Associations between outdoor fungal spores and childhood and adolescent asthma hospitalizations

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Background: Childhood asthma is a significant public health problem and severe exacerbations can result in diminished quality of life and hospitalization.

Objective: We sought to examine the contribution of outdoor fungi to childhood and adolescent asthma hospitalizations.

Methods: The Melbourne Air Pollen Children and Adolescent study is a case-crossover study of 644 children and adolescents (aged 2-17 years) hospitalized for asthma. The Melbourne Air Pollen Children and Adolescent study collected individual data on human rhinovirus infection and sensitization to *Alternaria* and *Cladosporium* and daily counts of ambient concentrations of fungal spores, pollen, and air pollutants. Conditional logistic regression models were used to assess associations with increases in spore counts while controlling for potential confounding and testing interactions.

Results: Exposure to *Alternaria* (adjusted odds ratio [aOR], 1.07; 95% CI, 1.03-1.11), *Leptosphaeria* (aOR, 1.05; 95% CI, 1.02-1.07), *Coprinus* (aOR, 1.04; 95% CI, 1.01-1.07), *Drechslera* (aOR, 1.03; 95% CI, 1.00-1.05), and total spores (aOR, 1.05; 95% CI, 1.01-1.09) was significantly associated with child asthma hospitalizations independent of human rhinovirus infection. There were significant lagged effects up to 3 days with *Alternaria*, *Leptosphaeria*, *Cladosporium*, *Sporormiella*, *Coprinus*, and *Drechslera*. Some of these associations were significantly greater in participants with *Cladosporium* sensitization.

Conclusions: Exposures to several outdoor fungal spore taxa, including some not reported in previous research, are associated with the risk of child and adolescent asthma hospitalization, particularly in individuals sensitized to *Cladosporium*. We need further studies to examine cross-reactivity causing asthma exacerbations. Identifying sensitization to multiple fungal allergens in children with asthma could support the design and implementation of more effective strategies to prevent asthma exacerbations. (J Allergy Clin Immunol 2017;139:1140-7.)

Key words: *Outdoor fungi, asthma, hospitalization, child, adolescent, case-crossover design*

Asthma is a significant global public health problem, estimated to affect approximately 334 million people and 11% to 14% of the world's children.[1] The prevalence of asthma in Australia is comparable, with 9.9% of adults and 10.4% of children having current asthma.[2] More than 50% of asthma hospitalizations involve children and adolescents aged between 0 and 15 years.[3] Severe asthma exacerbations have adverse impacts on quality of life and long-term respiratory health and incur significant costs for families and the public health system.[4] Environmental triggers of sudden-onset child asthma exacerbations that result in hospitalizations can include respiratory viruses[5] (especially human rhinovirus [HRV]),[6] pollen,[7] indoor fungi (related to house mold/dampness),[8] and air pollutants (such as particulate matter, ozone, and nitrogen dioxide).[9]

There has been limited research examining the contributions of outdoor fungi to asthma exacerbations. It is proposed that airborne fungi can trigger asthma exacerbation because of their allergenic protein structure and their small size (often 1-20 μm in diameter), which allows them to lodge deep in the airways and lungs, causing inflammation

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Abbreviations used
aOR: Adjusted odds ratio
HRV: Human rhinovirus
MAPCAH: Melbourne Air Pollen Children and Adolescent Health
OR: Odds ratio
$PM_{2.5}$: Particulate matter up to 2.5 μm in diameter
PM_{10}: Particulate matter up to 10 μm in diameter

and allergic response.[10] A number of ecological studies have examined the association between outdoor fungal spores and child and adolescent asthma hospitalizations[11] and the predominant outdoor fungi taxa most often reported are *Alternaria* spp and *Cladosporium* spp. To date, only 1 study has examined this association at an individual level in children sensitized to fungi[12] and no studies have controlled for respiratory viral infections (particularly HRV) in individuals, both being strong risk factors for child asthma exacerbations.[13,14] It may be possible to improve asthma management if we gain a better understanding of the contribution that fungal spores make to severe asthma exacerbations while accounting for potential confounders. We also need to understand potential interactions between exposures to outdoor fungi spores, being sensitized to fungi, and having HRV infection that may be associated with severe asthma exacerbations, to allow identification of high-risk groups.

Fungal exposure is ubiquitous, but the species (taxa) and numbers of fungi present in the air that we breathe depend on regional variations in vegetation, temperature, relative humidity, and seasonal changes.[15] As a result, numbers can fluctuate within short time frames. This makes studying their effects on relatively rapid-onset events, such as asthma exacerbations, difficult. The case-crossover design has been used more broadly in environmental epidemiology to study the effects of short-term ambient exposures on the risk of rapid-onset events in individuals.[16] There are no currently published studies that have used this approach to examine the association between outdoor fungal spore exposure and childhood and adolescent asthma exacerbations.

The aim of this case-crossover study was to examine the associations between outdoor fungal spores and asthma hospitalizations, and whether the associations were modified by sex, fungal sensitization status, presence of HRV infection, or age group.

METHODS

Study design and population

The Melbourne Air Pollen Children and Adolescent Health (MAPCAH) study is a case-crossover study of 644 children and adolescents (aged 2-17 years) with "incident" asthma admitted to the Royal Children's Hospital in Melbourne, Australia, between September 2009 and December 2011. This study used a bidirectional time-stratified case-crossover design that has been shown to be well suited for studying the effects of transient short-term exposures (fungi spore release, air pollution changes) on the risk of short-onset events (asthma exacerbation requiring hospitalization) in individuals.[17] Because cases serve as their own controls, there is less risk of confounding due to stable individual characteristics (ie, age, sex, behavioral factors).[16] The hospital admission date was set as the index case, and the referent control dates were the same day of the week within the same month and year as the index case. Bidirectional control sampling is valid because this approach reduces potential biases related to possible time or seasonal trends.[18,19] For each admission date (case) and referent control days, we compared the daily level of fungi, grass pollen, air pollution, and meteorological variables.

This study was approved by the Royal Children's Hospital Ethics Committee and the La Trobe University Human Ethics Committee, and all participating parents/guardians provided written informed consent.

