New Field-Compatible Method for Collection and Analysis of β-glucan in Fungal Fragments

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INTRODUCTION

Several epidemiological studies have shown an association between dampness or visible mold and respiratory illness of humans. However, no clear cause-and-effect relationship has yet been established with fungal spore-targeted exposure assessment. In our previous laboratory studies (Górny et al. 2002; Cho et al., 2005), we found that large quantities of small fungal fragments are released together with intact spores from contaminated surfaces. We hypothesize that submicrometer sized fungal fragments may contribute to the adverse health effects. The lack of suitable sampling and analysis methods, however, has hindered the exposure assessment to fungal fragments. To fill this gap, we have developed a field-compatible sampling and analysis protocol for fungal fragments and field-tested it in outdoor and indoor environments.

MATERIAL AND METHODS

The sampling system consists of two sharp-cut cyclones ($PM_{2.5}$ and PM_1) and an after-filter that separates the aerosolized fungal propagules into three size fractions: 1. >2.5 μm (spores); 2. 1-2.5 μm (combination of spores and fragments), and 3. <1 μm (submicrometer fragments). Two portable optical particle counters and two fine particle counters are utilized to measure the total number of collected spores and fragments into each size fraction.

As fragments cannot be analyzed by traditional microbiological methods, such as cultivation and microscopic counting, we have adopted a method for analyzing β-(1→3)-*D*-glucan as a surrogate for total fungal biomass. The analysis was performed using the kinetic chromogenic *Limulus* amebocyte lysate assay (LAL). Microscopic counting was used to confirm the absence of intact spores in the submicrometer size fraction.

Monodisperse PSL particles (0.54, 1.79, and 3.94 μm) and fungal propagules aerosolized from pure cultures of *Aspergillus versicolor* and *Stachybotrys chartarum* were used in the laboratory testing. Field-testing was performed in two environments: the outdoor air in Cincinnati and inside a mold-contaminated home in New Orleans.

RESULTS

Tests with PSL particles confirmed that the collection efficiency curves for the two cyclones had relatively sharp cut-sizes of 1.05 and 2.25 μm. Laboratory tests with *A. versicolor* and *C. chartarum* showed that the submicrometer sample did not contain any intact spores if the total number of particles collected into the PM1 cyclone was kept below $8x10^5$. This particle number was used as a threshold when estimating the optimum sampling time for the field samples allowing us to collect sufficiently high biomass of pure fungal fragments for β-glucan analysis.

B-glucan concentrations in the indoor air of a flooded home in New Orleans were about 10 times higher than those measured in the outdoor air in Cincinnati (Table 1). The ratio of the β-glucan concentration in the submicrometer size fraction to that in the spore size fraction ranged from 0.04 to 0.16.

Table 1. Concentration (average ± standard deviation) of β-glucan in fragment and spore size fractions in field samples.

Sampling site	β-glucan (pg/m^3)		β-glucan ratio*
	In submicro-meter fragments	In spores	
Outdoor Air	39±17	561±586	0.12±0.07
Indoor Air in New Orleans	371±237	6016±403	0.06±0.04

*β-glucan in submicrometer fragments / β-glucan in spores

CONCLUSIONS

The results indicate that a considerable amount of fungal biomass is below the size range of intact fungal spores. The new sampling method is a promising tool for collecting fungal fragments and spores in future field studies.

Keywords: *Fungi; Bioaerosol detection and identification; Bioaerosols and health effects*

