

Association between fungal spore exposure in inner-city schools and asthma morbidity



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ABSTRACT

Background: Home fungus exposures may be associated with development or worsening of asthma. Little is known about the effects of school/classroom fungus exposures on asthma morbidity in students.

Objective: To evaluate the association of school-based fungus exposures on asthma symptoms in both fungus-sensitized and nonsensitized students with asthma.

Methods: In this prospective study, 280 children with asthma from 37 inner-city schools were phenotypically characterized at baseline and followed-up for 1 year. Fungal spores were collected by using a Burkard air sampler twice during the school year. Clinical outcomes were evaluated throughout the school year and linked to classroom-specific airborne spore sampling. The primary outcome was days with asthma symptoms per 2-week period.

Results: Fungal spores were present in all classroom samples. The geometric mean of the total fungi was 316.9 spores/m³ and ranged from 15.0 to 59,345.7 spores/m³. There was variability in total fungus quantity between schools and classrooms within the same school. Mitosporales were the most commonly detected fungal grouping. Investigation of the individual mitosporales revealed that exposure to *Alternaria* was significantly associated with asthma symptom days in students sensitized to *Alternaria* (OR = 3.61, CI = 1.34–9.76, *P* = .01), but not in children not sensitized to *Alternaria* (OR = 1.04, CI = 0.72–1.49, *P* = .85). Students sensitized to *Alternaria* and exposed to high levels (≥75th percentile exposure) had 3.2 more symptom days per 2-week period as compared with students sensitized but exposed to lower levels.

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Conclusion: Children with asthma who are sensitized to *Alternaria* and exposed to this fungus in their classroom may have significantly more days with asthma symptoms than those who were sensitized and not exposed. Clinical Trial Registration: [Clinicaltrials.gov](https://clinicaltrials.gov) NCT01756391.

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Introduction

Asthma is the most common chronic disease among children. Poorly controlled asthma can cause disturbed sleep, limited activity, and missed school days. The environment is 1 of many factors that influence asthma morbidity.^{1,2} Several studies and meta-analyses have recognized that home fungus exposure may be associated with development or worsening of asthma.^{3–9} Children spend a large portion of their day in school; however, few studies have provided a comprehensive assessment of fungus in the school classroom and the possible effects on students with asthma.^{10–13} We have previously shown that inner-city school classrooms have highly diverse fungal populations and significant intra-classroom variation.¹⁴ The objective of this study was to evaluate the association of school-based fungal spore exposures on asthma symptom days in children with asthma.

Methods

Study Population

The School Inner City Asthma Study is a single-center longitudinal prospective study of a cohort of children with persistent asthma who attended schools in the Northeast United States from 2008 to 2013. It was designed to evaluate the role of indoor allergen and toxin exposures specific to the inner-city classroom environment and asthma morbidity. The study design has been previously reported.¹⁵ Briefly, inclusion criteria consisted of a history of physician-diagnosed asthma and current symptoms of cough, wheezing, shortness of breath, or whistling in the chest in the past 12 months; daily controller use; or unscheduled medical visits for asthma in the past year. The study population was made up of children, aged 4 to 13 years, attending an elementary school where permission for environmental sampling was obtained. Children were excluded from the study if they had a chronic lung disease other than asthma and cardiovascular disease. The study was approved by the Boston Children's Hospital institutional review board and the participating schools.

Each school year, approximately 75 students were recruited from 8 to 10 participating schools. Children were recruited during the spring and phenotypically characterized at a baseline visit in the summer before the academic year. Assessments included a detailed questionnaire, allergy testing, spirometry, and exhaled nitric oxide. Follow-up surveys were collected at 3, 6, 9, and 12 months after the baseline assessments (Fig 1).

Fungus Sensitization

Skin prick testing (MultiTest device; Lincoln Diagnostics, Decatur, Illinois) was performed with *Aspergillus*, *Alternaria tenuis*, *Penicillium*, and *Cladosporium* (Greer). Sensitization was defined as a wheal diameter ≥ 3 mm larger than the negative control read 15 minutes after placement.

