# FINAL PROGRESS REPORT

Project Title:

## Bioaerosols in Midwest Greenhouses and Respiratory Symptoms Among the Workers

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# ABSTRACT

Estimated economic impacts of the US Green Industry are 1,964,339 jobs and $147.8 billion in output, of which 27% is represented by the greenhouse and nursery sectors. These two sectors are rapidly growing components of American agriculture that present a variety of challenges for occupational safety and health. A large number of workers, many foreign born, encounter a variety of potential respiratory hazards in greenhouse work environments. Exposure to bioaerosols, including microorganisms of pathogenic, toxic and allergenic species and pollen in an enclosed environment could magnify concerns about respiratory hazards encountered elsewhere in traditional agriculture. Rates of bioaerosol exposures and respiratory disorders in greenhouse workers have been strikingly high in some countries. This problem has not been fully studied in the US. There is a lack of quantitative information on workers' exposure to bioaerosols and relevant respiratory symptoms, particularly in the Midwestern region of the United States.

The objectives of this CDC-NIOSH R03 pilot project were: (1) to investigate exposure levels to airborne culturable and total fungi, bacteria (total culturable bacteria and actinomycetes), endotoxin, and (1→3)-β-D-glucans in three Midwest greenhouses during summer and winter using multiple exposure assessment methods; (2) characterize the load of microorganisms on greenhouse floors and determine potential microbial source strengths of the floors for aerosolizing microbial biocontaminants, and (3) to estimate the prevalence of rhinitis, wheezing, asthma, and other respiratory symptoms/conditions among greenhouse workers. Stationary inhalable aerosol samples were collected from each greenhouse using Button Inhalable Aerosol Samplers. Control samples were collected from offices and nearby outdoor locations. A microbial source strength tester was used to examine the aerosolization potential of microbial contaminants from greenhouse floors. Additionally, surface samples were collected by sterile cotton swabs. Temperature, relative humidity, and wind velocity were recorded. Airborne culturable fungi, bacteria, and actinomycetes were analyzed in the extracts from field samples by cultivation in nutrient agar media. Endotoxin and (1→3)-β-D-glucan in the extracts from field samples were analyzed by specific kinetic chromogenic LAL assays. The prevalence of respiratory symptoms among greenhouse workers (n = 35) and control subjects (office workers; n = 14) was estimated with a standardized questionnaire.

The collected data indicate that workers employed in Midwest greenhouses may be exposed to elevated levels of inhalable culturable microorganisms (fungi and bacteria collectively on the order of $10^2 – 10^5$ CFU/m$^3$), endotoxin ($10^1$ to $10^3$ EU/m$^3$), and (1→3)-β-D-glucan ($10^1$ to $10^2$ ng/m$^3$); exposure levels are comparable to those previously measured in animal confinements and greenhouses of European countries. Seasonal variations were observed for some bioaerosol components. Fungi dominated in summer whereas bacteria and endotoxin dominated in winter. Levels of (1→3)-β-D-glucan in two seasons were not significantly different. The prevalence of respiratory symptoms was generally higher among greenhouse workers compared to controls. Although the crude/unadjusted Prevalence Ratios ranged from 1.2 – 4.4, the differences were not statistically significant based on Fisher's Exact Tests. To conclude, greenhouses in the Midwestern United States are a significant source of workers' exposures to airborne microbial contaminants. Elevated levels of airborne microbial contaminants

were observed in three greenhouses with significant seasonal variations. The contaminants were likely to have originated from the greenhouse work areas as indicated by the indoor to outdoor ratios. Lack of correlations between exposure variables assessed by various methods indicates that employing several exposure assessment methods and investigating multiple bioaerosol exposure matrices would be more informative. The prevalence of self-reported respiratory symptoms was generally higher among greenhouse workers compared to controls; on the other hand the differences were not statistically significant, possibly due to the small sample size and relatively low statistical power of the study. Overall, we believe our pilot data on exposure levels and prevalence of respiratory symptoms among the greenhouse workers warrants further study in a larger group of subjects.

Bioaerosol exposure levels coupled with assessment of workers' respiratory symptoms described in this project report will be relevant in improving specific methods and tools to assess worker exposures to inhalable biohazards and in advancing the understanding of the pathophysiology of respiratory disorders among greenhouse workers. The approach developed in this pilot project for bioaerosol exposure matrix can be applied in the future for assessing exposure in future large scale studies in greenhouses as well as in other agricultural occupational environments. The pilot data collected in this small research project can be utilized later for future sample size calculations for larger studies on respiratory symptoms among greenhouse workers in the United States.

# SECTION 1

# HIGHLIGHTS/SIGNIFICANT FINDINGS

The collected data indicate that workers employed in Midwest greenhouses may be exposed to elevated levels of inhalable culturable microorganisms (fungi and bacteria collectively on the order of $10^2 – 10^5$ CFU/$m^3$), endotoxin ($10^1$ to $10^3$ EU/$m^3$), and (1→3)-β-D-glucan ($10^1$ to $10^2$ ng/$m^3$); exposure levels are comparable to those previously measured in animal confinements and greenhouses of European countries.

Seasonal variations were observed for some bioaerosol components. Fungi dominated in summer whereas bacteria and endotoxin dominated in winter. Levels of (1→3)-β-D-glucan in two seasons were not significantly different.

Microbial source strength tester demonstrated aerosolizable culturable fungi and bacteria from floor surfaces at the range of $10^4$ - $10^5$ CFU/$m^2$ and $10^5 – 10^6$ CFU/$m^2$. Microbial source strength tester data also demonstrated a mean concentration of aerosolizable endotoxin from floor surfaces ranged from 1,297.2 to 9,018.2 EU/$m^2$ and (1→3)-β-D-glucan from floor surfaces as 1,009.1 to 5,369.9 ng/$m^2$.

The contaminants were likely to have originated from the greenhouse work areas as indicated by the indoor to outdoor ratios. Significant positive correlations were found between the following airborne contaminants: (1→3)-β-D-glucan and culturable fungi ($r = 0.420$; $p < 0.05$), endotoxin and culturable bacteria ($r = 0.551$; $p < 0.05$), and (1→3)-β-D-glucan and endotoxin ($r = 0.487$; $p < 0.05$), whereas a significant negative correlation was observed between airborne culturable fungi and airborne culturable bacteria ($r = 0.551$; $p < 0.05$). No significant correlations ($p > 0.05$) were found when microbial source tester data (aerosolizable biocontaminats from floors) and Button Sampler data (biocontaminants in air) were compared for all biocontaminants. Lack of correlations between exposure variables assessed by various methods indicates that employing several exposure assessment methods and investigating multiple bioaerosol exposure matrices would be more informative.

Significant negative correlations were observed between culturable bacteria and temperature ($r = - 0.793$; $p < 0.05$) and between endotoxin and temperature ($r = - 0.733$; $p < 0.05$). On the other hand, significant positive correlations were observed between culturable fungi and temperature ($r = 0.370$; $p < 0.05$), endotoxin and air velocity ($r = 0.490$; $p < 0.05$), and (1→3)-β-D-glucan and air velocity ($r = 0.387$; $p < 0.05$).

The prevalence of respiratory symptoms was generally higher among greenhouse workers compared to controls. Although the crude/unadjusted Prevalence Ratios ranged from 1.2 – 4.4, the differences were not statistically significant based on Fisher's Exact Tests. A modified Poisson regression analysis for unadjusted as well as adjusted estimates also consistently showed PRs > 1, though none of the comparisons reached statistical significance. Also, none of respiratory outcomes were found to be significantly associated with the total number of lifetime hours worked in a greenhouse. Thus, with respect to prevalence of respiratory symptoms, none of the comparisons between workers and controls reached statistical significance, although there was a discernible trend observed. The trend showed a higher prevalence of respiratory symptoms among workers in most of the comparisons. This suggests that exposure in greenhouses might indeed be associated with adverse respiratory outcomes; however, this finding needs to be corroborated in a larger group of subjects. The pilot data collected in this study can be

utilized later for future sample size calculations for a larger study on respiratory symptoms among greenhouse workers in the United States. Overall, we believe our pilot data on exposure levels and prevalence of respiratory symptoms among the greenhouse workers warrants further study in a larger group of subjects.

## TRANSLATION OF FINDINGS

Bioaerosol exposure levels coupled with assessment of workers' respiratory symptoms described in this project report will be relevant in improving specific methods and tools to assess worker exposures to inhalable biohazards and in advancing the understanding of the pathophysiology of respiratory disorders among greenhouse workers. The approach developed in this pilot project for bioaerosol exposure matrix can be applied in the future large scale studies in greenhouses, nurseries, as well as in other agricultural occupational environments. The pilot data collected in this small research project can be utilized later for future sample size calculations for larger studies on respiratory symptoms among greenhouse workers in the United States.

## OUTCOMES/RELEVANCE/IMPACT

The summarized key findings of this R03 project are: (1) high concentrations of airborne microbial contaminants were present in three Midwest greenhouses with significant seasonal variations; (2) the sources of the contaminants were primarily from the greenhouse work areas as indicated by the indoor/outdoor ratios; (3) lack of correlation between exposure variables assessed by various methods indicated that employing several different exposure assessment methods and investigating multiple bioaerosol exposure matrices would be more informative than relying on a single method; (4) the prevalence of self-reported respiratory symptoms was generally higher among greenhouse workers compared to controls; on the other hand the differences were not statistically significant, likely due to low statistical power of the study. will be relevant for the following program portfolio of NIOSH: Sector program (Agriculture, Forestry and Fishing); Cross sector program (Exposure assessment); Mission (Quality research activities that will lead to reductions in occupational illnesses among all workers); Strategic Goal 1 (Develop or improve exposure assessment strategies..); and Strategic Goal 2 (Develop or improve specific methods and tools to assess worker exposures to critical occupational agents). As the obtained data involves assessment of bioaerosols relevant to respiratory allergy and asthma and identification and advancement of knowledge regarding occupational aeroallergens, the application responds to the following cross-sector programs in the NIOSH Program Portfolio: Health Hazard Evaluation, Respiratory Diseases, and Immune and Dermal Diseases. The collected data are also relevant to research goals relevant to the NORA National Agriculture, Forestry, and Fishing Agenda of CDC-NIOSH (2008), particularly, Strategic Goal 5 (Improve the health and well-being of agricultural workers…). Intermediate Goals 5.2 (Reduce acute and chronic respiratory disease caused, or exacerbated by, agricultural exposures including asthma…diseases of the respiratory system) and 5.4 (Reduce illness and disease due to environmental and infectious exposures in agriculture..) Bioaerosol and aeroallergen exposure assessment in greenhouses is related to all these topics. The pilot data can be utilized later for future sample size calculations to assess respiratory symptoms among greenhouse workers in other large scale studies.

# SECTION 2

# SCIENTIFIC REPORT

## BACKGROUND

Greenhouse operations are an important sector of the environmental horticulture industry, also known as the "Green Industry". Greenhouse environmental conditions are characterized by enclosed spaces, high temperature, high humidity, high concentrations of airborne microorganisms, microbial growth in potting materials, soil, and plants, and frequent use of biological and chemical pesticides (Zuskin et al., 1993). Hall et al. (2005) reported that the estimated economic impacts of the U.S. Green Industry are 1,964,339 jobs and $147.8 billion in output, of which 27% was represented by the greenhouse and nursery sector. A report of the work group convened by the National Institute for Occupational Safety and Health (1995), which identified priorities for hired farm workers' occupational health surveillance and research, considered greenhouse workers as one of the two top priorities for both upper and lower respiratory tract problems.

