactivation rates from UVGI irradiation. The chamber was constructed of 1.27 cm thick Lucite<sup>®</sup> plastic (Lucite International Ltd, Southampton, UK), and the volume was estimated to be  $0.8 \text{ m}^3$  ( $0.93 \text{ m} \times 0.93 \text{ m} \times 0.93 \text{ m}$ ). Compressed outdoor air provided the supply air for the chamber. Supply air was filtered through a  $20 \text{ cm} \times 3.8 \text{ cm}$  (length  $\times$  inner diameter) cylindrical polyvinyl chloride tube containing equal volumes of size 6–14 mesh activated carbon (Fisher Scientific, Pittsburgh, Pa.) and indicating desiccant (W.A. Hamond Drierite Company Ltd., Xenia, Ohio). Two Nalgene (0.635 cm ID) tubes supplied outdoor air that was metered by a rotameter (Gilmont Instruments, Barrington, Ill.). Before entering the reactor, incoming outdoor air was passed through two bacterial air vents (Pall Corp., Ann Arbor, Mich.) to prevent bioaerosol contamination from outdoor air. The chamber air was mixed by a 42 W fan (Model 707; Caframo Ltd., Warton, Ontario, Canada).

Four low-pressure mercury vapor ultraviolet lamps (30 W each; Model G30T8; Osram-Sylvania, Hanover, Mass.) that ran the entire height of the chamber were installed in the corners of the chamber. UV-C lamps were wrapped in eight layers of aluminum filter mesh screens (Research Products Co., Madison, Wis.) to attain the desired experimental UVGI fluence rate. Lamps were operated for more than 100 h before their use in these experiments, and lamps were allowed to warm up for 15 min before each UV experiment to achieve desired operating temperature.

#### *Full-scale room*

A full-scale  $87\text{-m}^3$  test room established at the Joint Center for Energy Management's Larson Building Systems Laboratory, University of Colorado at Boulder was used for this study and was described previously (Kreider and Brandemuehl 1991; Xu et al. 2003, 2005). This room is capable of maintaining a

stable temperature in the range 15–35 °C, and a relative humidity (RH) in the range between 20% and 100%. The temperature and RH for all experiments were maintained at room ambient conditions. Temperature was in the range 20–25 °C and RH in the range 20–35%. The room has a natural infiltration rate of 0.1–0.3 air changes per hour (ACH or h<sup>-1</sup>). It is equipped with a computer-controlled ventilation system. For commercial buildings 15–25% of the indoor recirculated airflow is comprised of outside air (Kowalski et al. 2003), and some ventilation systems may have full-time 100% outdoor air. The HVAC system used in this study supplied 100% outdoor air, which is typical for hospital settings. Panel filters designed for general air filtration (Model 5700, AAF Int., Louisville, Ky.) were installed inside the “mixing box” (where outdoor air was mixed with recirculating air).

In separate tracer gas studies, it was determined that the aerosol mixing in the chamber approximated completely mixed conditions (Xu and Miller 1999). During testing, the ventilation’s supply and exhaust airflow were balanced to achieve exhaust airflow greater than the supply such that there was a negative pressure of 12 Pa within the room relative to the surrounding laboratory. The negative pressure was continuously monitored and maintained using pressure gauges and ventilation system feedback-control loops.

A modern UVGI system (Lumalier, Memphis, Tenn.) was installed in the room and consisted of five fixtures, four mounted in each of the corners of the room and one hung from the center of the ceiling. The center fixture was rated at 72 W, consisting of four lamps. The corner fixtures were rated at 36 W each, containing two lamps installed with parabolic aluminum reflectors on the back of the fixtures. Each fixture was equipped with concentric black louvers of 1.9-cm spacing. The fixtures were installed so that the lower edge was located 2.1 m above the floor, and the top was 10 cm below the ceiling. This placement created a 30-cm wide band of UVGI in the upper level of the room.

#### ***In-duct UVGI system and ductwork***

Experiments were conducted to investigate the effectiveness of a ductwork UVGI system (Dust Free<sup>®</sup>, Royse City, Tex.) for inactivating bioaerosols. Dust Free<sup>®</sup>’s BioFighter Triad HO system operated on 120 V with the single bulb configuration (0.5 m in length) was tested. A UV output of 0.73 mJ s<sup>-1</sup> cm<sup>-2</sup> was reported by the manufacturer (measured at 0.3 m from the lamp at 10 °C in a 0.6 m × 0.6 m duct at a velocity of 2.5 m s<sup>-1</sup>). This system was previously tested against *Serratia marcescens*, an extremely environmentally-sensitive UV-susceptible microorganism, and provided 95–99% single-pass efficiency (Dust Free 2004). The Dust Free<sup>®</sup> equipment was installed directly into the Larson Building Systems Laboratory’s ventilation system ductwork, which provided the ventilation for the 87-m<sup>3</sup> room used in the upper-room air UVGI experiments. The germicidal lamp array test section was placed in the room’s supply air intake ducting. The ventilation system’s return and supply fan were operated to modulate airflow to achieve the necessary

range of operating air stream velocities within the test section and air sampling locations. The system was operated in a 100% outdoor air configuration.

