

Airborne Microorganisms, Endotoxin, and (1 → 3)-β-D-Glucan Exposure in Greenhouses and Assessment of Respiratory Symptoms Among Workers

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Objectives: Greenhouse operations are an important sector of the horticulture industry, also known as the Green Industry. The objectives of this study were (i) to investigate exposure levels to airborne culturable fungi, bacteria (total culturable bacteria and actinomycetes), endotoxin, and (1 → 3)-β-D-glucan in three Midwest greenhouses during summer and winter using multiple exposure assessment methods; (ii) characterize the load of microorganisms on greenhouse floors and determine potential microbial source strengths of the floors for aerosolizing microbial biocontaminants, and (iii) to estimate the prevalence of rhinitis, wheezing, asthma, and other respiratory symptoms/conditions among greenhouse workers.

Methods: Stationary inhalable aerosol samples were collected from each greenhouse using But-ton 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 Limulus amoebocyte lysate assays. The prevalence of respiratory symptoms among greenhouse workers ($n = 35$) and control subjects (office workers; $n = 14$) was estimated with a standardized questionnaire.

Results and Conclusions: 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 – 10^3 EU m^{-3}), and (1 → 3)-β-D-glucan (10^1 – 10^2 ng m^{-3}). Seasonal variations were observed for some bioaerosol components. The prevalence of self-reported respiratory symptoms was generally higher among greenhouse workers compared to controls; however, the differences were not statistically significant, likely due to the relatively low statistical power of the study.

Keywords: allergens; bioaerosols; endotoxin; fungi; glucans; greenhouses; microorganisms; respiratory symptoms

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INTRODUCTION

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 US 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 [NIOSH (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 USA 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 1-s forced expiratory volume (FEV₁) and flow rates at 50% and 25% of the vital capacity (FEF₅₀ and FEF₂₅)] related to their working conditions in closed greenhouse spaces. In another collaborative study, scientists from the USA and Europe (Monsó *et al.*, 2003) investigated risk factors for respiratory symptoms in European and Californian farmers involved in various animal and crop farming 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 odds ratio 1.59, 95% confidence interval: 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 ~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). Unlike molds, endotoxin has also been recognized as a causative factor in the etiology of occupational lung diseases, including non-allergic asthma 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, studies showing associations between bioaerosol exposures and respiratory diseases among greenhouse workers are particularly lacking. Except for the studies of Monsó *et al.* (2002), Radon *et al.* (2002), Madsen (2006), and Madsen *et al.* (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 study 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 US Green Industry workers.

The specific aims of this study were

1. Investigate inhalable spatial exposure levels of airborne culturable fungi, bacteria (total culturable bacteria and actinomycetes), endotoxin, and (1→3)-β-D-glucan in three Midwestern greenhouses through air sampling.
2. Characterize the load of biological particles on greenhouse floors and determine potential microbial source strengths of the floors for aerosolizing biocontaminants through sampling with a microbial source tester and sterile cotton swabs.

3. Estimate the prevalence of rhinitis, wheezing, asthma, and other respiratory disease symptoms among greenhouse workers using a standardized questionnaire.

