The Effect of Outdoor Fungal Spore Concentrations on Daily Asthma Severity

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The relationship between day-to-day changes in asthma severity and combined exposures to community air pollutants and aeroallergens remains to be clearly defined. We examined the effects of outdoor air pollutants, fungi, and pollen on asthma. Twenty-two asthmatics ages 9–46 years were followed for 8 weeks (9 May–3 July 1994) in a semirural Southern California community around the air inversion base elevation (1,200 ft). Daily diary responses included asthma symptom severity (6 levels), morning and evening peak expiratory flow rates (PEFR), and as-needed β-agonist inhaler use. Exposures included 24-hr outdoor concentrations of fungi, pollen, and particulate matter with a diameter <10 μm (PM_{10}; maximum = 51 μg/m³) and 12-hour day-time personal ozone (O_3) measurements (90th percentile = 38 ppb). Random effects longitudinal regression models controlled for autocorrelation and weather. Higher temperatures were strongly protective, probably due to air conditioning use and diminished indoor allergens during hot, dry periods. Controlling for weather, total fungal spore concentrations were associated with all outcomes: per minimum to 90th percentile increase of nearly 4,000 spores/m³, asthma symptom scores increased 0.36 (95% CI, 0.16–0.56), inhaler use increased 0.33 puffs (95% CI, -0.02–0.69), and evening PEFR decreased 12.1 l/min (95% CI, -1.8–22.3). These associations were greatly enhanced by examining certain fungal types (e.g., *Alternaria*, basidiospores, and hyphal fragments) and stratifying on 16 asthmatics allergic to tested deuteromycete fungi. There were no significant associations to low levels of pollen or O_3, but inhaler use was associated with PM_{10} (0.15 inhaler puffs/10 μg/m³; $p<0.02$). These findings suggest that exposure to fungal spores can adversely effect the daily respiratory status of some asthmatics. *Key words*: *Alternaria*, asthma, basidiomycete, epicutaneous allergen skin tests, epidemiology, longitudinal data analysis, ozone, panel study, particulate air pollution. *Environ Health Perspect* 105:622–635 (1997)

Although the importance of aeroallergens as triggers of asthma has been known for many years, particularly among asthmatics with demonstrable allergy (*1*), there is a lack of specific information on the relationship between the level of exposure to specific aeroallergens and daily asthma status. However, there is a relative abundance of literature concerning the inflammatory responses to respiratory allergens among allergic asthmatics and the importance of anti-inflammatory therapy for the treatment of allergic asthma (*2*). The present study focuses on the impact of fungal spores on acute asthma severity, a topic that has not been well investigated. Several published studies addressing this issue involving individual asthmatics have not utilized time–series analytic techniques to detect acute exposure–response relationships (*3–5*); other studies have involved correlations between aggregated exposure and response data (*6–9*). Additionally, more research has been called for regarding the synergistic or antagonistic effects on asthma status caused by different ambient airborne contaminants including interactions between air pollutants and aeroallergens (*10*).

The present study was designed to investigate the potential interactive and independent effects of ozone (O_3) and outdoor aeroallergens (pollens and molds) on daily asthma severity. This was carried out using the panel study design, which is characterized by longitudinal analyses of daily data for a panel of asthmatics, with each subject acting as his or her own control. This study was conducted in the small Southern California community of Alpine, California, over an 8-week period from 9 May through 3 July 1994. The time period was selected to maximize both O_3 and fungal spore exposures, and followed the peak spring pollen season for the area. This study represents the second in a series of epidemilogic studies utilizing personal passive O_3 exposures. The first study found that 12 asthmatic children followed daily for 6 weeks showed notably stronger adverse effects from personal O_3 than from outdoor stationary site O_3 and found that outdoor airborne fungal spores adversely effected asthma symptom levels and as-needed bronchodilator use (*11*). In a recently published O_3 modeling study, Liu et al. (*12*) utilized personal O_3 data from the panel of this paper and an additional panel from the fall of 1994 in Alpine; Liu et al. describe prediction models and factors that determine personal ozone exposure levels.

Materials and Methods

Study population and allergy testing. The community targeted by this study was chosen in order to maximize the ability to isolate the independent effect of O_3 from particulate air pollutants with which O_3 is commonly found to be highly intercorrelated. It is a semirural inland community of Southern California located around the small town of Alpine, whose residents live at or above the base of the air inversion zone (1,200 feet in elevation). It has had a permanent governmental air pollution monitoring site since 1981, with data showing high levels of transported and locally generated ozone but low levels of particulate air pollution relative to more urbanized regions of the United States (*13*).

The study protocols were approved by the institutional review boards of San Diego State University and the Southern California Permanente Medical Group. Informed written consent was obtained from all subjects and one of their legal guardians if they were under the age of 18 years. Recruitment of subjects was done by advertisements and through four schools in the Alpine area in addition to referrals from

the Kaiser Permanente Health Plan, Inc., Southern California Region, San Diego Area, Department of Allergy. Eligibility criteria of the study included 1) physician-diagnosed asthma with a minimum history of 1 year; 2) asthma exacerbations during several weeks of the warm seasons (March through October) requiring the use of prescribed asthma medications; 3) ages between 9 and 49 years; 4) a home and school or work address in the Alpine area; and 5) absence of patient/parental or indoor household smoking. After excluding two noncompliant subjects, the study population included 22 symptomatic asthmatics, 9 adults ages 24–46 years and 13 children ages 9–14 years.

Classification of allergy among subjects was based on the presence or absence of positive allergen reactivity as assessed using skin prick (epicutaneous) tests (SPT) (Bayer Corp., Allergy Products, Spokane, WA) for various pollens or molds common to the study area (*14*). Positive SPT reactivity was defined as a skin wheal greater than 3 mm or with a diameter greater than 50% of a histamine dihydrochloride control while in the presence of a negative saline control. The SPTs for locally relevant pollens consisted of standardized allergen extracts of nine trees, Bermuda grass, one grass mix, and two weed mixes. There were five SPTs for fungal spore taxa: *Alternaria alternata* (extract labeled *Alternaria tenuis*), *Cladosporium cladosporiodes* (extract labeled *Hormodendrum cladosporiodes*), *Helminthosporium interseminatum*, *Aspergillus* species mix (*A. fumigatus, A. terreus, A. niger, A. nidulans*), and *Penicillium* species mix (*P. digitatum, P. expansum, P. glaucum, P. roseum, P. notatum*). Subjects were also skin tested for house dust mites [*Dermatophagoides farinae* (*Der f*) and *D. pteronyssinus* (*Der p*)].

Outcomes. Within 2 weeks prior to the start of the study, participants were instructed on study procedures and administered a background interview. Subjects were trained and then tested on the proper use of a peak flow meter (Assess, Healthscan Products, Inc., Cedar Grove, NJ). After the start of the study, weekly follow-ups at the subject's homes were done to collect personal O_3 passive samplers, to clarify the accuracy of diary response information, and to discuss any problems with the study procedures. Daily diary responses were given concerning asthma symptom severity, and three morning and three evening peak expiratory flow rate maneuvers (PEFR) were recorded. Asthma symptoms (cough, wheeze, sputum production, shortness of breath, and chest tightness) were rated by the subjects in terms of their combined severity on a scale from 0 to 5. The six levels of asthma severity were classified as 0, no asthma symptoms present; 1, asthma symptoms present but caused no discomfort; 2, asthma symptoms caused discomfort but did not interfere with daily activities or sleep; 3, asthma symptoms interfered somewhat with daily activities or sleep; 4, asthma symptoms interfered with most activities and may have required that the participants stay home in bed, return home early from school or work, or call a doctor or nurse for advice; 5, asthma symptoms required either going to a hospital, emergency room, or outpatient clinic. Subjects were instructed to record PEFR measurements prior to using their inhalers. The highest of the three PEFR maneuvers was retained for the analysis. Peak flows were invalidated if the same value was recorded for the three successive maneuvers or if they did less than three maneuvers. Data were checked for repeated entries from day to day of the same PEFR level as an indication that maneuvers may not have been performed. Weekly home visits were used to check the performance of PEFR maneuvers in the presence of potentially invalid entries for the prior week. The other main outcome recorded by the subjects at the end of each day included the number of β-adrenergic agonist inhaler puffs used on an as-needed basis in the past 24 hr. The inhaler use variable is intended to reflect the level of daily asthma severity among subjects using this approach to therapy, thereby complementing the symptom variable. The presence or absence of upper or lower respiratory tract infections was also recorded by subjects.