Fungal Air Sampling and Analysis

Burkard Indoor Recording Air Samplers (Burkard Mfg. Co., Rickmansworth, Herts, UK) were used to collect airborne spores of all fungi, including common indoor molds, in each classroom linked to the student. The segment of the slide representing the school day (8:00 AM until 4:00 PM) was marked and a portion scanned at 1000×

magnification, and all fungal spores encountered were identified and counted. Raw counts were converted to airborne concentrations using the sampler flow rate, exposure time, and percentage of the collection surface analyzed. Results were reported as spores per cubic meter of air (spores/m³) for the 8-hour collection period. Two consecutive 8-hour days were averaged for each classroom. This method has been previously described.^{14,16}

A total fungi category was calculated as the sum of all fungus groupings. The 3 largest fungal categories were mitosporae, basidiosporae, and ascosporae. The mitosporae category consisted of *Alternaria*, *Botrytis*, *Cladosporium*, *Bipolaris*, *Epicoccum*, *Penicillium*/*Aspergillus*, *Periconia*, *Pithomyces*, *Stachybotrys*, and “other mitosporae.” *Penicillium* and *Aspergillus* were reported together because they are too similar in morphology to differentiate by direct microscopy. The basidiosporae category consisted of basidiosporae small hyaline, *Coprinus*, *Ganoderma*, and “other basidiosporae.” The ascosporae category consisted of *Leptosphaeria*, *Xylariaceae*, *Chaetomium*, *Diatrype*-like, *Paraphaeosphaeria michotii*, and “other ascosporae.” More than 25 genera of fungi were measured.

Outcome Measure

The primary outcome was asthma symptom days per 2-week period (ASD) as used in prior urban home-based studies.^{17–20} It is the primary outcome in many pediatric asthma studies,^{19,21,22} including those that investigate the impact of environmental exposures on asthma.^{9,23–25} This way of ascertaining asthma symptom days is validated, and it allows comparability across studies.

The ASD comprises the following variables in the 2 weeks before each follow-up survey: (1) number of days with wheezing, chest tightness, or cough; (2) number of days on which the child had to slow down or discontinue play activities because of wheezing, chest tightness, or cough; (3) nights with wheezing, chest tightness, or cough leading to disturbed sleep. The largest result of these 3 variables was used as the outcome of ASD, with a range between 0 to 14 days.

Secondary outcome measurements included the following: number of days the child missed school because of asthma; health care use, defined as the number of hospitalizations and unscheduled health care visits for asthma; number of days the caregiver changed plans because of the child's asthma; number of nights the caregiver lost sleep because of the child's asthma; and lung function based on percentage of predicted pre-bronchodilator forced expiratory volume in 1 second.

Statistical Analysis

Geometric means were calculated for each fungal spore grouping and total spores. To account for a high frequency of zero values, as was the case where certain fungal groups were rarely recovered, a value of 1 spore/m³ was added to all concentrations and then subtracted from the calculated geometric mean.²⁶ Asthma symptom days were linked to the closest measured fungal exposures from the student's classroom during the academic school year. A priori, we defined fungal exposure as a binary “high” vs “low” exposure based on whether the measured grouping was greater than or equal to the 75th percentile of exposure or below. We used generalized estimating equations to model asthma symptom days (binomial family, logit link) with an exchangeable correlation structure, robust variance estimates, and an