MATERIALS AND METHODS

Sampling sites

Three greenhouses in the Midwestern USA (Paris, KY; Lawrenceburg, IN; and Cincinnati, OH) were selected for our study. Our selection criteria were greenhouses should specialize in the production of ornamental plants and at least 10 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 study was conducted between the years 2008 and 2009. The job duties of the workers included: transplanting plants, which was done all day; watering plants, which was done two to 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. Common flowering plants in the greenhouses were *Zinnia*, *Petunia*, *Marigold*, *Celosia*, *Vinca*, *Begonia*, *Rose*, and *Impatiens* in summer and different varieties of *Pansy* (dynamite clear mix, delta true blue, violet, etc.) and *Viola* in winter. We also observed beddings of different varieties of plant transplants in winter. A specific compartment of 15.24–22.86 × 30.48–60.96 m 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 ~5 to 7 h per one work shift—four from the corners of the greenhouses and one from the center. Polycarbonate filters (porosity: 3.0 μm) were used in the Button samplers. This relatively large pore size was selected to avoid pressure drop in the pumps and a previous study reported that polycarbonate filter with 3.0-μm porosity demonstrated ~99 to 100% physical collection efficiency for bacteria (Burton *et al.*, 2007). 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). Altogether 30 samples (5 samples × 3 greenhouses × 2 seasons = 30 samples; $n = 30$) were collected. Additionally, three samples were collected from the major working locations (3 samples × 3 greenhouses × 2 seasons = 18 samples; $n = 18$) and one from the office area (control). One additional control outdoor sampling was performed exactly outside the greenhouses within a 5 m distance in the nearby ambient air [to assess indoor to outdoor (I/O) ratios for various biocontaminants]. Altogether six office area samples (1 sample × 3 greenhouses × 2 seasons) and six outdoor samples (1 sample × 3 greenhouses × 2 seasons) were collected. 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⁻¹ 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 were measured using thermohygrometer pens (Fisher Scientific, Pittsburgh, PA, USA) and air velocity using anemometers (TSI Inc., Shoreview, MN, USA).

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 American Industrial Hygiene Association (AIHA) protocol for surface and bulk sampling for viable fungi and bacteria (AIHA, 2005). In brief, floor surfaces (~1 cm²) were swabbed with wet sterile cotton wool-tipped sticks soaked with sterile 0.1% peptone with 0.01% Tween 80 and preserved in airtight sterile tubes. Altogether 30 surface samples were collected by sterile cotton swabs (5 samples × 3 greenhouses × 2 seasons = 30 samples; $n = 30$). Fungi, bacteria, and actinomycetes from these cotton swabs were extracted similarly as from the Button sampler filters 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; Górný *et al.*, 2002; Grinshpun *et al.*, 2002; Sivasubramani *et al.*, 2004a,b; 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⁻¹. The source tester was applied tightly to the floor surfaces (11 × 11 cm) for 15 min. 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 source tester samples were collected from each greenhouse and altogether 30 samples were collected (5 samples × 3 greenhouses × 2 seasons = 30 samples; *n* = 30).

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 min in a vortex mixer and then reshaking it in an ultrasonic cleaner for 15 min. 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 microliters (for fungi) or 100 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 at 50°C for 2 weeks for the development of actinomycetes colonies (Reponen *et al.*, 1998). The numbers of colonies on each plate (colony forming units or CFUs) were counted and converted into airborne concentration (colony forming units per cubic meter) or concentration on surfaces

(colony forming units per square meter). 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⁻¹ and sampling time as 6 h) was 35 CFU m⁻³ for Button sampler and 8264 CFU m⁻² for the microbial source tester. The corresponding LLODs for culturable bacteria and actinomycetes were 69 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 were 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 1 h. For source tester samples, the extracts were centrifuged at 7000 r.p.m. (5204 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 amoebocyte lysate (LAL) assay (Pyrochrome, Associates of Cape Cod, East Falmouth, MA, USA). Endotoxin concentrations in liquid extracts were converted into endotoxin units per cubic meter for air samples and endotoxin units per square meter for microbial source tester samples, which were derived from the air flow rates, sampling duration, and area sampled. The LLOD for endotoxin in suspension was 0.05 EU ml⁻¹.

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 nanograms per cubic meter for air samples and nanograms per square meter 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⁻¹.

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 greenhouse workers (exposed group; *n* = 35) and office workers (non-exposed group; *n* = 14) in three participating greenhouses and

neighboring greenhouses, which were also engaged in growing of flowering plants. 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 (GMs) and geometric standard deviations (GSDs) 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 nonparametric Spearman's test.

For the respiratory symptoms questionnaire data, descriptive statistics were generated and the exposed

and non-exposed groups were compared using Fisher's exact test for categorical variables and equality of means test for continuous variables. Prevalence ratios (PR) were estimated as measures of association. 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 (continuous variable), and smoking status (lifelong non-smoker, ex-smoker, and 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, USA). A two-tailed *P*-value that was <0.05 was defined as statistically significant.