Environmental variables. Twenty-four hour samples of airborne particulate matter of aerodynamic diameter <10 μm (PM_{10}) were measured with a tapered-element oscillating microbalance (TEOM) (*15*) located centrally at a monitoring station in Alpine and operated by the San Diego Air Pollution Control District (SDAPCD site). Sampling, analysis, and data processing protocols were carried out as part of the ongoing project (Epidemiological Investigation to Identify Chronic Health Effects of Ambient Air Pollution in Southern California) conducted at the University of Southern California (California Air Resources Board Project No. A033-186) (*13*). The PM_{10} data was collected and made available for this study by Sonoma Technology Inc., Santa Rosa, CA.

Personal O_3 exposures were measured using the Harvard personal passive sampler, which was worn daily by each subject over a 12-hr period, approximately between the hours of 8 A.M. and 8 P.M. This time period is relevant for outdoor exposures to peak O_3 levels. The personal O_3 sampler is a small device (2 cm diameter by 3 cm in length), which is clipped to the subject's clothes at chest level. Filters are coated with a potassium carbonate and sodium nitrite solution. Nitrite ion is oxidized by O_3 to nitrate ion (NO_3) (*16*). Ozone concentrations are determined by the amount of nitrate measured by ion chromatography. Comparisons of ozone concentrations between a standard continuous monitor and the passive sampler yielded excellent agreement (*16*); however, differences due to face velocity effects and O_3 depletion at body surfaces were found (*17,18*). The sampling methodology and validation of these samplers is fully described elsewhere (*16–18*). Continuous monitoring of outdoor ozone was conducted using UV photometry at the SDAPCD site. Weather variables measured at the SDAPCD site included hourly temperature and relative humidity.

Measurements of aeroallergens were made using the Burkard 7-day recording volumetric pollen and fungal spore collector with a sample flow rate of 10 l/min (Burkard Manufacturing Co., Rickmansworth Hertfordshire, England). Placement of the pollen and fungal spore sampler at the SDAPCD site ensured no nearby upwind obstructions and was 4 m above the ground. To prepare the sampler for pollen and fungal spore collection, Melenix tape (Burkard Manufacturing Co.) was placed on the mounted drum of the sampler and coated with silicone solution evenly applied with an artist's brush. After 7 days of continuous sample collection, the tape was cut into seven 24-hour segments from 8 A.M. to 8 A.M. and mounted onto microscope slides. The various fungal spores and pollen grains were then counted and identified using a compound microscope at 500× and 1000×. The pollen and fungal spore counts were then converted into spores per cubic meter of air for each 24-hour period.

Analytic variables for the total fungal spore and total pollen concentrations were derived by summing the daily concentrations of all identified and unidentified types of these two allergen groups. Other fungal variables were created by summing spore concentrations across genera related to the commonly assessed fungal SPTs as follows: 1) a test fungi variable was the sum of concentrations of each of the five fungal genera represented by the five fungal SPTs; 2) a nontest fungi variable was the total fungal spore concentration minus the test fungi concentration; 3) a positive SPT fungi variable was the sum of fungal spore concentrations of those five genera represented by the five fungal SPTs to which a subject tested positive; and 4) a negative SPT plus nontest fungi variable

was total fungal spore concentration minus the positive SPT fungi concentration. The assumption here was that a positive SPT to a fungal extract was an indication of allergy to the genus, although cross-reactivity patterns within single genera have not been fully described and are not likely to be 100%.

Analysis. The analysis began with descriptive statistics, which included time plots for the examination of possible trends in the data. Exposure correlation matrices were constructed to assess the amount of intercorrelation that could imply potential confounding or multicollinearity in the regression analyses.

Regression analyses were based on the general linear mixed model, which estimates both fixed and random effects (SAS MIXED procedure) (19) and is particularly suitable for longitudinal data analysis of serially correlated data in individuals (20). The SAS MIXED procedure optimizes a restricted maximum likelihood (REML) function by using the Newton-Raphson algorithm (19,21). Repeated daily measurements over time in every research subject constitutes a cluster of observations. The random effects modeling accounts for the heterogeneity from one such cluster of observations to another. This approach is applicable to this study because the periodicity of attacks and etiology of asthma is highly heterogeneous among asthmatics. For example, modeling each subject as a random effect using this procedure would accommodate the variation in the use of anti-inflammatory medications that could block an asthmatics respiratory response to an aeroallergen. This modeling is accomplished by essentially treating the subject's cluster of observations as a sample of intercepts and slopes (regression parameters) from some underlying population distribution. It is conceptually a set of separate regression models on repeated measurements over time for each individual. Every subject can therefore act as his or her own control. Anthropomorphic differences such as age, height, and gender that could potentially lead to differences in lung function were controlled for by estimating separate random intercepts for each individual. This allows the evaluation of the fixed effects of interest (aeroallergens and pollutants). For each modeled exposure, a common regression slope and its standard error were estimated.

The mixed model is a linear model that can account for correlated responses and the dependence of repeated observations in a single individual. The serial correlation must be accounted for to prevent bias in the estimation of statistical significance. The Akaike's information criterion (AIC, REML log likelihood—the number of covariance parameters) (19) was used to assess the fit of the various mixed models having the same

fixed effects, but with versus without modeling autocorrelation of the residual error terms. It was consequently found that the best models included first order autoregressive parameters to control for significant autocorrelation of residual errors. In addition to examining the relationship of asthma responses to same day exposures, levels of environmental factors were lagged 1 day prior to the day in which the diary was completed and compared to response levels to account for delayed effects.

Although symptom scores and as-needed β-agonist inhaler doses are ordinal outcome variables with non-normal distributions, residual errors from regressions using these ordinal variables in the models were found to be approximately normal, as indicated by normal probability plots and means of zero. In the regression analyses, maximum temperature and average relative humidity were examined for confounding and interaction, and days of the week trends in the outcome and environmental variables were tested to assess potential bias in the estimation of effects.

The estimates of effect were rescaled to represent the effect of a multiple unit change in exposure in its daily concentra-

tion (e.g., change in PEFR per increase of 100 ppb O_3). Standard errors of these estimates are also given. For the individual fungal types (e.g., *Alternaria*), this scale reflects the effect at the 90th percentile of the distribution for that fungal variable (i.e., the difference between the upper decile and the minimum concentration). This will allow a standardized view of effects for the fungal concentration ranges measured in this study. These effect estimates will be referred to in this paper as 90th percentile effects. Since concentrations for any particular group can be much higher in other geographic areas or during different time periods in the region of study, we will also present results for fungi as the amount of change in the response variable per increase of 1,000 fungal spores/m^3 of air. This is useful because it is largely unknown what the magnitude of effects or thresholds are for different fungal taxa, although it is expected to vary based upon individual sensitivity and fungal species (22). It should be cautioned, however, that for some fungal taxa with measured concentrations less than 1,000 spores/m^3, this effect magnitude would constitute an extrapolation beyond the estimated regression slope. For the

Table 1. Characteristics of individual participants in the spring 1994 Alpine Panel Study

Subject	Gender	Age	Medications	Tested fungal spore allergies[a]	Mean symptom score ± SD	Inhaler puffs ± SD
Pediatric subjects						
1	Male	14	Al, Be*, Cr, Th*	A, As, C, H, P,	0.22 ± 0.57	0.00 ± 0.00
2	Male	13	Al, Be	A, As, C, P	1.46 ± 1.06	1.34 ± 0.94
3	Female	14	Al, Cr*	None	1.08 ± 1.09	0.59 ± 1.04
4	Female	11	Al	A, As, C, H, P	0.95 ± 1.35	0.69 ± 1.10
5	Male	11	Al, Be*	A, As, H. P	0.86 ± 0.86	2.27 ± 1.98
6	Female	10	Al	A, As, C, H, P	0.38 ± 0.97	0.09 ± 0.35
7	Female	13	Al, Be,	A, As, C, H, P	1.10 ± 1.33	1.80 ± 3.49
8	Male	15	Al*, Be, Cr*	None	1.32 ± 0.83	0.93 ± 0.81
9	Male	10	Al, Tr, Th	A, As, C, H, P	0.79 ± 1.32	4.55 ± 4.39
10	Male	13	Al	A, C, H, P	1.69 ± 0.70	0.62 ± 1.01
11	Male	12	Al, Be*	A, As, C, H, P	1.91 ± 0.86	0.09 ± 0.36
12	Female	12	Al	None	2.22 ± 0.42	1.27 ± 1.27
13	Female	11	Al, Cr*	A	1.89 ± 0.98	7.24 ± 2.36
Adult subjects						
14	Male	37	Al, Be	None	1.09 ± 1.08	1.02 ± 1.04
15	Female	25	Al*, Be*	A	0.66 ± 0.98	0.90 ± 1.65
16	Female	44	Al, Be	None	0.95 ± 0.92	2.50 ± 2.60
17	Female	47	Al, Be*	None	2.02 ± 0.98	1.11 ± 1.47
18	Female	38	Be*	A	1.48 ± 1.18	0.00 ± 0.00
19	Female	36	Cr, Tb	A, H	1.46 ± 0.91	2.23 ± 1.72
20	Male	38	Al, Be*	A, P	2.71 ± 0.53	4.13 ± 1.63
21	Female	24	Al	A, As, C, H, P	2.46 ± 0.59	6.77 ± 1.44
22	Female	33	Tb	A, C, H	0.81 ± 1.33	0.30 ± 0.73