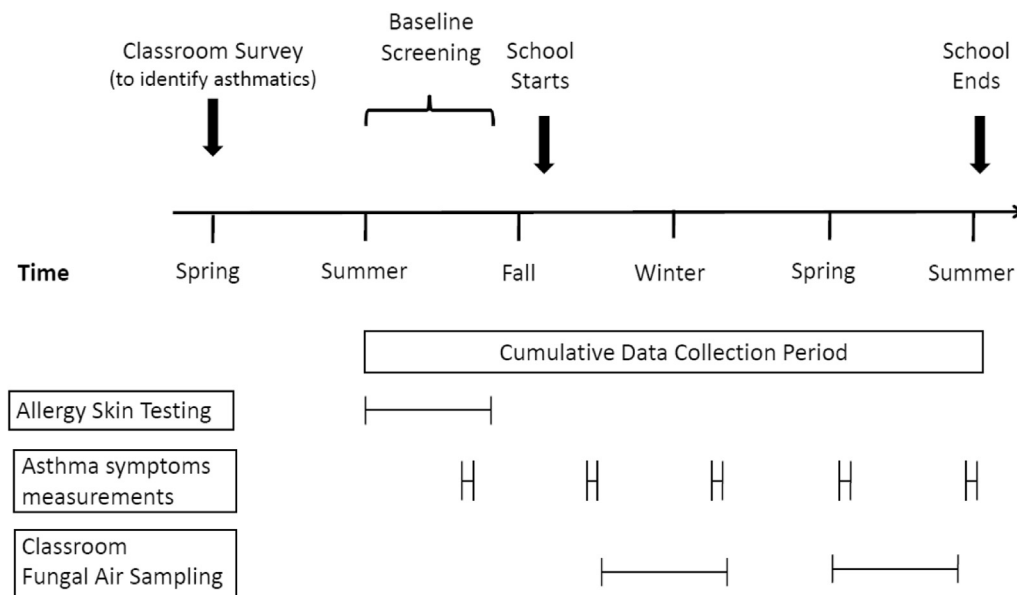


Figure 1. Annual schema for recruitment, screening, and study procedures.

overdispersion parameter. Independent variables included fungal exposure, fungal sensitization, interaction term of exposure and sensitization, age, sex, race, season, mouse allergen, and endotoxin level. Fungal sensitization was defined as any sensitization to fungus for models with the larger fungal categories; within specific spore categories, fungal sensitization was based on specific sensitizations for the specific exposure models (ie, *Alternaria* sensitization interaction with *Alternaria* exposure on the health outcome). Season was defined as the number of days since school started and was modeled with linear and quadratic terms. For each model we reported the effect of fungal exposure for subjects stratified by their sensitization status. Our strategy for statistical testing was as follows: We first investigated the relationship between total fungus and the 3 large fungal groupings (mitospores, ascospores, and basidiospores) and asthma symptom days. In the event of a significant association between asthma symptom days and exposure to a larger fungus category, we then tested the association between asthma symptom days and each of the constituent fungi from that larger category. Analyses were performed using STATA 13.1 (StataCorp. 2013. *Stata Statistical Software: Release 13*, College Station, TX: StataCorp LP.). All tests were 2-tailed, and $P < .05$ was considered significant.

Results

In total, 351 students from 38 schools completed the baseline phenotypic screening and were enrolled in the study. Of those, 280 children from 37 schools (mean age, 7.9 years; 146 male and 134 female) met the requirements of having allergy testing, classroom fungal exposure data, and follow-up symptom data during the school year. The baseline characteristics of the study population are found in Table 1. Most students were black or Hispanic. Forty percent of students were from homes with an annual household income less than \$25,000, and 72% of students were from homes with an annual household income less than \$45,000. Eighty-one percent of students had a family history of asthma. More than half (55%) of students used maintenance asthma medication. Approximately 1 in 5 (18.9%) of the students were sensitized to at least 1 fungus, with *Aspergillus* (9.9%) being the most common, followed by *Alternaria* (9.2%), *Cladosporium* (7.0%), and *Penicillium* (4%). The mean number of asthma symptom days per 2-week period was 2.9.

