Science of the Total Environment 466–467 (2014) 725–732

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Size-selective assessment of agricultural workers' personal exposure to airborne fungi and fungal fragments

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HIGHLIGHTS

- Farmers' exposure to airborne fungal contaminants was size-selectively assessed.
- Most of the collected airborne fungal contaminants were larger than 1.8 μm.
- A significant correlation between airborne fungi and (1 → 3)-β-D-glucan was found.

ARTICLE INFO

Article history:
Received 22 March 2013
Received in revised form 23 July 2013
Accepted 30 July 2013
Available online 25 August 2013

Editor: Lidia Morawska

Keywords:
Size
Fungi
Fungal fragments
(1 → 3)-β-D-glucan
Agricultural farms

ABSTRACT

Fungi are ubiquitous agents that cause human respiratory diseases. Very few studies have size-selectively assessed farmers' exposure to fungi and fungal fragments in agricultural settings. In this study, a two-stage bio-aerosol cyclone personal sampler was employed to collect airborne fungi and fungal fragments size-selectively at corn, swine, poultry, and mushroom farms. The collected air samples were analyzed for culturable fungi, fungal spores, viable fungi and (1 → 3)-β-D-glucan. The results show that the median concentrations ranged from 3.2×10^5 to 1.3×10^8 spores/m^3 for total fungal spores, from 1.3×10^5 to 5.1×10^7 spores/m^3 for total viable fungi, from 1.9×10^3 to 1.5×10^7 CFU/m^3 for total culturable fungi, and from 4.3×10^3 to 2.4×10^6 pg/m^3 for total (1 → 3)-β-D-glucan. The aerodynamic sizes of most of the collected fungal contaminants were larger than 1.8 μm. Total (1 → 3)-β-D-glucan significantly correlated with total fungal spores ($r = 0.65$, $p < 0.001$), total viable fungi ($r = 0.68$, $p < 0.001$) and total culturable fungi ($r = 0.72$, $p < 0.001$). Total (1 → 3)-β-D-glucan significantly correlated with *Aspergillus*/*Penicillium*, *Alternaria*, and *Cladosporium*. *Alternaria* and *Botrytis* were also found to highly correlate with (1 → 3)-β-D-glucan at the size <1 μm, which was less than the expected spore sizes (the mean measured aerodynamic sizes were 18.5 μm for *Alternaria* and 6.1 μm for *Botrytis*); therefore, *Alternaria* and *Botrytis* might release small fragments that could enter the deep lung and cause respiratory diseases.

1. Introduction

Previous studies have shown that exposure to dust and fungi can induce respiratory diseases in agricultural workers (Dosman et al., 2004; Hameed and Khodr, 2001; Radon et al., 2002). As many as 80 out of 69,000 known fungal species have been recognized as allergens, which are associated with IgE-induced allergic respiratory diseases (Horner et al., 1995). People exposed to fungi are at high risk for asthma and respiratory diseases (Bush et al., 2006; Crimi et al., 2001; Edwards et al., 2012). Both secondary metabolites (e.g. mycotoxins) and components of fungal cell walls (e.g. (1 → 3)-β-D-glucan) have been known to induce toxic or immunologic reactions (Levetin, 1995; Husman, 1996; Robbins et al., 2000).

Humid and warm conditions favor fungal growth when sufficient nutrients are present. Taiwan is a small island located in the sub-tropical climatic zone. The weather is warm and humid and suitable for fungal growth. In addition, agricultural production is one of the major industries of Taiwan. According to the Directorate-General of Budget, Accounting and Statistics, about 3 million people out of 23 million residents live and work in agricultural environments in Taiwan (Taiwan Directorate-General of Budget, 2013). Many farming activities, including harvesting, plowing, soil preparation, seeding, and animal feeding are conducted in these agricultural environments. Lacey and Dutkiewicz (1994) found that significant numbers of airborne fungi originate from plant surfaces, hay and grain storage, and compost. Fungi-contaminated materials are often disturbed, resulting in release of fungal contaminants in the air when these farming activities are performed. Previous studies have shown that agricultural workers are at high risk of exposure to microorganisms (Lee et al., 2006; Lis et al., 2008; Molocznik, 2002; Radon et al., 2002). High microbial concentrations in swine farms were also reported

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http://dx.doi.org/10.1016/j.scitotenv.2013.07.104

in Taiwan (Chang et al., 2001). These publications suggest that research on farmers' respiratory diseases should examine Taiwanese farmers' exposure to fungal contaminants.

Most airborne fungi are about 2–10 μm in aerodynamic diameter (Reponen et al., 2001). Li and Kuo (1993) found that about 70–85% of indoor fungal particles found in Taiwan are in the inhalable size range. Lis et al. (2008) reported that on average, about 73% of fungal aerosols found in the air at agricultural facilities were in the respirable size range. In addition to fungal spores, many large hyphal fragments (Glikson et al., 1995; Li and Kendrick, 1995) and submicron fungal fragments (30 nm–1 μm) (Cho et al., 2005) comprise a large portion of total airborne fungal particles. These studies also reported that the number of fungal fragments is always higher than that of fungal spores. Most studies investigating the association between respiratory symptoms and concentration of fungal spores have found only weak associations or no association (Cooley et al., 1998; Sorenson, 1999). Chew et al. (2003) found that the airborne concentrations of fungal spores are not necessarily greater in mold-contaminated buildings, which indicates that measurement of airborne fungal spores is not an adequate method to assess fungal exposure.

