ABSTRACT

MATTHEW STIEGEL: A Comparison of Indoor Fungal Spore Concentrations between Naturally Ventilated and Mechanically Ventilated Dormitories at Duke University (Under the direction of Dr.Michael Flynn, Dr.David Leith, Dr.Wayne Thomann, and Wiley Schell)

It is well recognized that the quality of air a person breathes can have a significant impact on their health. "Clean" indoor air is especially important since people in the US spend 88% of their time in the indoor environment. Some research has identified links between dampness, indoor microbial growth, and health effects in single-family homes, but little research has been conducted concerning community living areas, such as college dorms and large apartment buildings. The U.S. Census Bureau estimated that in 2007 there were 17.9 million students enrolled in colleges around the United States of which 1/3 live in on-campus housing. In this study we tried to address three general questions: one, are there differences between indoor fungal spore concentrations and outdoor fungal spore concentrations, two, are indoor fungal concentrations different between naturally ventilated dormitories and mechanically ventilated dormitories, and three, what effect do open windows have on the indoor fungal concentration? We sampled outdoors and inside four dormitories at Duke University, one with natural ventilation and three with mechanical ventilation. Our results indicate that the indoor fungal concentration in each of the dorms was less than the outdoor concentration. Natural ventilation produces indoor fungal levels that are significantly higher than those observed in mechanically ventilated dormitories. Open windows can have an effect on the indoor fungal spore concentrations in mechanically ventilated dorms.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to the following people for all the help they offered during this project: Dr. Wayne Thomann (Director of Occupational and Environmental Safety, Duke University), Dr. Michael Flynn (Professor, University of North Carolina at Chapel Hill), Dr. David Leith (Professor, University of North Carolina at Chapel Hill), Wiley Schell (Assistant Professor of Medicine, Duke University Medical Center), Eric Tassone, Maryanne Boundy, and Tricia Drummey.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I
INTRODUCTION

It is well recognized that the quality of air a person breathes can have a significant impact on their health (Jacob et al, 2002; Jones, 1999; Kilburn, 2003;O'Connor et al, 2004; Pasanen, 2001; Santilli, 2002; Spengler & Sexton, 1983; Storey et al, 2004). Since the majority of people in the US spend 88% of their time in the indoor environment, it is easy to recognize the importance of having "clean" indoor air (Jones, 1999; Kim et al, 2007; Sundell et al, 1995; Sundell, 1999; Sundell, 2004). Certain indoor pollutants, such as volatile organic compounds and environmental tobacco smoke, have well known health impacts that include asthmatic conditions, chronic obstructive pulmonary disease, and even different forms of cancer (D'Amato, 1994; Jones, 1999; Kim et al, 2007; Mendell et al, 2002; Nielson et al, 2007; Rehwagen et al, 2003; Sundell, 2004). Less is known about the health impacts of fungal spores but it has been shown that they contribute to coughing, rhinitis, and wheezing in susceptible populations and are also a major cause of asthma (Bush et al, 2006; Fisk et al, 2007; Kim et al, 2007; Mendell et al, 2002; Mudarri & Fisk, 2007). These health effects are important to note because the incidence of allergic and asthmatic diseases has doubled in developed countries over the past two decades and it has been estimated that 4.6 million people in the US have asthma that can be attributed to mold and dampness in the home, with the resulting economic impact of close to $3.5 billion per year ($U.S. 2004) (CDC, 2007; Fanger, 2006; Fisk & Rosenfield, 1997; Mudarri and Fisk, 2007).

The US energy crisis of the late 1970's and early 1980's led to the development of homes and buildings that were more energy efficient. Increases in the use of better

insulation products, "tight" building designs, and newer building products were an important aspect to energy efficiency. These changes led to an increase in energy efficient housing, but also a decrease in the flushing and dilution effects that are associated with natural ventilation and outdoor air infiltration common in houses built in previous decades. The changes also created an indoor environment where airborne contaminants could build up to concentrations that were significantly higher than those observed outdoors (Brager & de Dear, 2000Jones, 1998; Jones, 1999; Samfield, 1996; Teichman, 1995). Thus, the need for appropriate ventilation accelerated the use of the mechanical heating and air-conditioning systems for ventilation in building design, making mechanical ventilation the primary source for the removal of indoor contaminants and the introduction of "clean" air. (Maroni, 1995; Samfield, 1996).

Dampness, open windows, high indoor levels of relative humidity, and faulty central heating, ventilation and air conditioning (HVAC) systems can all contribute to increased levels of indoor fungal spores. The primary source of fungal spores in the indoor environment is outdoors, unless there is fungal growth in the building itself. Fungal spores have the ability to grow and reproduce in the indoor environment, typically only needing a small amount of moisture and a carbon source (Armstrong & Liaw, 2002; Bornehag et al, 2001). High indoor humidity as well as areas of water intrusion, leaking pipes, etc. can contribute to a "damp" environment. Wet or damp surfaces in a home or office building can promote fungal spore proliferation in the indoor environment. Windows that are open to the outside can also contribute to the indoor fungal spore count. Open windows allow the transfer of outdoor fungal spores, which are known in the

Southeastern U.S. to exist at higher outdoor concentrations than indoor concentrations, into the indoor environment (Shelton et al, 2002).

A multitude of factors can contribute to indoor fungal growth, however some of these factors can be controlled by an HVAC system. Mechanical ventilation of living areas conditions and filters air making it more pleasant for occupants as well as providing a "cleaner" environment when compared to outdoor air (Burge et al, 2000; Fisk et al, 2001; Moritz et al, 2001). Mechanical ventilation can also help prevent the intrusion of outdoor fungal spores into the indoor environment if positive relative pressure is produced within the building. The indoor environment in a mechanically ventilated building often has windows that cannot be opened; in effect creating a sealed interior that is somewhat isolated from direct contact with the outdoor environment. HVAC systems do bring in a certain percentage of outdoor air that is used to replace, or dilute, "contaminated" indoor air, but a properly functioning HVAC system filters and removes a large majority of fungal spores from the outdoor air (Burge et al, 2000). Thus, it would seem that the intrusion of fungal spores into the indoor environment from outside should only occur with an improperly working HVAC system or by infiltration of outdoor air through doors, etc. Naturally ventilated environments, lacking the mechanical filtration present in HVAC systems, could exhibit fungal concentrations that are more similar to outdoor concentrations. However, there are few studies that compare fungal spore concentrations in mechanically ventilated buildings to naturally ventilated buildings.