A NIOSH-funded collaborative project involving scientists from the United States and Croatia studied respiratory health of greenhouse workers in Croatia (Zuskin et al. 1993) and found that greenhouse workers developed acute and chronic respiratory symptoms as well as lung function impairment (significant reduction in $FEV_1$, $FEF_{50}$, and $FEF_{25}$) related to their working conditions in closed greenhouse spaces. In another collaborative study, scientists from the United States and Europe (Monsó et al., 2003) investigated risk factors for respiratory symptoms in European and Californian farmers and found that flower growing was significantly associated with asthma, toxic pneumonitis, and chronic bronchitis. In the above-mentioned study, working inside greenhouses appeared to be a significant risk factor for chronic bronchitis among non-smokers (adjusted OR 1.59, 95% CI: 1.02-2.48), who reported a 15% prevalence of respiratory symptoms if working inside, significantly higher than the 9% prevalence reported in non-smoking farmers from outdoor agricultural environments.

The associations between airborne fungi and respiratory allergies and asthma were reported by previous investigators (Malling, 1986; Strachan, 1988). Allergic sensitization to many fungal species, including *Cladosporium*, *Penicillium*, *Aspergillus*, and *Alternaria*, was reported in about 20% of the greenhouse flower growers in Spain by Monsó et al. (2002). In addition to airborne fungi, high concentrations of bacteria, actinomycetes, and endotoxin have been reported in the air of farm workplaces (Krysinska-Traczyk et al., 2004; Skorska et al., 2005; Lee et al, 2006a). Thermophilic bacteria and spore-forming actinomycetes are well-known sources of allergens (Douwes et al., 2003). Endotoxin is a cell wall component derived from Gram-negative bacteria, and is composed of lipopolysaccharides as a main ingredient. Inhaled endotoxin contributes significantly to the induction of airway inflammation and dysfunction (Pirie et al., 2003); many occupational studies have shown positive associations between endotoxin exposure and respiratory disorders including asthma-like symptoms, chronic airway obstruction, byssinosis, bronchitis, and increased airway responsiveness (Madsen, 2006). Endotoxin has also been recognized as a causative factor in the etiology of occupational lung diseases including non-allergic asthma (unlike molds) and organic dust toxic syndromes (Douwes et al., 2003). Little information is available on the health effects of (1→3)-β-D-

glucan in agricultural environments; however, its association with dry cough, cough associated with phlegm, hoarseness, and atopy has been reported in indoor environments (Rylander et al., 1998; Rylander, 1999; Rylander et al., 1999; Thorn and Rylander, 1998).

Even though previous researchers have reported occupational allergy and asthma from molds in greenhouses, little information is available on the exposure levels of bioaerosols in greenhouses, which are associated with respiratory diseases. Except for the studies of Monsó et al. (2002), Radon et al. (2002), and Madsen (2006; 2009, endotoxin only) in European countries and a recent study on airborne fungi in two Connecticut greenhouses by Li and LaMondia (2010), to our knowledge, no recent study has attempted to measure exposure levels to health-related bioaerosols in American greenhouses. The purpose of this project was to collect pilot data for an ensuing large scale research project to examine the associations between the exposures to different bioaerosol components and workplace characteristics with the respiratory symptoms and allergic sensitizations among the U. S. Green Industry workers.

## SPECIFIC AIMS

The specific aims originally proposed for this research project were:

1. *Investigate spatial, temporal, and task-specific exposures to airborne pollen, molds, bacteria, actinomycetes, endotoxin, and (1→3)-β-D-glucan in three greenhouses through air sampling.*
2. *Characterize the load of biological particles on the greenhouse floors and hard surfaces and determine potential source strengths of these surfaces for aerosolizing biological particles.*
3. *Estimate the prevalence of rhinitis and other respiratory symptoms among greenhouse workers using a standardized questionnaire.*

We have accomplished all tasks as proposed in the original grant proposal. We are presenting all findings in this scientific report except for the analysis report on airborne pollen because the levels of pollen in most air samples were below the limit of detections possibly due to the low air volume sampled by the inhalable aerosol samplers.

## METHODOLOGY

***Sampling sites***

Three greenhouses in the Midwestern United States (Paris, Kentucky; Lawrenceburg, Indiana; and Cincinnati, Ohio) were selected for our study. Our selection criteria were: greenhouses should specialize in the production of ornamental plants, and at least ten employees of the greenhouse should be involved in flowers or plants growing during spring and summer. We initially contacted 103 greenhouses in Kentucky, Indiana, and Ohio; nine agreed to participate and finally three greenhouses matching the selection criteria for the project were selected. The job duties of the workers included: transplanting plants, which was done all day; watering plants, which was done two-three times a week, mostly on sunny days; frequent moving and sticking of plants; and handling of mulch and pot filling with mulch and soil. A specific compartment of 50-75 ft × 100-200 ft was selected in each greenhouse for sampling of bioaerosols and recording of environmental factors.

***Air sampling for bioaerosols and recording of environmental parameters***

Long-term inhalable aerosol samples were collected using five Button Inhalable Aerosol Samplers (SKC, Inc., Eighty Four, PA, USA) simultaneously for approximately 5-7 hours per one work shift - four from the corners of the greenhouses and one from the center. The samplers were placed at a height of 1.5 m from the ground. The sampling was conducted in winter (when concentrations of outdoor airborne fungi are typically low) and again in summer (when concentrations are typically high). Additionally, three samples were collected from the major working locations, one from the office area (control) and one from the nearby ambient air [to assess indoor to outdoor (I/O) ratios for various biocontaminants]. Thus, altogether 60 samples (30 samples in winter of 2008-2009 and 30 samples in summer of 2009) were collected to investigate the seasonal and spatial variation of inhalable bioaerosols. Air samples were collected at a flow rate of 4 L/min continuously using a personal pump (BGI Inc., Waltham, MA, USA). Fungi, bacteria, and actinomycetes were extracted from the Button sampler filters as described below and the extracts were analyzed for endotoxin and (1→3)-β-D-glucan.

During each air sampling experiment, temperature and relative humidity was measured using thermohygrometer pens (Fisher Scientific, Pittsburgh, PA, USA) and air velocity using anemometers (TSI Inc., Shoreview, MN).

***Characterizing the load of surface-laden and aerosolizable microbial contaminants on greenhouse floors***

Conventional surface samples from greenhouse floors (near air sampling locations) were collected by sterile cotton swabs following the AIHA protocol for surface and bulk sampling for viable fungi and bacteria (AIHA, 2005). In brief, floor surfaces (1 $cm^2$) were swabbed with wet, sterile cotton wool-tipped sticks soaked with sterile 0.1% peptone with 0.01% Tween 80 and preserved in air-tight sterile tubes. Fungi, bacteria, and actinomycetes from these cotton swabs were extracted similarly as from the Button sampler filters and and cultivated on nutrient agar media. Extracts of cotton swabs were not analyzed for endotoxin and (1→3)-β-D-glucan.

Conventional surface sampling, however, cannot measure the aerosolization potential of different microbial biocontaminants. For that reason we used a microbial source tester (previously referred as Fungal Spore Source Strength Tester or FSSST; Grinshpun et al., 2002; Górny et al., 2002; Sivasubramani et al., 2004a, 2004b; Niemeier et al., 2006; Seo et al., 2008; Adhikari et al., 2009) to measure the potential of aerosolization of culturable fungi, bacteria, actinomycetes, endotoxin, and (1→3)-β-D-glucan from the floors of greenhouses. The microbial source tester is equipped with two pumps (pull and push) and the BioSampler (SKC Inc.) operating at a flow rate of 12.5 L/min. The source tester was applied tightly to the floor surfaces (11 cm × 11 cm) for 15 minutes. The process inside the source tester cap stimulated release of biocontaminants from the surface. The BioSampler liquid was analyzed for culturable fungi, bacteria, actinomycetes, endotoxin, and (1→3)-β-D-glucan. Five surface cotton swab samples and five source tester samples were collected from each greenhouse.

***Analysis of samples***

**Extraction.** Field sample processing, cultivation, and mounting were performed according to our previous protocols (Adhikari et al.; 2003; 2009; Lee et al., 2006a). Briefly, the polycarbonate filter was removed from the sampler using sterile forceps inside a biosafety hood, and placed in a sterile pyrogen-free tube with a 10 mL extraction solution (filtered sterile pyrogen-free water: 1 L, peptone: 1 g, Tween 80: 500 µL) freshly filtered through 0.2 µm porous filter. The extraction was performed by shaking the tube

for 2 minutes in a Vortex mixer and then re-shaking it in an ultrasonic cleaner for 15 minutes. Cotton swabs were extracted in the same solution following similar steps. The extracts were stored at -20°C for several days until analysis. Source tester suspensions (from BioSamplers) were stored at -20°C until analysis.

**Cultivation.** Two hundred μL (for fungi) or one hundred μL (for bacteria and actinomycetes) aliquots from the suspension were immediately cultivated on specific media in Petri plates (malt extract agar supplemented with streptomycin sulphate for fungi and tryptic soy agar supplemented with cycloheximide for bacteria and actinomycetes) to recover culturable fungi, bacteria, and actinomycetes. The culture plates were incubated for 3-7 days for fungi, and 18-24 h for bacteria. The bacterial plates were further incubated for two weeks for the development of actinomycetes colonies. The numbers of colonies on each plate (colony forming units or CFUs) were counted and converted into airborne concentration ($CFU/m^3$) or concentration on surfaces ($CFU/m^2$). The lower limit of detection (LLOD) for culturable fungi (derived from the limit of one colony per plate and calculated accounting for the sampled area, air flow rate as 4 L/min, and sampling time as 6 h) was 35 $CFU/m^3$ for Button sampler and 8,264 $CFU/m^2$ for the microbial source tester. The corresponding LLODs for culturable bacteria and actinomycetes were 69 $CFU/m^3$ and 16,529 $CFU/m^3$.

**Endotoxin and (1→3)-β-D-glucan analysis.** For endotoxin extraction, 1.0 mL of the extracts and microbial source tester suspensions was further sonicated for 1 h. For (1→3)-β-D-glucan, 0.5 mL extracts were combined with 0.5 mL of 0.6 M NaOH solution and vigorously shaken for one hour. For source tester samples the extracts were centrifuged at 7,000 rpm (5,204×g) for 1 min. Supernatants were collected for analysis.

For endotoxin measurement, the sonicated extracts from the Button Sampler and supernatants of the microbial source tester (from Biosampler) suspensions were analyzed with the endotoxin-specific kinetic chromogenic *Limulus* Amebocyte Lysate (LAL) assay (Pyrochrome, Associates of Cape Cod, East Falmouth, MA). Endotoxin concentrations in liquid extracts were converted into $EU/m^3$ for air samples and $EU/m^2$ for microbial source tester samples, which were derived from the air flow rates, sampling duration, and area sampled. The lower limit of detection (LLOD) for endotoxin in suspension was 0.05 EU/mL.

For (1→3)-β-D-glucan measurements, sonicated extracts from the Button Sampler and supernatants of Biosampler suspensions were analyzed with the (1→3)-β-D-glucan-specific kinetic chromogenic LAL assay (Glucatell, Associates of Cape Cod), as previously described by Lee et al. (2006b) and Iossifova et al. (2007). The (1→3)-β-D-glucan concentration values were presented in $ng/m^3$ for air samples and $ng/m^2$ for microbial source tester samples derived similarly as described above for endotoxin. For (1→3)-β-D-glucan, the LLOD in suspension was 2.53 pg/mL.