Abbreviations: Al, albuterol; Tr, triamcinolone acetonide; Be, beclomethasone dipropionate; Cr, Cromolyn; Th, theophyllin; Tb, terbutaline; A, *Alternaria alternata*; As, *Aspergillus* mix; C, *Cladosporium cladosporioides*; H, *Helminthosporium interseminatum*; P, *Penicillium* mix.
Indicates that the medication was prescribed for daily use, with the subject taking it as prescribed during the study, in addition to as-needed β-adrenergic agonist inhalers (Al and Tb). Albuterol was both as-needed and prescribed daily where indicated by.
[a]Positive fungal spore allergies tested with multitest epicutaneous punctures.

summed fungal variables defined above, effects are expressed as response change per 1,000 fungal spores/m³, which is within their observed concentration range.

In addition to the mixed model analysis of the panel data, autoregressive linear regression analyses of each individual's time–series data were performed to explore the possibility that particular subjects were responding to environmental exposures. This was accomplished using the SAS AUTOREG procedure (SAS Institute, Cary, NC) to estimate the autoregressive parameter(s) that were selected on the basis of controlling autcorrelation of the residual error as described above.

Statistical significance of fixed effects was attributed to *p*-values <0.05 for two-sided *t* statistics. The 0.05 level of significance was appropriately used as a test of significance rather than a more conservative Bonferroni correction: *p*<0.05/*k* tests, since there is ample evidence from experimental and nonexperimental studies that positive associations were to be expected for air pollutants and aeroallergens. Bonferroni or other multiple testing corrections are appropriate where little or no *a priori* evidence for effects is available. Despite the expectation of adverse effects, significance testing was based upon a two-sided critical value. It was also expected that the use of a single outdoor monitoring site for aeroallergen expo-

sures would lead to exposure misclassification and inflation of variance estimates. Therefore, models with *p*-values <0.1 were indicated in tables for individual fungal types and for autoregressive models testing effects in individual subjects.

Results

Outcomes. The characteristics of the 22 subjects are shown in Table 1. Subjects in this study were using a variety of as-needed β-agonist inhalers and daily prescribed medications, and 11 subjects took only as-needed β-agonist inhalers. Percent predicted PEFR (*23,24*) calculated from the average evening PEFRs were below 88% for both the pediatric and adult groups. There was little difference overall between the average levels of evening and morning PEFR, with a diurnal difference of only 3% higher levels in the evening than in the morning. Sixteen subjects were allergic to at least one of the five fungal SPTs; of these, all were allergic to *Alternaria*, and three were only allergic to *Alternaria*. All nine subjects allergic to *Aspergillus* were also allergic to *Penicillium*, but two subjects were allergic to *Penicillium* but not *Aspergillus*. This is important in the analysis of effects of these two spore types on the asthma outcomes, including the positive SPT variable, since *Penicillium* spores cannot be distinguished from *Aspergillus* under

the microscope. This required that responses from all 11 subjects be regressed on the summed concentrations of *Aspergillus* and *Penicillium*. All subjects were SPT positive to at least one pollen extract and 16 subjects were allergic to one or both dust mite antigens (*Der f* and *Der p*).

Daily diaries were obtained for a total of 1,218 person-days of observations from a possible 1,250 person-days. During this 8-week study, noncompliance with diary completion accounted for 32 person-days. There were 1,152 person-days of observation for asthma symptoms and 883 person-days of observation for as-needed β-agonist inhaler use. There were two subjects who only took prescribed inhalers daily (beclomethasone dipropionate) and did not use as-needed β-agonist inhalers (subjects 1 and 18, Table 1), accounting for 112 days not included in the analysis of as-needed β-agonist inhaler use. The remaining observations unavailable for analysis were in error or missing largely because subjects failed to enter a zero and, instead, left the field blank, despite repeated instructions to enter zeros if there were no symptoms or if they did not require as-needed β-agonist inhalers. Subjects were not allowed to recall events more than 1 day in the past; therefore, blank observations were considered missing. There were a total of 1,114 person-days of observation for morning PEFR and 1,107 person-days of observation for evening PEFR, with most of the remaining missing observations (104 and 111 person-days, respectively) for times when subjects did not perform maneuvers or made invalid entries. All of the PEFR data was invalidated for one subject who frequently did not perform the peak flow maneuvers properly despite retraining (subject 3, who used the spitting technique rather than a forced expiratory maneuver over several seconds).

There were no significant day-of-week trends in any of the outcome variables, but asthma symptom scores and as-needed inhaler use were slightly lower on average for Saturdays than for the rest of the week (*p*<0.12). Inclusion of a day-of-week indicator variable in the regression models for these variables did not confound any of the associations reported below. Also, all three of the outcomes were significantly worse during the reported presence versus absence of respiratory infections on 43 versus 1,094 person-days of observation, respectively (*p*<0.02). However, none of the associations reported below were confounded by the presence of respiratory infections.

Correlations between the three outcomes were tested with Spearman's rank correlation. The correlation of PEFR to both asthma symptom scores and as-needed

Table 2. Descriptive statistics for daily air pollution, aeroallergen, and weather measurements, 9 May–3 July 1994, Alpine area of San Diego County

Exposure	Number obs	Mean ± SD	Minimum/maximum	90th percentile
O₃ outdoor site 1-hr max (ppb)[a]	56	88 ± 25	46/147	119
O₃ outdoor site 12-hr (ppb)[b]	56	64 ± 17	34/103	84
O₃ personal 12-hr (ppb)[c]	1046	18 ± 14	0/80	38
PM₁₀ 24-hr (µg/m³)	41	26 ± 11	6/51	52
Temperature 1-hr maximum (°F)	56	75 ± 11	55/101	88
Relative humidity 24-hr (%)	56	68 ± 19	20/98	90
Total fungal spores 24-hr/m³[c]	55	2,959 ± 1,200	655/6,032	4,538
Alternaria 24-hr/m³	55	99 ± 66	0/296	190
Cladosporium 24-hr/m³	55	1,430 ± 668	247/3094	2,220
Helminthosporium 24-hr/m³	55	21 ± 17	0/78	49
Aspergillus and *Penicillium* 24-hr/m³	55	38 ± 47	0/204	120
Coprinus 24-hr/m³	55	36 ± 60	0/374	85
Periconia 24-hr/m³[c]	54	64 ± 55	0/233	127
Botrytis 24-hr/m³	55	36 ± 48	0/211	85
Total ascospores 24-hr/m³[c]	53	113 ± 93	0/423	232
Total basidiospores 24-hr/m³[c]	54	94 ± 96	7/458	190
Hyphal fragments 24-hr/m³	55	203 ± 108	21/416	352
Rusts 24-hr/m³	55	36 ± 23	0/99	70
Unidentified spores 24-hr/m³	55	717 ± 393	56/1,832	1,226
Pollen grains 24-hr/m³	55	216 ± 144	11/611	410

Abbreviations: obs, observations; SD, standard deviation; PM₁₀, particulate matter of aerodynamic diameter <10 µm.
[a]The stationary site outdoor monitor was the Alpine site operated by the San Diego Air Pollution Control District.
[b]Levels for the 12-hr sampling period were averaged from 8 A.M. to 8 P.M.; levels for the 24-hr sampling period were averaged from 12 A.M. to 12 P.M. for PM₁₀, temperature, and relative humidity and from 8 A.M. to 8 A.M. for fungal spores and pollen grains.
[c]The above statistics exclude high outliers for 12-hr personal O₃ (13 obs from 90 to 176 ppb); total fungal spores and total basidiospores (1 obs due to a gastromycete contaminant at 2,741 spores/m³); *Periconia* (1 obs at 402 spores/m³); total ascospores (2 obs at 677 and 881 spores/m³).

inhaler use was near zero, but symptom scores and as-needed inhaler use were moderately correlated ($R = 0.55$, $p<0.0001$).