#### **Experimental studies**

Three experimental studies were performed to evaluate the efficacy of UV-C lamps in different configurations for inactivating fungal spores or vegetative bacterial cells. The objectives and description of the studies are given below.

#### ***Intrinsic inactivation rate of airborne fungal spores to UVGI***

Experiments were conducted in the pilot-scale chamber to estimate the intrinsic inactivation rate of airborne fungal spores to UVGI. After purging the chamber with clean air, airborne fungal spores were continuously generated (for usually 15 min) to raise the concentration in the chamber to a suitable level for detection. Two box fans (48-cm diameter, Model 3723, Lasko Inc., West Chester, Pa.) were turned on to ensure mixing. No ventilation was provided during this period and the UVGI lamps were kept off. Once the fungal spore aerosolization was stopped, sampling of the airborne fungal spores was initiated. Also in some of the experiments, UVGI lamps were turned on at this time. Samples were collected 4 or 5 separate times with duplicate liquid impingers as the concentration decayed over a 14 or 26 min period. For each sample, the impingers were operated for 3–5 min. The shorter decay time (14 min) was used for experiments in which UVGI lamps were operating, while the longer decay time (26 min) was used for tests with UVGI lamps not in operation. After the final sample was collected, the chamber was purged again with the clean air. Decay experiments were conducted without the UVGI system operating to measure removal of airborne fungal spores by deposition, exfiltration, and natural die-off. No ventilation was provided during these experiments.

A completely-mixed room model was used to estimate the inactivation rate of UVGI as previously described (Miller-Leiden et al. 1996; Xu et al. 2003, 2005). In summary, the least-squares method was used to fit lines to the log-transformed concentrations measured over the decay period, and inactivation rates were inferred by subtracting the slope of the lines for the no-UVGI experiments from those with UVGI. The UVGI inactivation rate has units of ACH or h<sup>-1</sup>.

#### ***Upper-room air UVGI inactivation rate of airborne fungal spores***

The objective of this study was to characterize the potential of an existing upper-room air UVGI system as previously described for inactivating airborne fungal spores. This UVGI system had already been tested against *Mycobacteria spp.* and bacterial spores (Xu et al. 2003). After cleaning the room by outside air, airborne fungal spores were generated continuously (for usually 75 min) to raise the concentration in the room to a suitable level for detection. An optical particle counter (OPC; Model 237B, Pacific Sci. Instruments, Grants Pass, Ore.) was

used to measure the total number of aerosolized particles. Fungal spores were released into the center of the room, approximately 1.5 m above the floor, between the ventilation exhaust and supply. The box fans were used to ensure mixing, and the lights, including the UVGI system, were kept off. After 75 min, aerosolization was stopped. In some of the experiments, UVGI lamps were turned on after fungal spore aerosolization. Air samples were collected at three different times at approximately 30 min intervals, as the concentration decayed over 90 min, with duplicate liquid impingers located underneath the ventilation exhaust at 1.6 m above the floor. Thus, in total, six air samples were collected over the decay period. Experiments were conducted without the UVGI system operating to measure removal of fungal spores by deposition, exfiltration, and natural die-off. A completely-mixed room model was used to estimate the inactivation rate of UVGI as described above.

#### ***In-duct UVGI single pass efficiency for inactivating fungal spores and bacterial vegetative cells***

Prior to each experiment, the room was purged with outdoor air. Bioaerosol was aerosolized for 90 min into the outdoor air intake at a constant rate. Sampling was conducted during the last 30 min of aerosolization. Air samples were collected using liquid impingers in duplicate inside the outdoor air duct and downstream of the germicidal lamp array in the supply air duct. Well-mixed conditions were induced in the ducts due to the operation of a centrifugal blower (560 r/min, 5500 W) that was located just after the outdoor air intake.

One of the major environmental factors impacting UVGI lamp performance in ventilation ductwork applications is the air stream velocity, which is directly related to the exposure duration. Test air stream velocities were selected to be representative of environmental conditions typical of ventilation system return and supply ductwork. Velocities in ventilation ductwork are typically lowest at air handler coils and highest in supply ductwork. For these experiments, tests were performed at  $2.2 \text{ m s}^{-1}$  ( $440 \text{ ft min}^{-1}$ ) and  $5.1 \text{ m s}^{-1}$  ( $1000 \text{ ft min}^{-1}$ ) to represent these two conditions. For each velocity, two experiments were conducted in series, the first with power provided to ductwork UVGI lamps and the second without the lamps in operation. Weather conditions varied during the course of the experiments. Outside mean temperatures were from 15 to 24 °C, and relative humidity was in the range between 18% and 88%.