***Estimating the prevalence of rhinitis and other respiratory disease symptoms among greenhouse workers using a standardized questionnaire***

The prevalence of respiratory symptoms was investigated using a cross-sectional study design. A standardized questionnaire was used to collect data from the workers (exposed group; n = 35) and office workers (non-exposed group; n = 14) in three participating greenhouses and neighboring greenhouses. The symptoms were recorded only in summer and were related to exposure and work status in greenhouses. The majority of the questions were adapted from the American Thoracic Society

Epidemiology Standardization Project (Ferris, 1978) and the more recent "Initial Questionnaire of the NIOSH Occupational Asthma Identification Project" developed by Dr. Lee Petsonk from the Division of Respiratory Disease Studies of NIOSH that includes materials collected from the ATS-DLD-78-A questionnaire (see: http://www.cdc.gov/niosh /asthwww.txt). Moreover, we relied on a validated asthma questionnaire developed by Delclos et al. (2006) for use among healthcare workers.

Information on the symptoms of past and present respiratory illnesses, including episodes of cough and phlegm, wheezing and breathlessness, sinus problems, pneumonia, acute and chronic bronchitis and emphysema, and allergic conditions, such as allergic rhinitis and asthma, were self-reported by each participant. A physician's diagnosis of rhinitis (hay fever), asthma, bronchitis and emphysema, pneumonia, and sinus trouble was recorded. For example, asthmatics were defined as those who answered affirmatively to the questions: "Have you ever had asthma?", and "Was it confirmed by a doctor?" We also collected detailed occupational histories of the workers including self-reported occupational airborne exposures to various bioaerosols, such as pollen and molds. Tobacco smoking history included number of cigarettes, cigars, or amount of pipe tobacco smoked per day; age started or quit regular tobacco smoking; and inhalation of tobacco smoke. Family history of chronic bronchitis, emphysema, asthma, lung cancer, and other pulmonary conditions was ascertained for each worker participating in the study.

***Statistical analyses***

Geometric means and geometric standard deviations were determined for all microbial contaminants and environmental factors. Log-transformation was used to achieve normality of data. Differences between seasonal exposure levels were determined by the paired *t*-test. Correlations between different bioaerosol exposure variables, environmental variables and bioaerosol exposure levels, as well as between surface sampling and microbial source tester-generated data and concentration levels of microbial contaminants were measured using the non-parametric Spearman's test.

For the respiratory symptoms questionnaire data, descriptive statistics were generated and the exposed and non-exposed groups compared using Fisher's Exact Test for categorical variables and Equality of Means Test for continuous variables. We investigated the association of different respiratory outcomes with working status in a greenhouse. Next, we investigated the association of the respiratory outcomes (treated as binary variables, respiratory outcome = yes/no) with exposed/non-exposed status (binary variable) using a modified Poisson regression approach that incorporates estimation of robust error variances. Covariates/potential confounders considered in these analyses included gender, age (as a continuous variable), and smoking status (lifelong non-smoker, ex-smoker, current smoker). We also investigated whether respiratory outcomes were associated with the total number of lifetime hours worked in a greenhouse. We fitted several multivariable regression models for each outcome variable (treated as binary variables, respiratory outcome = yes/no), with total number of hours worked (continuous variable) as the predictor variable, after adjusting for age, gender, and tobacco smoking.

All statistical analyses were performed with SPSS, STATA, and SAS Version 9.1 (SAS Inc., Cary, NC). A two-tailed p-value that was less than 0.05 was defined as statistically significant.

# RESULTS

***Culturable fungi, bacteria, and actinomycetes in air and on floor surfaces of greenhouses***

Geometric means (GMs) and geometric standard deviations (GSDs) for concentrations of inhalable culturable fungi and bacteria are presented in Table 1 (airborne) and Table 2 (aerosolizable from floors). Mean concentrations of airborne culturable fungi in three greenhouses ranged on the order of $10^2$ to $10^4$ CFU/$m^3$. On the other hand, microbial source strength tester demonstrated aerosolizable culturable fungi from floor surfaces at the range of $10^3$-$10^5$ CFU/$m^2$. Cotton swab samples showed a concentration of <LLOD to 500 CFU/$m^2$ in summer and <LLOD to 400 CFU/$m^2$ in winter (n = 30). Air samples collected from office areas and nearby ambient air mostly demonstrated lower concentrations on the order of <LLOD (in winter) to $10^3$ CFU/$m^3$.

Similar to fungi, mean concentrations of airborne culturable bacteria in three greenhouses ranged on the order of $10^2$ to $10^4$ CFU/$m^3$. On the other hand, microbial source strength tester data demonstrated a higher concentration range for aerosolizable culturable bacteria: $10^5$ – $10^6$ CFU/$m^2$. Cotton swab samples showed a concentration of <LLOD to 21,600 CFU/$m^2$ in summer and <LLOD to 41,700 CFU/$m^2$ in winter (n = 30). Air samples collected from office areas and nearby ambient air mostly demonstrated relatively lower concentrations on the order of <LLOD (in winter) to $10^4$ CFU/$m^3$.

Culturable actinomycetes were mostly below LLODs during summer. Mean concentrations of inhalable culturable actinomycetes in three greenhouses were: 14 - 127 CFU/$m^3$ in winter and <LLOD - 45 CFU/$m^3$ in summer from five simultaneous measurements with Button Samplers (n = 30). Except for one office area in a selected Kentucky greenhouse in winter (358 CFU/$m^3$), culturable actinomycetes were never observed in other office areas and nearby outdoor environments. Aerosolizable actinomycetes levels from greenhouse floors demonstrated all mean concentrations were <LLOD during summer and <LLOD to 13,323 CFU/$m^2$ in winter (n = 30). All cotton swab samples (n = 30) collected during summer and winter demonstrated <LLOD values.

***Endotoxin and (1→3)-β-D-glucan in air and on floor surfaces of greenhouses***

Geometric means (GMs) and geometric standard deviations (GSDs) for concentrations of inhalable endotoxin and (1→3)-β-D-glucan are presented in Table 1 (airborne) and Table 2 (aerosolizable from floors). Mean concentrations of inhalable endotoxin levels in greenhouses ranged on the order of $10^1$ to $10^3$ EU/$m^3$ in air. Mean concentrations of aerosolizable endotoxin from floor surfaces ranged from $10^3$ to $10^4$ EU/$m^2$. Cotton swab samples were not analyzed here. Samples collected from office areas (n = 6) showed a concentration ranged of $10^1$ to $10^2$ EU/$m^3$ and samples collected from nearby ambient air showed a concentration range of 0.57 to 9.00 EU/$m^3$.

On average, concentrations of inhalable (1→3)-β-D-glucan in three greenhouses ranged on the order of $10^1$ to $10^4$ ng/$m^3$. Microbial source strength tester data demonstrated a mean concentration of aerosolizable (1→3)-β-D-glucan from floor surfaces as $10^3$ to $10^4$ ng/$m^2$. Cotton swab samples were not analyzed for (1→3)-β-D-glucan. Samples collected from office areas (n = 6) showed a concentration range of 4.63 to 42.42 ng/$m^3$ and samples collected from nearby ambient air during winter ranged from 0.61 to 22.37 ng/$m^3$.

***Seasonal variation of airborne fungi, bacteria, (1→3)-β-D-glucan, and endotoxin exposure levels***

Seasonal variation of inhalable airborne fungi, bacteria, (1→3)-β-D-glucan, and endotoxin exposure levels measured by five Button Samplers in three greenhouses are presented in Fig. 1. The data on actinomycetes were excluded because they were below the lower limit of detection in most cases. Significantly higher ($p < 0.05$ in paired t-test) concentrations of fungi were observed in summer whereas opposite trends – significantly higher concentrations in winter - were observed for culturable bacteria and endotoxin. No significant difference ($p > 0.05$ in paired t-test) was observed for (1→3)-β-D-glucan levels in two seasons.

***Airborne microbial contaminants in greenhouses versus nearby ambient locations and indoor to outdoor ratios***

Outdoor airborne microorganisms, particularly fungi, often influence the levels of airborne microbial concentrations in indoor environments (Burge, 1990). I/O ratios >1 for the microbial contaminant levels indicate potential contamination sources inside the indoor environment. In the present study, one control outdoor sampling was performed exactly outside the greenhouses within a five-meter distance. Airborne concentrations of culturable fungi, bacteria, (1→3)-β-D-glucan and endotoxin in summer and winter seasons for the nearby outdoor air are presented in Table 1. The concentrations of outdoor fungi, bacteria, and actinomycetes in winter were < LLODs, whereas their indoor concentrations in both greenhouses and offices were relatively high (see Table 1). Therefore, the I/O ratios for fungi, bacteria, and actinomycetes during winter were much greater than 1. The I/O ratios for (1→3)-β-D-glucan in winter ranged from 3.0 to 70.5 in greenhouses and 3.6 to 42.0 in offices. For endotoxin, the ratios in winter ranged from 7.12 to 267.20 in greenhouses and 11.16 to 45.41 in offices.

I/O ratios for all types of biocontaminants were lower during summer. The I/O ratios for fungi in summer ranged from 1.5 to 2.1 in greenhouses and 0.5 to 0.7 in offices. The summer ratios for bacteria ranged from 0.7 to 1.9 in greenhouses and 1.0 to 1.1 in offices. The ratios for (1→3)-β-D-glucan in summer ranged from 3.04 to 70.50 in greenhouses and 3.6 to 41.9 in offices. For endotoxin, the ratios in summer ranged from 0.9 to 19.7 in greenhouses and 2.2 to 57.5 in offices.

***Relationship between bioaerosol exposure variables***

Nonparametric Spearman's correlation coefficients were calculated between different bioaerosol exposure variables because the data were not normally distributed. Significant positive correlations were found between the following airborne contaminants: (1→3)-β-D-glucan and culturable fungi ($r = 0.420$; $p < 0.05$), endotoxin and culturable bacteria ($r = 0.551$; $p < 0.05$), and (1→3)-β-D-glucan and endotoxin ($r = 0.487$; $p < 0.05$), whereas a significant negative correlation was observed between airborne culturable fungi and airborne culturable bacteria ($r = 0.551$; $p < 0.05$). These significant relationships are presented in Fig. 2a and 2b. It is interesting to note that when correlations between aerosolizable biocontaminants on floors were examined significant correlations were only found between culturable bacteria and fungi ($r = 0.561$; $p < 0.05$), bacteria and endotoxin ($r = 0.729$; $p < 0.05$), and fungi and endotoxin ($r = 0.594$; $p < 0.05$). No significant correlations ($p > 0.05$) were found when microbial source tester data (aerosolizable biocontaminats from floors) and Button Sampler data (biocontaminants in air) were compared for all biocontaminants. Data from cotton swab samples were not used for this comparison because they were <LLOD in most cases.