Exposures. Out of a potential of 1,232 person-days of observation for personal O_3 samples, 117 observations were not obtained because the badge had not been worn, and 56 observations were invalidated due to other field errors by subjects or analytical problems. In addition, 13 personal O_3 samples were more than four times the standard deviation of the sample distribution above the mean (≥90 ppb, 12-hr average), leaving 1,046 samples for analysis (Table 2). We believe that these high measurements represent laboratory or analytic error because they are higher than outdoor concentrations and they were sampled during weekdays when subjects spent a majority of time indoors. In fact, after removing these in our ozone modeling study noted above, the expected higher concentrations on the weekends than on weekdays became clearer because subjects spent higher fractions of time outdoors on weekends (12). The possibility of indoor point sources for these values is remote since none of the subjects were operating electrostatic air filters, and exposure to copier machines would have to occur over long periods of time to account for such high levels. The limit of detection (LOD), defined as three times the standard deviation of the blanks (61 unexposed samplers), was 16.7 ppb. Given that subjects spent most of their time indoors during the 12-hr daytime sampling period (mean duration 71%), a majority (55%) of the personal O_3 samples were at or below the LOD. There were 53 (5.1%) personal O_3 samples that were 0 ppb. The frequent number of low personal O_3 measurements is in part attributable to lower levels of O_3 found indoors compared to outdoors, as previously reported (17). However, some of these observations may have been due to subjects not uncapping the sampler; but this could not be verified in the present study. The distribution of personal O_3 was markedly log-normal (skewness 1.1), and geometric mean (13 ppb) and standard deviation (2.6 ppb) was lower than arithmetic levels (Table 2). Consequently, a log transformation was tested along with untransformed data in the regression analysis of O_3. In contrast, the skewness for the outdoor 12-hr mean O_3 was 0.2 (Shapiro-Wilk statistic 0.95). The geometric mean personal O_3 exposure concentration was 22% of the arithmetic 12-hr mean O_3 at the outdoor stationary site (64 ppb). In addition, a high

Table 3. Correlation matrix of air pollution, aeroallergens, and weather, Alpine area of San Diego County, 9 May–3 July 1994[a]

	PM_{10}	Personal O_3[a]	Stationary site O_3	Fungal spores	Pollen	Maximum temperature	Minimum relative humidity
PM_{10}	1.00 (41)	0.15[†] (760)	0.55[†] (41)	0.21 (40)	0.59[†] (40)	0.66[†] (41)	-0.49[**] (41)
Personal O_3		1.00 (1,046)	0.18[†] (1,046)	-0.07[*] (1,026)	0.23[†] (1,026)	0.08[**] (1,046)	-0.02 (1,046)
Stationary site O_3			1.00 (56)	0.20[*] (55)	0.67[†] (55)	0.80[†] (56)	-0.74[†] (56)
Fungal spores				1.00 (55)	0.24 (55)	0.18 (55)	-0.27[*] (55)
Pollen					1.00 (55)	0.56[†] (55)	-0.59[†] (55)
Maximum temperature						1.00 (56)	-0.88[†] (56)
Minimum relative humidity							1.00 (56)

PM_{10}, particulate matter of aerodynamic diameter <10 μm.
[a]Sample sizes are given in parentheses; ozone (O_3) variables are 12-hr daytime exposures. Pearson's R correlations were used except for personal O_3 (Spearman's rank correlations were used because of its markedly log-normal distribution).
[*]$p<0.05$; [**]$p<0.01$; [†]$p<0.001$.

Table 4. Correlation matrix of fungal types, Alpine area of San Diego County, 9 May–3 July 1994[a]

	Alternaria	Cladospor	Helminth	Asp-Pen	Coprinus	Pericon	Botrytis	Ascospore	Basidio	Hyphae	Rusts	Spores NI
Alternaria	1.00 (55)	0.65[†] (55)	0.56[†] (55)	0.24 (55)	0.064 (55)	0.14 (55)	0.51[†] (55)	-0.09 (53)	-0.01 (54)	0.52[†] (55)	0.42[**] (55)	0.49[†] (55)
Clado		1.00 (55)	0.55[†] (55)	0.29[*] (55)	0.34[*] (55)	0.22 (55)	0.64[†] (55)	0.16 (53)	0.21 (54)	0.50[†] (55)	0.25 (55)	0.66[†] (55)
Helminth			1.00 (55)	0.16 (55)	0.28[*] (55)	0.04 (55)	0.38[**] (55)	-0.08 (53)	0.12 (54)	0.35[**] (55)	0.061 (55)	0.23 (55)
Asp-Pen				1.00 (55)	0.24 (55)	0.33[*] (55)	0.034 (55)	-0.00 (53)	0.09 (54)	0.24 (55)	0.19 (55)	0.27[*] (55)
Coprinus[b]					1.00 (55)	0.08 (55)	0.34[*] (55)	0.44[**] (53)	0.78[†] (54)	0.08 (55)	0.01 (55)	0.08 (55)
Pericon						1.00 (55)	0.18 (55)	-0.07 (53)	-0.08 (54)	0.48[†] (55)	0.43[†] (55)	0.35[**] (55)
Botrytis							1.00 (55)	0.18 (53)	0.36[**] (54)	0.44[†] (55)	0.32[*] (55)	0.43[**] (55)
Ascospore								1.00 (53)	0.35[*] (52)	-0.15 (53)	-0.09 (53)	0.08 (53)
Basidio									1.00 (54)	-0.19 (54)	-0.11 (54)	-0.05 (54)
Hyphae										1.00 (55)	0.48[†] (55)	0.72[†] (55)
Rusts											1.00 (55)	0.41[**] (55)
Spores NI												1.00 (55)

Abbreviations: Clado, Cladosporium; Helminth, Helminthosporium; Asp-Pen, Aspergillus-Penicillium; Pericon, Periconia; Basidio, total basidiospores; NI, not identified.
[a]Sample sizes are shown in parentheses; Pearson's R correlations were used except for Aspergillus-Penicillium, Coprinus, and Botrytis, where Spearman's rank correlations were used because of their markedly log-normal distributions.
[b]Coprinus was the major identified basidiospore and is included in the total basidiospore variable.
[*]$p<0.05$; [**]$p<0.01$; [†]$p<0.001$.

degree of interindividual variability in personal O_3 concentrations was found, as presented in our previous paper (*12*).

Due to equipment malfunction, 15 days were missing for PM_{10}. Levels of PM_{10} were low as expected with the maximum 24-hr average being only 51 µg/m³ (Table 2). The distribution of PM_{10} was approximately normal (skewness 0.1, Shapiro-Wilk statistic 0.98). On 1 day (15 May) most of the Burkard tape adhesive surface was lost.

Levels of fungi were moderately high but pollen was low, given that the spring pollen season in the region had nearly ended at the beginning of the study (Table 2). Pollen levels were dominated by one pollen from the native plant *Adenostoma* (mean 125 grains/m³). The most common spore was *Cladosporium* (mean 1,430 spores/m³). *Alternaria* (mean 99 spores/m³; maximun 296 spores/m³) was only 3.3% of the average total fungal concentration. Concentrations of one of the SPT fungi, *Helminthosporium*, was low (mean 21 spores/m³). *Coprinus* was one basidiospore examined separately because it was one-third of the total basidiospore concentration (Table 2). Three fungal taxa (*Aspergillus-Penicillium*, *Coprinus*, and *Botrytis*) showed markedly log-normal distributions because of frequent zeros or low concentrations (skewness >1.5) and had geometric means of 15–16 and geometric standard deviations (SD) of 4–5. One extreme outlier for the basidiospores of 2,818 spores/m³ (>7 times the SD and >29 times the interquartile range) was due to an unusual peak in gastromycetes (puffballs) of 2,741 spores/m³. Because the 90th percentile of gastromycetes was zero and the nature of puffballs is to release large bursts of spores, it is likely that this observation was due to a puffball release near the Burkard spore trap. Therefore, the observation was dropped because it was not relevant to the regional analysis of health effects. A notable effect of this single observation on the regression analysis is described in Results. As compared with the fungal types reported in Table 2, smaller concentrations (means <20 spores/m³) were found for numerous other fungal taxa (Smuts, *Epicoccum*, *Oidium*, *Ganoderma*, *Bovista*, *Stemphylium*, and others). They are grouped together with the other spore types into the nontest fungi variable.