Definitions

Outcome. The case definition for inclusion in the study was an asthma admission on a given day with a principal International Classification of Diseases, Tenth Revision diagnosis code of asthma (J45) identified through the admissions department and confirmed at discharge.

Primary exposure: Ambient levels of fungal spores. Daily ambient fungal spore counts were measured using a volumetric spore trap (Burkard, United Kingdom) located on the rooftop of the Earth Sciences building at the Parkville campus of the University of Melbourne, which is located approximately 2 km from the Royal Children's Hospital and meets the guidelines of the World Allergy Organization.[20] Briefly, collection involved drawing 10 L of air per minute continuously across a microscope slide that had been coated with an adhesive. Particles in the air stuck to the slide as it moved past the inlet at 2 mm/h. The fungal spores were identified and counted by a trained technician (E.L.) and related to the volumes of air sampled, giving concentrations per cubic meter of air, averaged over the 24 hours. Identifiable fungal spores were classified into taxa: *Alternaria*, *Cladosporium*, *Ganoderma*, *Leptosphaeria*, *Pleospora*, *Sporormiella*, *Pithomyces*, smuts, *Coprinus*, *Drechslera*, *Stemphylium*, and *Periconia*.

HRV infection. Viral respiratory illness was assessed using nasal throat swabs that were collected on admission. HRV was identified using multiplex PCR methods as described by Erbas et al.[7]

Fungal sensitization. At admission, *fungal sensitization* was defined as a positive skin prick test result with a mean wheal diameter of 3 mm or greater to the fungi *Alternaria* or *Cladosporium* (Hollister-Stier, Spokane, Wash; Alyostal, Antony, France). *Controls* were defined as positive histamine (10 mg/mL) and negative saline.

Outdoor air quality, pollen, and weather data. The 24-hour average daily concentrations of particulate matter up to 2.5 μm in diameter ($PM_{2.5}$) and up to 10 μm in diameter (PM_{10}) ($\mu g/m^3$), the daily maximum 1-hour average nitrogen dioxide (NO_2) level (parts per billion), and the daily maximum 4-hour average ozone (O_3) level (parts per billion) were obtained from routine air quality monitoring stations in Melbourne. The Bureau of Meteorology provided data on daily maximum and minimum temperatures (degrees Celsius), rainfall (mm), and daily relative humidity (percentage) for the study period. Daily ambient pollen counts were collected and measured using the same method of capture as described for fungal spores. Pollen was considered here as they have been identified as triggers for respiratory admissions in Melbourne.[21]

Age groups. Participants were stratified into age groups: (1) 2 to 5, 6 to 10, 11 to 15, and 16 to 18 years and (2) 2 to 14 and 15 to 18 years.

Statistical analysis

Correlations between fungal spore taxa were assessed using Spearman rank correlation coefficients. We used conditional logistic regression models for binary outcomes to evaluate the association between the fungi taxa (continuous variable) and asthma hospitalization. Analyses were done for the same day (lag 0) and lagged fungal spore exposure (up to 3 days—multilag and cumulative).

Maximum temperature and relative humidity were included as a priori confounders in all adjusted models because these factors have been shown to be associated with fungal spore production and dispersion[15] and asthma exacerbation.[22] Models were adjusted for possible confounding variables: HRV infection (yes/no), grass, tree, and weed pollen (continuous), sensitization to *Alternaria* (yes/no), sensitization to *Cladosporium* (yes/no), and air pollutants: $PM_{2.5}$, PM_{10}, NO_2, or O_3 (continuous and stratified into low <75th percentile or high >75th percentile), and were retained in the model if they

TABLE I. MAPCAH participants' characteristics

Characteristic	n	n (%)
Total	644	
Boys		407 (63.2)
Girls		237 (36.8)
Age (y), median ± SD (range)		5.2 ± 3.3 (2-17)
Age group (y)	644	
2-5		416 (64.6)
6-10		164 (25.5)
11-15		58 (9.0)
16-18		6 (0.9)
Age group (y)	644	
2-14		635 (98.6)
15-18		9 (1.4)
	n tested	**n (%) yes**
Infected with HRV	642	447 (69.6)
Sensitized to *Alternaria*	630	56 (8.9)
Sensitized to *Cladosporium*	630	41 (6.5)
Sensitized to *Alternaria* or *Cladosporium*	630	86 (13.7)

changed the estimated associations by 10% or more or were statistically significant ($P < .05$) in the adjusted models.

We assessed whether fungal taxa were independently associated using mutually adjusted multifungal models. Analyses were stratified by sex, HRV infection status at admission, sensitization to *Alternaria*, sensitization to *Cladosporium*, exposure to high versus low levels of individual air pollutants, and age group to identify possible effect modification. If there was statistical evidence of effect modification, interaction terms were included in the regression models. Because the statistical power to test for significant interaction was lower than to test for the main effect, we set significance value of *P* interaction at less than .1 to avoid missing any important interactions.[23] All results in the tables are presented as odds ratios (ORs) and 95% CIs. The OR can be interpreted as per unit increase from the 75th to 90th percentiles of fungal spore counts except for the fungi taxa *Ganoderma* and *Sporormiella* where the 75th and 90th percentiles were both 0 and the OR are reported for single-spore increases. In the time series graphs, daily counts of participants were smoothed using Locally Weighted Scatterplot Smoothing. All statistical analyses were performed using Stata IC 13.1 (StataCorp, College Station, Tex).

RESULTS

MAPCAH participants' characteristics

The participants recruited (n = 644) were predominantly boys (63.2%); the median age of all participants was 5.2 years (range, 2-17 years), with 90% younger than 10 years. Most (69.6%) participants had HRV infection detected at admission. Skin prick tests for fungal sensitization were obtained for 630 participants, and it was found that 8.9% were sensitized to *Alternaria* only, 6.5% to *Cladosporium* only, and 13.7% to *Alternaria* and *Cladosporium* (Table I).