RESULTS

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

GMs and GSDs for concentrations of inhalable culturable fungi and bacteria from three greenhouses (combined data are presented because all greenhouses were engaged in growing mostly common flowering plants) are presented in Table 1 (airborne) and Table 2 (aerosolizable from floors). Mean concentrations of inhalable culturable fungi in three greenhouses were 429–3584 CFU m⁻³ in winter and 3939–9947 CFU m⁻³ in summer from five simultaneous measurements with Button samplers. Additional three measurements near major work areas in greenhouses showed a mean concentration range of 2427–4597 CFU m⁻³ in summer and 846–10 535 CFU m⁻³ in winter. On the other hand, microbial source strength tester demonstrated aerosolizable culturable fungi from floor surfaces at the range of 6612–41 322 CFU m⁻² in summer and 14 876–16 529 CFU m⁻² in winter. Cotton swab samples showed a concentration of <LLOD to 500 CFU m⁻² in summer and <LLOD to 400 CFU m⁻² in winter. Air samples collected from office areas and nearby ambient air mostly demonstrated lower concentrations on

Table 1. Airborne culturable fungi, 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 Sampling season	Culturable fungi (CFU m ⁻³)			Culturable bacteria (CFU m ⁻³)			Endotoxin (EU m ⁻³)			(1→3)-β-D-glucan (ng m ⁻³)						
	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	4605 (2.43)	2886 (1.72)	1943 (1.49)	2889 (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)	4.4 (2.14)	17.3 (2.51)	12.9 (2.07)	20.1 (1.36)	18.9 (1.35)
Winter	1060 (2.89)	3685 (3.27)	345 (18.70)	>LLOD	3859 (2.55)	1451 (4.87)	2059 (4.07)	<LLOD	115.6 (5.60)	153.1 (19.81)	34.2 (6.34)	6.9 (3.09)	19.4 (3.42)	29.1 (29.89)	12.2 (3.10)	11.2 (2.05)

the order of <LLOD (all outdoor samples and one office area sample collected in winter were <LLOD) to 5904 CFU m⁻³.

Similar to fungi, mean concentrations of inhalable culturable bacteria in three greenhouses were 429–3584 CFU m⁻³ in winter and 3939–9947 CFU m⁻³ summer from five simultaneous measurements with Button samplers. Additional three measurements near major work areas in greenhouses showed a concentration range of 136–4160 CFU m⁻³ in summer and 188–4912 CFU m⁻³ in winter. On the other hand, microbial source strength tester data demonstrated a higher concentration range for aerosolizable culturable bacteria: 119 008–952 066 CFU m⁻² in summer and 164 353–409 917 CFU m⁻² in winter. 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. Air samples collected from office areas and nearby ambient air mostly demonstrated relatively lower concentrations on the order of <LLOD (in winter) to 5904 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 to 45 CFU m⁻³ in summer from five simultaneous measurements with Button Samplers. 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. All cotton swab samples collected during summer and winter demonstrated <LLOD values.

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

Temperature, relative humidity, and wind velocity recorded in three greenhouses are presented in Table 3. GMs and 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 three greenhouses were 14.4–855.2 EU m⁻³ in winter and 8.2–38.9 EU m⁻³ in summer from five simultaneous measurements with Button samplers. Additional three measurements near major work areas in greenhouses showed a concentration range of 2.7–5034.6 EU m⁻³ in winter and 6.5–80.2 EU m⁻³ in summer. Mean concentrations of aerosolizable endotoxin from floor surfaces ranged from 1297.2 to 154 148.8 EU m⁻² in summer and 2599.0

Table 2. Aerosolizable culturable fungi, 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 (<i>n</i> = 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)	1415.1 (27.17)	191.4 (17.76)
Winter	12 289 (2.12)	134 650 (2.98)	2653.0 (4.64)	2294.9 (2.01)

to 9018.2 EU m⁻² in winter. Cotton swab samples were not analyzed here. Samples collected from office areas showed a concentration range of 6.3–245.7 EU m⁻³ and samples collected from nearby ambient air showed a concentration range of 0.6–9.0 EU m⁻³.