***Relationship between temperature, relative humidity, air velocity, and airborne microbial contaminants***

Temperature levels in participating greenhouses ranged from 27.1 to 41.5°C (mean ± SD = 30.9±4.2°C) during summer and 9.7 to 17.3°C (mean ± SD = 13.3±2.4°C) during winter. The relative humidity levels were 32.0% to 65.0% (mean ± SD = 54.8±12.8 %) during summer and 55.0% to 86.4% (mean ± SD = 66.4±4.1 %) during winter. It is interesting to note that although temperature during winter was significantly lower when compared to summer ($p<0.05$; paired t test), there was no significant difference between the relative humidity levels of the two seasons ($p>0.05$; paired t test). The air velocity in winter ranged from <LLOD to 0.8 m/s (mean ± SD = 0.14±0.24 m/s) and 0.06 to 0.75 m/s (mean ± SD = 0.28±0.24 m/s) in summer. Spearman's correlation coefficients were calculated to elucidate the relationships between the exposure levels of inhalable airborne fungi, bacteria, (1→3)-β-D-glucan, endotoxin, and temperature relative humidity, and air velocity in greenhouses. As illustrated in Fig. 2b and 2c, significant negative correlations were observed between culturable bacteria and temperature ($r = - 0.793$; $p <0.05$) and between endotoxin and temperature ($r = - 0.733$; $p <0.05$). On the other hand, significant positive correlations were observed between culturable fungi and temperature ($r = 0.370$; $p <0.05$), endotoxin and air velocity ($r = 0.490$; $p <0.05$), and (1→3)-β-D-glucan and air velocity ($r = 0.387$; $p <0.05$).

***Prevalence of self-reported respiratory symptoms among greenhouse workers***

Selected personal characteristics (including gender, birthplace, education, age, household income, height, weight, cigarette smoking behavior, etc.) of the study participants in the respiratory symptoms questionnaire survey are summarized in Table 4. All subjects were White, except an office worker who self-identified as American Indian/Alaska Native. As presented in Table 4, all statistical comparisons between the exposed and unexposed groups resulted in two-sided p-values >0.05.

The prevalence of selected self-reported respiratory outcomes among the workers and the controls are given in Table 5. Prevalence estimates were generally higher among workers compared to controls. Although the crude/unadjusted prevalence ratios (PRs) ranged from 1.2 – 4.4, the differences were not statistically significant based on Fisher's Exact Test (all p-values in Table 5 are > 0.10).

Adjusted PRs (Barros et al., 2003) for selected respiratory outcomes are summarized in Table 6. Poisson regression analysis for unadjusted as well as adjusted estimates showed PRs > 1, though none of the comparisons reached statistical significance.

## DISCUSSION

To estimate health risks caused by airborne microbial contaminants in greenhouse confinements, bioaerosol sampling should be performed in a way that adequately reflects workers' inhalation exposure. Our previous study in agricultural confinements (Adhikari et al., 2004) indicated that personal inhalable exposure of agricultural workers to airborne fungi could be adequately assessed by placing several Button Inhalable Aerosol Samplers simultaneously operating in a static stationary mode throughout the work site. We have followed this sampling strategy in the present study to assess exposure levels to various airborne microbial contaminants. The Button Sampler was selected because it has a high sampling efficiency at both high and low wind speeds for particles with aerodynamic diameters <70 μm, and there is no significant difference between the sampling

efficiencies of the Button Sampler in the stationary and personal modes (Aizenberg et al., 1998).

The present study demonstrates that workers employed in Midwestern greenhouses are exposed to high concentrations of inhalable culturable microorganisms, endotoxin, and (1→3)-β-D-glucan. There are no internationally accepted occupational exposure limits for airborne concentrations of microorganisms, endotoxin, and (1→3)-β-D-glucan in occupational environments. Therefore, the results of our study should be evaluated with respect to results from similar studies. The average concentrations of airborne fungi in three greenhouses (from four corners and center) in our study were on the order of $10^3 – 10^4$ CFU/m$^3$ in summer and $10^2 – 10^3$ CFU/m$^3$ in winter. A study by Radon et al. (2002) investigated bioaerosols in Spanish greenhouses, which revealed median airborne concentration of culturable fungi to be $8.3 \times 10^4$ CFU/m$^3$. Monsó et al. (2002) investigated workers' exposure to airborne microorganisms, total dust, and endotoxin in 34 greenhouses in Spain. Median concentrations of airborne fungi in their study were $5 \times 10^3$ CFU/m$^3$. The mean concentrations of airborne fungi in our study were comparable to the levels of airborne fungi previously reported from greenhouses in Spain. The levels were also comparable to the culturable fungal concentrations observed in animal confinements (swine, poultry, and dairy), but lower than in grain harvesting places in Ohio (Lee et al., 2006a). Levels of airborne culturable fungi were significantly higher than our previous results on airborne culturable fungi in Cincinnati homes, where concentration levels were between 0 and 1,362 CFU/m$^3$ (Lee et al., 2006c). Levels of aerosolizable culturable fungi from floors, as detected with the microbial source tester, were notably higher than the levels obtained from cotton swab samples indicating that the source tester could be a more appropriate method for testing aerosolizable culturable fungi on surfaces. Significantly higher concentrations of airborne culturable fungi were found in summer as compared to winter, probably because of the favorable growth temperatures for fungi in summer.

The mean concentrations of airborne culturable bacteria (from four corners and the center) were on the order of $10^2 – 10^3$ CFU/m$^3$ in summer and $10^3 – 10^5$ CFU/m$^3$ in winter. Monsó et al. (2002) reported median concentration of culturable bacteria as 2,300 CFU/m$^3$ in 34 Spanish greenhouses. Radon et al. (2002) reported levels of culturable bacteria in other greenhouses in Spain <LLOD to $1.1 \times 10^6$ CFU/m$^3$. Consequently, exposure levels to culturable bacteria found in our study are comparable to greenhouses in Spain. The levels are also comparable to dairies and poultry farms but lower than swine farms in Ohio (Lee et al., 2006a). Tsi and Macher (2005) previously reported a median concentration of 91 CFU/m$^3$ of culturable bacteria in one hundred office buildings in the United States. Levels of culturable bacteria in our study were thus notably higher than previously measured in office buildings. Similar to fungi, levels of culturable bacteria collected with the microbial source tester were significantly higher than those obtained from cotton swab samples indicating that cotton swab samples may not be as efficient as the microbial source tester in estimating culturable bacterial levels on surfaces.

Exposure levels of inhalable endotoxin in the three greenhouses investigated in our study (from four corners and center) were generally high [14.35 – 855.16 EU/m$^3$ ≈ 1.4 – 85.5 ng/m$^3$ in winter and 8.20 – 38.90 EU/m$^3$ ≈ 0.8 – 3.8 ng/m$^3$ in summer) and much higher than reference measurements in nearby outdoor locations (see Table 1). Environmental bacteria and endotoxin in mulch, soil, the phyllosphere, and contaminated

irrigation water can be aerosolized by wind, spray, and mechanical disturbances (Monn and Koren, 1999) in a greenhouse. Previous studies from European countries have reported data on endotoxin exposures among greenhouse workers. In comparing endotoxin concentrations among greenhouses and poultry and swine confinements, Radon et al. (2002) found low endotoxin levels in the greenhouses (0.05 – 12.68 ng/$m^3$). However, Madsen (2006) compared endotoxin levels in ambient air and biofuel plants with greenhouses in Denmark and found significantly higher levels in the greenhouses (median: 13.2 EU/$m^3$). Monso et al. (2004) reported 0.17 – 0.89 ng/$m^3$ endotoxin exposures among the greenhouse workers dealing with flowers and ornamental plants in Spain. In a Dutch study conducted in cucumber and paprika nurseries, the levels of endotoxin exposure were between 36 and 650 EU/$m^3$ (Spaan et al., 2006). Madsen et al. (2009) found endotoxin exposure levels between 0.5 and 400 ng/$m^3$ (median = 32 ng/$m^3$) in cucumber and tomato nurseries of Denmark. Thus, the endotoxin exposure levels we found in three greenhouses were very similar to those measured in European greenhouses, and sometimes exceeded previously reported levels particularly in winter. Endotoxin exposure levels in greenhouses were considerably higher than typical airborne levels in home environments. For example, recent measurements of airborne endotoxin levels in non-moldy Cincinnati homes have revealed a geometric mean of 4.2 EU/$m^3$ (Reponen et al., 1010).

Monsó (2004) argued that the relatively low level of endotoxin found inside greenhouses indicates that inhaled endotoxin may not be clinically significant as a trigger of the respiratory symptoms reported by greenhouse workers. We included endotoxin measurements in this study for two reasons. First, there is no available information on the endotoxin concentrations in U.S. greenhouses. Second, a recent genetic study showed that high endotoxin exposure is associated with a decreased risk of allergic sensitization (as found previously among pig farmers by Portengen et al., 2005); however low exposure has been found to be associated with an increased risk (Simpson et al., 2006). Consequently, lower airborne concentrations of endotoxin in greenhouses may have clinical importance in terms of respiratory health of workers.

Exposure levels to inhalable (1→3)-β-D-glucan in the greenhouses in our study were relatively high (7.67 – 71.27/$m^3$ in winter and 16.87 – 26.40 ng/$m^3$) with respect to previous measurements in home environments. For example, the geometric mean of airborne (1→3)-β-D-glucan concentrations measured in non-moldy homes in Cincinnati were much lower: 0.92 ng/$m^3$ (Lee et al., 2006), 1.0 ng/$m^3$ (Crawford et al., 2009) and 1.5 ng/$m^3$ (Reponen et al., 2010). The airborne concentrations of (1→3)-β-D-glucan were also higher than the reference values in nearby outdoor locations in most cases.

Thorn and Rylander (1998) found that exposure to (1→3)-β-D-glucan was associated with an increased prevalence of atopy and decreased $FEV_1$. On the other hand, recently published studies (Schram-Bijkerk et al., 2005; Iossifova et al., 2007) have indicated that (1→3)-β-D-glucan may have a protective effect against atopic wheeze in children. Thus, similar to endotoxin, our study of airborne (1→3)-β-D-glucan in greenhouses is also necessary for a critical understanding of the respiratory health hazards of workers. To our knowledge, no published reports are available which focus on this issue. We believe that the information on exposure levels of endotoxin and (1→3)-β-D-glucan in greenhouses would be valuable in understanding the respiratory health hazards in these environments.

There was considerable variability in seasonal exposure levels to culturable fungi and bacteria (see Fig. 1). Significantly higher levels of culturable fungi were observed during

summer, whereas bacteria showed an opposite trend, i.e., a significantly higher concentration during winter. For fungi, we can explain this difference by temperature, which is more favorable for fungi during summer. For bacteria, it is difficult to explain this seasonal variability with respect to temperature and relative humidity only. Further research on bacterial ecology and diversity in greenhouse environments is necessary to understand the seasonal differences in airborne levels of bacteria. In contrast to our findings with respect to airborne concentrations, the maximum plausible aerosolizable fractions of culturable bacteria and fungi from the floors of greenhouses did not demonstrate any significant seasonal differences. This observation is explainable because floors were not the only source for airborne microbial contaminants in the greenhouses. In addition, no significant correlations were observed between airborne and aerosolizable fungi and bacteria. This observation supports our previous findings in flood-affected homes, where no conclusive correlations were found between the three environmental monitoring methods (Adhikari et al., 2010).