A correlation matrix for the air pollutant, summed aeroallergen, and weather variables is presented in Table 3. There were several modest but statistically significant correlations of the air pollutants (PM_{10} and O_3) with the aeroallergens (total pollen and total fungal spores), suggesting a potential for

aeroallergens to confound or modify associations between air pollutants and respiratory health outcomes, or vice versa. This was due in large part to shared meteorological determinants with higher levels of these variables during warm, dry weather, although many fungi in the region of study, particularly the ascomycetes and basidiomycetes (*14*), sporulate during damp, cool periods when pollution levels are lower. Correlations of the outdoor environmental variables to personal O_3 exposure measurements were low, again suggesting that personal activity patterns such as indoor residence time dominated the exposure profile (*12*).

The basidiomycetes and ascomycetes were not significantly correlated with the remaining nontest spores as a group or with the test fungi variable ($p>0.15$). Table 4 shows a correlation matrix for the specific fungal types examined, which further supports this finding. A small correlation was found between basidiospores and ascospores ($R = 0.35$) and between basidiospores and one of the deuteromycetes, *Botrytis*. The

deuteromycetes (*Periconia, Botrytis*, and the five test fungi) were moderately correlated with each other in general. Two test fungi (*Alternaria* and *Cladosporium*) were moderately correlated to hyphal fragments, rusts, and unidentified spores with the exception of *Cladosporium* and rusts. Higher levels of ascomycetes and basidiomycetes were found during the cooler, damper period during the first 3 weeks of the study, which contributed to a lack of temporal correlation with the other spore types, many of which like the deuteromycetes of *Alternaria* and *Cladosporium* sporulate in response to low humidity (*25*).

Regression models, symptom severity. A major determinant of symptom severity was maximum temperature, with hotter temperatures being associated with lower symptom levels (Table 5, model A; symptom scores decreased by 0.2 for each 10°F increase in temperature). Temperatures were warm for all of the study period after 26 May; for the period before 26 May, 12-hr daytime average temperatures never

Table 5. Asthma symptom severity score: selected regression models, the Alpine Asthma Panel Study, 9 May–3 July 1994

Model	Variables	Estimated coefficient[a] ± SE	*p*-value
All subjects (*n* = 22)			
A	Maximum temperature	-0.021 ± 0.003	0.00001
B	Total fungal spores	0.070 ± 0.026	0.009
C	Total fungal spores	0.093 ± 0.026	0.0003
	Maximum temperature	-0.024 ± 0.003	0.00001
D	Test fungi[b]	0.158 ± 0.042	0.0002
E	Nontest fungi[c]	0.139 ± 0.053	0.009
Subjects allergic to fungal spores tested (*n* = 16)			
F	Total fungal spores	0.108 ± 0.031	0.0006
G	Positive SPT fungi[d]	0.159 ± 0.062	0.01
H	Negative SPT and nontest fungi[e]	0.134 ± 0.044	0.003
Pediatric subjects			
I	Total fungal spores (*n* = 13 subjects)	0.102 ± 0.033	0.002
J	Positive SPT fungi (*n* = 10 allergic subjects)	0.134 ± 0.069	0.05
K	Negative SPT and nontest fungi (*n* = 10 allergic subjects)	0.107 ± 0.062	0.09
Adult subjects			
L	Total fungal spores (*n* = 9)	0.072 ± 0.041	0.08
M	Positive SPT fungi (*n* = 6 allergic subjects)	0.296 ± 0.125	0.02
N	Negative SPT and nontest fungi (*n* = 6 allergic subjects)	0.135 ± 0.057	0.02

SPT, allergy skin prick tests; SE, standard error.
[a]Regression coefficients for fungal exposures and their standard errors are × 1,000 fungal spores per m³; models C–N controlled for maximum temperature (°F) and involve exposures on the same day as symptom reporting.
[b]Fungal spore concentrations of those five genera represented by the SPTs, namely, *Alternaria, Helminthosporium, Cladosporium, Aspergillus,* and *Penicillium.*
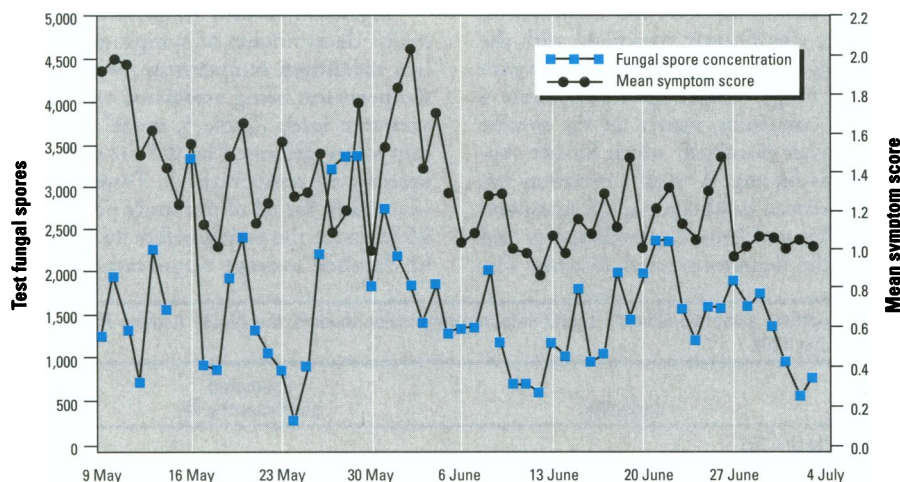[c]Total fungal spore concentration minus the five genera represented by SPTs.
[d]Fungal spore concentration of those five genera tested to which subjects showed a positive SPT.
[e]Total fungal spore concentration minus the concentrations of the five genera tested to which subjects showed a positive SPT.

dropped below 53°F. Total fungal spore concentrations were positively associated with asthma symptom scores, and the magnitude of the relationship was greater when controlling for temperature (Table 5, model C). The remaining models in Table 5 control for temperature. The magnitude of the association with asthma symptoms was similar for test fungi and nontest fungi (Table 5, models D and E, respectively). For an increase of 1,000 test or nontest fungal spores/m³, asthma symptom scores significantly increased by 0.16 and 0.14, respectively. A time–series plot of mean

symptom scores adjusted for temperature versus test fungal spore concentration illustrates the relationship, but note that the regression analysis was based upon individual time–series data and not on data aggregated across subjects as shown in Figure 1.

The analysis was then restricted to the 16 subjects with a positive SPT to at least one of the five fungal genera tested. The largest parameter estimate was found for positive SPT fungi, as opposed to total spore concentrations or to negative SPT plus nontest fungi. However, differences were small and the standard error of the estimate was larger

for the positive SPT fungi (Table 5, model G vs. models F and H, respectively). There were no significant effects of fungal spores (including total, test, and nontest fungi) on asthma symptom scores in models including the six individuals with negative SPTs for the five fungal taxa ($p<0.2$; models not shown). The analysis was further subdivided into pediatric and adult groups. Again, for both age groups, the largest parameter estimate was for positive SPT fungi, but higher standard errors were also found (Table 5, models J and M). The largest parameter estimate of any of the models for fungi in Table 5 was for positive SPT fungi for the six fungal allergic adults (model M; asthma symptom scores increased 0.3/1,000 spores/m³, $p<0.02$). However, 95% confidence limits clearly include the point estimates for the other models (0.05–0.54/1000 spores/m³). In contrast to the model for asthma symptom scores among all nine adults in relation to total fungal spore levels (Table 5, model L), symptoms among the six fungal allergic adults were significantly associated with total fungal spore levels (scores increased 0.14/1,000 spores/m³; $p<0.02$; model not shown). Again however, 95% confidence limits overlap considerably with other estimates (0.04–0.23/1,000 spores/m³).

Neither total pollen nor *Adenostoma* pollen levels were associated with asthma symptom severity. Also, personal O_3, stationary site outdoor O_3, and PM_{10} were not associated with symptoms. Log-transformed personal O_3 was also not associated with symptoms. No interaction or confounding between these environmental variables and fungal spores or weather variables was found in relation to symptoms.