On average, concentrations of inhalable (1 → 3)-β-D-glucan in three greenhouses ranged from 7.7 to 71.2 ng m⁻³ in winter and 16.9 to 26.4 ng m⁻³ in summer from five simultaneous measurements with Button samplers. Additional three measurements near major work areas in greenhouses showed a concentration range of 1.5–51 110.6 ng m⁻³ in winter and 5.0–55.8 ng m⁻³ in summer. Microbial source strength tester data demonstrated a mean concentration of aerosolizable (1 → 3)-β-D-glucan from floor surfaces as 1009.1–1801.8 ng m⁻² in summer and 1318.0 to 5369.9 ng m⁻² in winter. Cotton swab samples were not analyzed for (1 → 3)-β-D-glucan. Samples collected from office areas showed a concentration range of 4.6–42.4 ng m⁻³ and samples collected from nearby ambient air during winter ranged from 0.6 to 22.4 ng m⁻³.

Seasonal variation in bioaerosol 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 LLOD 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.

Indoor versus outdoor exposures to bioaerosols

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. 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 > 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). No significant correlations (*P* > 0.05) were found when microbial source tester data (aerosolizable biocontaminants 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.

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 ⁻¹)		
	Four corners and center (n = 15); GM (GSD)	Office area (n = 3); GM (GSD)	Nearby ambient air (n = 3); GM (GSD) ^a	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)

^aGM and GSD were calculated for summer data only; range is presented for winter data as one point was below 0°C.

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. This difference was statistically significant ($P < 0.05$; paired t -test). The relative humidity levels were 32.0–65.0% (mean ± SD = 54.8 ± 12.8%) during summer and 55.0–86.4% (mean ± SD = 66.4 ± 4.1%) during winter (no significant difference; $P > 0.05$; paired t -test). The air velocity in winter ranged from <LLOD to 0.8 m s⁻¹ (mean ± SD = 0.14 ± 0.24 m s⁻¹) and 0.06 to 0.75 m s⁻¹ (mean ± SD = 0.28 ± 0.24 m s⁻¹) in summer. 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 study subjects

All subjects, except an office employee (control subject) who self-identified as American Indian/Alaska Native, were Whites. The worker and the control groups did not differ significantly with respect to mean age in years (40.1; 44.5), proportion of males (57.1; 28.6), proportion of ever smokers (45.7; 35.7), and proportion of current smokers (17.1; 21.4). Other sociodemographic characteristics that included birthplace, education, household income, and anthropometric measures height and weight also did not differ significantly between groups (data not shown).

The prevalence of selected self-reported respiratory outcomes among the workers and the controls are given in Table 4. Prevalence estimates were generally higher among workers compared to controls. Although the crude/unadjusted PRs ranged from 1.2 to 4.4, the differences were not statistically significant based on Fisher's exact test. A modified Poisson regression analysis for unadjusted as well as adjusted estimates (Barros and Hirakata, 2003) also consistently showed PRs >1, though none of the comparisons reached statistical significance (data not shown). Also, none of respiratory outcomes were found to be significantly associated with the total number of lifetime hours worked in a greenhouse (data not shown).