Recent media reports have stressed the need for healthy indoor air, and more specifically air that is "mold-free"(New York Times, 2008; Pokhnel-Willett, 2008;

Science Daily, 2008). This publicity has stimulated the public's interest and has created an interesting debate. Two important questions have arisen from the discussion: one, how can we define an acceptable, healthy, level of fungal spores in the indoor environment and two, how can we minimize exposures to fungal spores in the indoor environment? Seemingly, these questions could be answered through a series of scientific studies. However, after years of research there are no governmental regulations and only a few recommendations that pertain to fungal exposure limits in any environment. Part of this problem stems from the fact that there are still large gaps within the scientific knowledge concerning mold, exposures and health effects.

These areas of concern are compounded further when the incredible variability of fungal spore concentrations in the outdoor environment is considered (Horner et al, 2004; Hyvarinen et al, 2001; Pei-Chih, 2000). Statistically significant differences of outdoor fungal spore concentrations have been observed through spatial and temporal variations (Hyvarinen et al, 2001; Shelton et al, 2002). Extreme fluctuations in outdoor fungal spore concentrations complicates the discussion even more because it introduces a level of variability to the overall debate that was not previously present. Hypothetically, a sample taken in a naturally ventilated home or office building might be statistically different than a second sample taken in the same location but only a few hours later. This variation is a direct cause of the introduction of outdoor air into the indoor environment. Accordingly, this variation also makes it extremely difficult to establish a definitive limit on the number of fungal spores that may be present in the indoor environment.

If an acceptable exposure limit cannot be established, or even if one is established, then how can we minimize the potential for exposure to fungal levels in the

indoor environment? In theory this seems like an easy goal to accomplish, but realistically, this is quite difficult. The American Society of Heating, Refrigeration, and Air-Conditioning Engineers (ASHRAE) offers guidelines and recommendations with respect to almost every operational aspect of an HVAC system, but they do not have a formalized statement with respect to IAQ (ASHRAE, 2008). Any efforts to minimize or manage exposures is greatly impacted by the aforementioned variability in outdoor fungal spore levels, the ongoing debate on whether full-time mechanical ventilation is better than complete natural ventilation or if a combination of the two would be optimal, and the variability in the methodologies that can accurately and precisely quantify the exposure. A multitude of different instruments and methods have been used in the attempt to capture and enumerate fungal spores. In many respects this has only added to the inability to set a standard for "enumerating fungal aerosols"(Lee et al, 2004). Lee et al, 2004 describe the standardization of a methodology for enumerating airborne fungal spores as necessary "to avoid inappropriate test interpretation".

The sample equipment used for collecting fungal spores is divided into two main categories, viable and total. Instruments used for the collection of viable fungal spores also collect non-viable spores, fungal fragments and other particulate matter that is present in the air, but the use of a selective growth medium stimulates the growth of some types of viable spores, effectively excluding the ability for enumeration and identification of everything else. In contrast, instruments that collect the total fungal spore burden retain the ability to identify and count viable spores, non-viable spores, and fungal fragments. This is important to note because the health effects of fungal spore inhalation are not exclusive to viable fungal spores. Fungal fragments and non-viable spores also

play a role in allergic reactions and sensitization after inhalation (Gorny, 2002). Looking at the total fungal spore burden in the air can provide a more complete picture of potential exposure within the space.

Some research has identified links between dampness, indoor microbial growth, and health effects in single-family homes, but little research has been conducted concerning community living areas, such as college dorms and large apartment buildings. The U.S. Census Bureau estimated that in 2007 there were 17.9 million students enrolled in colleges around the United States of which 1/3 live in on-campus housing (U.S. Census Bureau, 2007; U.S. Department of Education, 2008). Even if those numbers are over-estimations, the potential health impact of fungal contamination in a dorm-living situation is substantial. The ubiquitous prevalence of fungal spores in the outdoor environment combined with the fact that the majority of people's time is spent in the indoor environment, and the lack of research concerning indoor fungal spore concentrations raises the need for studying the presence of indoor fungal spores in dormitories. Building the knowledge base on indoor fungal concentrations and sources of fungal spores will help investigators prevent fungal contamination problems and potential health impacts for inhabitants. This study attempts to compare fungal spore concentrations between naturally ventilated and mechanically ventilated dormitories at a large university in the Southeastern United States.

CHAPTER II
MATERIALS AND METHODS

Sample Location and Data Collection. We chose four dormitories on the campus of Duke University (Durham, NC) based on the type of ventilation, natural versus mechanical. One dormitory (A) lacks a mechanical HVAC system while the other three, B, C, and D, have an HVAC system. Dormitories A and B have identical external structural characteristics, similar floor plans, and are located adjacent to one another. Dormitories C and D are identical in every aspect and are adjacent to one another but differ in location and structure when compared to dormitories A and B. Sample locations (i.e. rooms) within these dormitories were selected using a random number generator. See Table 1.

All samples were collected using Air-O-Cell cassettes (Zefon International) (Figure 1) and a Zefon Bio-Pump (Zefon International) (Figure 2), calibrated to 15 L/min, for 10 minutes (Figure 3). The Bio-Pump was calibrated at least three times per sampling period (the beginning, middle and end of the sampling period) or at least every 10 samples. Calibration of the pump was accomplished through the use of a ball-type rotameter that had been calibrated with a Bios DryCal DC-1 (BIOS International Corp.), which is an electronic primary standard. The use of an Air-O-Cell as a particle collection device is based on the principle of inertial impaction and slit sampling. A vacuum pump is attached to the Air-O-Cell and pulls air from outside the Air-O-Cell through an orifice, and then into the Air-O-Cell cassette. Particulate matter in the ambient air is accelerated as it enters the orifice. The accelerated air then impacts a small slide containing an

adhesive collection medium. This medium is not selective in nature and collects viable fungal spores, non-viable spores, fungal fragments, and a variety of other particulate matter that may be present in the air. The adhesive collection medium is not a culture medium. The 50% particle cut point size for the Air-O-Cell is approximately an aerodynamic particle diameter of 2.6µm at a flow rate of 15LPM (Zefon).

The Air-O-Cell cassette was attached directly to the Zefon Bio-Pump. Samples in dormitory A were taken in the middle of the room at breathing-zone height. Samples in dormitories B, C, and D were taken directly under the airflow from a supply vent at breathing-zone height. Samples in dormitories B, C and D were only taken after the air-handling unit (AHU) had been running for one hour. Each sample was given a unique identification number and was collected between 7am and 12:30pm from June 15, 2007-July 31, 2007. Outdoor samples were taken at the beginning and end of the sampling period. Rooms with open windows or window-unit air conditioners were noted.