The prevalence, quantity, and range of the fungal groupings with $\geq 20\%$ prevalence are summarized in Table 2. Fungal spores were present in all 438 classroom samples. The geometric mean of the total fungi was 316.9 spores/m³ and ranged from 15.0 to 59,345.7 spores/m³. Mitospores were the most commonly detected fungal grouping. Within this group *Cladosporium*, *Penicillium/Aspergillus*, and *Alternaria* were the most prevalent. *Cladosporium* had the highest geometric mean of 29.3 spores/m³ and was found in 93% of samples. Regarding the traditional fungi associated with dampness and moisture damage, *Penicillium/Aspergillus* were detected in 86% of samples with a geometric mean of 18.9 spores/m³. *Alternaria* was not as prevalent or abundant. It was found in 22% of samples with a geometric mean of 0.6 spores/m³. When including only classroom samples with *Alternaria* present, the geometric mean was

Table 1
Baseline Characteristics of the Study Population (n = 280)

Male	146	(52.1%)
Age, years—mean (range)	7.9	(4–13)
Race		
White	13	(4.6%)
Black	97	(34.6%)
Hispanic	101	(36.1%)
Other	69	(24.6%)
Annual household income		
<\$25,000	112	(40.0%)
<\$45,000	170	(71.7%)
Family history of asthma	227	(81.1%)
Asthma medications		
SABA only	126	(45.0%)
ICS or montelukast	154	(55.0%)
Allergen sensitization rates		
ANY fungus	53	(18.9%)
<i>Aspergillus</i>	27	(9.9%)
<i>Alternaria</i>	25	(9.2%)
<i>Penicillium</i>	13	(4.8%)
<i>Cladosporium</i>	19	(7.0%)
Maximum symptoms days (past 2 weeks)		
0–1 Days	147	(52.5%)
2–3 Days	56	(20.0%)
4–9 Days	48	(17.1%)
10–14 Days	29	(10.4%)
Mean (SD)	2.9	(±4.1)

Abbreviations: FEV₁, forced expiratory volume in 1 second; ICS, inhaled corticosteroids; SABA, inhaled short-acting beta-agonist.

Table 2
Distribution of the Most Common Fungal Groupings in Classrooms^a

Fungal grouping	Geomean (spore/m ³)	Detectable ^b	Min (spore/m ³)	Max (spore/m ³)
Total fungi	316.9	100%	15.0	59,345.7
Mitospores ^c	71.8	98%	0	59,100.0
<i>Cladosporium</i>	29.3	93%	0	1525.7
<i>Penicillium/Aspergillus</i>	18.9	86%	0	58,894.0
<i>Alternaria</i>	0.6	22%	0	47.0
Basidiospores ^d	18.8	81%	0	16,401.5
Other basidiospores ^d	8.0	70%	0	2445.5
Basidiospores small hyaline ^d	5.1	51%	0	16,017.5
<i>Coprinus</i> ^d	1.0	27%	0	149.6
<i>Ganoderma</i> ^d	0.8	25%	0	403.9
Ascospores ^e	4.5	57%	0	8721.9
Other ascospores	3.1	46%	0	8612.3
Hyphae	70.9	99%	0	571.2
Unidentifiable spores	33.6	98%	0	563.7
Smut spores (Ustilaginomycetes)	12.1	83%	0	639.4
<i>Bispora</i>	0.8	27%	0	127.0
Rust spores (Pucciniomycetes)	0.6	23%	0	29.8
Myxomycetes	0.6	22%	0	291.7

^an = 438 classroom samples from 37 schools; limited to >20% detectable.

^bDetectable is defined as percentage of classrooms in which a particular fungal type was identified at least once.

^cMitospores = *Alternaria*, *Botrytis*, *Cladosporium*, *Bipolaris*, *Epicoccum*, *Penicillium/Asp*, *Periconia*, *Pithomyces*, *Stachybotrys*, and other mitospores.

^dBasidiospore = Basidiospores small hyaline, *Coprinus*, *Ganoderma*, other basidiospores.

^eAscospore = *Leptosphaeria*, *Xylariaceae*, *Chaetomium*, *Diatrype*-like, *Paraphaeosphaeria michotii*, other ascospores.

7.5 spores/m³ (range, 3–47 spores/m³). The other major fungal groupings were basidiospores and ascospores. Basidiospores were detected in 81% of classroom samples and ascospores in 57% of samples. There was substantial variability in total fungus quantity between schools and classrooms within the same school (Fig 2).