Regression models, peak expiratory flow rates. Evening PEFR, but not morning PEFR, was inversely associated with fungal spore concentrations. As with symptoms, there was a major effect on evening PEFR from maximum temperature, with hotter temperatures being associated with higher evening PEFR (Table 6, model A; increase of 5.2 l/min/10°F; $p<0.003$). Similar to the association between asthma symptoms and fungi, the magnitude of the effect of total fungal spores on PEFR was greater when controlling for temperature, and this effect became statistically significant (Table 6, model C). The magnitude of the inverse association to PEFR was similar for both the test fungi and nontest fungi (Table 6, models D and E, respectively). For an increase of 1,000 test or nontest fungal spores/m³, PEFR decreased by 4.3 and 6.2 l/min, respectively ($p<0.05$).

A subset analysis involving the 16 subjects who had at least one positive SPT to the test fungi showed the largest effect from positive

Figure 1. Time plot of daily mean asthma symptom scores and test fungal spore concentration: spring 1994 Alpine Panel Study. Asthma symptom scores are averaged across 22 subjects and adjusted for maximum hourly temperature. Test fungal spore concentrations are in spores/m³ air and include summed concentrations of those five genera represented by the allergy skin prick tests, i.e., *Alternaria*, *Helminthosporium*, *Cladosporium*, *Aspergillus*, and *Penicillium*.

Table 6. Evening peak expiratory flow rate (PEFR) and as-needed β-agonist inhaler use: selected regression models, the Alpine Asthma Panel Study, 9 May–3 July 1994

Model	Variables	Estimated coefficient ± SE[a]	p-value
Peak expiratory flow rate			
A	Maximum temperature	0.52 ± 0.17	0.003
B	Total fungal spores	-2.37 ± 1.34	0.08
C	Total fungal spores	-3.10 ± 1.35	0.03
	Maximum temperature	0.63 ± 0.18	0.0003
D	Test fungi[b]	-4.33 ± 2.20	0.05
E	Nontest fungi[c]	-6.17 ± 2.74	0.03
As-needed β-agonist inhaler use			
F	Maximum temperature	-0.010 ± 0.007	0.14
G	Relative humidity	0.007 ± 0.004	0.06
H	Total fungal spores	0.074 ± 0.046	0.12
I	Total fungal spores	0.085 ± 0.047	0.07
	Relative humidity	0.008 ± 0.004	0.07
J	Test fungi	0.161 ± 0.070	0.02
K	Nontest fungi	0.150 ± 0.094	0.12

[a]Regression coefficients for fungal exposures and their standard errors (SE) are × 1,000 fungal spores/m³ and involve exposures on the same day as PEFR measurements (l/min) in 21 subjects, or the same day for which the number of inhaler puffs were reported in 20 subjects; regression models C–E for PEFR controlled for maximum temperature (°F) and regression models I–K for inhaler use controlled for mean relative humidity (%).
[b]Fungal spore concentrations of those five genera represented by the allergy skin prick tests (*Alternaria*, *Helminthosporium*, *Cladosporium*, *Aspergillus*, and *Penicillium*).
[c]Total fungal spore concentration minus the five genera represented by skin prick tests.

SPT fungi (-5.5 l/min/1,000 spores), as compared with total spore concentrations (-2.8 l/min/1,000 spores). However, these effects were borderline statistically significant ($p<0.08$). There were no significant effects of fungal spores on PEFR in a group analysis of the six individuals with negative SPTs for fungi. A subset analysis of the effect of fungal spore concentrations on PEFR among pediatric and adult groups showed that although nearly all parameter estimates were negative, the only statistically significant model was for the effect of total fungal spore concentrations on pediatric subjects (-3.1 l/min/1,000 spores; $p<0.05$).

Similar to results for symptoms, there were no associations of morning or evening PEFR to total pollen, *Adenostoma* pollen, personal O_3, stationary site outdoor O_3, or PM_{10}. No interaction or confounding between these environmental variables and fungal spores or weather variables was found in relation to PEFR.

Regression models, as-needed inhaler use. As-needed β-agonist inhaler use varied inversely with maximum temperature (Table 6, model F; -0.1 puffs/10°F; $p<0.14$) and positively with average relative humidity (0.07 puffs/10%; $p<0.06$). The magnitude and statistical significance of an association between inhaler use and total fungal spore concentrations alone was greater when controlling for relative humidity (Table 6, model I vs. model H) or for temperature. Inclusion of temperature in the regression did not improve the fit of the model as well as humidity. The magnitude of the association to inhaler use was similar for test fungi and nontest fungi, but only the test fungi were statistically significant (Table 6, models J and K). For an increase

of 1,000 test fungal spores/m^3, inhaler use increased by 0.16 puffs ($p<0.02$).

A subset analysis of the 16 fungal allergic subjects again showed the largest effect estimate from positive SPT fungi (0.20 puffs/1,000 spores/m^3; $p<0.07$), although the p-value was smaller for total spore concentrations (0.11 puffs/1,000 spores/m^3; $p<0.001$). There were no significant associations between inhaler use and fungal spores in the six individuals with negative SPTs for the five fungal taxa ($p<0.5$). The only statistically significant effects on inhaler use among adults were for positive SPT fungi on the 6 fungal allergic adults (0.44 puffs/1,000 spores/m^3; $p<0.05$). However, the largest effect on inhaler use in the pediatric group was for negative SPT plus nontest fungi among the fungal allergic (0.36 puffs/1,000 spores/m^3; $p<0.003$) as compared to total spore concentrations for either all children or fungal allergic children (0.14 and 0.17 puffs/1,000 spores/m^3, respectively; $p<0.02$). The lower 95% confidence limit for negative SPT plus nontest fungi just includes these point estimates for total fungi (0.12–0.59/1,000 spores/m^3). Unlike results for asthma symptoms, positive SPT fungal concentrations were not significantly associated with inhaler use among pediatric subjects ($p<0.37$).

Total pollen, *Adenostoma* pollen, personal O_3, and stationary site outdoor O_3 were not associated with as-needed β-agonist inhaler use. No interaction or confounding between these environmental variables and fungal spores or weather variables was found in relation to inhaler use. However, in contrast to the other outcomes, inhaler use was associated with PM_{10} concentrations. Controlling for average relative humidity,

inhaler puffs increased by 0.15/10 μg/m^3 of PM_{10} ($p<0.02$). No interaction or confounding between PM_{10} and fungal spores was found.

Regression analysis of individual fungal types. To assess the potential effects of the different types of test and nontest fungi on asthma status, regression models for each of the three outcomes included the following fungal types: 1) each the five separate SPT fungi except *Aspergillus* and *Penicillium*, which had to be combined as discussed above; 2) total basidiospores; 3) the major identified basidiospore, *Coprinus*; 4) total ascospores; and 5) the major nontest spores other than the basidiospores or ascospores (the deuteromycetes *Periconia* and *Botrytis*, hyphal fragments, rusts, and unidentified taxa). Regressions for each of the five test fungi involved subjects allergic to the spore type, whereas regressions for the nontest fungal types involved all 22 subjects (Table 7). One extreme outlier for the basidiospores due to gastromycete contamination discussed above and two high ascospore outliers >677 spores/m^3 (>8 times the interquartile range) were excluded from the regression analysis because they had a great influence on parameter estimates and residual errors and led to negative results for basidiospores and unexpected significant inverse associations for ascospores. The same was found for one *Periconia* outlier (402 spores/m^3; >5 times the interquartile range), which when included in regressions, halved the regression parameter estimates.