UVGI single pass efficiency in the ventilation system ductwork was characterized in terms of the percentage of airborne fungal spores or vegetative bacterial cells that could not be cultured after UVGI exposure (Miller-Leiden et al. 1996; Hernandez et al. 1999; Xu et al. 2003, 2005). The single-pass efficiency was quantified by comparing the bioaerosol concentration upstream and downstream of the tested UV-C lamps using the following equation:

$$[1] \quad \text{single pass efficiency(\%)} = \left(1 - \frac{C_{\text{downstream}}}{C_{\text{upstream}}}\right) \times 100\%$$

where  $C_{\text{downstream}}$  was the average culturable bioaerosol concentration measured in the supply air duct downstream of the UVGI lamps ( $\text{CFU m}^{-3}$ ); and  $C_{\text{upstream}}$  was the average culturable bioaerosol concentration measured in the outdoor air duct upstream of the UVGI lamps ( $\text{CFU m}^{-3}$ ). In addition, control experiments were performed with the UVGI lamps off to show that the difference in bioaerosol concentrations between sampling points in the UV experiments was due to the effects of UV radiation only.

#### **Bioaerosol generation and sampling**

The response of aerosolized pure cultures of healthy, viable fungal spores to UVGI was evaluated. *Aspergillus versicolor* (American Type Culture Collection (ATCC) 52173) was used in this study because its spores have been successfully used as subpathogenic fungal surrogates in previous bioaerosol studies (Reponen et al. 1996; Grinshpun et al. 2003). *Aspergillus versicolor* spore dimensions as measured using microscopy in this study were 5–7  $\mu\text{m}$  in diameter.

*Mycobacterium parafortuitum* (ATCC 19689) was aerosolized in in-duct UVGI single-pass efficiency experiments. *Mycobacterium parafortuitum* is a non-motile, rod-shaped bacterium, 2–4  $\mu\text{m}$  long (Wayne and Kubica 1986). It grows rapidly on standard bacterial culture media and produces smooth pale yellow colonies that disperse readily in water.

Fungi were grown on malt extract agar (MEA) (Difco Laboratories, Detroit, Mich.) at 24 °C for 7 to 10 d. Just before aerosolization, *A. versicolor* spores were removed from plate surfaces by aseptic shaking with 3-mm glass beads (Fisher Sci., Pittsburgh, Pa.), and were suspended in sterile water with addition of 0.1% Tween<sup>®</sup> 80 (Sigma, St. Louis, Mo.).

Bacteria were grown on soybean-casein digest agar (SCDA) (Difco Laboratories, Detroit, Mich.) at 37 °C for 3 d. Just before aerosolization, bacteria cells were removed from agar plate surfaces by aseptic scraping with a sterile glass rod, and were suspended in a 15 mM phosphate buffer solution (PBS; 10 mM sodium phosphate buffer; 5 mM NaCl; pH 7.2).

Active fungal spore and bacterial stocks (>90% viable) were suspended in deionized water or PBS at  $10^7$  to  $10^9$  fungal spores per mL or  $10^8$  to  $10^{11}$  bacterial cells per mL, and aerosolized using a six-jet Collison nebulizer (CN 25, BGI Inc., Waltham, Mass.) with a 250 mL reservoir. The nebulizer was operated at 138 kPa (20 psi), by a compressed air cylinder in series with an air supply system that includes a dehumidifier, a HEPA filter, and a regulator (Model 3074, TSI Inc., St. Paul, Minn.).

Bioaerosol was sampled with swirling liquid impingers (BioSampler<sup>®</sup>, SKC Inc., EightyFour, Pa.). Before each experiment, bioaerosol samplers were completely disassembled, washed thoroughly with ethanol and distilled, deionized water, and autoclaved at 203 kPa (2 atm) and 120 °C for 15 min. Bioaerosol was collected in 20 mL of sterile PBS using swirling liquid impingers. Two liquid impingers were operated simultaneously to collect bioaerosol for 1 to 30 min, depending on the experimental scenario. All air samples were collected at an airflow rate of  $12.5 \text{ L min}^{-1}$  with high-flow sampling pumps

(Model 1023-1 01 Q, Gast Manufacturing Inc., Benton Harbor, Mich.) that were regulated with rotameters (Model 7400, King Instruments Inc., Garden Grove, Calif.). The pumps and rotameters were calibrated using a primary flow meter (Dry Cal, DC-Lite, Butler, N.J.). When sampling within the ventilation system ductwork, isokinetic sampling was conducted using plastic shrouds that were manufactured and attached to the inlet of each impinger to ensure that the velocity in the inlet was equal to the supply and outdoor air duct velocity. All liquid impingers were covered with opaque material to protect collected bacterial cells and fungal spores from UVGI.