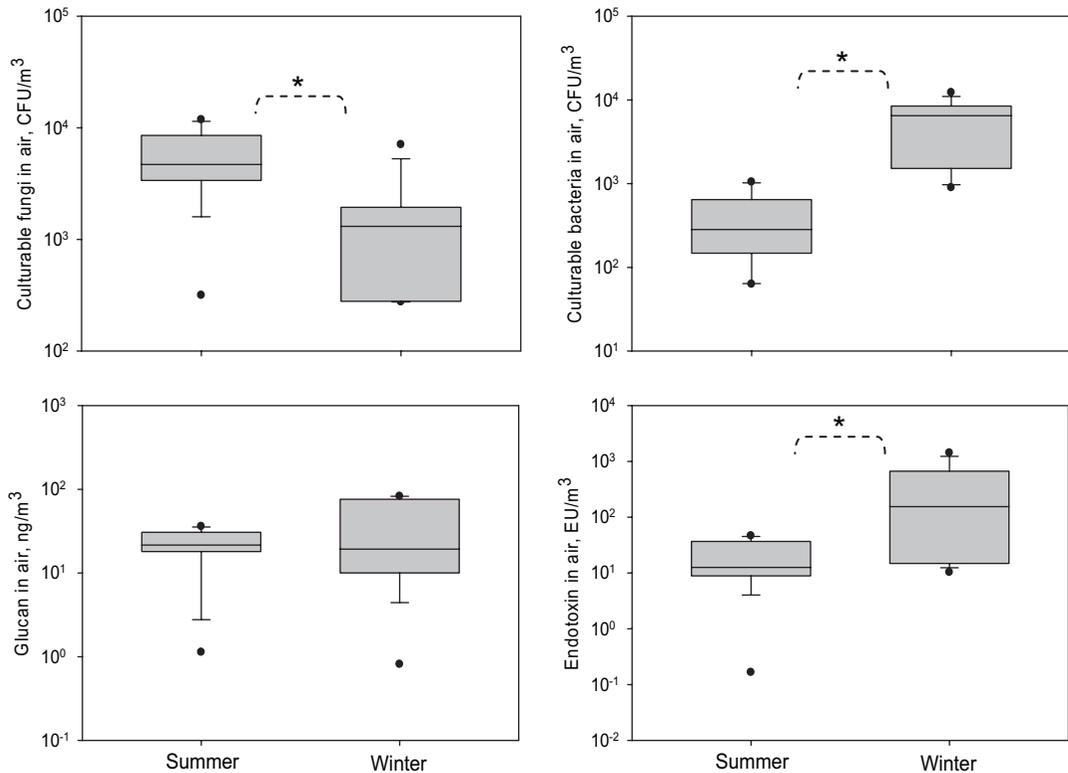


Fig. 1. Seasonal variation of airborne fungi, bacteria, (1→3)-β-D-glucan, and endotoxin exposure levels in three Midwest greenhouses. The lower and upper boundaries of the box specify the 25th and 75th percentiles, respectively. The line within the box indicates the median and the whiskers above and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

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 $<70 \mu\text{m}$, and there is no significant difference between the sampling efficiencies of the Button Sampler in the stationary and personal modes (Aizenberg *et al.*, 1998). Recently, Hansen *et al.* (2010), who measured bioaerosol expo-

sure in a tomato growing greenhouse during biopesticide applications, reported differences in bioaerosol exposures levels when measured with personal and stationary samplers. However, this study used three stationary samplers versus one personal sampler and a filtration based sampling method was used. Direct personal sampling of bioaerosols among the workers would certainly reflect the exposures more precisely; however, we found that the employers of the greenhouses were reluctant to allow workers to carry air sampling set-up during their various activities.

The present study demonstrates that workers employed in Midwestern greenhouses are exposed to elevated airborne 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 mean concentrations of airborne fungi in our study were comparable to the levels

Table 4. Prevalence of respiratory symptoms among greenhouse workers and controls

Two-sided <i>P</i> -value ^a	Symptom	Workers (<i>n</i> = 35)		Controls (<i>n</i> = 14)		Crude PR
		<i>n</i>	%	<i>n</i>	%	
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 ^b	7	21.2	1	7.1	3.0
0.659	Cough ^{b,c}	5	15.2	1	7.7	1.97

^aTwo-sided *P*-value based on Fisher's exact test.

^bFor hay fever and cough, *n* = 33 for workers.