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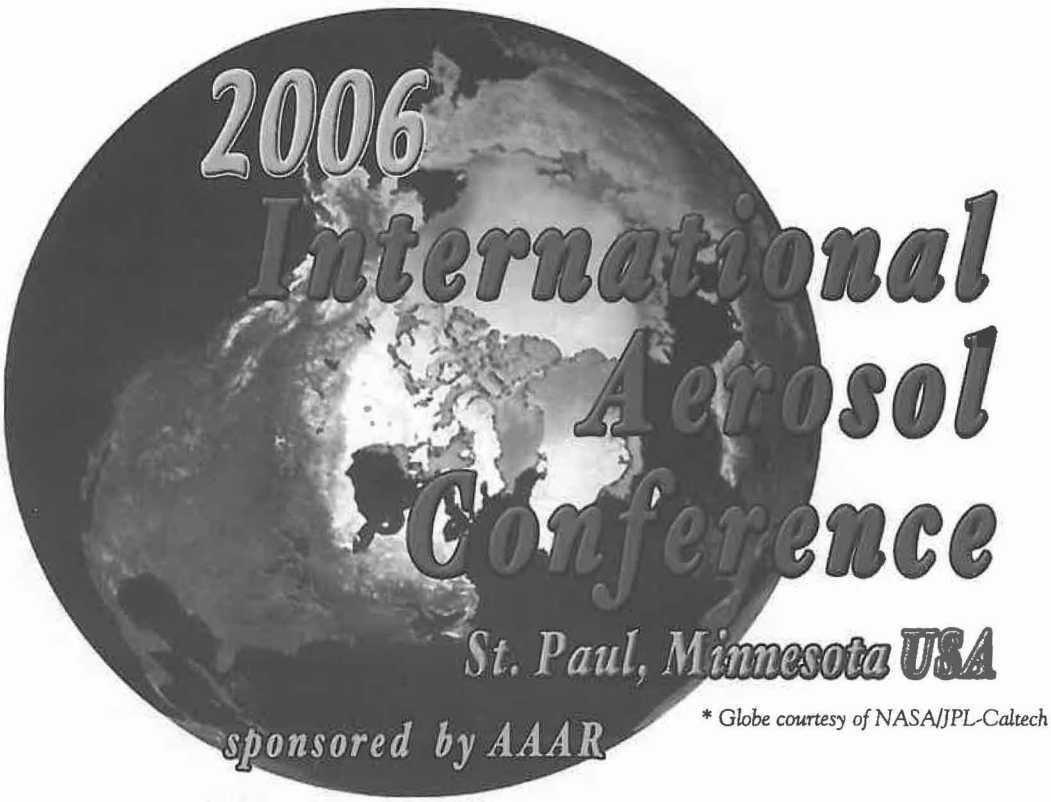

* Globe courtesy of NASA/JPL-Caltech

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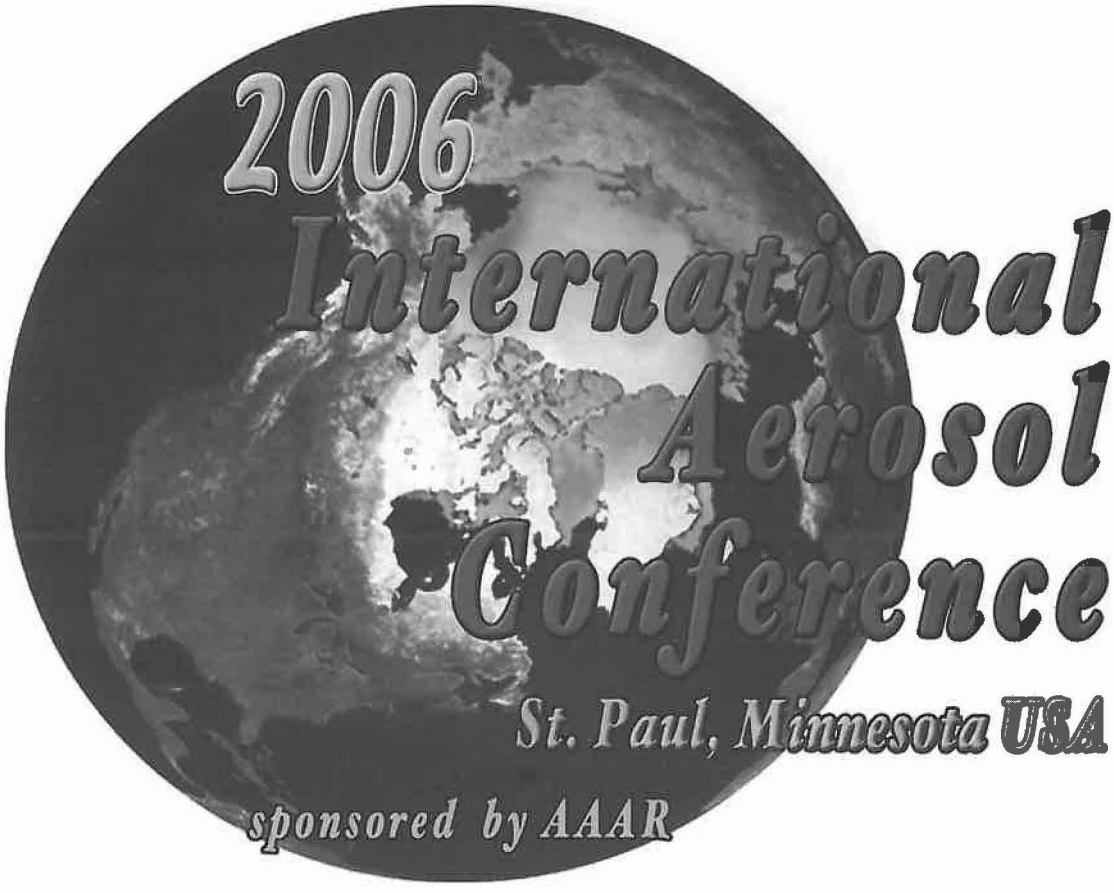

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