### Bioaerosol quantification

Two independent methods were used for bioaerosol quantification: (a) culturing and (b) direct microscopy in accordance with previously described methods (Hernandez et al. 1999; Pecchia and Hernandez 2001).

### Culturing

Concentrations of fungal spores and bacteria vegetative cells were determined by plating of liquid-capture air samples and were expressed as colony forming units (CFUs) per m<sup>3</sup> of air. Non-diluted liquid was plated, according to manufacturer's recommendations onto nutrient-rich MEA or SCDA medium for quantification using a spiral-dispensing method (Spiral Biotech Inc., Bethesda, Md.). Plates were incubated at 24 °C for 7–10 d for fungal spores, and at 37 °C for 2–3 d for bacteria. All colonies were counted. All plating was performed in indirect dimmed light and all incubations were carried out in the dark to control for potential photoreactivation. At least three replicates of each sample were plated.

### Direct microscopy

Number concentrations of total bacteria cells were determined using direct microscopy (Hernandez et al. 1999). Fungal spore in-room number concentrations, however, could not be determined using this method because the detection limit was too high. Direct microscopy was used only to determine the number concentration of fungal spores in solution prepared for aerosolization. Bacteria cells were stained and enumerated with 4',6-diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, Mo.), a DNA-binding fluorescent stain. Fungal spores were stained with acridine orange (AO) (Molecular Probes, Eugene, Ore.). Some of the sampler reservoir contents containing the bacteria and fungal spore solution prepared for aerosolization were stained and incubated under appropriate conditions. After incubation, the solution was passed through 25-mm polycarbonate membrane filters (Poretics Inc., Livermore, Calif.). Pore diameters of 0.22 µm and 0.4 µm were used for bacterial cells and fungal spores, respectively. Microorganisms retained on the filter surface were mounted using low fluorescence immersion oil containing an antifadent (CitiFluor Ltd., Leicester, England), and were examined under 1100× magnification using a Nikon Eclipse E400 epifluorescent microscope fitted with a mercury lamp and polarizing filters. Ten random microscopic fields were counted per slide. All counts were reported as the average of all

microscopic fields counted. In accordance with the statistical criteria previously reported, counts from aliquots having coefficients of variation greater than 30% were discarded, and new sample aliquots were stained and counted until the distribution of microorganisms immobilized was judged to be uniform by these criteria (Hernandez et al. 1999).

### UV fluence rate

#### *UV fluence rate in the pilot-scale chamber*

An optometer (Model P9710; Gigahertz Optik, Newburyport, Mass.) was used to measure the UV fluence rate from 360 degrees. Ultraviolet measurements were taken at 25 evenly spaced locations within the room near the ceiling, the middle, and near the floor of the chamber. Three UV lamp configurations were investigated: all lamps in operation (120 W), half lamps in operation (60 W), and one lamp in operation (30 W). For 50% UVGI, two lamps were operated: the northwest and southeast lamp. For 25% UVGI, only the southeast lamp was operated.

#### *Upper-room air UV fluence rate*

The 360-degree optometer was used to characterize the UVGI distribution in the 87-m<sup>3</sup> room. The optometer was mounted on a pole, and was positioned in such a manner that it was approximately 23 cm below the ceiling. To determine the room-average fluence rate, a floor grid of 54 squares of equal size (60.96 cm × 60.96 cm) was used. The UV fluence rate was measured at the centre of each square. The UV fluence rates from all the sites were summed and that value was divided by the number of sites to determine the average UV fluence rate.

#### *In-duct UV fluence rate*

The 360-degree optometer was utilized for measuring the UV fluence rate since bioaerosols in the duct may be exposed to UVGI from all angles, particularly if the duct is significantly reflective to UV. Measurements in the supply air duct were conducted at three configurations: (a) static, (b) air stream velocity of 2.2 m s<sup>-1</sup>, and (c) air stream velocity of 5.1 m s<sup>-1</sup>. The measurements were taken 0.3 m away from the UV lamps and at two heights: 0.13 m and at 0.48 m from the bottom of the supply air duct, which was 0.71 m tall.