^cFor cough, *n* = 13 for controls.

previously reported from greenhouses in Spain (Monsó *et al.*, 2002; Radon *et al.*, 2002). The mean concentrations of airborne fungi in our study were comparable to the levels of airborne fungi previously reported from greenhouses in Spain. However, we have to keep in mind that filter-based personal sampling methods were used by the previous researchers, which collected total airborne fungi, whereas we collected the inhalable fraction of total airborne fungi using the Button Samplers. The levels were also comparable to the culturable fungal concentrations observed in animal confinements (swine, poultry, and dairy; Lee *et al.*, 2006a) but lower than in grain harvesting places in Ohio (Lee *et al.*, 2006a). 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 collected from the same adjacent floor areas 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 culturable bacteria found in our study were comparable to greenhouses in Spain (Monsó *et al.*, 2002; Radon *et al.*, 2002). The levels were also comparable to dairies and poultry farms but lower than swine farms in Ohio (Lee *et al.*, 2006a). 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.

The mean concentrations 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). Monsó (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 office and home environments (Dong and Yao, 2009; Reponen *et al.*, 2010).

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 US greenhouses. Second, a recent 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, airborne endotoxin in greenhouses may have clinical importance in terms of respiratory health of workers.

The mean concentrations of inhalable (1→3)-β-D-glucan in the greenhouses in our study were relatively high (7.67–71.27 ng m⁻³ in winter and 16.87–26.40 ng m⁻³) with respect to previous measurements in home environments. For example, the GM of airborne (1→3)-β-D-glucan concentrations measured in non-moldy homes in Cincinnati was much lower: 0.92 ng m⁻³ (Lee *et al.*, 2006b), 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 FEV₁. 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.

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 $\gg 1$ 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 not always consistent. 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 office areas being located adjacent to greenhouses, movement of workers from work areas to office areas, and other sources of microbial contamination in offices. 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 found significant positive correlations between culturable fungi and bacteria with (1→3)-β-D-glucan and endotoxin, 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 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 between culturable bacteria and fungi is interesting to note, which could be further explored in a large-scale study. Various internal sources of microorganisms can be responsible for this disparity. It may be due to the opposite effect of temperature in the growth of certain fungal and bacterial species as indicated by positive and negative correlation coefficients, respectively. We found significant negative correlation between endotoxin and temperature 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 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 biocontaminants 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 sug-

gests 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 USA. 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 and 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 (Fleiss, 1981). 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 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 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 small sample size and relatively low statistical power of the study.