Fungal spore distribution

The most prevalent fungi taxa detected were *Cladosporium* (44%), *Leptosphaeria* (14%), and *Alternaria* and smuts (11% each) of the total fungi spore count (Table II). The remaining fungal taxa accounted for 1% to 7% each. The daily fungal spore counts and daily participants enrolled in the MAPCAH study over the study period showed seasonal variation (Fig 1). During the study period, fungal spore concentrations were relatively low (median, 0-3) for most of the year, with *Cladosporium*, *Leptosphaeria*, *Alternaria*, *Pleospora*, *Sporomelia*, and *Periconia* peaking during spring (September 1 to November 30); smuts, *Coprinus*, and *Drechslera* peaking in summer (December 1 to February 28); *Ganoderma* and *Pithomyces* peaking in autumn (March 1 to May 31); and none peaking during winter. Spring accounted for 54% and summer for 33% of the total fungi spore count for the study period. Most fungi taxa appeared highly correlated in this study except for *Ganoderma* and *Sporomelia* (see Table E1 in this article's Online Repository at www.jacionline.org).

The lower fungal spore concentrations in the period September 2009 to September 2010 correlated with the end of a major drought (high maximum temperatures and low rainfall) that affected Melbourne. The period September 2010 to December 2011 was characterized by above-average rainfall and lower temperatures across Melbourne, which stimulated fungal spore growth (see Fig E1 in this article's Online Repository at www.jacionline.org).

Unadjusted analyses

In unadjusted models, *Alternaria* (OR, 1.05; 95% CI, 1.03-1.08)), *Leptosphaeria* (OR, 1.04; 95% CI, 1.02-1.06), *Pleospora* (OR, 1.12; 95% CI, 1.01-1.25), and *Drechslera* (OR, 1.02; 95% CI, 1.00-1.04) were significantly associated with increased odds of asthma hospital admission (Table III).

Adjusted analyses

In separate adjusted models for each fungus, 4 fungal taxa were significantly associated with asthma hospitalizations: *Alternaria* (adjusted odds ratio [aOR], 1.07; 95% CI, 1.03-1.11), *Leptosphaeria* (aOR, 1.05; 95% CI, 1.02-1.07), *Coprinus* (aOR, 1.04; 95% CI, 1.01-1.07), Drechslera (aOR, 1.03; 95% CI, 1.00-1.05), and total spores (aOR, 1.05; 95% CI, 1.01-1.09) (Table III). Because HRV infection was strongly associated with asthma hospitalization risk (OR, 5.1; 95% CI, 4.1-6.3; $P < .001$) and grass pollen is a potential confounder for asthma exacerbation, each model was adjusted for HRV infection and grass pollen in addition to the 2 *a priori* confounders: maximum temperature and relative humidity. None of the other covariates changed the association between the fungal spores and asthma hospitalization by more than 10%.

When stratified by sex, HRV infection status, sensitization to *Alternaria* or sensitization to *Cladosporium*, exposure to high versus low levels of individual air pollutants, and age group and assessed as effect modifiers in the adjusted models, we found that the associations between *Alternaria*, *Coprinus*, *Drechslera*, and *Stemphylium* and asthma hospitalizations were greater in participants who were sensitized to *Cladosporium.* Associations with *Alternaria*, *Coprinus*, *Drechslera*, and *Sporormiella* in children sensitized to *Alternaria* were significant, but *Alternaria* sensitization fitted as an interaction term was not significant (Table IV). When we stratified the data by presence of HRV infection at admission, we found that among the fungal species assessed, *Sporormiella*, *Ganoderma*, and *Pithomyces* count were modified by HRV infection because stronger associations were seen with hospitalization in those with an HRV infection (interaction $P < .05$) (see Table E2 in this article's Online Repository at www.jacionline.org). Air pollutants did not appear to act as effect modifiers. We also fitted multifungal regression models and found

TABLE II. Summary statistics for fungal spore counts and other environmental factors from September 2009 to December 2011 in Melbourne

Fungal spore species (number of spores/m^3 air tested)	N (d)	Mean ± SD	Geometric mean	Minimum	25%	Median	75%	90%	Maximum	Frequency % of total
Cladosporium	852	12.6 ± 45.9	6.3	0	0	3	8	28	907	43.8
Leptosphaeria	852	4.0 ± 13.4	4.3	0	0	0	2	8	203	14.0
Alternaria	852	3.3 ± 8.0	3.4	0	0	0	3	9	98	11.4
Smuts	852	3.2 ± 14.8	5.7	0	0	0	0	6	224	11.3
Coprinus	852	1.9 ± 6.3	3.3	0	0	0	1	5	133	6.6
Drechslera	852	1.4 ± 4.7	2.6	0	0	0	1	3	58	5.0
Periconia	852	0.7 ± 1.9	2.0	0	0	0	0	2	19	2.3
Pleospora	852	0.5 ± 1.5	2.0	0	0	0	0	2	25	1.9
Ganoderma	852	0.4 ± 2.2	2.5	0	0	0	0	0	42	1.3
Pithomyces	852	0.3 ± 1.2	1.7	0	0	0	0	1	23	1.0
Stemphylium	852	0.3 ± 0.9	1.6	0	0	0	0	1	7	0.9
Sporormiella	852	0.1 ± 0.7	1.6	0	0	0	0	0	9	0.5
Total spores	852	28.6 ± 65.5	13.4	0	2	9.5	25.5	72	949	
Meteorological variables	**N (d)**	**Mean ± SD**	**Geometric mean**	**Minimum**	**25%**	**Median**	**75%**	**90%**	**Maximum**	**Frequency % of total**
Maximum temperature (°C)	851	21.2 ± 5.91		10.8	16.5	20.3	24.9	29.5	43.6	
Rainfall (mm)	824	2.3 ± 6.6		0	0	0	1.4	11.8	82.4	
Relative humidity (%)	849	51.3 ± 15.9		8	41	49	61	73	96	
Pollutant	**N (d)**	**Mean ± SD**	**Geometric mean**	**Minimum**	**25%**	**Median**	**75%**	**90%**	**Maximum**	**Frequency % of total**
$PM_{2.5}$ (μg/m^3)	831	4.9 ± 3.1		−0.8	2.9	4.2	6.2	8.8	20.2	
PM_{10} (μg/m^3)	832	17.7 ± 7.0		3.5	12.7	16.45	21.4	26.2	60.1	
Nitrogen dioxide (ppm)	847	9.5 ± 4.1		0.4	6.5	9	12.4	15.1	23.8	
Ozone (ppm)	816	13.7 ± 5.5		−0.2	10.25	13.5	17.05	20.3	32.1	
Grass pollen	**N (d)**	**Mean ± SD**	**Geometric mean**	**Minimum**	**25%**	**Median**	**75%**	**90%**	**Maximum**	**Frequency % of total**
	824	10.8 ± 27.3		0	0	0	5	34	207	

that *Alternaria* and *Leptosphaeria* continued to be independently associated (see Table E3 in this article's Online Repository at www.jacionline.org).