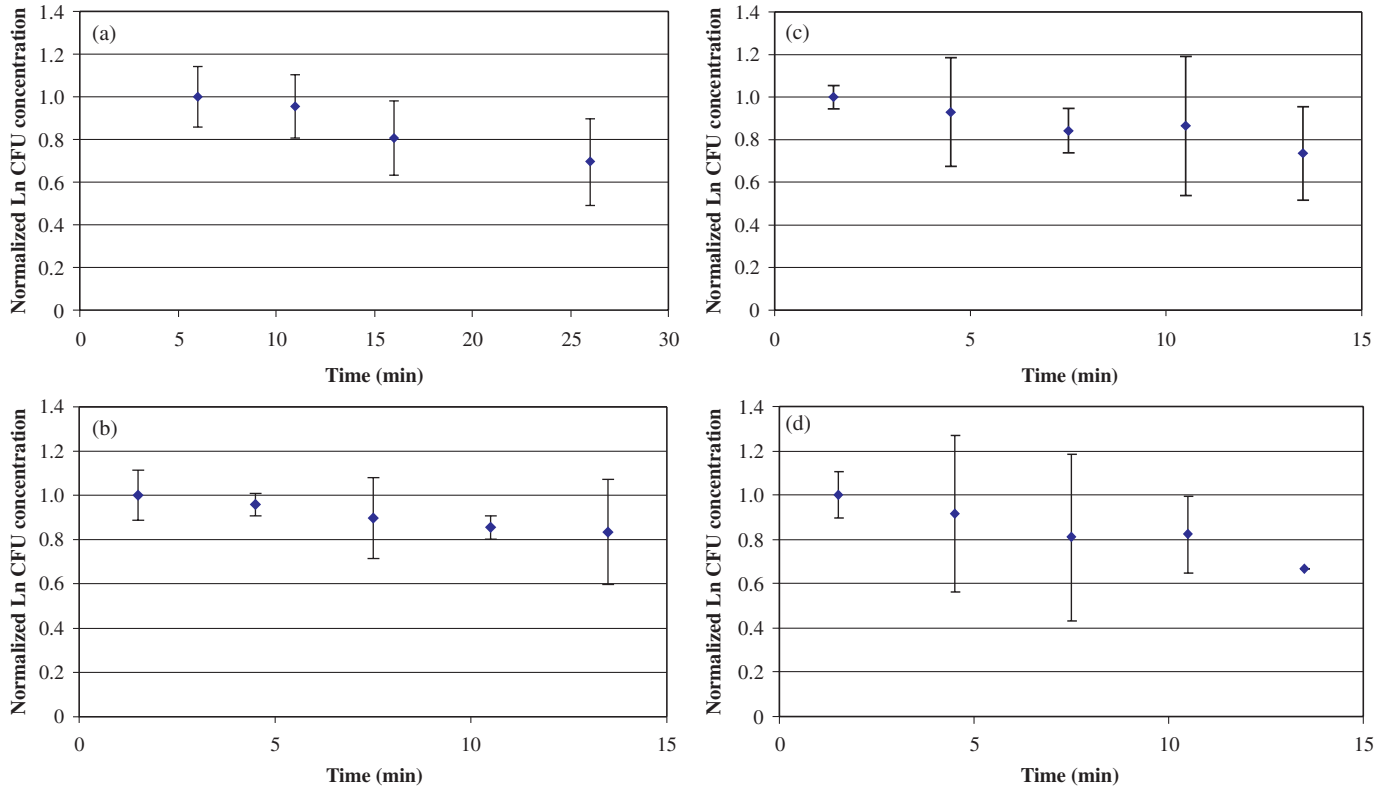
## Results

### UVGI fluence rates

#### *Pilot-scale chamber UV fluence rate*

Results are presented as area averages and the standard deviation of repeat measurements is presented in parentheses. The average chamber fluence rate when all four lamps were operated (total power of 120 W) was 10.6 (0.8) µJ s<sup>-1</sup> cm<sup>-2</sup>. The average fluence rate when two of the lamps were operated (60 W) was 7.0 (1.1) µJ s<sup>-1</sup> cm<sup>-2</sup>. The average fluence rate with only one lamp operating was 2.7 (1.7) µJ s<sup>-1</sup> cm<sup>-2</sup>. The averaged UV fluence rate was significantly higher at the top of the chamber compared to the bottom due to one lamp not being totally wrapped with aluminum mesh screens at the top of the chamber. For four lamps operating, the average UV fluence rate at the

**Fig. 1.** Summary of typical data from UVGI inactivation rate experiments for *A. versicolor* in the pilot-scale chamber. Data are for experiments with varying number of UV lamps operating: (a) no lamps; (b) four lamps; (c) two lamps; and (d) one lamp. Plots report normalized natural log culturable concentrations (CFU m<sup>-3</sup>) versus exposure time. The error bars represent the standard deviation in replicate plates used to obtain the culturable concentration. Note for (a) x-axis is from 0 to 30 min and for (b–d) x-axis is from 0 to 15 min.



bottom of the chamber was 4.9 (0.5)  $\mu\text{J s}^{-1} \text{cm}^{-2}$ , at the middle level (0.46 m from the chamber floor) was 7.3 (4.0)  $\mu\text{J s}^{-1} \text{cm}^{-2}$ , and at the top was 17.7 (2.1)  $\mu\text{J s}^{-1} \text{cm}^{-2}$ . For two lamps operating, the average UV fluence rate at the bottom of the chamber was 1.6 (0.1)  $\mu\text{J s}^{-1} \text{cm}^{-2}$ , at the middle level was 3.9 (0.5)  $\mu\text{J s}^{-1} \text{cm}^{-2}$ , and at the top was 16 (0.8)  $\mu\text{J s}^{-1} \text{cm}^{-2}$ . For one lamp operating, the average UV fluence rate at the bottom of the chamber was 1.0 (0.2)  $\mu\text{J s}^{-1} \text{cm}^{-2}$ , at the middle level was 2.1 (0.6)  $\mu\text{J s}^{-1} \text{cm}^{-2}$ , and at the top was 3.7 (1.9)  $\mu\text{J s}^{-1} \text{cm}^{-2}$ .