Sample Processing and Analysis. After sampling, the Air-O-Cells were taken to the Duke Medical Mycology Lab (Duke University, Durham, NC). We removed the impact slides from within the Air-O-Cells, mounted them on microscope slides and dyed them with a lactofuchsin stain. The unique identification number of each Air-O-Cell sample was noted on each slide. The entire area of impact on the slide, or "trace", was visually screened under 200x magnification, followed by reading 20% of the impact trace at 400x magnification (Figure 4). The trace is defined as the area on the slide within the adhesive medium where particulate impaction occurs. 200x magnification was used to locate and identify fungal spores that were 10um in length or greater while 400x magnification was

used for all spores that were less than 10um in length. All readings at 400x were extrapolated into a "full" trace reading. The sample counts were then converted into the number of spores per cubic meter of air, which is the standard format for reporting fungal spore concentrations. The following QA/QC measures were observed to maintain accurate identification and enumeration techniques: unique identifiers were used on all samples to avoid overlap, ongoing training for all lab technicians that counted and identified fungal spores, and oversight of all samples by the resident expert. All data were entered into an Access (Microsoft Corp., Redmond, WA) database that was developed to facilitate data entry and integrity.

Statistical methods. Samples were analyzed separately using descriptive statistics. Shapiro-Wilk's tests and Q-Q plots were used to determine normality. The original fungal concentrations did not fit a normal distribution and were transformed by natural logarithm before proceeding with analysis. Statistical methods used were unpaired t-tests assuming unequal variances and analysis of variance (ANOVA). Differences were considered to be statistically significant with an *alpha* value of 0.05. Statistical analysis was accomplished with R (R Foundation for Statistical Computing, Vienna, Austria) and StatPlus: mac (AnalystSoft, Washington, DC).

CHAPTER III
RESULTS

The mean and median total fungal spore concentrations in the four dormitories decreased from the naturally ventilated dorm, A, to the mechanically ventilated dormitories, B-D. The total outdoor fungal spore concentrations were elevated when compared to the four dormitories. Descriptive statistics for the four dorms are presented in Table 2. Each Air-O-Cell sample was analyzed for the presence of 26 different categories of fungal spores. The number of detections, rank, and mean concentration are presented in Tables 3.a, 3.b, and 4. *Aspergillus/Penicillium*, basidiospores, and *Cladosporium* were chosen for comparison through statistical analysis because they are known to be the most predominant genera found in the outdoor environment and their presence in the indoor environment can be indicative of an outdoor contribution of fungal spores and/or indoor fungal contamination. Descriptive statistics for *Aspergillus/Penicillium*, basidiospores, and *Cladosporium* for the four dorms are presented in Table 5.

The original fungal concentrations were tested for normality using Shapiro-Wilk's tests (Table 6.a.). These results indicated that the original data did not fit a normal distribution so a natural logarithm transformation was performed, followed by secondary Shapiro-Wilk's tests (Table 6.b.). The data from dormitories C and D were not statistically different so they were combined to strengthen the statistical comparisons with dormitories A and B. This combined data is categorized with the notation "C/D".

All comparisons where the data sets are managed independently are noted as "dormitory C" and "dormitory D". We looked at differences in total concentrations of outdoor fungal spore counts and indoor fungal spore counts between the dorms. The means of the outdoor fungal spore concentrations were significantly higher ($P<0.0001$) than the means of the indoor fungal spore counts for the four dorms (Table 7). Results of an ANOVA analysis of spore ct/m^3 values between the four dormitories indicated significant differences ($P<0.0001$) among means (Table 8). The ANOVA analysis had two main conclusions: the mean indoor fungal spore concentration for dormitory A was significantly higher than the mean indoor fungal spore concentrations for dormitories B, C, and D, and the mean indoor fungal spore concentration for dormitory B was significantly higher than mean indoor fungal spore concentrations for dormitories C/D. Indoor/outdoor ratios for the total fungal spore concentration, *Aspergillus/Penicillium*, basidiospores, and *Cladosporium* concentrations were calculated and are presented in Figure 5.

We also compared indoor fungal concentrations between rooms that had open windows and those that had closed windows. The naturally ventilated dorm, A, exhibited no statistical difference between the open and closed windows. Dormitory B, a mechanically ventilated dorm, also had no statistically significant differences between rooms with open windows and rooms with closed windows. Dormitories C and D, both mechanically ventilated, did show a statistically significant difference ($P<0.0003$) between rooms with open windows versus rooms with closed windows. The mean indoor fungal spore concentrations for rooms with open windows were significantly higher than for rooms with closed windows. These results can be seen in Table 9 and Figure 6.

CHAPTER IV
DISCUSSION

We have already discussed the need for more data concerning multi-unit housing and the use of natural versus mechanical ventilation. In this study we attempted to address three general questions: one, are there significant differences between indoor fungal spore concentrations and outdoor fungal spore concentrations, two, are indoor fungal spore concentrations different between naturally ventilated dormitories and mechanically ventilated dormitories, and three, what effect do open windows have on the indoor fungal spore concentration? We hypothesized that the indoor and outdoor fungal concentrations would be different, especially among the mechanically ventilated dormitories. A cursory look at the descriptive statistics shows that there are large differences in the mean indoor fungal spore concentrations within the four dormitories when compared to the mean outside fungal spore concentration. On further examination the indoor fungal spore concentrations were shown to be significantly lower ($P<0.0001$) than the outdoor concentrations for all four dormitories. This result was not surprising for the mechanically ventilated dormitories, but was somewhat surprising for the naturally ventilated dormitory. Initially, we thought that the indoor and outdoor fungal spore concentrations in dormitory A, with natural ventilation, would be fairly similar but somewhat lower indoors. Our results show that the indoor and outdoor fungal spore concentrations were significantly different in mean concentrations.

There are a few possible explanations for this difference. Natural distribution of fungal spores is accomplished through the air and they have the ability to stay airborne with minimal wind current. Fungal spores are also more easily disturbed and entrained

into the air when compared to larger airborne constituents. The majority of the rooms that were sampled had not had inhabitants for almost a month prior to sampling. Nonetheless, we still found large concentrations of fungal spores in the majority of these rooms, even in rooms that did not have open windows. This finding demonstrated that it was almost impossible to predict which rooms had been disrupted through the opening of a door, intrusion of outdoor air through leaky windows and cracks in the wall, or the movement of occupants, etc. However, the impact of the settling of fungal spores and/or the lack of disturbance or introduction of airborne fungal spores in the rooms of dormitory A were great enough to create a statistically significant difference between the indoor and outdoor mean concentrations.