Lag analysis

We undertook a lag analysis up to day 3 including cumulative 4-day lags. In adjusted regression models, *Alternaria* (Lag1, Lag2, cumulative lag), *Leptosphaeria* (cumulative lag), *Sporormiella* (Lag2), *Coprinus* (Lag1, cumulative lag), *Drechslera* (Lag1, Lag2, cumulative lag), *Stemphylium* (Lag1), *Periconia* (Lag3), and total spores (Lag3 and cumulative lag) were associated with increased odds of asthma admissions (Table V).

DISCUSSION

Using a case-crossover approach, we found that exposures to spores of several outdoor fungal taxa, including some that, individually, have not been reported in previous research, were associated with the risk of asthma exacerbations in children and adolescents. *Alternaria*, *Leptosphaeria*, *Coprinus*, and *Drechslera* spores were associated with asthma hospitalizations on the day of exposure. In addition, there were associations with *Alternaria*, *Coprinus*, *Drechslera*, and *Stemphylium* 1 day after exposure, associations with *Alternaria* and *Drechslera* 2 days after exposure, and associations with *Periconia* and total spores 3 days after exposure. Associations were found with *Alternaria*, *Leptosphaeria*, *Coprinus*, *Drechslera*, and total spores after 4-day cumulative exposure. All these spores have been shown to be associated with aeroallergen production except *Leptosphaeria*.[24]

Our findings build on previous research that some ambient fungal spores are associated with child and adolescent asthma hospitalization. *Alternaria* is commonly documented in the childhood asthma hospitalizations[25-28] and severe asthma[10,29] literature. It has been associated with bronchial hyperreactivity in children sensitized to *Alternaria* in inland rural Australia.[30] *Coprinus* was associated with an increased risk of child asthma hospital admission.[25] Individually, *Leptosphaeria* and *Drechslera* have not been commonly reported as being associated with child asthma hospitalizations. Newson et al[25] found no significant association between *Leptosphaeria* and *Drechslera* and child asthma hospital admissions. However, their inclusion in fungal phylum (Ascomycetes) for analysis may provide some evidence of their effect. Atkinson et al[31] grouped *Leptosphaeria* into the Ascomycetes and elevated counts of this phylum were significantly associated with child asthma emergency department attendances. *Coprinus* is in the Basidiomycetes and elevated levels of spores from this phylum were associated with child asthma emergency department attendances and hospitalizations.[31] In Auckland, New Zealand, Hasnain's study[32] correlated elevated ambient *Leptosphaeria* levels with high regional prevalence of allergic respiratory diseases although asthma was not the specific outcome of interest. *Leptosphaeria* may be a significant and underidentified aeroallergen in temperate environments in the southern hemisphere.

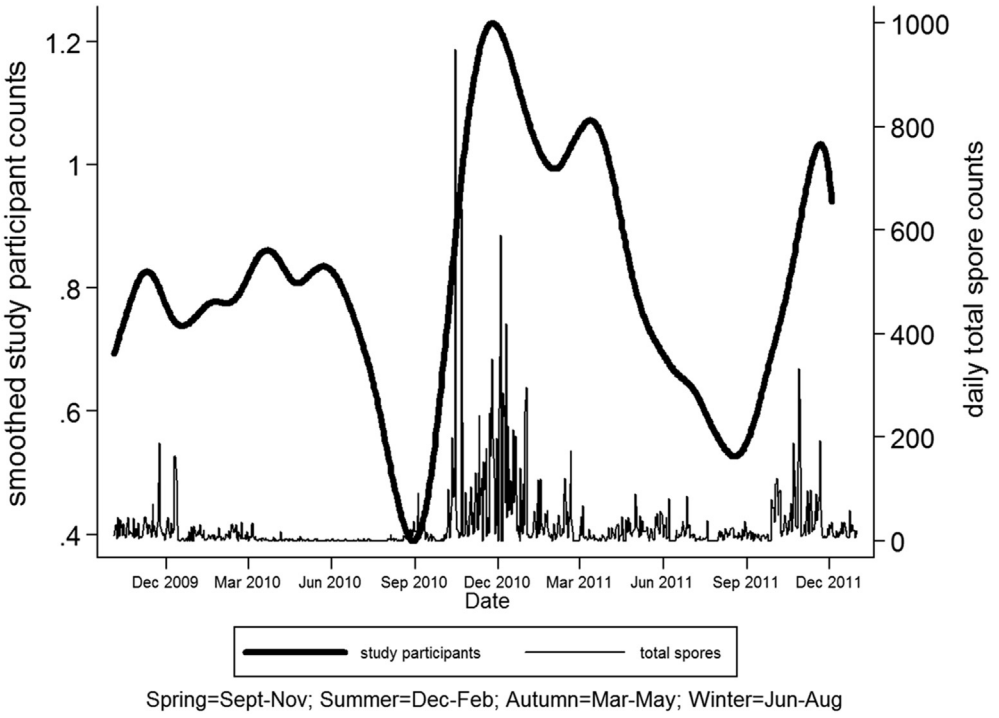

FIG 1. Daily counts of total fungi spores and study participants admitted to Royal Children's Hospital (smoothed) for the period September 2009 to December 2011.