#### Upper-room air UV fluence rate

The optometer measurements when all five fixtures of the upper-room air UVGI system were operating (218 W) resulted in an average UV fluence rate of 26 (1)  $\mu\text{J s}^{-1} \text{cm}^{-2}$ . The propagated standard deviation of three sets of UV irradiance measurements is presented in parentheses.

#### In-duct UV fluence rate

The UV fluence rates measured at three different airflow conditions were not statistically significantly different (Student's *t* test: two-sample assuming unequal variances,  $\alpha = 0.05$ ;  $p = 0.19$ ). The average UV fluence rate at two different heights in the supply air duct and 0.3 m away from the UV lamps was as follows: at 0.13 m height the UV fluence rate was

1.3 (0.1)  $\text{mJ s}^{-1} \text{cm}^{-2}$  and at 0.48 m height the UV fluence rate was 0.9 (0.1)  $\text{mJ s}^{-1} \text{cm}^{-2}$ . The propagated standard deviation of 12 UV fluence rates taken at the different heights is presented in parentheses.

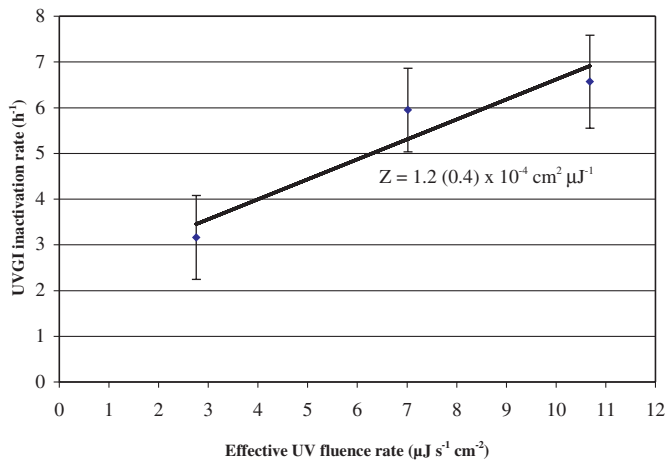
#### Intrinsic UVGI inactivation rate for airborne fungal spores

Data from the experiments used to determine intrinsic inactivation rates induced by UVGI are presented in Fig. 1. The standard error (SE) in the linear regression is reported and propagation of errors was applied to determine the uncertainty in the intrinsic inactivation rates. The removal rate of fungal spores due to deposition, exfiltration, and natural die-off was  $11.0 \pm 1.3 \text{ h}^{-1}$ . When all UV lamps were turned on, the observed inactivation rate was  $17.5 \pm 1.5 \text{ h}^{-1}$ . The intrinsic inactivation rate due to four lamps operating was then  $17.5 - 11.0 = 6.5 \text{ h}^{-1}$ . When half of the UV lamps were turned off, the observed inactivation rate was  $16.9 \pm 1.3 \text{ h}^{-1}$ , so the intrinsic inactivation rate due to two lamps operating was then  $16.9 - 11.0 = 5.9 \text{ h}^{-1}$ . When only one of the UV lamps was operating, the observed inactivation rate was  $14.1 \pm 1.3 \text{ h}^{-1}$ , so the intrinsic inactivation rate was  $14.1 - 11.0 = 3.1 \text{ h}^{-1}$ .

#### Z value

The Z value is a term adopted from the medical community. First order reaction rate coefficients were normalized against

**Fig. 2.** Intrinsic UVGI inactivation rates plotted as a function of UV fluence rates for *A. versicolor* as determined from pilot-scale chamber experiments. Z value is also presented and is derived from a least squares linear fit to the data. Error bars represent standard error in the inactivation rates.