There were also compositional and numerical differences between the categories of fungal spores found in the indoor environments of the four dormitories and the outdoor environment. Table 3.a. shows that the outdoor environment was predominately composed of ascospores, basidiospores, myxomycetes/smut, *Penicillium/Aspergillus* spores, and *Cladosporium* spores. These categories of fungal spores were detected in more than half of the outdoor samples and their mean concentrations are much higher than the indoor means of the four dormitories. We chose to further investigate the relationship of *Aspergillus/Penicillium* spores, *Cladosporium* spores and basidiospores in the indoor environment based on their prevalence in the outdoor samples. Tables 3.a. and 3.b. show that mean indoor concentrations for these three categories of spores were always lower than the outdoor concentration, which was expected. One commonly accepted method for analyzing the differences between indoor and outdoor concentrations of fungal spores it to look at the indoor/outdoor ratio. An indoor/outdoor

ratio that is greater than 0.5 lends support to the hypothesis that a majority of the fungal spores in the indoor environment are coming from the outdoor environment. In our ratio comparison between the four dormitories and across the total mean concentrations, *Aspergillus/Penicillium*, *Cladosporium* and basidiospores produced one ratio that was greater than 0.5. This ratio occurred in the *Aspergillus/Penicillium* comparison for Dormitory C; however, this result is not a reliable measure of indoor contribution of fungal spores from the outdoor environment because there were 4 spores/m^3 meter in the indoor environment and 7 spores/m^3 in the outdoor environment, which are extremely low (Table 5).

There is a very important consideration to note when looking at the relationship between indoor fungal spore counts and the ability mechanical ventilation to "clean" the air of spores. Dormitory B has seven different HVAC systems, each controlled independently of one another. This configuration likely introduced considerable variability into our measurements. That is, on the day of sampling, we ensured that the HVAC system for the rooms we were sampling had been running for at least an hour before the first sample was taken. We had no way of controlling the amount of time that the individual HVAC system in our "sample area" had been running in the previous days, weeks and months. Theoretically, individual control of these units could have caused one unit to run for an extended period before we sampled, compared to another unit that was only activated to address the requirements of our sampling protocol. Any system that had been operational for a longer period than one one-hour minimum requirement would have more time to filter the air from the area it supplies as compared to the unit that had just been turned on. We would expect that the air from the longer running unit would be

"cleaner" than the air from the other unit. While we did not evaluate the direct impact of this inter-unit operational difference, we did compare the differences between dormitory B and dormitories C and D in an effort to assess this impact.

Dormitories C and D each have two HVAC units that run in conjunction with one another. We documented that these HVAC units had been on for at least a week prior to our sampling and they were not controlled by individual input. We hypothesized that the indoor fungal spore concentrations within these two dormitories to be lower than those of dormitories A and B. The results of the ANOVA analysis support our hypothesis that there is a difference between the dormitories A, B, and C/D (Table 8). The ANOVA also helps to support our hypothesis that mechanically ventilated dormitories would have lower indoor fungal spore concentrations than a naturally ventilated dormitory. The fungal spore concentrations in Dormitory A were different from dormitories B and C/D because A relies on natural ventilation and B, C and D rely on mechanical ventilation. The difference in fungal spore concentrations between dormitory B and dormitories C/D can partially be attributed to the previously discussed differences in the run-time of their respective HVAC units and the differences in the control of these units.

Another important aspect in the relationship between indoor fungal spore concentrations and the type of ventilation was the presence of open windows (Table 9). Buildings that have central HVAC systems often have windows that cannot be opened, or are only to be opened in cases of emergency. This building characteristic is partially based on the assumption that outdoor contaminants and increased levels of humidity will be introduced into the indoor environment and be detrimental to the "controlled" indoor environment. More often than not fungal spores will comprise a significant fraction of

these outdoor contaminants. We compared the indoor fungal spore concentrations in rooms that had closed windows to indoor fungal spore concentrations of rooms that had open windows across the four dormitories (Figure 9). We expected that the results for dormitory A would show no statistically significant difference between rooms with open and closed windows, while dormitories B, C and D would show statistically significant differences.

The results from dormitory A displayed no statistically significant difference in fungal spore concentration between rooms with either open and closed windows, as did the results for dormitory B. Dormitories C and D did have statistically significant differences between rooms with open windows and rooms with closed windows. The results for dormitory A were expected because the dorm is naturally ventilated. The lack of a difference between open and closed windows in dormitory B once again shows how variable the indoor fungal concentrations can be with independently running HVAC systems and how this data collection is only a snapshot in time. The results from dormitories C and D lend support to the hypothesis that a mechanically ventilated indoor environment that has not been exposed to the outdoor environment through an open window can produce low levels of indoor fungal spores.

The interpretation of our results was partially based on a comparison between the outdoor fungal spore concentrations and the indoor fungal spore concentrations. Outdoor samples are usually taken as a control and used as a baseline measurement for fungal spores that might be present indoors. Investigators often look at average outdoor concentrations, compare them with indoor samples, and then make a generalized statement based on differences in numerical counts. We used this approach in creating

the indoor/outdoor ratios, however our data indicates that caution needs to be taken when employing this technique. Table 5 compares our indoor and outdoor mean fungal spore concentrations for three fungal species in the four dormitories. There is a noticeable difference between the average outdoor concentrations and the average concentrations by species. This difference is important to note because it shows the marked variations that can occur in outdoor fungal spore counts, especially when one considers the composition of the total outdoor sample. For example, the average outdoor count for dormitory A was 14,567 spores/m^3 and the *Aspergillus spp./Penicillium spp.* outdoor count was 2,016 spores/m^3. Dormitory D had an average outdoor count for total spores of 34,923 spores/m^3 and an *Aspergillus spp./Penicillium spp.* outdoor count of 82 spores/m^3. If we only looked at the ratios displayed in Figure 5, we would not have discovered the significant variability in the mixture of fungal species/spores in the outdoor environment. Thus, we could have misinterpreted the relative contribution of the outdoor fungal spore counts into the indoor environment.