Airborne endotoxin concentration levels between summer and winter seasons were found to differ. Air samples showed a significantly higher endotoxin concentration in winter (see Fig. 1) as compared to summer. When all data were combined, no significant differences were observed between the aerosolizable endotoxin levels of summer and winter from greenhouse floors ($p>0.05$). Higher concentrations of airborne and aerosolizable endotoxin in winter corroborate our previous findings in flood-affected homes (Adhikari et al., 2010). Unlike endotoxin, there was no significant variation in airborne (1→3)-β-D-glucan exposure levels between summer and winter (see Fig.1). This observation is consistent with the results of our previous study conducted in flood-affected homes (Adhikari et al., 2010). On the other hand, aerosolizable (1→3)-β-D-glucan from floors showed a significantly higher concentration of (1→3)-β-D-glucan on floor surfaces during winter compared to summer, which may be explained by contamination from plant materials in greenhouses. From visual observations, we found that dry plant materials were more common on the floors of greenhouses during winter. When endotoxin and (1→3)-β-D-glucan concentrations in air and aerosolizable from floors were compared, no significant correlations were observed. This disparity with respect to all four biocontaminants indicates that employing several exposure assessment methods and investigating multiple bioaerosol exposure matrices is more informative than employing a single exposure assessment method for microbial contaminants present in greenhouses, and probably in other indoor agricultural confinements as well.

In most cases, we found that the concentrations of microbial contaminants in greenhouses were higher when compared to nearby outdoor locations. I/O ratios for fungi, bacteria, and actinomycetes were always >>1 in winter, indicating that sources of microbial contamination were primarily from the indoor greenhouse work areas. When we compared exposure levels in greenhouse work areas and office areas, our results were mixed. In most cases, biocontaminant concentration levels in office areas were lower, though at times were comparable or even higher. This disparity is likely due to the locations of office areas being adjacent to greenhouses, and movement of workers from work areas to office areas. Although we collected only one control sample from the office areas, we believe any potential future large scale study would require more samples from greenhouse offices.

We examined correlations between various biocontaminants because both (1→3)-β-D-glucan and endotoxin can be potential immunolomodulators for various atopic

respiratory diseases and fungi and bacteria can grow in common environmental conditions. We found significant positive correlations between culturable fungi and bacteria with (1→3)-β-D-glucan and endotoxin (Fig. 2a), possibly because fungi and bacteria act as source of (1→3)-β-D-glucan and endotoxin, respectively. The significant positive correlation between (1→3)-β-D-glucan and endotoxin (Fig. 2a) is important because both can act as immunomodulators of various atopic respiratory diseases. We previously observed this kind of significant correlation in flood affected homes of New Orleans (Adhikari et al., 2010). The inverse correlation (Fig. 2b) between culturable bacteria and fungi is interesting to note and suggests an antagonistic ecological relationship between the species of culturable fungi and bacteria in greenhouses, which could be further explored in a large-scale study. It may also be due to the opposite effect of temperature in the growth of certain fungal and bacterial species (Fig. 2b) as indicated by positive and negative correlation coefficients, respectively. We found significant negative correlation between endotoxin and temperature (Fig. 2c) possibly because culturable bacteria acted as major sources of endotoxin. Notable significant positive correlations between air velocity and concentrations of airborne endotoxin and (1→3)-β-D-glucan (Fig. 2c) were observed. Particles containing these biocontaminants may remain in the air for a longer period due to the movement of air. This observation also suggests that future potential studies on bioaerosols in agricultural confinements would benefit from the inclusion of air velocity measurements. When microbial source tester data were analyzed, we found significant correlations between bacteria and fungi, bacteria and endotoxin, and fungi and endotoxin. No significant correlation was found between (1→3)-β-D-glucan and endotoxin. Thus, interrelationships between airborne biocontamiants and aerosolizable biocontaminants may differ and the complex ecology of microorganisms on surfaces, as stated previously, may account for this disparity.

With respect to prevalence of respiratory symptoms, none of the comparisons between workers and controls reached statistical significance, although there was a discernible trend observed. The trend showed a higher prevalence of respiratory symptoms among workers in most of the comparisons. This suggests that exposure in greenhouses might indeed be associated with adverse respiratory outcomes; however, this finding needs to be corroborated in a larger group of subjects. The pilot data collected in this study can be utilized later for future sample size calculations for a larger study on respiratory symptoms among greenhouse workers in the United States. Based on the observed differences in the prevalence of respiratory symptoms in this study, we estimated sample sizes required to achieve 80% power to detect a difference between exposed and non-exposed groups at the 5% level of significance for several respiratory symptoms/conditions. For example, total sample sizes of 146, 80, and 189 will be needed for the symptoms/conditions ‘wheeze’, ‘phlegm’, and ‘hayfever’, respectively. Overall, we believe our pilot data on exposure levels and prevalence of respiratory symptoms among the greenhouse workers warrants further study in a larger group of subjects.

## CONCLUSIONS

Elevated concentrations of airborne microbial contaminants were observed in three greenhouses with significant seasonal variations. Sources of the contaminants were primarily from the greenhouse work areas as indicated by the I/O ratios. Lack of correlations between exposure variables assessed by various methods indicates that

employing several exposure assessment methods and investigating multiple bioaerosol exposure matrices would be more informative. The prevalence of self-reported respiratory symptoms was generally higher among greenhouse workers compared to controls; on the other hand the differences were not statistically significant, possibly due to the low power of the study.

## REFERENCES


Adhikari A, Martuzevicius D, Reponen T, Grinshpun SA, Cho SH, Sivasubramani SK, Zhong W, Levin L, Kelley AL, St. Clair HG, LeMasters G. (2003) Performance of the Button Personal Inhalable Sampler for the measurement of outdoor aeroallergens. Atmos Environ ; 37: 4723-33.

Adhikari A, Reponen T, Lee SA, Grinshpun SA. (2004) Assessment of human exposure to airborne fungi in agricultural confinements: personal inhalable sampling versus stationary sampling. Ann Agric Environ Med; 11:269-77

Adhikari A, Jung J, Reponen T, Lewis JS, DeGrasse EC, Grimsley LF, Chew GL, Grinshpun SA. (2009) Aerosolization of fungi, (1→3)-β-D glucan, and endotoxin from flood-affected materials collected in New Orleans homes. Environ Res; 109:215-24.

Adhikari A, Lewis JS, Reponen T, DeGrasse EC, Grimsley LF, Chew GL, Iossifova Y, Grinshpun SA. (2010) Exposure matrices of endotoxin, (1→3)-β-D-glucan, fungi, and dust mite allergens in flood-affected homes of New Orleans. Sci Total Environ; in press.

American Indsutrial Hygiene Association. Field Guide for the Determination of Biological Contaminants in Environmental Samples. 2nd Edn. Hung LL, Miller JD, Dillon HK (Eds.) 2005. AIHA, Fairfax, Virginia.

Aizenberg V, Bidinger E, Grinshpun SA, Willeke K, Hamed A, Tabakoff W. (1998) Air flow and particle velocities near a personal aerosol sampler with curved, porous aerosol sampling surface. Aerosol Sci Technol; 28: 247–58.

Barros AJ, Hirakata VN. (2003) Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. BMC Med Res Methodol; 3:21.

Crawford C, Reponen T, Lee T, Iossifova Y, Levin L, Adhikari A, Grinshpun SA. (2009) Temporal and spatial variation of indoor and outdoor airborne fungal spores, pollen, and (1→3)-β-D-glucan. Aerobiologia; 25:147-58.

Delclos GL, Arif AA, Aday L, Carson A, Lai D, Lusk C, Stock T, Symanski E, Whitehead LW, Benavides FG, Antó JM. (2006) Validation of an asthma questionnaire for use in healthcare workers. Occup Environ Med; 63:173-9.

Douwes J, Thorne P, Pearce N, Heederik D. (2003) Bioaerosol health effects and exposure assessment: progress and prospects. Ann Occup Hyg; 47:187-200.

Ferris BG. (1978) Epidemiology Standardization Project. II. Recommended respiratory disease questionnaires for use with adults and children in epidemiological research. Am Rev Respir Dis; 118(part 2 of 2 parts): 7-53.

Grinshpun SA, Górny RL, Reponen T, Willeke K, Trakumas S, Hall P, Dietrich DF. (2002) New method for assessment of potential spore aerosolization from contaminated surfaces. In: Proceedings of the Sixth International Aerosol Conference, September 8–13, Taipei, Taiwan; 2:767-8.

Górny RL, Reponen T, Willeke K, Robine E, Boissier M, Grinshpun SA. (2002) Fungal fragments as indoor biocontaminants. Appl Environ Microbiol; 68:3522-31.

Hall CR, Hodges AW, Haydu JJ. Economic Impacts of the Green Industry in the United States. 2005. Available at: http://www.ufei.org/files/pubs/EconomicImpactsofthe USGreen%20Industr(NUCFACfinalreport).pdf (accessed on July 14, 2010).

Iossifova Y, Reponen T, Bernstein D, Levin L, Zeigler H, Kalra H, Campo P, Manuel Villareal M, Lockey J, Khurana Hershey GK, LeMasters G. (2007) House dust (1→3)-β-D-glucan and wheezing in infants. Allergy; 62:504-13.

Krysinska-Traczyk E, Skorska C, Prazmo Z, Sitkowska J, Cholewa G, Dutkiewicz J. (2004) Exposure to airborne microorganisms, dust and endotoxin during flax scutching on farms. Ann Agric Environ Med; 11:309-17.

Lee SA, Adhikari A, Grinshpun SA, McKay R, Shukla R, Reponen T. (2006a) Personal exposure to airborne dust and microorganisms in agricultural environments. J Occup Environ Hyg; 3:118-30.

Lee T, Grinshpun SA, Kim KY, Iossifova Y, Adhikari A, Reponen T. (2006b) Relationship between indoor and outdoor airborne fungal spores, pollen, and (1→3)-β-D-glucan in homes without visible mold growth. Aerobiologia; 22:227-36.

Lee T, Grinshpun SA, Martuzevicius D, Adhikari A, Crawford CM, Reponen T. (2006c) Culturability and concentration of indoor and outdoor airborne fungi in six single family homes. Atmos Environ; 40:2902-10.

Li D-W, LaMondia J (2010) Airborne fungi associated with ornamental plant propagation in greenhouses. Aerobiologia; 26:15–28.

Madsen AM. (2006) Airborne endotoxin in different background environments and seasons. Ann Agric Environ Med; 13:81-6.

Madsen AM, Hansen VM, Nielsen SH, Olsen TT. (2009) Exposure to dust and endotoxin of employees in cucumber and tomato nurseries. Ann Occup Hyg; 53:129-38.

Malling HJ. (1986) Diagnosis and immunotherapy of mould allergy. IV. Relation between asthma symptoms, spore counts and diagnostic tests. Allergy; 41:342-50.

Monn C, Koren HS. (1999) Bioaerosols in ambient air particulates: a review and research needs. Rev Environ Health; 14:79-89.

Monsó E. (2004) Occupational asthma in greenhouse workers. Curr Opinion Pulmonary Med; 10:147-50.

Monsó E, Magarolas R, Badorrey I, Radon K, Nowak D, Morera J. (2002) Occupational asthma in greenhouse flower and ornamental plant growers. Am J Respir Crit Care Med; 165:954-60.

Monsó E, Schenker M, Radon K, Riu E, Magarolas R, McCurdy S, Danuser B, Iversen M, Saiki C, Nowak D. (2003) Region-related risk factors for respiratory symptoms in European and Californian farmers. Eur Respir J; 21:323-31.

National Institute for Occupational Safety and Health (NIOSH). New Directions in the Surveillance of Hired Farm Worker Health and Occupational Safety: A Report of the Work Group Convened by NIOSH, May 5, 1995, to Identify Priorities for Hired Farm Worker Occupational Health Surveillance and Research. Available at: http://www.cdc.gov/niosh/hfw-index.html#contents (accessed on July 14, 2010).