average UV fluence rates and reported as Z value. In accordance with accepted convention for UV inactivation in air (Riley et al. 1976; Riley 1988, Riley and Nardell 1989) the Z value was calculated by following equation:

$$[2] \quad Z \text{ value} = \frac{\text{IR}_{\text{UV}}}{I_{\text{UV}}}$$

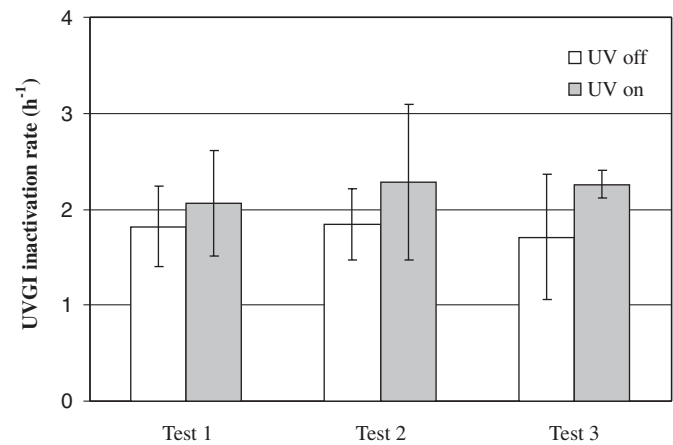
where  $Z$  ( $\text{cm}^2 \mu\text{J}^{-1}$ ) is Z-value response;  $\text{IR}_{\text{UV}}$  ( $\text{s}^{-1}$ ) is the inactivation rate due to UVGI, and  $I_{\text{UV}}$  is average UV fluence rate ( $\mu\text{J s}^{-1} \text{cm}^{-2}$ ). Because the Z value is directly proportional to the UVGI inactivation rate, a higher Z value indicates a lower resistance of the microorganism to inactivation by UV radiation, while a lower Z value indicates a higher resistance to inactivation by UV radiation.

The response of *A. versicolor* as judged by intrinsic inactivation rates was nearly linear as the UV was increased from 25% to 100% of its total power (Fig. 2). The Z value was determined from these data. The least squares method was used to fit a line to the plot of inactivation rate versus UV fluence rate. The slope of the line ( $\pm$ SE of the regression line), which represents the Z value, was calculated to be  $1.2 \pm 0.4 \times 10^{-4} \text{ cm}^2 \mu\text{J}^{-1}$ .

### Upper-room air UVGI inactivation rate for airborne fungal spores

Data used to determine the inactivation rates for airborne fungal spores of *A. versicolor* induced by upper-room air UVGI are presented in Fig. 3. Regression lines (not presented in figure) were used to estimate the inactivation rates based on methods described above. Standard errors in the regression lines are reported and propagation of errors was applied to determine the uncertainty in the inactivation rates. The removal rate of fungal spores due to deposition, exfiltration, and natural die-off was  $1.8 \pm 0.07 \text{ h}^{-1}$ . When all five fixtures of the upper-room

**Fig. 3.** Upper-room air UVGI inactivation rates for *A. versicolor*. Error bars represent standard error in the inactivation rates.



air UVGI system were turned on (216 W), the observed inactivation rate was on average from three trials found to be  $2.2 \pm 0.33 \text{ h}^{-1}$ . There was a significant difference between the inactivation rate with UVGI on and UVGI off (Student's  $t$  test: assuming unequal variances,  $\alpha = 0.05$ ;  $p = 0.007$ ). The inactivation rate due to upper-room air UVGI only was then  $2.2 - 1.8 = 0.4$  ( $\text{SE} = 0.22$ )  $\text{h}^{-1}$ .

### In-duct UVGI single-pass efficiency for inactivating bioaerosols

The fully operated in-duct UVGI system reduced the concentration of culturable *M. parafortuitum* in the supply air duct on average 87% at  $2.2 \text{ m s}^{-1}$ . No significant difference was found between normalized culturable concentrations of bacteria in the outdoor air duct measured with and without UV-C lamps operating (Student's  $t$  test assuming unequal variances,  $\alpha = 0.05$ ;  $p = 0.41$ ) at this air stream velocity. When the velocity increased to  $5.1 \text{ m s}^{-1}$ , no significant difference was found between culturable *M. parafortuitum* in the supply air duct and outdoor air duct with UV-C lamps operating (Student's  $t$  test assuming unequal variances,  $\alpha = 0.05$ ;  $p = 0.32$ ). Also, at the air stream velocity of  $5.1 \text{ m s}^{-1}$ , no significant difference was found between culturable *M. parafortuitum* in the room air and outdoor air duct with UV-C lamps operating.

The fully operated in-duct UVGI system reduced the concentration of culturable *A. versicolor* spores in the supply air duct by 75% at the airstream velocity of  $2.2 \text{ m s}^{-1}$ . When the velocity increased to  $5.1 \text{ m s}^{-1}$ , no significant difference was found between culturable fungal spore concentrations upstream and downstream from UV-C lamps operating (Student's  $t$  test assuming unequal variances,  $\alpha = 0.05$ ;  $p = 0.17$ ).