The magnitudes of effect on all three outcomes by *Alternaria* and *Helminthosporium* were notably greater than the other test spores *Cladosporium* and *Aspergillus-Penicillium* when estimates of effect were

Table 7. Effects of specific fungal types on three asthma outcomes, the Alpine Asthma Panel Study, 9 May–3 July 1994[a]

Fungal types (90th percentile-minimum)	Asthma symptom scores		Evening peak expiratory flow		As-needed β-agonist inhaler	
	Change/1,000 particles/m^3 ± SE	Change/90th percentile-mininum ± SE	Change/1,000 particles/m^3 ± SE	Change/90th percentile-mininum ± SE	Change/1,000 particles/m^3 ± SE	Change/90th percentile-mininum ± SE
Total fungi (3,883 spores/m^3)	0.09 ± 0.03[‡]	0.36 ± 0.10[‡]	-3.10 ± 1.35[**]	-12.06 ± 5.23[**]	0.08 ± 0.05[*]	0.33 ± 0.18[*]
Alternaria[b] (190 spores/m^3)	1.41 ± 0.55[**]	0.27 ± 0.10[**]	-40.47 ± 27.05	-7.69 ± 5.14	1.49 ± 0.98	0.28 ± 0.19
Cladosporium[b] (1,973 spores/m^3)	0.19 ± 0.07[**]	0.36 ± 0.14[**]	-6.16 ± 3.70[*]	-12.16 ± 7.30[*]	0.23 ± 0.10[**]	0.45 ± 0.20[**]
Helminthosporium[b] (49 spores/m^3)	5.57 ± 2.15[†]	0.27 ± 0.10[†]	-201.2 ± 117.8[*]	-9.86 ± 5.77[*]	6.68 ± 3.11[**]	0.33 ± 0.15[**]
Aspergillus-Penicillium[b] (120 spores/m^3)	0.46 ± 0.79	0.06 ± 0.10	-68.90 ± 44.15	-8.27 ± 5.30	-1.29 ± 1.17	-0.16 ± 0.14
Total basidiospores (183 spores/m^3)	1.52 ± 0.37[‡]	0.28 ± 0.07[‡]	3.70 ± 19.68	0.68 ± 3.60	-0.00 ± 0.67	-0.00 ± 0.12
Total ascospores (232 spores/m^3)	-0.03 ± 0.33	-0.01 ± 0.08	-23.08 ± 16.48	-5.35 ± 3.82	-0.85 ± 0.53	-0.20 ± 0.12
Coprinus (85 spores/m^3)	2.31 ± 0.53[‡]	0.20 ± 0.04[‡]	15.12 ± 28.19	1.28 ± 2.40	0.81 ± 0.99	0.07 ± 0.08
Periconia (127 spores/m^3)	2.33 ± 0.57[‡]	0.30 ± 0.072[‡]	-75.90 ± 29.87[**]	-9.64 ± 3.79[**]	1.84 ± 1.03[*]	0.23 ± 0.13[*]
Botrytis (85 spores/m^3)	1.81 ± 0.66[†]	0.15 ± 0.06[†]	-21.07 ± 34.18	-1.79 ± 2.90	2.24 ± 1.22[*]	0.19 ± 0.10[*]
Hyphae (331 fragments/m^3)	0.84 ± 0.32[†]	0.28 ± 0.10[†]	-53.90 ± 16.27[‡]	-17.84 ± 5.39[‡]	1.25 ± 0.56[**]	0.41 ± 0.18[**]
Rusts (70 spores/m^3)	2.04 ± 1.29	0.14 ± 0.09	-113.6 ± 66.6[*]	-7.95 ± 4.66[*]	3.73 ± 2.23[*]	0.26 ± 0.16[*]
Unidentified spores (1,170 spores/m^3)	0.10 ± 0.08	0.12 ± 0.09	-9.07 ± 4.15[**]	-10.61 ± 4.85[**]	0.28 ± 0.14[**]	0.33 ± 0.16[**]

SE, standard error.
[a]All models control for weather.
[b]Regressions included allergic subjects only (*Alternaria*, 16 subjects; *Cladosporium*, 10 subjects; *Helminthosporium*, 11 subjects; *Aspergillus-Penicillium*, 11 subjects; 1 subject allergic to all 5 fungi; and 1 subject only allergic to *Alternaria* did not use as-needed β-agonist inhalers); remaining models include all 22 subjects.
[*]$p<0.1$; [**]$p<0.05$; [†]$p<0.01$; [‡]$p<0.001$.

expressed per 1,000 spores/m³ (Table 7). However, differences were small when estimates of effect were expressed for 90th percentile increases in spore concentrations. None of the models were significant for *Aspergillus-Penicillium* and only borderline significant effects were found for the remaining test fungi in relation to PEFR ($p<0.1$). Effects of *Alternaria* were significant only for symptoms, although the direction and magnitude of parameter estimates for PEFR and inhaler use were suggestive of adverse effects.

Asthma symptom scores among all 22 subjects were strongly associated with basidiospore levels (symptom scores increased 1.5/1,000 spores/m³; $p<0.00005$, controlling for temperature). The magnitude of this effect was the same for a regression involving only the 16 subjects with positive skin tests for fungi (model not shown). Asthma symptom scores were also strongly associated with basidiospore levels on the day prior to the day of asthma symptom reporting, suggesting a possible lag effect as well (symptom scores increased 1.0/1,000 spores/m³; $p<0.004$, controlling for temperature; model not shown). However, neither PEFR nor as-needed β-agonist inhaler use were associated with basidiospore levels. The magnitude of the basidiospore effect was not larger than many of the other fungal types when estimates of effect were expressed as a 90th percentile effect, similar to what was found for *Alternaria* and *Helminthosporium*. The slope of effect was larger still for the basidiospore genus *Coprinus* (symptom scores increased 2.3 vs. 1.5/1,000 spores/m³, respectively), whereas the 90th percentile effect was similar again. None of the outcome variables were associated with ascospore concentrations. Notably large slopes were found for the other nontest fungal types, including the deuteromycetes *Periconia* and *Botrytis*, hyphal fragments, rusts, and unidentified taxa. Again, 90th percentile effects were not much different than other fungal types, with the possible exception of hyphal fragments and PEFR (-18 l/min decrease for 331 fragments/m³). Interestingly, the most consistently significant effects across all outcomes were from the hyphal fragments.

Given that there was a decreasing linear trend in asthma symptoms and basidiospores, it is possible that the association found was due to temporal confounding. Therefore, the analysis was split into two time periods: an earlier period of cool, damp weather prior to 27 May, having higher basidiospore concentrations and symptom scores, and a later period of warm, dry weather, having lower basidiospore concentrations and symptom scores. The early period showed an effect of basidiospore on asthma symptoms, which

was somewhat smaller than the analysis for the total period (symptom scores increased 1.0/1,000 spores/m³; $p<0.02$, controlling for temperature). There was no significant effect for the later time period. This indicates that the effect of basidiospores was largely isolated to the first 3 weeks when levels of sporulation were higher. However, the effect of total spore concentrations on asthma symptoms was statistically significant ($p<0.02$) for both time periods (symptom scores increased 0.12 and 0.09 /1,000 spores/m³ for the earlier and later period, respectively).

Individual regression analysis. Autoregressions for 7 of 13 pediatric asthmatics showed statistically significant and large changes in asthma symptom scores from basidiospores (2.5–6.0/1,000 basidiospores/m³), but none of the 9 adults showed significant autoregressions (Table 8). The autoregressions showed far fewer significant effects from the test spores for either symptom scores, PEFR, or inhaler use. One male adult subject not allergic to test spores (subject 14) showed unexpected inverse associations between symptoms and test spores ($p<0.1$) and between inhaler use and test spores ($p<0.05$). Two pediatric subjects who were not allergic to test spores (subjects 3 and 8) and one adult nonaller-

gic subject (subject 17) showed significant or borderline significant effects from test fungi on one or two of the three outcomes. This suggests that either the SPTs were falsely negative or other nontest fungi were driving the associations. The two pediatric subjects in question showed marked effects on symptoms scores from basidiospores (symptom scores increased 6.0 and 3.9 /1,000 basidiospores/m³). Coregression of test spores with basidiospores in autoregression models for these two subjects showed that for pediatric subject 3 the regression parameter for symptoms and test spores was markedly reduced (0.19/1,000 spores/m³; $p<0.3$) and the significant effect of basidiospores was relatively unchanged. For the other subjects in question, coregression did not alter effects of either test spores or basidiospores.