Niemeier RT, Sivasubramani SK, Reponen T, Grinshpun SA. (2006) Assessment of fungal contamination in moldy homes: comparison of different methods. J Occup Environ Hyg; 3:262-73.

Pirie RS, Collie DD, Dixon PM, McGorum BC. (2003) Inhaled endotoxin and organic dust particulates have synergistic proinflammatory effects in equine heaves (organic dust-induced asthma). Clin Exp Allergy; 33:676-83.

Portengen L, Preller L, Tielen M, Doekes G, Heederik D. (2005) Endotoxin exposure and atopic sensitization in adult pig farmers. J Allergy Clin Immunol; 115:797-802.

Radon K, Danuser B, Iversen M, Monso E, Weber C, Hartung J, Donham K, Palmgren U, Nowak D. (2002) Air contaminants in different European farming environments. Ann Agric Environ Med; 9:41-8.

Reponen T, Singh U, Schaffer C, Vesper S, Johansson E, Adhikari A, Grinshpun SA, Indugula R, Ryan P, Levin L, LeMasters G. (2010) Visually observed mold and moldy odor versus quantitatively measured microbial exposure in homes. Sci Total Environ; in press.

Rylander R, Norrhall M, Engdahl U, Tunsater A, Holt PG. (1998) Airways inflammation, atopy, and (1→3)-beta-D-glucan exposures in two schools. Am J Respir Crit Care Med; 158:1685-7.

Rylander R. (1999) Indoor air-related effects and airborne (1→3)-beta-D-glucan. Environ Health Perspect; 107, Suppl 3:501-3.

Rylander R, Fogelmark B, McWilliam A, Currie A. (1999) (1→3)-beta-D-glucan may contribute to pollen sensitivity. Clin Exp Immunol; 115:383-4.

Schram-Bijkerk D, Doekes G, Douwes J, Boeve M, Riedler J, Ublagger E, von Mutius E, Benz MR, Pershagen G, van Hage M, Scheynius A, Braun-Fahrlander C, Waser M, Brunekreef B. (2005) Bacterial and fungal agents in house dust and wheeze in children: the PARSIFAL study. Clin Exp Allergy; 35:1272-8.

Seo SC, Reponen T, Levin L, Borchelt T, Grinshpun SA. (2008) Aerosolization of particulate (1→3)-β-D-glucan from moldy materials. Appl Environ Microbiol; 74:585-93.

Simpson A, John SL, Jury F, Niven R, Woodcock A, Ollier WE, Custovic A. (2006) Endotoxin Exposure, CD14 and Allergic Disease: An Interaction between Genes and the Environment. Am J Respir Crit Care Med; 174:386-92.

Sivasubramani SK, Niemeier RT, Reponen T, Grinshpun SA. (2004a) Fungal spore source strength tester: laboratory evaluation of a new concept. Sci Total Environ; 329: 75-86.

Sivasubramani SK, Niemeier RT, Reponen T, Grinshpun SA. (2004b) Assessment of the aerosolization potential for fungal spores in moldy homes. Indoor Air; 14:405-12.

Skorska C, Sitkowska J, Krysinska-Traczyk E, Cholewa G, Dutkiewicz J. (2005) Exposure to airborne microorganisms, dust and endotoxin during processing of peppermint and chamomile herbs on farms. Ann Agric Environ Med; 12:281-8.

Spaan S, Wouters IM, Oosting I, Doekes G, Heederik D. (2006) Exposure to inhalable dust and endotoxins in agricultural industries. J Environ Monit; 8:63-72.

Strachan DP. (1988) Damp housing and childhood asthma: validation of reporting of symptoms. Br Med J; 297:1223-6.

Thorn J, Rylander R. (1998) Airways inflammation and glucan in a rowhouse area. Am J Respir Crit Care Med; 157:1798-803.

Tsai FC, Macher JM (2005) Concentrations of airborne culturable bacteria in 100 large US office buildings from the BASE study. Indoor Air; 15 (Suppl 9): 71–81.

Zuskin E, Schachter EN, Mustajbegovic J. (1993) Respiratory function in greenhouse workers. Int Arch Occup Environ Health; 64:521-6.

Table 1. Airborne culturable fungi, culturable bacteria, endotoxin, and (1→3)-β-D-glucan in work areas, office areas, and nearby ambient locations in three Midwest greenhouses during summer and winter.

| Microbial contaminants | Culturable fungi (CFU/m$^3$) | | | | Culturable bacteria (CFU/m$^3$) | | | | Endotoxin (EU/m$^3$) | | | | (1→3)-β-D-glucan (ng/m$^3$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sampling season | Four corners and center (n=15); GM (GSD) | Near work areas (n=9); GM (GSD) | Office area (n=3) GM (GSD) | Nearby ambient air (n=3) GM (GSD) | Four corners and center (n=15); GM (GSD) | Near work areas (n=9); GM (GSD) | Office area (n=3) GM (GSD) | Nearby ambient air (n=3) GM (GSD) | Four corners and center (n=15); GM (GSD) | Near work areas (n=9); GM (GSD) | Office area (n=3) GM (GSD) | Nearby ambient air (n=3) GM (GSD) | Four corners and center (n=15); GM (GSD) | Near work areas (n=9); GM (GSD) | Office area (n=3) GM (GSD) | Nearby ambient air (n=3) GM (GSD) |
| **Summer** | 4,605 (2.43) | 2,886 (1.72) | 1,943 (1.49) | 2,889 (1.84) | 296 (2.49) | 487 (4.59) | 290 (2.42) | 338 (1.34) | 12.2 (3.91) | 23.3 (2.52) | 37.8 (3.29) | 4.37 (2.14) | 17.3 (2.51) | 12.9 (2.07) | 20.1 (1.36) | 18.93 (1.35) |
| **Winter** | 1,060 (2.89) | 3,685 (3.27) | 345 (18.70) | <LLOD | 3,859 (2.55) | 1,451 (4.87) | 2,059 (4.07) | <LLOD | 115.6 (5.60) | 153.1 (19.81) | 34.2 (6.34) | 6.84 (3.09) | 19.35 (3.42) | 29.1 (29.89) | 12.2 (3.10) | 11.15 (2.05) |

Abbreviations: GM = Geometric mean; GSD = Geometric standard deviation; LLOD = Lower limit of detection

Table 2. Aerosolizable culturable fungi, culturable bacteria, endotoxin, and (1→3)-β-D-glucan isolated from the floors of work areas in greenhouses using the microbial source tester during summer and winter.

| Microbial contaminants / Sampling season | Aerosolizable microbial contaminants from greenhouse floors (n = 15) | | | |
|---|---|---|---|---|
| | Culturable fungi (CFU/$m^2$) GM (GSD) | Culturable bacteria (CFU/$m^2$) GM (GSD) | Endotoxin (EU/$m^2$) GM (GSD) | (1→3)-β-D-glucan (ng/$m^2$) GM (GSD) |
| **Summer** | 13,585 (2.48) | 126,170 (5.67) | 1,415.1 (27.17) | 191.4 (17.76) |
| **Winter** | 12,289 (2.12) | 134,650 (2.98) | 2,653.0 (4.64) | 2294.9 (2.01) |

Abbreviations: GM = Geometric mean; GSD = Geometric standard deviation

Table 3. Temperature, relative humidity, and wind velocity in work areas, office areas, and nearby ambient locations in three Midwest greenhouses during summer and winter.

| Environmental factors | Temperature (°C) | | | Relative humidity (%) | | | Wind velocity (m/s) | | |
|---|---|---|---|---|---|---|---|---|---|
| Sampling season | Four corners and center (n=15); GM (GSD) | Office area (n=3) GM (GSD) | Nearby ambient air (n=3) GM (GSD)* | Four corners and center (n=15); GM (GSD) | Office area (n=3) | Nearby ambient air (n=3) | Four corners and center (n=15); GM (GSD) | Office area (n=3) | Nearby ambient air (n=3) |
| **Summer** | 30.7 (1.13) | 26.65 (1.04) | 28.39 (1.02) | 53.2 (1.36) | 46.5 (1.30) | 49.3 (1.38) | 0.007 (18.23) | 0.005 (3.29) | 0.342 (2.53) |
| **Winter** | 13.1 (1.20) | 18.39 (1.11) | - 2.8 to 7.9 | 65.7 (1.15) | 42.5 (1.44) | 49.7 (1.16) | 0.183 (2.74) | 0.010 (5.00) | 0.307 (3.88) |

Abbreviations: GM = Geometric mean; GSD = Geometric standard deviation; *GM and GSD were calculated for summer data only; range is presented for winter data as one point was below 0°C.

Table 4. Selected characteristics of the study population participating in the respiratory symptoms questionnaire survey.

| Variable | Subgroups | Workers | | Controls | | Two-sided p-value |
|---|---|---|---|---|---|---|
| | | n | % | n | % | |
| Gender | Males | 20 | 57.1 | 4 | 28.6 | 0.114[a] |
| | Females | 15 | 42.9 | 10 | 71.4 | |
| | Totals | 35 | 100.0 | 14 | 100.0 | |
| | | | | | | |
| Birthplace | Urban | 23 | 65.7 | 8 | 57.1 | 0.539[a] |
| | Rural | 10 | 28.9 | 6 | 42.9 | |
| | Missing | 2 | 5.7 | 0 | 0.0 | |
| | Totals | 35 | 100.3 | 14 | 100.0 | |
| | | | | | | |
| Education | No formal education | 0 | 0.0 | 1 | 7.1 | 0.386[a] |
| | High school grad or GED | 9 | 25.7 | 3 | 21.4 | |
| | College or vocational/technical training | 18 | 51.4 | 5 | 35.7 | |
| | 4-year college graduate | 7 | 20.0 | 4 | 28.6 | |
| | Graduate/Medical/Law degree | 1 | 2.9 | 1 | 7.1 | |
| | Totals | 35 | 100.0 | 14 | 99.9 | |
| | | | | | | |
| Age (quartiles) | Q1 (≤28 years) | 12 | 34.3 | 1 | 7.1 | 0.232[a] |
| | Q2 (29-43 years) | 7 | 20.0 | 5 | 35.7 | |
| | Q3 (44-50 years) | 8 | 22.9 | 4 | 28.6 | |
| | Q4 (>50 years) | 8 | 22.9 | 4 | 28.6 | |
| | Totals | 35 | 100.1 | 14 | 100.0 | |
| | | | | | | |
| Age (continuous) | Means | 40.1 years | | 44.5 years | | 0.222[b] |
| | | | | | | |
| Household income | <$14,999/year | 1 | 2.9 | 1 | 7.1 | 0.151[a] |
| | $15,000-$29,999/year | 5 | 14.3 | 0 | 0.0 | |
| | $30,000-$60,000/year | 14 | 40.0 | 5 | 35.7 | |
| | >$60,000/year | 10 | 28.6 | 8 | 57.1 | |
| | Missing | 5 | 14.3 | 0 | 0.0 | |
| | Totals | 35 | 100.1 | 14 | 99.9 | |
| | | | | | | |
| Height (Quartiles) | Q1 | 8 | 22.9 | 7 | 50.0 | 0.148[a] |
| | Q2 | 10 | 28.6 | 1 | 7.1 | |
| | Q3 | 10 | 28.6 | 2 | 13.3 | |
| | Q4 | 7 | 20.0 | 4 | 28.6 | |
| | Totals | 35 | 100.1 | 14 | 99.0 | |