CHAPTER V
CONCLUSION

These results and this discussion point to a variety of conclusions. We have seen that indoor and outdoor fungal counts can have a large amount of variation dependent on a variety of known and unknown characteristics. Natural ventilation does produce indoor fungal spore levels that are significantly higher than those observed in mechanically ventilated dormitories. Open windows can have an effect on the indoor fungal spore concentrations in mechanically ventilated dorms. But we still have not come to a conclusion on how to define an acceptable level of indoor fungal spores. Indirectly, this study tried to address that situation but the significant variation shown from indoor concentrations to outdoor concentrations, natural versus mechanical ventilation, and open versus closed windows only complicates the issue. The results do show that variation between fungal spore concentrations is not only regional, as discussed in other publications, but also very localized (Hyvarinen et al, 2001; Shelton et al, 2002).

Future research needs to include multi-unit comparisons that could help in addressing the variation associated with different HVAC units in the same building. We also need to look at enhanced filtration and indoor fungal spore variations. This study did not look at the differences between the filtration systems of dormitories B, C and D, but further examination of these systems might shed light on the variation experienced in dormitory B. Finally, we need to conduct seasonal sampling that would produce a more comprehensive fungal profile. Our sampling was a snapshot in time, thus limiting our ability to make conclusive statements with regard to overall fungal spore concentrations.

Table 1:

Number of Samples per Dormitory

Dormitory	HVAC	Floor *	# of Samples
A	No	-2	8
		-1	5
		0	24
		1	48
		2	67
		3	51
		4	10
		Total	213
B	Yes	-2	10
		0	32
		1	53
		2	66
		3	47
		4	10
		5	4
		Total	222
C	Yes	-2	3
		1	14
		2	29
		3	27
		Total	73
D	Yes	-2	3
		1	15
		2	28
		3	28
		Total	74

*-2 indicates outdoor sample, -1 indicates sub-basement, 0 indicates basement, 1 indicates first floor, 2 indicates second floor, 3 indicates third floor, 4 indicates fourth floor, 5 indicates fifth floor

Table 2:

Descriptive Statistics for Fungal Spore Concentrations of the Outdoors, Dormitories A, B, C, and D

Statistic	*Outdoor (ct/m3)*	*Dormitory A (ct/m3) - Natural Ventilation*	*Dormitory B (ct/m3) - Mechanical Ventilation*	*Dormitory C (ct/m3) - Mechanical Ventilation*	*Dormitory D (ct/m3) - Mechanical Ventilation*
Mean	20812	4436	576	197	205
Standard Error	2407	580	87	89	41
Median	23083	694	253	25	84
Standard Deviation	11792	8307	1267	743	341
Sample Variance	139054429	69005730	1605822	551934	116199
Minimum	1041	7	0	0	0
Maximum	40308	74022	14355	5014	2160
Confidence Level (95.0%)	4979	1144	172	177	81

Table 3.a:

Frequency of Fungal Species Detection in the Outdoors, Dormitory A, and Dormitory B

Outdoors			Dormitory A			Dormitory B		
Species	Frequency of Detects	Mean*	Species	Frequency of Detects	Mean*	Species	Frequency of Detects	Mean*
Ascospores	0.75	2938.78	Ascospores	0.97	1221.4	myxomycete/smut	0.90	36.02
Basidiospores	0.75	6791.39	Basidiospores	0.96	777.7	Basidiospores	0.86	226.87
myxomycete/smut	0.75	1365.61	Cladosporium	0.96	1450.2	Cladosporium	0.85	145.14
Penicillium/Aspergillus	0.71	1085.94	myxomycete/smut	0.90	335.4	Ascospores	0.62	91.98
Cladosporium	0.67	4544.89	Penicillium/Aspergillus	0.86	552.3	other	0.33	16.15
Alternaria	0.63	83.33	Alternaria	0.53	10.4	Alternaria	0.33	4.55
Bipolaris/Dsechslera	0.63	21.44	rusts	0.39	33.9	Penicillium/Aspergillus	0.31	40.28
Curvularia	0.42	7.11	Bipolaris/Dsechslera	0.37	6.2	Bipolaris/Dsechslera	0.19	4.57
hyphal fragments	0.38	1.17	hyphal fragments	0.33	0.6	Epicoccum	0.19	1.58
Pithomyces	0.38	7.44	other	0.33	12.5	hyphal fragments	0.17	0.21
other	0.33	24.72	Curvularia	0.16	1.5	Curvularia	0.13	0.92
Epicoccum	0.29	50.78	Pithomyces	0.15	1.6	Pithomyces	0.09	0.78
rusts	0.29	67.83	Chaetomium	0.14	3.3	rusts	0.08	1.93
Cercospora	0.25	28.56	Pestalotiopsis	0.08	2.1	Ulocladium	0.05	0.51
Chaetomium	0.25	838.11	Cercospora	0.06	1.2	Ganoderma	0.04	1.92
Ganoderma	0.25	31.11	Nigrospora	0.05	10.0	Cercospora	0.03	0.32
Nigrospora	0.21	3.39	powdery mildew	0.05	13.9	Nigrospora	0.03	0.72
Ulocladium	0.21	2.28	Spegazzinia	0.04	0.5	Chaetomium	0.02	0.76
powdery mildew	0.13	11.50	Ganoderma	0.02	0.9	Spegazzinia	0.02	0.17
Spegazzinia	0.13	1.17	Tetraploa	0.02	0.1	Stemphylium	0.02	0.32
Fusarium	0	0	Stemphylium	0.01	0.4	Fusarium	0.01	0.22
Pestalotiopsis	0	0	Ulocladium	0.01	0.1	powdery mildew	0.01	0.07
Stachybotrys	0	0	Epicoccum	0.01	0.1	Torula	0.01	0.07
Stemphylium	0	0	Stachybotrys	0	0.0	Pestalotiopsis	0	0
Tetraploa	0	0	Fusarium	0	0.0	Stachybotrys	0	0
Torula	0	0	Torula	0	0.0	Tetraploa	0	0

*ct/m^3

Table 3.b:

Frequency of Fungal Species Detection in Dormitory C and Dormitory D

Dormitory C			Dormitory D		
Species	Frequency of Detects	Mean*	Species	Frequency of Detects	Mean*
other	0.64	23.36	other	0.45	19.55
Basidiospores	0.53	59.07	myxomycete/smut	0.44	18.41
Alternaria	0.47	11.07	Cladosporium	0.35	20.25
Ascospores	0.44	42.56	Basidiospores	0.30	103.77
Bipolaris/Dsechslera	0.44	9.49	Ascospores	0.28	20.61
myxomycete/smut	0.44	15.27	Alternaria	0.24	2.85
Cladosporium	0.43	38.39	Bipolaris/Dsechslera	0.24	2.39
hyphal fragments	0.13	0.16	hyphal fragments	0.11	0.14
Curvularia	0.07	1.26	Curvularia	0.10	0.69
Epicoccum	0.07	0.50	Penicillium/Aspergillus	0.07	5.25
Penicillium/Aspergillus	0.07	3.70	Epicoccum	0.03	0.20
Nigrospora	0.01	0.19	Cercospora	0.01	0.10
Pithomyces	0.01	0.10	Chaetomium	0.01	0.10
Stemphylium	0.01	0.10	Ganoderma	0.01	0.10
Fusarium	0.01	0.10	Pestalotiopsis	0.01	0.10
Cercospora	0	0	Stemphylium	0.01	0.10
Chaetomium	0	0	Tetraploa	0.01	0.10
Ganoderma	0	0	Nigrospora	0	0
Pestalotiopsis	0	0	Pithomyces	0	0
rusts	0	0	rusts	0	0
Spegazzinia	0	0	Spegazzinia	0	0
Stachybotrys	0	0	Stachybotrys	0	0
Tetraploa	0	0	Fusarium	0	0
Torula	0	0	Torula	0	0
Ulocladium	0	0	Ulocladium	0	0
powdery mildew	0	0	powdery mildew	0	0

*ct/m^3

Table 4:

Frequency of Fungal Species Detection, Sorted Alphabetically

	Outdoors		Dormitory A		Dormitory B		Dormitory C		Dormitory D	
Species	Frequency of Detects	Mean*	Frequency of Detects	Mean*	Frequency of Detects	Mean*	Frequency of Detects	Mean*	Frequency of Detects	Mean*
Alternaria	0.63	83.33	0.53	10.4	0.33	4.55	0.47	11.07	0.24	2.85
Ascospores	0.75	2938.78	0.97	1221.4	0.62	91.98	0.44	42.56	0.28	20.61
Basidiospores	0.75	6791.39	0.96	777.7	0.86	226.87	0.53	59.07	0.30	103.77
Bipolaris/Dsechslera	0.63	21.44	0.37	6.2	0.19	4.57	0.44	9.49	0.24	2.39
Cercospora	0.25	28.56	0.06	1.2	0.03	0.32	0.00	0.00	0.01	0.10
Chaetomium	0.25	838.11	0.14	3.3	0.02	0.76	0.00	0.00	0.01	0.10
Cladosporium	0.67	4544.89	0.96	1450.2	0.85	145.14	0.43	38.39	0.35	20.25
Curvularia	0.42	7.11	0.16	1.5	0.13	0.92	0.07	1.26	0.10	0.69
Epicoocum	0.29	50.78	0.01	0.1	0.19	1.58	0.07	0.50	0.03	0.20
Fusarium	0.00	0.00	0.00	0.0	0.01	0.22	0.01	0.10	0.00	0.00
Ganoderma	0.25	31.11	0.02	0.9	0.04	1.92	0.00	0.00	0.01	0.10
hyphal fragments	0.38	1.17	0.33	0.6	0.17	0.21	0.13	0.16	0.11	0.14
myxomycete/smut	0.75	1365.61	0.90	335.4	0.90	36.02	0.44	15.27	0.44	18.41
Nigrospora	0.21	3.39	0.05	10.0	0.03	0.72	0.01	0.19	0.00	0.00
other	0.33	24.72	0.33	12.5	0.33	16.15	0.64	23.36	0.45	19.55
Penicillium/Aspergillus	0.71	1085.94	0.86	552.3	0.31	40.28	0.07	3.70	0.07	5.25
Pestalotiopsis	0.00	0.00	0.08	2.1	0.00	0.00	0.00	0.00	0.01	0.10
Pithomyces	0.38	7.44	0.15	1.6	0.09	0.78	0.01	0.10	0.00	0.00
powdery mildew	0.13	11.50	0.05	13.9	0.01	0.07	0.00	0.00	0.00	0.00
rusts	0.29	67.83	0.39	33.9	0.08	1.93	0.00	0.00	0.00	0.00
Spegazzinia	0.13	1.17	0.04	0.5	0.02	0.17	0.00	0.00	0.00	0.00
Stachybotrys	0.00	0.00	0.00	0.0	0.00	0.00	0.00	0.00	0.00	0.00
Stemphylium	0.00	0.00	0.01	0.4	0.02	0.32	0.01	0.10	0.01	0.10
Tetraploa	0.00	0.00	0.02	0.1	0.00	0.00	0.00	0.00	0.01	0.10
Torula	0.00	0.00	0.00	0.0	0.01	0.07	0.00	0.00	0.00	0.00
Ulocladium	0.21	2.28	0.01	0.2	0.05	0.51	0.00	0.00	0.00	0.00

*ct/m3

Table 5:

Mean Concentrations (ct/m^3) of Fungal Spores by Species for the Sampling Related to Dormitories A, B, C, and D

Fungal Species	Dormitory							
	A		B		C		D	
	Indoor *	Outdoor *	Indoor *	Outdoor *	Indoor *	Outdoor *	Indoor *	Outdoor *
Total	4436 (7-74022)	14567 (3907-33752)	576 (0-14355)	20579 (8368-29047)	205 (0-2160)	24134 (1041-37752)	195 (0-5014)	34923 (26361-40308)
Aspergillus spp./ Penicillium spp.	552 (0-5593)	2016 (0-6420)	40 (0-1227)	342 (140-747)	4 (0-93)	7 (0-33)	5 (0-127)	82 (0-153)
Basidiospores	778 (0-10560)	2183 (527-4933)	227 (0-4033)	10477 (2680-14773)	59 (0-667)	11398 (407-29893)	104 (0-3800)	27782 (21820-33380)
Cladosporium spp.	1450 (0-43633)	3038 (0-11347)	145 (0-1807)	5750 (0-12567)	38 (0-587)	8429 (187-21673)	20 (0-387)	2067 (1527-2900)

*arithmetic mean(ct/m^3) and range(ct/m^3)

Table 6.a:

Results of the Shapiro-Wilk's Tests before Natural Logarithm Transformation

Dormitory	Total*	Aspergillus/Penicillium*	basidiospores*	Cladosporium*
Outdoor	0.947(0.257)	0.586(0.000)	0.856(0.004)	0.795(0.000)
A	0.549(0.000)	0.640(0.000)	0.576(0.000)	0.355(0.000)
B	0.379(0.000)	0.321(0.000)	0.448(0.000)	0.550(0.000)
C	0.597(0.000)	0.293(0.000)	0.562(0.000)	0.488(0.000)
D	0.257(0.000)	0.267(0.000)	0.190(0.000)	0.364(0.000)