TABLE III. Associations between fungi taxa and asthma hospitalizations—unadjusted and adjusted models

Fungi species	Unadjusted model, OR (95% CI) (N = 644)	Adjusted* model, aOR (95% CI) (N = 644)
Cladosporium	1.01 (0.99-1.03)	1.02 (0.98-1.04)
Leptosphaeria	**1.04 (1.02-1.06)**†	**1.05 (1.02-1.07)**†
Alternaria	**1.05 (1.03-1.08)**†	**1.07 (1.03-1.11)**†
Smuts	0.99 (0.97-1.00)	0.98 (0.95-1.01)
Coprinus	1.03 (1.00-1.06)	**1.04 (1.01-1.07)**‡
Drechslera	**1.02 (1.00-1.04)**‡	**1.03 (1.00-1.05)**‡
Periconia	1.00 (0.95-1.05)	1.00 (0.93-1.07)
Pleospora	**1.12 (1.01-1.25)**‡	1.05 (0.92-1.20)
Ganoderma§	0.96 (0.91-1.01)	0.98 (0.94-1.02)
Pithomyces	1.01 (0.95-1.07)	1.02 (0.95-1.11)
Stemphylium	1.01 (0.94-1.08)	1.04 (0.96-1.13)
Sporormiella§	1.06 (0.97-1.08)	1.06 (0.96-1.18)
Total spores	1.03 (1.00-1.06)	**1.05 (1.01-1.09)**‡

OR and aOR per increase from 75th to 90th percentile. Statistically significant results are in boldface.
*Adjusted for HRV status, relative humidity, maximum temperature, and grass pollen.
†$P < .001$.
‡$P < .05$.
§OR and aOR for unit increase per fungal spore when the change from 75th to 90th percentile is 0.

Our study also found that the risk of asthma hospitalization was still significant when children were exposed to *Alternaria*, *Leptosphaeria*, *Coprinus*, *Drechslera*, *Stemphylium*, and *Periconia* over a range of lag periods. This is similar to findings from Raphoz et al[33] who found associations with increased child asthma emergency department visits and *Cladosporium* and Deuteromycetes (possibly including *Alternaria*, *Coprinus*, *Drechslera*, and *Stemphylium* but these were not specified) at Lag3 to Lag5.[33] Newson et al[25] also found associations with *Alternaria*, *Cladosporium*, and *Drechslera* at Lag1, but our study did not find any associations with *Cladosporium*. No other studies examining child asthma hospitalizations and outdoor fungal spore exposure have reported on lagged effects.[11]

Our findings that grass pollen and air pollutants ($PM_{2.5}$, PM_{10}, NO_2, or O_3) did not change the effect estimates by more than 10% have been reported in other epidemiological studies examining the effect of fungi spores on child asthma hospitalizations.[31,34,35] However, to date, no comparable studies have accounted for individual factors such as HRV infection or fungal sensitization status that may interact with the effect of fungi exposure on asthma exacerbation. Our study examined potential confounding or effect modification by other individual and environmental factors. We included maximum temperature and relative humidity as *a priori* confounders because these were known to affect both spore production and were also associated with asthma exacerbations in children.[22] The analysis of other covariates in our modeling highlighted that HRV infection was strongly associated with asthma exacerbations, so we controlled for this factor in our modeling to reduce error variance. We interpreted the finding of HRV infection modifying the effects of *Sporormiella*, *Ganoderma*, and *Pithomyces* with caution because the relatively small number of participants with no HRV infection at admission and low fungal spore counts may contribute to these findings. Other individual factors such as age group, sex, or *Alternaria* sensitization status did not appear to act as effect modifiers. We also examined environmental covariates—ambient grass pollen, air pollutants ($PM_{2.5}$, NO_2, and O_3)—and found that they did not have significant confounding or effect modification. These analyses potentially reduced the possible bias in our findings.

Although we found significant associations between fungal spore taxa and children both sensitized and nonsensitized to

TABLE IV. Adjusted associations between fungi taxa and asthma hospitalizations stratified by *Alternaria* and *Cladosporium* sensitization status (yes/no) and with sensitization as an interaction term

Fungal spore species	N (d)	*Alternaria* sensitivity (n = 630)			*Cladosporium* sensitivity (n = 630)		
		Yes (n = 56), aOR* (95% CI)	No (n = 574), aOR* (95% CI)	*P* interaction	Yes (n = 41), aOR* (95% CI)	No (n = 588), aOR* (95% CI)	*P* interaction
Cladosporium	852	1.19 (0.97-1.46)	1.02 (0.99-1.04)	.71	1.02 (1.0-1.03)	1.02 (1.0-1.03)	.72
Leptosphaeria	852	1.04 (0.98-1.12)	**1.04 (1.02-1.07)**†	.87	1.09 (0.85-1.4)	**1.05 (1.02-1.4)**†	.75
Alternaria	852	**1.18 (1.08-1.29)**‡	**1.06 (1.02-1.10)**†	.62	**1.25 (1.07-1.45)**†	**1.06 (1.02-1.45)**†	**.002**
Smuts	852	0.83 (0.61-1.11)	0.98 (0.95-1.02)	.67	0.62 (0.32-1.03)	0.98 (0.95-1.03)	.18
Coprinus	852	**1.65 (1.13-2.4)**†	1.02 (0.99-1.05)	.29	**1.15 (1.08-1.22)**‡	1.03 (0.99-1.22)	**.021**
Drechslera	852	**1.14 (1.00-1.29)**†	1.02 (1.0-1.05)	.95	**1.24 (1.1-1.4)**‡	1.02 (0.99-1.4)	**<.001**
Periconia	852	**0.67 (0.46-0.99)**†	1.04 (0.97-1.11)	.20	1.14 (0.99-1.29)	0.99 (0.92-1.29)	.53
Pleospora	852	1.30 (0.92-1.83)	1.04 (0.9-1.2)	.63	0.90 (0.51-1.59)	1.07 (0.93-1.59)	.38
Ganoderma§	852	0.98 (0.84-1.13)	1.00 (1.0-1.0)	.91	1.00 (0-7.0)	0.98 (0.94-7.0)	**<.001**
Pithomyces	852	1.17 (1.01-1.04)†	1.01 (0.93-1.09)	.21	0.73 (0.36-1.48)	1.03 (0.96-1.48)	.98
Stemphylium	852	0.95 (0.63-1.41)	1.05 (0.97-1.13)	.62	**1.42 (1.06-1.9)**†	1.02 (0.94-1.9)	**.015**
Sporormiella§	852	**1.50 (1.03-2.1)**†	1.05 (0.94-1.17)	.87	1.64 (0.94-2.85)	1.06 (0.95-2.85)	.25
Total spores	852	1.24 (0.94-1.63)	**1.04 (1.0-1.08)**†	.48	1.06 (0.99-1.14)	**1.05 (1.0-1.14)**†	.30