Continued in next page

| | | | | | | |
|---|---|---|---|---|---|---|
| Height (continuous) | Means | 68.0 in. | | 66.6 in. | | 0.401[b] |
| Weight (quartiles) | Q1 | 9 | 25.7 | 4 | 28.6 | 0.408[a] |
| | Q2 | 8 | 22.9 | 5 | 35.7 | |
| | Q3 | 10 | 28.6 | 1 | 7.1 | |
| | Q4 | 8 | 22.9 | 4 | 28.6 | |
| | Totals | 35 | 100.1 | 14 | 100.0 | |
| Weight (continuous) | Means | 169.9 lbs. | | 171.6 lbs. | | 0.945[b] |
| Ever smoked cigarettes? | Yes | 16 | 45.7 | 5 | 35.7 | 0.750[a] |
| | No | 19 | 54.3 | 9 | 64.3 | |
| | Totals | 35 | 100.0 | 14 | 100.0 | |
| Current smoker? | Yes | 6 | 17.1 | 3 | 21.4 | 0.702[a] |
| | No | 29 | 82.9 | 11 | 78.6 | |
| | Totals | 35 | 100.0 | 14 | 100.0 | |
| Smoking status | Current smoker | 6 | 17.1 | 3 | 21.4 | 0.679[a] |
| | Ex-smoker | 10 | 28.8 | 2 | 14.3 | |
| | Lifelong never smoker | 19 | 54.3 | 9 | 64.3 | |
| | Totals | 35 | 100.2 | 14 | 100.0 | |
| Cigarettes per day (current smokers) | Means | 14.8 | | 12.3 | | 0.644[b] |

[b] Two-sided p-value based on Equality of Means t Test (equal variances not assumed).
Abbreviations: cig = cigarette; grad: graduate; GED: General Educational Development graduate

Table 5. Prevalence of respiratory symptoms among greenhouse workers and controls.

| Two-sided p-value[a] | Symptom | Workers (n = 35) | | Controls (n = 14) | | Crude PR[b] |
|---|---|---|---|---|---|---|
| | | n | % | n | % | |
| 1.000 | Ever had asthma | 3 | 8.6 | 1 | 7.1 | 1.2 |
| 0.202 | Chest ever wheezy or whistling | 15 | 42.9 | 3 | 21.4 | 2.0 |
| 0.133 | Usually bring up phlegm | 11 | 31.4 | 1 | 7.1 | 4.4 |
| 0.196 | Usually bring up phlegm first thing in morning | 9 | 25.7 | 1 | 7.1 | 3.6 |
| 0.405 | Hay fever[c] | 7 | 21.2 | 1 | 7.1 | 3.0 |
| 0.659 | Cough[c, d] | 5 | 15.2 | 1 | 7.7 | 1.97 |

[a] Two-sided p-value based on Fisher's Exact Test
[b] PR = Prevalence Ratio
[c] For Hay fever and Cough, n = 33 for workers
[d] For Cough, n = 13 for controls

Table 6. Adjusted prevalence ratios for selected respiratory outcomes among greenhouse workers and controls using a modified Poisson regression approach.

| Symptom | Model* | Prevalence ratio | 95% CI | p-value |
|---|---|---|---|---|
| Chest ever wheezy or whistling | 1 | 2.00 | 0.68 – 5.92 | 0.21 |
| | 2 | 2.25 | 0.74 – 6.79 | 0.15 |
| Usually bring up phlegm | 1 | 4.53 | 0.63 – 32.51 | 0.13 |
| | 2 | 1.83 | 0.26 – 12.90 | 0.54 |
| Hay fever | 1 | 2.97 | 0.37 – 24.10 | 0.30 |
| | 2 | 2.12 | 0.24 – 18.89 | 0.50 |
| Cough | 1 | 1.97 | 0.25 – 15.28 | 0.52 |
| | 2 | 1.37 | 0.12 – 16.04 | 0.80 |

* Model 1: Unadjusted; Model 2: Adjusted for age, gender, and smoking status

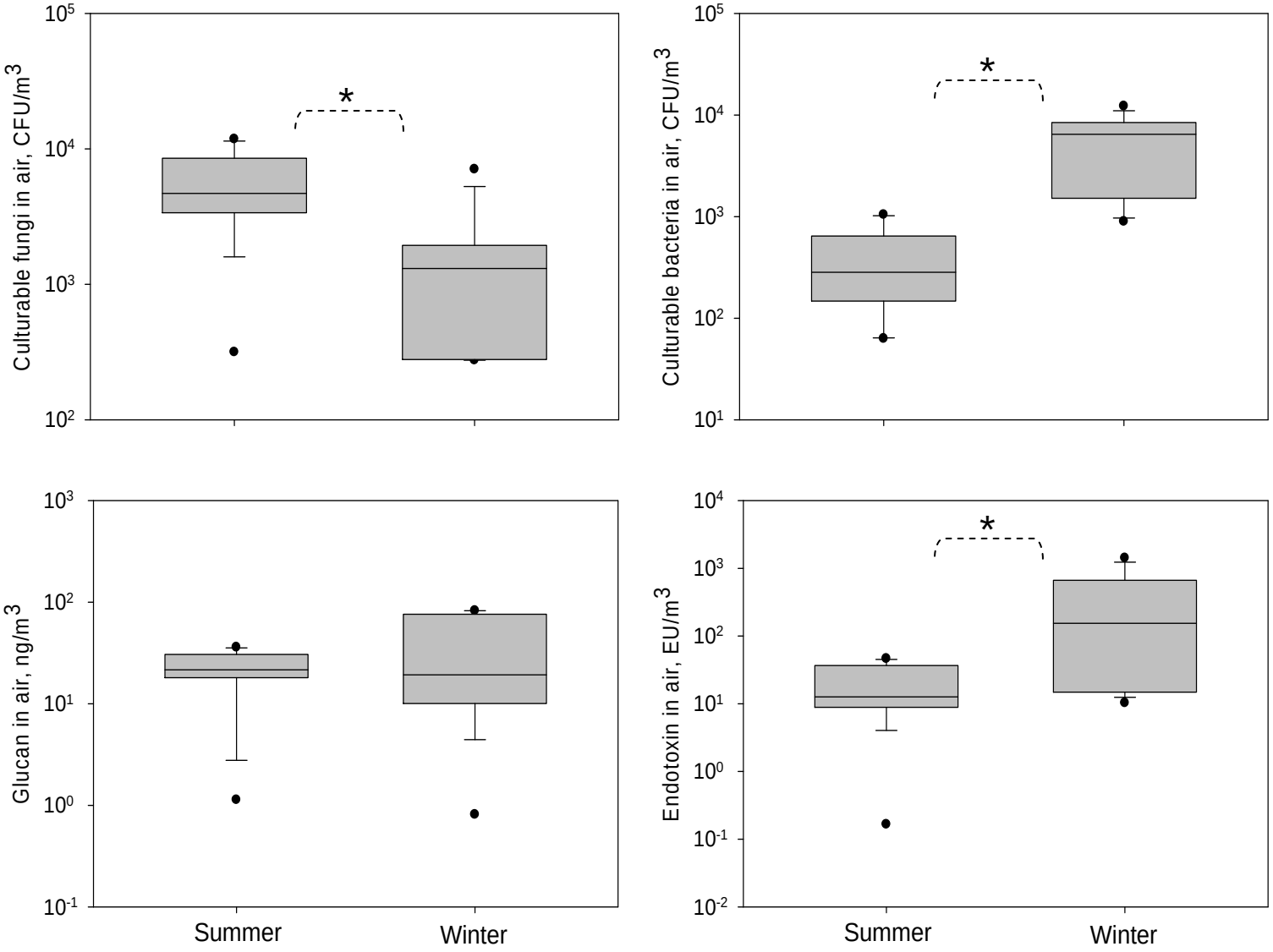



Figure 1. Seasonal variation of airborne fungi, bacteria, (1→3)-β-D-glucan, and endotoxin exposure levels in three Midwest greenhouses. The lower and upper boundaries of the box specify the 25th and 75th percentiles, respectively. The line within the box indicates the median and the whiskers above and below the box indicate the 95th and 5th percentiles, respectively. * Indicates statistically significant difference (paired t-test: $p < 0.05$).

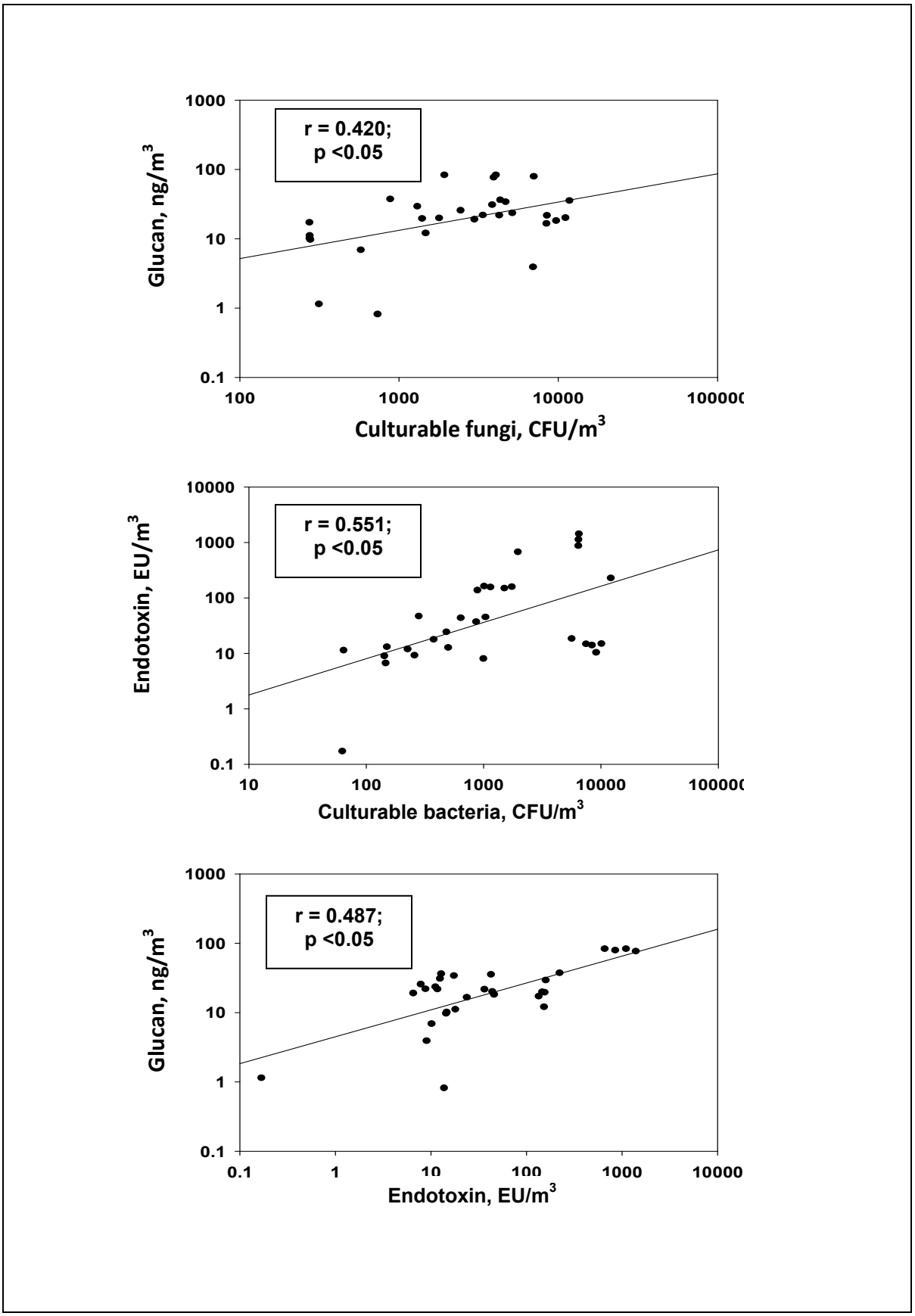
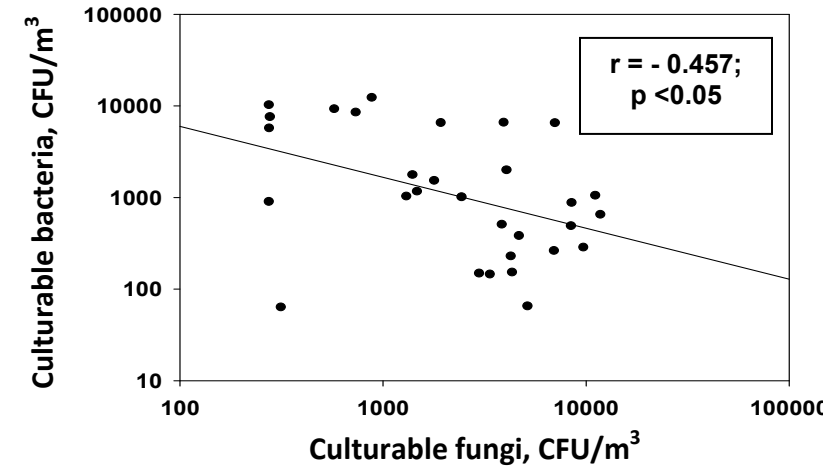
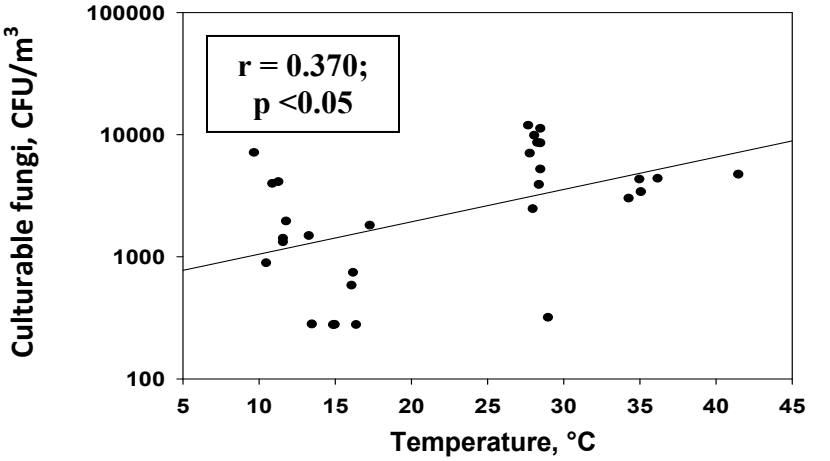