For children with high exposure to total fungi, the odds of having an increase in asthma symptom days was elevated, although not statistically significantly, for fungus-sensitized children (odds ratio [OR] = 1.74, 95% confidence interval [CI] = 0.89–3.40, *P* = .10), but was not elevated in children who were not sensitized to fungus (OR = 0.91, 95%CI = 0.63–1.30, *P* = .6). The association between asthma symptoms days and mitospore exposure significantly varied by sensitization status (interaction *P* value = .04). A significant association was seen between exposure to mitospores and maximum symptom days in students with fungal sensitization (OR = 2.00, 95% CI = 1.06–3.76, *P* = .03), but not in students without fungal sensitization (OR = 0.94, 95% CI = 0.65–1.36). None of the secondary outcomes were significantly associated with mitospore exposure.

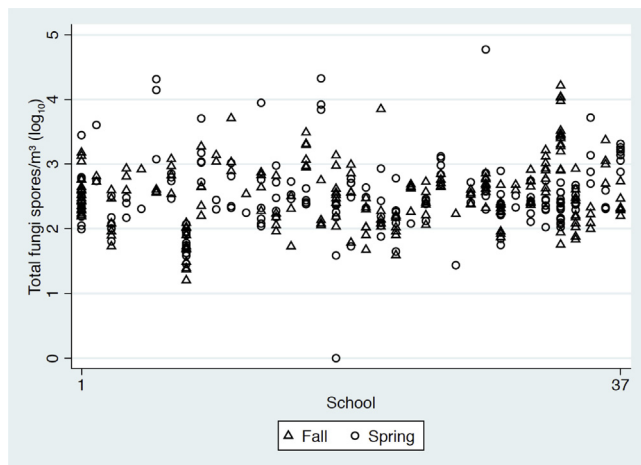


Figure 2. Substantial variability exists in total fungus quantity between schools and classrooms within the same school. Total fungus (spores/m³) was not significantly higher in the fall, with a geometric mean of 340.2 (SD = 3.2) and 307.3 (SD = 3.5) in the spring; *P* = .39.

No significant findings were made for exposure to ascospores (fungus sensitized OR = 1.17, 95% CI = 0.57–2.39, *P* = .67; fungus not-sensitized OR = 1.12, 95% CI = 0.79–1.60, *P* = .52) or basidiospores (fungus sensitized OR = 1.12, 95% CI = 0.61–2.05, *P* = .72; fungus not-sensitized OR = 0.91, 95% CI = 0.64–1.29, *P* = .60).

Further investigation of the individual mitospores showed that exposure to higher levels of *Alternaria* was significantly associated with asthma symptom days in students sensitized to *Alternaria* (OR = 3.61, CI = 1.34–9.76, *P* = .01), but not in children not sensitized to *Alternaria* (Table 3). The association between asthma symptom days and *Alternaria* exposure significantly varied by sensitization status (interaction *P* = .02). This finding indicates that students sensitized to *Alternaria* and exposed to high levels were estimated to have 3.2 more symptom days per 2-week period as compared with students sensitized but exposed to low levels (5.3 vs 2.1 symptom days) after adjusting for covariates. No difference in asthma symptom days was seen for children not sensitized to *Alternaria*, regardless of exposure (OR = 1.04; 95% CI = 0.72–1.49, *P* = .85). None of the secondary outcomes were significantly associated with *Alternaria* exposure (eTable 1). Finally, no associations were found between asthma symptoms and exposure to *Cladosporium* or *Penicillium/Aspergillus*, regardless of sensitization to these fungi (Table 3).

Discussion

This prospective study demonstrates that school classroom fungal exposure may be associated with increased asthma symptoms in sensitized children. Specifically, children with asthma who are sensitized to *Alternaria* and exposed to this fungus in their classroom have significantly more days with asthma symptoms than those who were sensitized and not exposed. No effect of exposure was seen in those not sensitized to *Alternaria*. These findings were significant even when adjusting for co-exposure with known asthma triggers, including mouse allergen and endotoxin, and when adjusted for variation in seasonal changes.