OR per increase in fungal spores from 75th to 90th percentile. Statistically significant results are in boldface.
*Adjusted for HRV infection status, maximum temperature, relative humidity, grass pollen.
†$P < .05$.
‡$P < .001$.
§aOR for unit increase per fungal spore when the change from 75th to 90th percentile is 0.

TABLE V. Adjusted associations between fungi taxa and asthma hospitalizations—Lags 1, 2, and 3 and cumulative lag (Lag0-Lag3)

n = 644 Fungi species	aOR (95% CI)			
	Lag1*	Lag2*	Lag3*	Cumulative lag*
Cladosporium	0.98 (0.94-1.0)	1.01 (0.99-1.03)	1.03 (1.00-1.06)	1.00 (0.99-1.01)
Leptosphaeria	1.01 (0.99-1.04)	1.02 (0.99-1.04)	1.03 (1.00-1.05)	**1.01 (1.00-1.02)**†
Alternaria	**1.06 (1.03-1.1)**†	**1.06 (1.02-1.09)**†	1.03 (0.99-1.08)	**1.02 (1.01-1.04)**†
Coprinus	**1.06 (1.02-1.09)**‡	1.02 (1.0-1.05)	0.99 (0.93-1.05)	**1.02 (1.01-1.04)**‡
Drechslera	**1.03 (1.0-1.05)**‡	**1.04 (1.0-1.08)**‡	1.01 (0.98-1.04)	**1.01 (1.00-1.02)**‡
Periconia	1.0 (0.94-1.06)	1.01 (0.92-1.1)	**1.06 (1.0-1.13)**‡	1.01 (0.99-1.04)
Stemphyllium	**1.08 (1.0-1.17)**‡	0.99 (0.91-1.08)	1.02 (0.95-1.1)	1.02 (0.99-1.05)
Sporormiella§	1.02 (0.93-1.12)	**1.08 (1.00-1.17)**‡	0.98 (0.90-1.06)	1.07 (0.95-1.20)
Total spores	1.01 (.96-1.05)	1.04 (1.0-1.09)	**1.05 (1.0-1.1)**‡	**1.02 (1.00-1.03)**‡

aOR per increase in fungi spores from 75th to 90th percentile. Statistically significant results are in boldface.
*Adjusted for HRV status, relative humidity, maximum temperature, and grass pollen.
†$P < .001$.
‡$P < .05$.
§aOR for unit increase per fungal spore when the change from 75th to 90th percentile is 0.

Alternaria and *Cladosporium*, the effect estimates were stronger in sensitized children. Although *Alternaria* sensitization was not significant when fitted as an interaction term, our results suggest that *Alternaria* sensitization may be significant when children are exposed to *Alternaria*, *Coprinus*, *Drechslera*, and *Sporormiella*. Our finding that the associations between *Alternaria*, *Coprinus*, *Drechslera*, and *Stemphylium* exposure were stronger in individuals with *Cladosporium* sensitization does not appear to fit the current understanding of the mechanisms of allergic asthma and may be due to a number of factors. Accurately detecting fungal sensitization may be complicated by the lack of standardization of testing reagents used, possible variation in the wheal pattern with different reagents, and possible difference in reaction to reagents in the same person between testing periods.[10] The possibility of some cross-reactivity between fungal allergens (proteins) may also contribute to these findings. *Cross-reactivity* is the ability of the immune system to recognize similarities between different allergens, such that antibodies produced against one allergen will also react against another, similar allergen. As 25 fungal taxa in the Ascomycetes and Basidiomycetes phyla have been officially identified as allergenic by the Nomenclature Subcommittee of the World Health Organization/International Union of Immunological Societies (www.allergen.org),[36] the phenomenon of cross-reactivity complicates the attribution of fungal sensitization and fungal exposure to asthma exacerbation. Recent reviews of the field by Crameri et al[36,37] summarized prominent cross-reactive fungal allergen structures on the basis of evidence to date, suggesting that the importance of fungal cross-reactivity and its clinical significance required further *in vitro* and *in vivo* research using fungal allergens. Other factors to consider may include the following: differing severity of sensitization between fungal atopic children; accurate sensitization in very young children may be difficult to detect by skin prick testing; the children not sensitized to fungi may be sensitized to other allergens that may trigger an asthma exacerbation, such as pollen or house dust mites; or the small sample may limit adequate power to overcome possible statistical errors.

The fungi spore distributions are similar to those recorded by Mitakakis and Guest[38] at the same site in Melbourne in 1993, except that the proportions of *Alternaria* were higher in this sample than in 1993 (11.3 % compared with 1.6%) and proportions of *Coprinus* lower (6.6% compared with 16.5%). The dominance of *Cladosporium*, *Leptosphaeria*, and *Alternaria* spores and low counts for many fungal taxa have been reported in other sites such as in the United Kingdom,[25,31] Canada,[33] and Sydney, Australia.[39] The lack of association with *Pleospora*, *Ganoderma*, *Pithomyces*, *Sporormiella*, and smuts could be due to a number of reasons: they have not been found to be allergenic in clinical/laboratory studies[25] and/or they are present in very low numbers even during their maximum sporulation time and so their dose is not high enough to elicit an allergic reaction and/or the low counts lacked the statistical power needed to detect effects.