### Discussion and conclusions

With the room air well mixed, the upper-room air UVGI system (all lamps operating, 216 W) provided an average  $0.4 \text{ h}^{-1}$  as an inactivation rate for *A. versicolor* spores, which is more than 40 times lower than for bacterium *M. parafortuitum* (Xu

et al. 2003). The fungal spore cell wall is a rigid structure quite distinct from the prokaryotic bacterial cell wall. It consists of a thick inner layer of chitin or cellulose and a thinner outer layer of glycoproteins (Maier et al. 2000). Also, a class of protein named histones found in fungal DNA is responsible for compacting DNA enough so that it will fit within a nucleus. Bacterial vegetative cells do not have histones, so environmental factors such as relative humidity can affect bacteria DNA conformation, and its sensitivity to UV radiation. The levels of UVGI provided by the modern upper-room air system that was installed in the room do not appear to be adequate to inactivate fungal spores.

To compare the pilot-scale chamber with the 87-m<sup>3</sup> room experiments conducted in this study, the inactivation rates for *A. versicolor* are expressed as UVGI clean-air delivery rates for microbiological aerosols (CADR<sub>m</sub>) (Foarde et al. 1999). The CADR<sub>m</sub> is calculated by multiplying the inactivation rate by the volume of UVGI to which the fungal spores were exposed. In the pilot-scale chamber tests, the intrinsic UVGI CADR<sub>m</sub> was estimated to be 5.2 m<sup>3</sup> h<sup>-1</sup>. To estimate the UVGI CADR<sub>m</sub> for the full-scale room tests, the volume of the upper-zone of the room (10.1 m<sup>3</sup>) was multiplied by the inactivation rate. The upper-room air UVGI CADR<sub>m</sub> was estimated to be 4.0 m<sup>3</sup> h<sup>-1</sup>, which agreed with the UVGI CADR<sub>m</sub> obtained in the pilot-scale chamber tests.

Data from the intrinsic study showed a linear relationship between UVGI inactivation rates and UV fluence rate. The *Z* value for *A. versicolor* was derived from this linear relationship and determined to be  $1.2 \pm 0.4 \times 10^{-4} \text{ cm}^2 \mu\text{J}^{-1}$ . Using the same pilot-scale chamber, a *Z* value for *M. parafortuitum* was estimated to be  $8.0 \pm 0.5 \times 10^{-4} \text{ cm}^2 \mu\text{J}^{-1}$  (Peccia and Hernandez 2001). Values obtained in our experiments agree well with the *Z* values previously reported for two different fungi *A. niger* and *A. terreus* of  $1 \times 10^{-4} \text{ cm}^2 \mu\text{J}^{-1}$  and  $2 \times 10^{-4} \text{ cm}^2 \mu\text{J}^{-1}$ , respectively (Riley 1988). Because fungal spores are not very susceptible to UV, higher levels of UVGI are needed to achieve significant inactivation of fungal spores. In this study the UV fluence rate varied from 3 to 11  $\mu\text{J s}^{-1} \text{ cm}^{-2}$ , resulting in a small range of inactivation rates. More measurements at higher UV fluence rates would be useful to achieve a robust estimate of the *Z* value.

Measuring UV fluence rate accurately is very important for these types of studies. In unpublished studies we compared chemical actinometers (Rahn et al. 1999), a radiometer (Model IL 1400A with SEL240/W detector, International Light Inc, Newburyport Mass.), and a 360-degree optometer (used in study presented here). Findings showed that chemical actinometers compared well with the radiometer (Xu et al. 2003). The average UV fluence rate with the chemical actinometers was  $54 \pm 20 \mu\text{J s}^{-1} \text{ cm}^{-2}$  while with the radiometer the average UV fluence rate was  $42 \pm 19 \mu\text{J s}^{-1} \text{ cm}^{-2}$  (Student's *t* test paired two samples for means showed no statistically significant difference at  $\alpha = 0.05$ ). Ultraviolet fluence rate measurements obtained using the radiometer and the 360-degree optometer disagreed by 30%. The average UV fluence rate with the radiometer was

$41 \pm 2 \mu\text{J s}^{-1} \text{ cm}^{-2}$  while with the 360-degree optometer the average UV fluence rate was  $26 \pm 1 \mu\text{J s}^{-1} \text{ cm}^{-2}$  (Student's *t* test paired two samples for means showed a statistically significant difference at  $\alpha = 0.05$ ); 30% higher average UV fluence rates will result in a 30% lower *Z* value.

Findings reported here showed that fungal spores were inactivated by in-duct UVGI, which has also been previously shown. VanOsdell and Foarde (2002) found that at a dose of 7509  $\mu\text{J cm}^{-2}$  fungal spores of *A. versicolor* were inactivated by 66–83%. In the experiments reported here, the culturable concentrations of *A. versicolor* and *M. parafortuitum* were reduced by 75% and 87%, respectively, in the room's supply air intake ducting, at an air stream velocity of 2.2 m s<sup>-1</sup>. The in-duct UVGI system was not effective at the higher air stream velocity of 5.1 m s<sup>-1</sup>. Increasing the air stream velocity through the supply air duct reduces the residence time of bioaerosol being exposed to in-duct UVGI (Beggs et al. 2000).

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