Students sensitized and exposed to *Alternaria* had 3.2 more symptom days per 2-week period as compared with students sensitized but not exposed to *Alternaria*. This difference would extrapolate to approximately 6 more asthma symptom days per month and approximately 50 more asthma symptom days per school year.

Table 3

Association of Classroom Fungal Exposure on Asthma Morbidity in Sensitized (Specific to Fungal Genus) Children with Asthma

	Sensitized to specific fungus		Not sensitized to specific fungus	
	OR (95% CI)		OR (95% CI)	
<i>Alternaria</i>				
Asthma symptom day				
Unadjusted ^a	4.02	(1.43–11.28)*	1.05	(0.74–1.48)
Adjusted	3.61	(1.34–9.76)**	1.04	(0.72–1.49)
<i>Cladosporium</i>				
Asthma symptom day				
Unadjusted ^a	1.70	(0.46–6.26)	1.19	(0.88–1.59)
Adjusted	1.45	(0.41–5.17)	1.10	(0.80–1.30)
<i>Penicillium/Aspergillus</i>				
Asthma symptom day				
Unadjusted ^a	0.48	(0.17–1.29)	0.80	(0.58–1.11)
Adjusted	0.81	(0.58–1.13)	0.49	(0.18–1.30)

^aP = .008.

**P = .01.

^aAdjusted for age, sex, race, season, classroom, mouse allergen, and classroom endotoxin levels.

Sensitization to *Alternaria* has been found to be significantly higher among patients with asthma than among subjects without asthma. Lehmann et al.²⁷ investigated the prevalence of fungal sensitization in 203 German pediatric asthma patients aged 1 to 17 years and found that *Alternaria* sensitization was the most common among fungi at 17%.²⁷ In the Inner-City Asthma Study, Pongracic et al.⁹ found that *Alternaria* was the most common fungus sensitization, as well, with 36% of 469 children with asthma sensitized to *Alternaria* species.⁹ In our study, *Alternaria* was the second most common fungal sensitization, with 9.2% of study participants having a positive skin prick test, only slightly behind *Aspergillus* with a sensitization prevalence of 9.9%. These differences in sensitization rates are likely related to varying environmental conditions regionally.

Our findings are consistent with the Inner-City Asthma Study (ICAS), which also found that home *Alternaria* exposure was 1 of a few fungi to have an effect on asthma symptom days.⁹ Importantly, we extend the ICAS home-based assessment to identify the school environment as a significant source of fungal exposure-related asthma morbidity. The greater effect size of our findings compared with ICAS (3.2 vs 1.3 asthma symptom days per 2-week period) may be related to methodologic differences between the studies, such as the mode of sampling (direct microscopy vs volumetric culture-based), but also may reflect the intense and prolonged exposure microenvironment of the school classroom.

In our study, “high fungus exposure” was defined as $\geq 75^{\text{th}}$ percentile, and “low fungal exposure” was defined as $< 75^{\text{th}}$ percentile. For *Alternaria*, which was found in just 22% of classroom samples, these cutoffs were essentially consistent with “exposure” vs “non-exposure.” *Alternaria* was present in lesser quantities and in fewer classrooms as compared with *Cladosporium* and *Penicillium/Aspergillus*. However, we did not find associations between asthma morbidity and total fungal spores or other prevalent fungi such as ascospores, basidiospores, *Penicillium/Aspergillus*, and *Cladosporium*.

Studies from other countries have identified exposure to fungi in classrooms as a risk factor for asthma or asthma morbidity.^{10–13,28–30} However, these were cross-sectional studies. The methods used to quantify fungus exposure included identification

of visible fungus, measurement of fungal metabolites in dust, culture-based volumetric sampling and direct microscopy with a shorter collection time (40 minutes). Our study is unique in that it is a prospective longitudinal cohort study conducted in the United States in multiple inner-city schools. The population comprised urban children with known asthma, and symptoms were evaluated at several time points during the year. We used continuous air sampling for 2 entire school days in 2 distinct seasons, which may reflect a more accurate exposure over long periods by averaging the short-term spore concentration peaks and valleys. Direct microscopy was used for spore identification and quantification because it provided the opportunity to identify the most spore types regardless of culturability or viability. The absences of a standardized fungal measurement technique and lack of exposure thresholds make assessing health risks of fungal exposure a challenge. Quantification methods vary from direct microscopy to culture-based volumetric sampling to measurement of fungal cell wall components such as ergosterol or beta-D-glucan. Even newer techniques for evaluating fungal diversity such as metagenomic sequencing may offer different insight into characterization of the fungal microbiome.