Strengths

This case-crossover design is well suited for studying the effects of transient short-term ambient exposures on the risk of rapid-onset events (ie, asthma exacerbation) in individuals. Because cases serve as their own controls, there is little risk of confounding due to stable individual characteristics (ie, age, sex, fungal sensitization, behavioral factors). The bidirectional selection of the control periods allows adjustment for seasonal trends. This is the first study in the Australasian region that has included daily measures of fungal spores and other environmental factors over a 2-year period. The sample was representative of the total daily child asthma admissions in Melbourne for that period[7]; hence, it can be considered to be generalizable to the young Melbourne population.

Limitations

Comparison of findings in this current report with findings in other studies is limited by significant variations in defining "exposure to fungi." Some studies report specific fungal taxa (aggregated and separately),[12,25] some report fungal phyla (grouping them by their reproductive processes: Ascomycetes/Basidiomycetes/Deuteromycetes),[31,40] and some as simply "total fungi."[41,42] The significant change in the fungi classification system in 2006,[43] with the incorporation of Deuteromycetes into Ascomycetes or Basidiomycetes phyla is important because some fungi species or taxa (eg, *Alternaria*, *Cladosporium*, and *Drechslera*) implicated in child asthma exacerbations were previously classified as Deuteromycetes but are now classified as either Ascomycetes or Basidiomycetes.

Exposure misclassification is a major limitation because the assessment of exposure to outdoor fungi was extrapolated from a single site. We cannot be certain that each child was exposed to the same levels counted at this single site and it is therefore impossible to gauge the generalizability of exposure. Levels of ambient fungi vary according to vegetation types and climate variations and there are no validated models currently available that enable us to assess the generalizability of exposure at outdoor and indoor levels. However, misclassification of fungal exposure is likely to be nondifferential; hence, it should bias the risk estimates toward null. Exposure assessment may also be limited by the absence of data on simultaneous indoor exposure to fungal spores, which can independently contribute to asthma exacerbation.[8,12] Indoor fungal spores constitute those produced within the indoor environment plus spores that enter the home through building openings. Previous research in homes in a nearby region found that spore counts were consistently higher outdoors during the warmer months, were higher indoors during the cold winter months, and were highly correlated.[44] To overcome potential confounding from outdoor fungi that moved indoors, we controlled for the climatic variables that most influence fungal spore production (maximum temperature and relative humidity). However, because we could not account for household conditions in relation to existing damp or mold, some asthma exacerbations attributed to outdoor fungal spores may be overestimated or the contribution of indoor spores to asthma exacerbations may be underestimated.

Our skin prick testing was limited to the 2 fungi taxa commonly associated with allergic asthma, *Alternaria* and *Cladosporium*, so we cannot be certain whether fungal sensitization to taxa other than these may be an important effect modifier.

Clinical implications

Identifying sensitization to multiple fungal allergens in children with asthma may help to guide improved asthma management. These findings may also contribute to informing future clinical trials of fungal immunotherapy of childhood asthma. Daily monitoring and reporting of high outdoor fungal spore levels could help reduce the risk of asthma exacerbations in high-risk children particularly if exposure reduction strategies are incorporated in their asthma management plans.

Conclusions

Children and adolescents with asthma exposed to ambient *Alternaria*, *Leptosphaeria*, *Coprinus*, and *Drechslera* in Melbourne, Australia, are at increased risk of being hospitalized with asthma independent of having HRV infection, *Alternaria* sensitization, and pollen and air pollution exposure. There are also associations with *Alternaria*, *Cladosporium*, *Coprinus*, *Drechslera*, *Stemphylium*, and *Periconia* over a range of lag periods before being hospitalized. The evidence of associations with *Leptosphaeria*, *Coprinus*, *Drechslera*, *Stemphylium*, and *Periconia* is new for this geographic region because these taxa have not been investigated before. Detecting sensitization to multiple fungal allergens may be important for asthma management. We need further studies to better understand the role of ambient fungi, fungal sensitization, and cross-reactivity in the causal pathway of asthma exacerbations from early childhood through to adolescence. This may permit identification of high-risk groups, support development of a public health warning system when relevant fungal spore counts are high, and support the design and implementation of more effective strategies to prevent asthma exacerbations.

HRV identification was performed by the Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia.

Clinical implications: Several outdoor fungi taxa, not previously reported before with children, are associated with childhood asthma hospitalizations. Identifying sensitization to multiple fungal allergens in children with asthma could guide asthma management.

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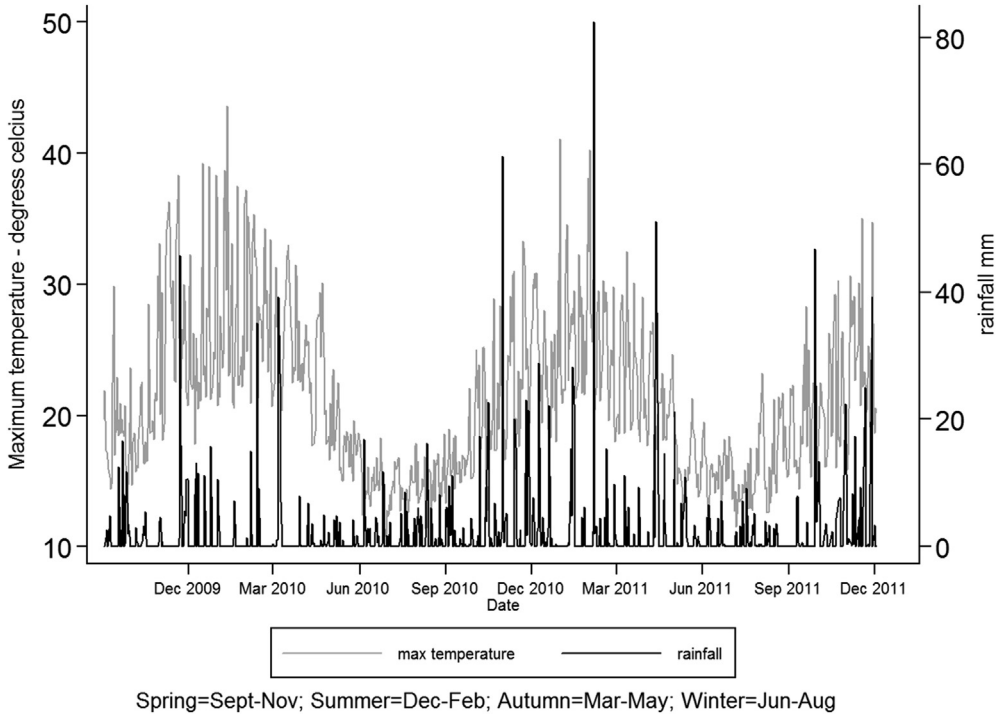

FIG E1. Maximum temperature and rainfall in Melbourne during the period September 2009 to December 2011.