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REFERENCES

- Adhikari A, Jung J, Reponen T *et al.* (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 *et al.* (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*; 408: 5489–98.
- Adhikari A, Martuzevicius D, Reponen T *et al.* (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 *et al.* (2004) Assessment of human exposure to airborne fungi in agricultural confinements: personal inhalable sampling versus stationary sampling. *Ann Agric Environ Med*; 11: 269–77.
- Aizenberg V, Biding E, Grinshpun SA *et al.* (1998) Air flow and particle velocities near a personal aerosol sampler with curved, porous aerosol sampling surface. *Aerosol Sci Technol*; 28: 247–58.
- AIHA (2005) Viable fungi and bacteria in air, bulk, and surface samples and Legionella. In Hung LL, Miller JD and Dillon HK, editors. *Field guide for the determination of biological contaminants in environmental samples*. 2nd edn. Fairfax, VA: American Industrial Hygiene Association. pp. 93–140.
- 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.
- Burge H. (1990) Bioaerosols: prevalence and health effects in the indoor environment. *J Allergy Clin Immunol*; 86: 687–701.
- Burton NC, Grinshpun SA, Reponen T. (2007) Physical collection efficiency of filter materials for bacteria and viruses. *Ann Occup Hyg*; 51: 143–51.
- Crawford C, Reponen T, Lee T *et al.* (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 *et al.* (2006) Validation of an asthma questionnaire for use in healthcare workers. *Occup Environ Med*; 63: 173–9.
- Dong S, Yao M. (2009) Exposure assessment in Beijing, China: biological agents, ultrafine particles, and lead. *Environ Monit Assess*; 170: 331–43.
- Douwes J, Thorne P, Pearce N *et al.* (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.
- Fleiss JL. (1981) *Statistical methods for rates and proportions*. 2nd edn. New York, NY: John Wiley & Sons, chapter 3.
- Górny RL, Reponen T, Willeke K *et al.* (2002) Fungal fragments as indoor biocontaminants. *Appl Environ Microbiol*; 68: 3522–31.
- Grinshpun SA, Górny RL, Reponen T *et al.* (2002) New method for assessment of potential spore aerosolization from contaminated surfaces. In Chiu-Sen Wang, editor. *Proceedings of the Sixth International Aerosol Conference*. Taipei, Taiwan: The Chinese Association for Aerosol Research, September 8–13. Vol. 2. Taipei, Taiwan. pp. 767–8.
- Hall CR, Hodges AW, Haydu JJ. (2005) Economic impacts of the green industry in the United States. Available at [http://www.ufe.org/files/pubs/EconomicImpactsoftheUSGreen%20Industr\(NUCFACfinalreport\).pdf](http://www.ufe.org/files/pubs/EconomicImpactsoftheUSGreen%20Industr(NUCFACfinalreport).pdf). Accessed 14 July 2010.
- Hansen VM, Winding A, Madsen AM. (2010) Exposure to bioaerosols during the growth season of tomatoes in an organic greenhouse using *Supresivit (Trichoderma harzianum)* and *Mycostop (Streptomyces griseoviridis)*. *Appl Environ Microbiol*; 76: 5874–81.
- Iossifova Y, Reponen T, Bernstein D *et al.* (2007) House dust (1→3)-β-D-glucan and wheezing in infants. *Allergy*; 62: 504–13.
- Krysinska-Traczyk E, Skorska C, Prazmo Z *et al.* (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 *et al.* (2006a) Personal exposure to airborne dust and microorganisms in agricultural environments. *J Occup Environ Hyg*; 3: 118–30.
- Lee T, Grinshpun SA, Kim KY *et al.* (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.
- 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 *et al.* (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 Opin Pulm Med*; 10: 147–50.
- Monsó E, Magarolas R, Badorrey I *et al.* (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 *et al.* (2003) Region-related risk factors for respiratory symptoms in European and Californian farmers. *Eur Respir J*; 21: 323–31.
- NIOSH. (1995) *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 14 July 2010.
- Niemeier RT, Sivasubramani SK, Reponen T *et al.* (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 *et al.* (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, Tielens M *et al.* (2005) Endotoxin exposure and atopic sensitization in adult pig farmers. *J Allergy Clin Immunol*; 115: 797–802.
- Radon K, Danuser B, Iversen M *et al.* (2002) Air contaminants in different European farming environments. *Ann Agric Environ Med*; 9: 41–8.
- Reponen TA, Gazonko SV, Grinshpun SA *et al.* (1998) Characteristics of airborne actinomycete spores. *Appl Environ Microbiol*; 64: 3807–12.

- Reponen T, Singh U, Schaffer C *et al.* (2010) Visually observed mold and moldy odor versus quantitatively measured microbial exposure in homes. *Sci Total Environ*; 408: 5565–74.
- 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 *et al.* (1999) (1→3)-beta-D-glucan may contribute to pollen sensitivity. *Clin Exp Immunol*; 115: 383–4.
- Rylander R, Norrhall M, Engdahl U *et al.* (1998) Airways inflammation, atopy, and (1→3)-beta-D-glucan exposures in two schools. *Am J Respir Crit Care Med*; 158: 1685–7.
- Schram-Bijkerk D, Doekes G, Douwes J *et al.* (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 *et al.* (2008) Aerosolization of particulate (1→3)-β-D-glucan from moldy materials. *Appl Environ Microbiol*; 74: 585–93.
- Simpson A, John SL, Jury F *et al.* (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 *et al.* (2004a) Assessment of the aerosolization potential for fungal spores in moldy homes. *Indoor Air*; 14: 405–12.
- Sivasubramani SK, Niemeier RT, Reponen T *et al.* (2004b) Fungal spore source strength tester: laboratory evaluation of a new concept. *Sci Total Environ*; 329: 75–86.
- Skorska C, Sitkowska J, Krysinska-Traczyk E *et al.* (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 *et al.* (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.
- Zuskin E, Schachter EN, Mustajbegovic J. (1993) Respiratory function in greenhouse workers. *Int Arch Occup Environ Health*; 64: 521–6.