Figure 2a. Significant relationships ($p < 0.05$) between culturable bacteria, culturable fungi, (1→3)-β-D-glucan, and endotoxin exposure levels during winter and summer in three Midwest greenhouses.



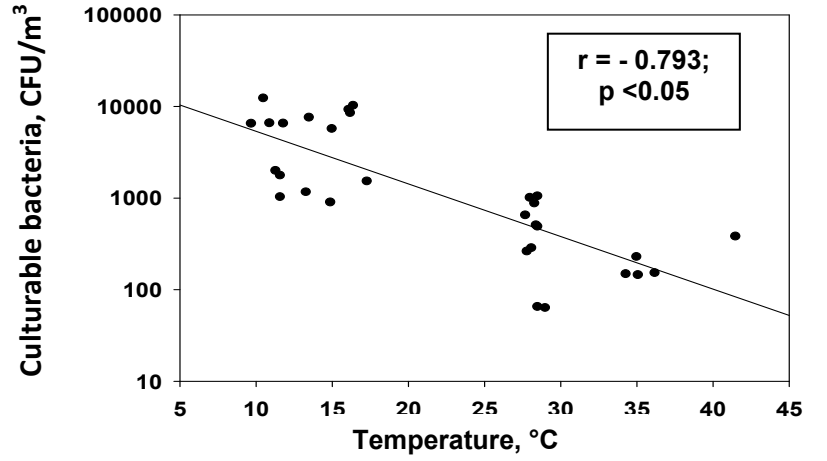
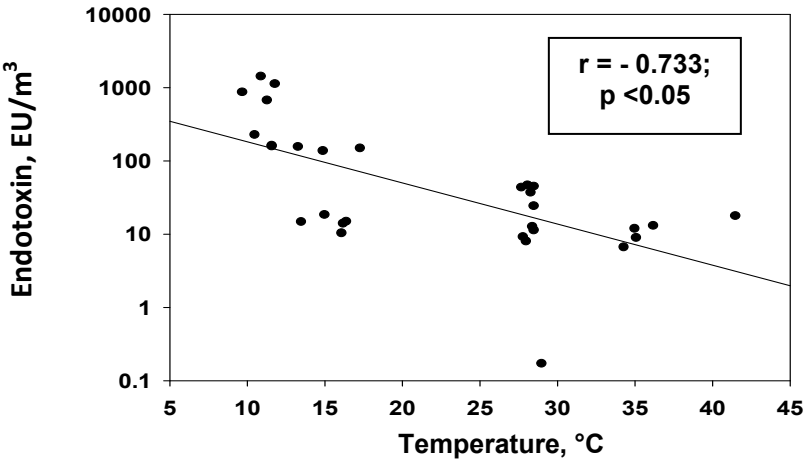
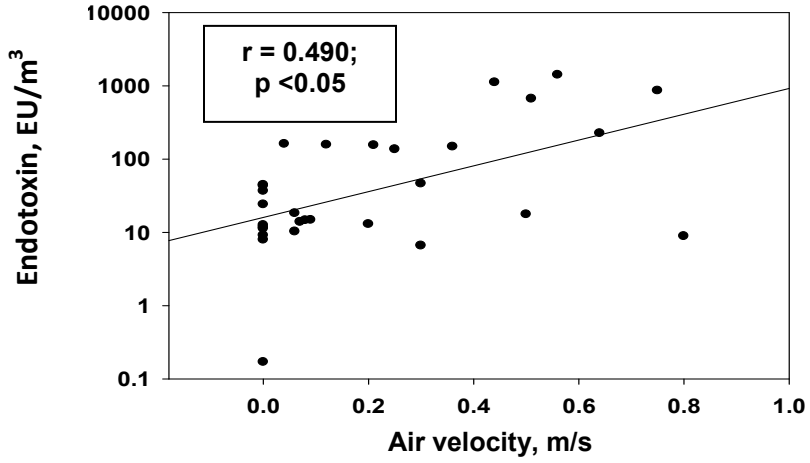




Figure 2b. Significant relationships ($p < 0.05$) between exposure variables during winter and summer in three Midwest greenhouses.



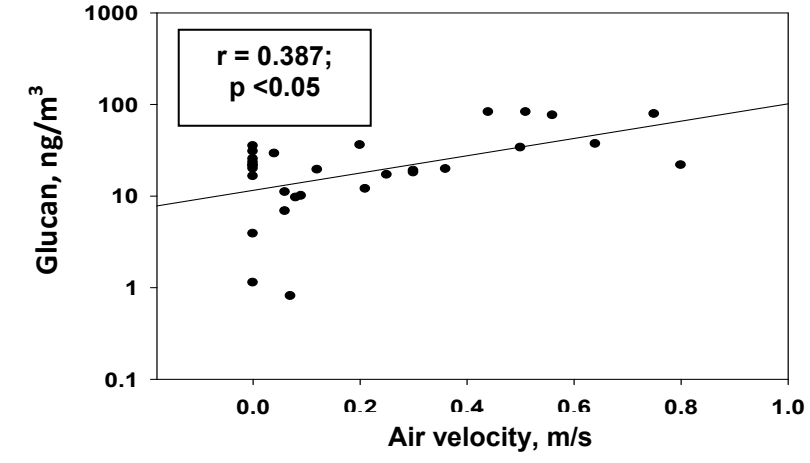




Figure 2c. Significant relationships ($p < 0.05$) between culturable bacteria, culturable fungi, (1→3)-β-D-glucan, and endotoxin exposure levels during winter and summer in three Midwest greenhouses.

# PUBLICATIONS

**Journal Article**

Adhikari A, Gupta J, Wilkins III JR, Olds RL, Indugula R, Cho,KJ, Li C, Yermakov M: [2010] Airborne microorganisms, endotoxin, and (1→3)-β-D-glucan exposure in greenhouses and assessment of respiratory symptoms among workers. Annals of Occupational Hygiene, in press.

**Proceeding**

Adhikari A, Olds RL, Gupta J, Wilkins III JR, Reponen T, Grinshpun SA, Indugula R, Cho KJ, Li C, Yermakov M: [2010] Airborne fungi, bacteria, actinomycetes, endotoxin, and (1→3)-β-D-glucan in Midwest greenhouses and respiratory symptoms among workers. Proc. of 110th General Meeting of the American Society for Microbiology; San Diego, California, Paper Q-897, May 22-27. (Available at: http://gm.asm.org /index.php/component/content/article/46-newsroom/180--exposures-to-inhalablemicroorganisms-and-workers-respiratory-disease-symptoms-in-greenhouse-confinements-session-086q-paper-q-897)

Program Director/Principal Investigator (Last, First, Middle): Adhikari, Atin

## Targeted/Planned Enrollment Table

**This report format should NOT be used for data collection from study participants.**

**Study Title:** Bioaerosols in Midwest Greenhouses and Respiratory Symptoms Among the Workers

**Total Planned Enrollment:** 60

| TARGETED/PLANNED ENROLLMENT: Number of Subjects | | | |
|---|---|---|---|
| **Ethnic Category** | **Females** | **Males** | **Total** |
| Hispanic or Latino | | | |
| Not Hispanic or Latino | | | |
| **Ethnic Category: Total of All Subjects *** | | | |
| **Racial Categories** | | | |
| American Indian/Alaska Native | 1 | | 1 |
| Asian | | | |
| Native Hawaiian or Other Pacific Islander | | | |
| Black or African American | | | |
| White | 24 | 24 | |
| **Racial Categories: Total of All Subjects *** | 25 | 24 | 49 |

* The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: Total of All Subjects."

## INCLUSION OF CHILDREN

Two children workers between the age of 18-21 years were participating in this project.

## MATERIALS AVAILABLE FOR OTHER INVESTIGATORS

The pilot data collected in this study can be utilized later by other investigators for future sample size calculations for a larger study on respiratory symptoms among greenhouse workers in the United States. Based on the observed differences in the prevalence of respiratory symptoms in this study, we estimated sample sizes required to achieve 80% power to detect a difference between exposed and non-exposed groups at the 5% level of significance for several respiratory symptoms/conditions. For example, total sample sizes of 187 (125 workers, 62 controls), 112 (75 workers and 37 controls), and 254 (169 workers and 85 controls) will be needed for the symptoms/conditions ‘wheeze’, ‘phlegm’, and ‘hay fever’, respectively. We have assumed a 2:1 worker to control ratio and have performed continuity correction to calculate the sample sizes.