TABLE E1. Correlations of fungal spore taxa and *P* values

Fungal spore taxa	Fungal spore taxa												
	Cladosporium	***Leptosphaeria***	***Alternaria***	**Smuts**	***Coprinus***	***Drechslera***	***Periconia***	***Pleospora***	***Ganoderma***	***Pithomyces***	***Stemphylium***	***Sporormiella***	**Total**
Cladosporium	1												
Leptosphaeria	0.4619	1											
	.0000												
Alternaria	0.4919	0.2576	1										
	.0000	.0000											
Smuts	0.3368	0.1307	0.28	1									
	.0000	.0001	.0000										
Coprinus	0.2524	0.4086	0.2686	0.0633	1								
	.0000	.0000	.0000	.0647									
Drechslera	0.4594	0.2943	0.4411	0.2147	0.2685	1							
	.0000	.0000	.0000	.0000	.0000								
Periconia	0.315	0.2156	0.2857	0.2123	0.212	0.3019	1						
	.0000	.0000	.0000	.0000	.0000	.0000							
Pleospora	0.245	0.3939	0.1663	0.1294	0.2619	0.1448	0.111	1					
	.0000	.0000	.0000	.0002	.0000	.0000	.0012						
Ganoderma	0.0253	0.1822	0.0618	−0.0251	0.245	0.0408	0.0467	0.1044	1				
	.4604	.0000	.0714	.465	.0000	.2341	.1735	.0023					
Pithomyces	0.1403	0.2014	0.219	0.0878	0.1862	0.2384	0.1753	0.1225	0.0336	1			
	.0000	.0000	.0000	.0103	.0000	.0000	.0000	.0003	.3278				
Stemphylium	0.3159	0.177	0.2912	0.0855	0.1471	0.2841	0.258	0.1557	0.022	0.1125	1		
	.0000	.0000	.0000	.0126	.0000	.0000	.0000	.0000	.5213	.001			
Sporormiella	0.1564	0.2054	0.1307	−0.0529	0.1759	0.1255	0.0359	0.0902	0.1453	0.0959	0.1641	1	
	.0000	.0000	.0001	.1229	.0000	.0002	.2954	.0084	.0000	.0051	.0000		
Total spores	0.8286	0.6258	0.6652	0.4044	0.4952	0.5567	0.4128	0.38	0.1856	0.2592	0.3571	0.1978	1
	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000	

TABLE E2. Adjusted associations between fungal taxa and asthma hospitalizations stratified by HRV infection status (yes/no) and with HRV infection status as an interaction term

	HRV infection present (n = 642), aOR (95% CI)*		
Fungal spore species	**No (n = 195)**	**Yes (n = 447)**	***P* interaction**
Cladosporium	0.98 (0.91-1.06)	1.09 (0.97-1.23)	.26
Leptosphaeria	1.03 (0.97-1.10)	**1.06 (1.01-1.11)**†	.25
Alternaria	1.05 (0.98-1.12)	**1.12 (1.00-1.26)**†	.80
Smuts	1.01 (0.97-1.06)	0.96 (0.92-1.01)	.66
Coprinus	1.04 (0.98-1.10)	1.08 (0.95-1.23)	.24
Drechslera	1.02 (0.97-1.06)	1.02 (0.97-1.07)	.63
Periconia	0.92 (0.79-1.07)	1.03 (0.92-1.16)	.36
Pleospora	1.01 (0.75-1.37)	1.06 (0.90-1.25)	.38
Ganoderma‡	0.82 (0.67-1.00)	1.07 (0.94-1.21)	**.01**
Pithomyces	1.05 (0.97-1.14)	0.91 (0.71-1.17)	**.03**
Stemphylium	0.86 (0.7-1.06)	1.13 (0.92-1.4)	.31
Sporormiella‡	0.79 (0.52-1.21)	**1.35 (1.05-1.74)**†	**.04**
Total spores	1.01 (0.92-1.11)	1.08 (0.96-1.2)	.59

aOR per increase in fungi spores from 75th to 90th percentile. Statistically significant results are in boldface.

*Adjusted for relative humidity, maximum temperature, and grass pollen.

†$P < .001$.

‡aOR for unit increase per fungal spore when the change from 75th to 90th percentile is 0.

TABLE E3. Adjusted multifungal models

Multifungal models*	OR (95% CI) (n = 644)
Alternaria	**1.06 (1.02-1.1)**†
Leptosphaeria	**1.04 (1.01-1.07)**‡
Alternaria	**1.06 (1.02-1.12)**‡
Coprinus	1.01 (0.97-1.06)
Alternaria	**1.09 (1.03-1.16)**‡
Drechslera	0.99 (0.95-1.03)
Leptosphaeria	**1.04 (1.01-1.07)**‡
Coprinus	1.03 (0.99-1.07)
Leptosphaeria	**1.04 (1.01-1.07)**‡
Drechslera	1.02 (0.99-1.05)
Coprinus	1.03 (0.99-1.07)
Drechslera	1.02 (0.99-1.05)

aOR per increase in fungi spores from 75th to 90th percentile. Statistically significant results are in boldface.

*Adjusted for HRV infection status, maximum temperature, relative humidity, and grass pollen.

†$P < .05$.

‡$P < .001$.