A limitation of our study is that it was created to evaluate asthma health effects from fungi and not necessarily to determine the source of the fungus exposure. Our key finding is that fungi are present in the classroom, and *Alternaria*, in particular, may be associated with a health effect in sensitized students with asthma. Whether the exposures to *Alternaria* spores were primarily caused by indoor growing spores vs outdoor spores that penetrated the indoor environment is unclear. Although we do not have outdoor sampling available, the variability in classroom spore count within the same school (Fig 2) and the strong correlation of the indoor classroom *Alternaria* count with asthma symptoms in children within those classrooms suggests that the indoor exposure is responsible. If the effect were attributable to outdoor *Alternaria* exposures alone, we would assume that all the sensitized children with asthma children within the same school would be exposed to comparable outdoor concentrations and have similar symptoms. Our sampling was done during the fall and spring in the northeastern United States, where conditions commonly include closed windows and reduced exposure to outdoor fungus. Although some of the spores may have been carried in from the outdoors, some of the higher concentrations include fungi more characteristically known as important in indoor environments, suggesting that likely direct indoor sources of fungi are present in these classrooms. Regardless of the source of *Alternaria* exposure, we have shown that the presence of *Alternaria* in the indoor classroom environment was associated with increased asthma symptom days in sensitized children with asthma. Furthermore, we recognize that the brief sampling time and variability of sampling date might not completely reflect the exposure. We acknowledge that our symptom measurement and outcome measurement may have been at different times and that this strategy might lead to exposure misclassification. Finally, determining the prevalence of fungal sensitization and quantifying exposure remains a challenge. Because of the lack of high-quality fungal extracts and the presence of cross-reactivity among fungi, the prevalence of fungal allergies is difficult to determine with certainty.³¹

In summary, *Alternaria* exposure in school classrooms may play an important role in asthma morbidity in inner-city children. Further research is necessary to determine whether efforts to decrease fungus exposure in classrooms can improve asthma outcomes in vulnerable children. A follow-up study by our laboratory is evaluating whether the use of air filters can reduce fungal levels and additionally whether a reduction in levels can reduce asthma morbidity (<https://clinicaltrials.gov/ct2/show/NCT02291302>).

Supplementary Data

Supplementary data related to this article can be found online at <https://doi.org/10.1016/j.anai.2019.03.011>.

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Supplementary Data

eTable 1

Secondary Outcomes for Exposure to *Alternaria* in Sensitized and Not Sensitized Children with Asthma

	Sensitized to <i>Alternaria</i>		Not sensitized to <i>Alternaria</i>	
	Coefficient	95% CI	Coefficient	95% CI
<i>Alternaria</i>				
Missed school days	IRR = 1.94	(0.88, 4.26)	IRR = 1.04	(0.66, 1.66)
Health care utilization	IRR = 2.04	(0.32, 12.97)	IRR = 1.28	(0.72, 2.26)
Caregiver changed plans	OR = 0.56	(0.12, 2.54)	OR = 1.04	(0.53, 1.63)
Caregiver lost sleep	OR = 3.05	(0.98, 9.51)	OR = 0.91	(0.60, 1.40)
FEV ₁ % predicted	B = -20.6	(-57.9, 16.7)	B = -7.7	(-35.5, 20.19)

Adjusted for age, sex, race, season, and classroom endotoxin levels.

Abbreviations: CI, confidence interval; IRR, incidence rate ratio; FEV₁, forced expiratory volume in 1 second; OR, odds ratio; β , beta coefficient.