Green et al. (2005) used halogen immunoassay to investigate the characteristics of allergic fungal genera and found that the immunostaining is localized at the germinated hyphal tips, septal junctions, basal regions, and the outer periphery of the conidia as well as around the entire conidia. In addition to commonly recognized species, airborne hyphae, fragmented conidia, and the conidia of a much more diverse range of genera are found as airborne allergens as well. Due to large quantity, small size, and immunoreactive potential of fungal fragments, the potential health effects of fungal fragments on the human body might be even worse than those of fungal spores. An epidemiological study shows that the association of allergic severity increases when the concentrations of fungal hyphae and spores are both included in the investigation (Delfino et al., 1997). This shows that both fungal spores and fragments should be considered for exposure assessment of airborne fungi.

Systematic investigations require an indicator for both fungal spores and fragments. About 47–60% of fungal cell walls consist of (1 → 3)-β-D-glucan in dry weight (Young and Castranova, 2005). Analyzing (1 → 3)-β-D-glucan is less time-consuming and labor-intensive than cultivation or microscopic counting of fungal spores, and it is commonly used as an indicator to evaluate human exposure to fungi (Douwes et al., 2006; Iossifova et al., 2008). Some scholars have suggested that (1 → 3)-β-D-glucan might be associated with airway inflammation and symptoms (Douwes, 2005); it is also found both in the fragment and spore size ranges (Seo et al., 2009). Therefore, we used (1 → 3)-β-D-glucan as an indicator to assess exposure to fungal spores and fungal fragments.

Rautiala et al. (1998) found that the concentrations of airborne allergens collected by stationary samplers are lower than those collected by personal samplers. Personal samplers provide more comprehensive data than stationary samplers. The present work used personal samplers to measure real farmers' exposure levels during farming activities. A two-stage bio-aerosol cyclone sampler was developed to collect fungal contaminants into three size fractions: <1.0 μm, 1–1.8 μm, and >1.8 μm (Lindsley et al., 2006). In contrast to many previous studies done by collecting fungal particles without size selection, we used this two-stage bio-aerosol cyclone personal sampler to collect fungal particles size-selectively to evaluate farmers' levels of exposure to these fungal contaminants. The associations of (1 → 3)-β-D-glucan with fungal spores and fungal fragments were investigated; the results could be used as a reference for future epidemiological studies on the health effects of fungal exposure in Taiwan.

2. Materials and methods

2.1. Personal sampling system

Field samples were collected using a personal sampling system (Lee et al., 2005, 2006), which was modified by connecting it to a two-stage bio-aerosol cyclone sampler (model BC221). This sampler consists of two screw-top 1.5 mL microcentrifuge tubes (Model 506–624, Fisher Scientific, USA) and a 37-mm filter holder with a 0.8 μm polycarbonate filter (Millipore Inc., Ireland). At an air flow rate of 3.5 L/min, the 50% cut-off diameters of the first and second tubes are 1.8 and 1.0 μm, respectively. The filter behind the two tubes is used to collect sample particles smaller than 1.0 μm. The size fractions of <1.0 μm, 1–1.8 μm, and >1.8 μm respectively represent fungal fragments, a mixture of fungal fragments and spores, and fungal spores. The aerosol collection performance of the sampler was presented by Lindsley et al. (2006). During sampling, airborne fungal spores and fragments were simultaneously sampled through the sampling probe and drawn through Tygon tubing to a two-stage bio-aerosol cyclone sampler. The collected samples were analyzed for fungal spores by microscopic counting, for culturable fungi by cultivation, and for (1 → 3)-β-D-glucan by Limulus Amebocyte Lysate (LAL) assay, as described below.

2.2. Description of agricultural farms

Four farms were included in this study: two types of animal confinements (swine and poultry), one corn farm, and one mushroom cultivation farm. The characteristics of these agricultural environments and farming activities are summarized in Table 1. The field tests were conducted during the following farming activities: animal feeding, routine examination and floor cleaning on a swine farm, egg collection and routine examination of facilities on a poultry farm, grain harvesting and routine investigation in a corn field, and routine examination of mushroom growth, mushroom harvesting, and waste handling on a mushroom farm. Each sampling lasted for about 60 min and took as much

Table 1
Environmental characteristics of agricultural farms.

Farm types	Sampling date	n[a]	Site dimensions	T (°C)[b]	RH (%)[c]	Farming activity
Corn	2010.3	6	110 m × 60 m	25–35	43–74	Grain harvesting, weeding, and routine investigation
Swine	2010.5	6	100 m × 58 m	28–32	51–73	Animal feeding, routine examination, vaccination and floor cleaning
Poultry	2010.7	6	72 m × 62 m	32–35	52–61	Animal feeding, egg collection and routine examination
Mushroom 1st[d]	2010.6	6	140 m × 50 m	28–33	50–69	Routine examination, mushroom harvesting, and handling of waste mushroom growth bags
Mushroom 2nd	2010.7	6	140 m × 50 m	29–32	57–60	Routine examination, and handling of waste mushroom growth bags
Mushroom 3rd	2010.8	6	140 m × 50 m	30–33	61–69	Routine examination, and handling of waste mushroom growth bags
Mushroom 4th	2010.8	6	140 m × 50 m	29–32	71–78	Routine examination, and handling of waste mushroom growth bags

[a] n = number of field samples.
[b] T: Temperature measured during sampling.
[c] RH: Relative humidity measured during sampling.
[d] 1st: First time sampling in the mushroom cultivation farm.