*test statistic(P-level)

Table 6.b:

Results of the Shapiro-Wilk's Tests after Natural Logarithm Transformation

Dormitory	Total*	Aspergillus/Penicillium*	basidiospores*	Cladosporium*
Outdoor	0.848(0.003)	0.875(0.008)	0.954(0.350)	0.708(0.000)
A	0.997(0.002)	0.93(0.000)	0.95(0.000)	0.974(0.0001)
B	0.938(0.000)	0.682(0.000)	0.901(0.000)	0.884(0.000)
C	0.937(0.002)	0.273(0.000)	0.812(0.000)	0.741(0.000)
D	0.943(0.003)	0.289(0.000)	0.605(0.000)	0.694(0.000)

*test statistic(P-level)

Table 7:

Results from Two Sample t-Tests Assuming Unequal Variances Comparing Indoor and Outdoor Concentrations

Dormitory	t statistic	t critical	P-Level
A	7.51	2.23	0.00002
B	28.26	2.08	3.39014E-18
C and D	10.22	2.45	0.00005

Table 8:

ANOVA Comparing Total Fungal Concentrations (ct/m^3) Between the Four Dormitories

Summary						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A	205	1420.014572	6.926900351	3.558054782		
B	212	1166.825026	5.503891633	1.754159647		
C and D	141	508.6944487	3.607762048	4.197170713		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	920.9901974	2	460.4950987	151.8048297	2.58807E-53	3.01196073
Within Groups	1683.574761	555	3.033468038			
Total	2604.564958	557				

Table 9:

Results from Two Sample t-Tests assuming Unequal Variances Comparing Open and Closed Windows

Dormitory	t statistic	t critical	P-Level
A	1.78	1.98	0.08
B	1.22	2.179	0.25
C and D	6.46	2.365	0.0003

Figure 1:

Complete Air-O-Cell and an "Exploded" Air-O-Cell

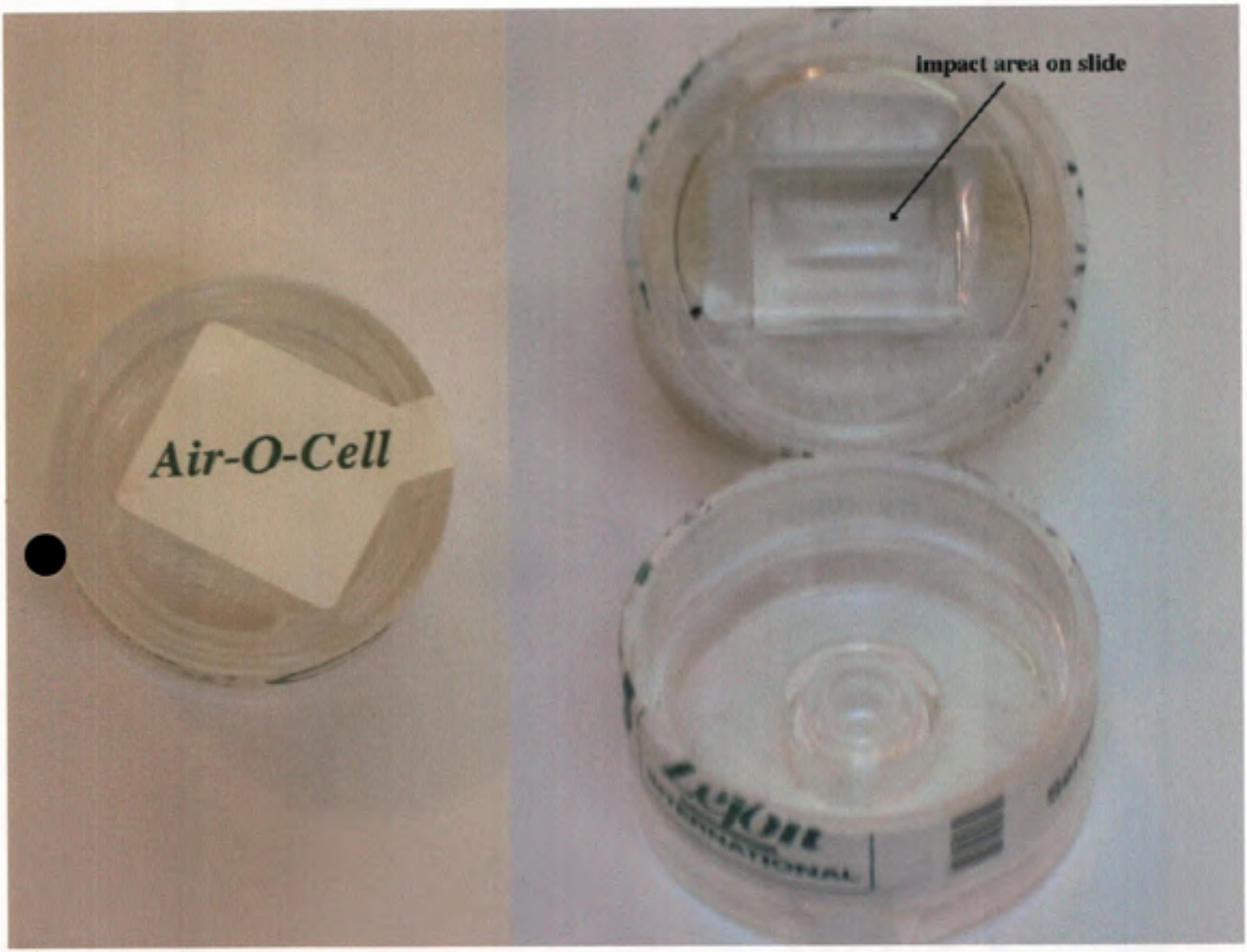


Figure 2:

Zefon Bio-Pump with Air-O-Cell Attached

Figure 3:

Zefon Bio-Pump with Rotameter

Figure 4:

Reading the "Trace"

Figure 5:

The Indoor and Outdoor Ratio Between Different Fungal Species

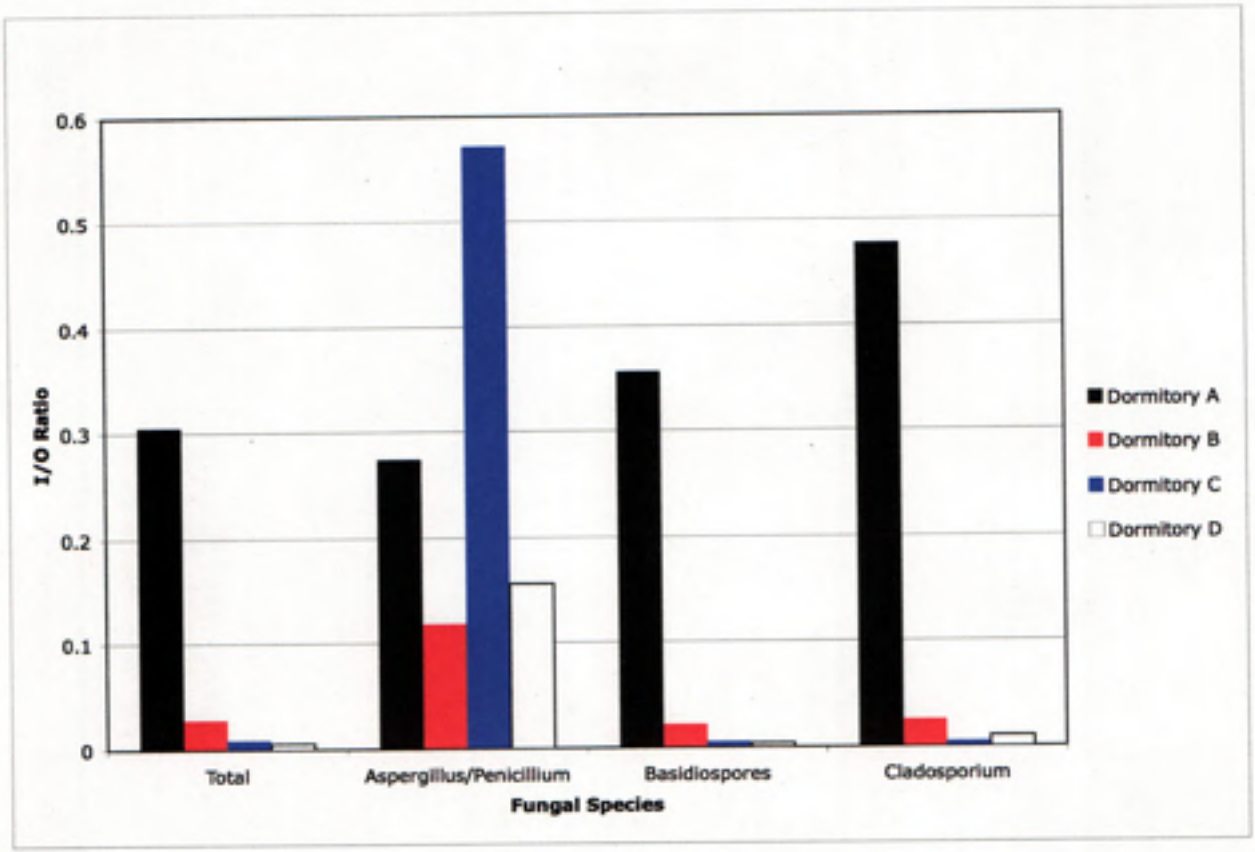
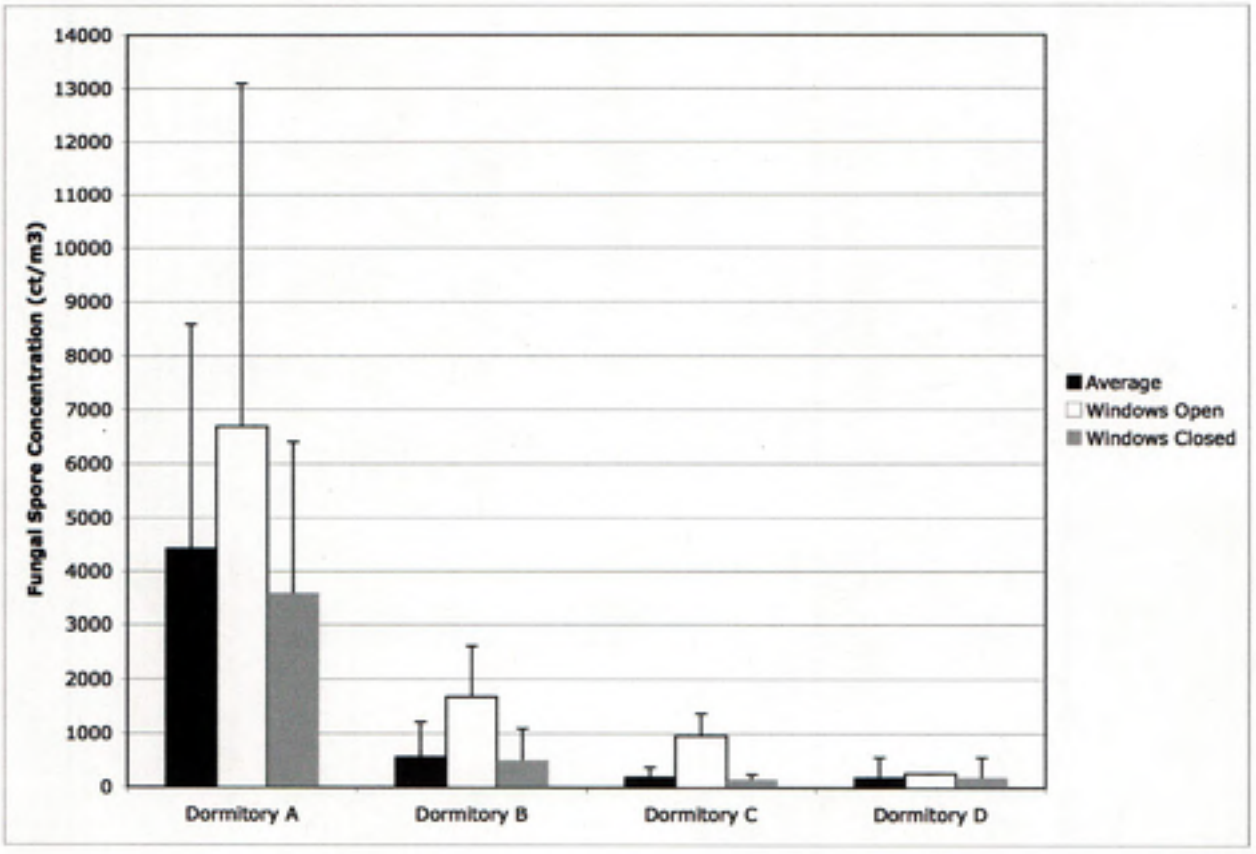


Figure 6:

Fungal Spore Concentrations Between Open/Closed Windows



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