Other individual autoregressions tested the relationship of the three outcomes to *Alternaria*, the nontest fungi variable, and the positive SPT fungi variable (results not shown). Autoregressions for pediatric subjects 6 and 11 and adult subjects 19 and 22 showed significant adverse effects on one to two outcomes in association with *Alternaria* and/or positive SPT fungi. Autoregressions for pediatric subjects 4 and 8 and adult sub-

Table 8. Results of autoregressive models for individual subjects: relationship of asthma outcomes to selected fungal exposures at 1,000 spores/m³ of air[a]

Subject	Change in symptom score from total basidiospores ± SE	Change in symptom score from deuteromycete test spores ± SE[b]	Change in peak expiratory flow rate from deuteromycete test spores ± SE	Change in as-needed inhaler use from deuteromycete test spores ± SE
Pediatric subjects				
1	-1.73 ± 0.93*	-0.15 ± 0.11	9.34 ± 6.34	–
2	1.31 ± 1.70	0.22 ± 0.19	-1.84 ± 4.90	0.17 ± 0.18
3	5.99 ± 1.50‡	0.35 ± 0.20*	–	-0.16 ± 0.23
4	1.66 ± 2.40	0.39 ± 0.27	-5.14 ± 5.45	0.19 ± 0.23
5	4.57 ± 1.50†	0.32 ± 0.17*	-3.32 ± 4.51	1.01 ± 0.58
6	5.98 ± 1.40‡	0.36 ± 0.16**	-30.51 ± 18.66	0.00 ± 0.06
7	-3.45 ± 2.20	-0.18 ± 0.27	-1.14 ± 9.20	-0.34 ± 0.58
8	3.90 ± 1.15†	0.38 ± 0.13†	0.54 ± 5.46	0.29 ± 0.15*
9	2.88 ± 1.80	0.25 ± 0.20	0.62 ± 5.45	0.80 ± 0.60
10	2.72 ± 1.20**	0.10 ± 0.16	-2.93 ± 10.00	0.36 ± 0.22
11	3.17 ± 1.08†	0.17 ± 0.13	-8.72 ± 9.12	0.00 ± 0.08
12	0.33 ± 0.75	-0.02 ± 0.08	-4.41 ± 3.46	0.02 ± 0.27
13	2.52 ± 1.20**	0.36 ± 0.14**	1.52 ± 4.35	0.27 ± 0.50
Adult subjects				
14	-0.97 ± 1.90	-0.41 ± 0.20*	4.98 ± 11.00	-0.45 ± 0.20**
15	1.61 ± 1.60	-0.09 ± 0.18	3.19 ± 9.11	-0.36 ± 0.47
16	0.95 ± 1.50	0.04 ± 0.17	-1.45 ± 15.88	0.51 ± 0.63
17	0.32 ± 1.80	0.08 ± 0.20	-7.06 ± 3.26**	-0.14 ± 0.30
18	1.64 ± 1.90	0.37 ± 0.20*	-0.77 ± 3.68	–
19	2.08 ± 1.18*	0.40 ± 0.12†	-3.33 ± 12.00	0.47 ± 0.28
20	-0.60 ± 0.77	0.07 ± 0.08	-2.39 ± 11.00	-0.09 ± 0.35
21	-0.09 ± 1.10	0.03 ± 0.13	5.94 ± 16.00	0.43 ± 0.32
22	1.17 ± 3.70	0.42 ± 0.22*	-5.79 ± 7.42	0.34 ± 0.15**

SE, standard error.
[a]Regression models control for weather and first order autocorrelation.
[b]Fungal spore concentrations of those five genera represented by the allergy skin prick tests, namely, *Alternaria, Helminthosporium, Cladosporium, Aspergillus,* and *Penicillium.*
*$p<0.1$; **$p<0.05$; †$p<0.01$; ‡$p<0.001$.

jects 19 and 22 showed significant adverse effects on one to two outcomes in association with nontest fungi (of these, only subject 8 showed significant effects from basidiospores). For the remaining subjects, the direction of effects was in the expected direction for most models (positive for symptoms and inhaler use and negative for PEFR; several $p<0.1$), thereby contributing informative data to the population analysis. Individual autoregressions for the relationship of personal O_3 to the three outcomes showed only one significant adverse effect on PEFR (decrease of 21.7 l/min/10 ppb, adult subject 6), but two significant protective effects on PEFR (increase of 6.6 l/min/10 ppb, pediatric subject 4; 23.4 l/min/10 ppb, adult subject 19). There was also one significant adverse effect in inhaler use (0.33 puffs/10 ppb, pediatric subject 12).

Discussion

Overview. The present study showed that outdoor fungal spore concentrations have an adverse effect on daily asthma severity, particularly in regard to reported asthma symptom levels. This finding was supported by adverse effects of fungal spores on evening PEFR and as-needed β-agonist inhaler use. The present findings are particularly relevant to patients with perennial asthma because subjects were selected on the basis of worsening asthma during the warm season. The magnitude of effect of outdoor fungi was marginally larger when the analysis focused on subjects with positive skin prick tests to five deuteromycete fungi commonly assessed in allergy practices, particularly when the explanatory variable was concentrations of those fungal taxa to which the subject had representative positive SPTs. These fungi included *Alternaria*, *Cladosporium*, *Helminthosporium*, *Aspergillus*, and *Penicillium* genera. However, there was evidence that other fungi may have an equal or greater effect on asthma severity, including basidiospores (notably *Coprinus*), *Periconia*, *Botrytis*, hyphal fragments, rusts, and unidentified taxa. Most of these different groups of fungi were not strongly correlated with each other, however some, most notably the deuteromycetes, hyphal fragments, and unidentified spores, tended to be moderately correlated ($R = 0.5$–0.7). Because the measurement of fungal exposures relied on a single outdoor stationary site, it is difficult to say which of the intercorrelated fungal types are driving the associations.

The only significant effect from air pollution was between as-needed inhaler use and PM_{10}. Despite an intense effort to collect personal O_3 data, no associations were detected. Although it would not have otherwise been unexpected for such low O_3

levels (Table 2), this contrasts with our earlier findings in a more urbanized area of San Diego county where similar personal O_3 concentrations were measured. Some of the possible reasons for this contrast are discussed below.

Effects of fungi. This is the second in a series of asthma panel studies by the present group of investigators, who are using longitudinal data analysis to examine the relationship between daily asthma status and outdoor fungal concentrations. Although the first study was conducted in a climatologically different region (coastal vs. inland San Diego county) and season (fall vs. late spring), results were consistent with the present findings regarding the effect of total fungal spore concentrations on symptom scores and on inhaler use (*11*). However, in the previous study, a greater magnitude of response for positive SPT fungal concentrations in comparison to total fungi was found only for inhaler use and not for asthma symptom score. Similar effect levels were found for the two studies for both asthma symptoms and inhaler use in relation to concentrations of fungi, which were not included in the SPT battery (same five fungal taxa used). This was exemplified by the effect of basidiospores on asthma symptoms among the 12 pediatric subjects in the previous study (symptom scores increased by 1.2/1,000 fungal spores/m³; $p<0.07$). Sample size, cohort, geographical, or seasonal factors may explain the few differences in results between the two studies, suggesting the need for additional longitudinal studies in more climatologically diverse areas in order to generalize the present findings to other populations of susceptible asthmatics.

Few other cohort studies have been directed at determining the importance of fungi to daily asthma severity, and results from those studies are limited. Klabuschnigg and colleagues (*3*) examined data collected daily from 40 asthmatics over a 6-week period in an Austrian summer camp. The authors graphically showed a relationship of asthmatic complaints to pollen and to *Cladosporium* concentrations; however, no statistical analyses were presented. Another study in the Netherlands involved measurements of colony-forming units of fungal spores outside of the homes of eight fungal allergic asthmatics; measurements were taken approximately once per month rather than on sequential days (*4*). This study showed significantly lower average PEFR on days when fungal spore concentrations were highest versus the lowest days (mean PEFR 354 vs. 400 l/min, respectively; $p<0.05$).

Many fungal spores are capable of reaching the conducting airways of the lungs, and

some have been found to produce inflammatory effects on the respiratory mucosa of allergic asthmatics, as demonstrated in bronchoalveolar lavage studies (*26*). Indirect evidence of allergic IgE-mediated responses to fungi was provided by Licorish et al. (*27*) who performed bronchial challenges on seven young adults with mild asthma using either *Alternaria* or *Penicillium* spores, depending upon the patient's allergy (approximately 2,000 and 10,000 spores/m³, respectively). Pulmonary function tests and clinical symptoms were consistent with both immediate and late onset asthmatic responses. Compared to other allergens, the relative importance of fungal spores with respect to IgE-mediated responses among allergic asthmatics is not known. Results of several successful experimental trials of mold immunotherapy suggests that fungal antigens are clinically important (*22*). The threshold concentration for inducing an asthmatic response by any fungal species is also unknown, but it is likely to vary based upon individual sensitivity and fungal species (*22*). The present study supports this view because results showed greater magnitudes of response when the fungal-specific allergies of the individual were accounted for and the magnitude of response per concentration of spores varied depending upon the spore type (e.g., basidiomycetes). The possibility that fungal allergens may have more potent effects in susceptible individuals compared to other allergens is supported by a study which examined 11 asthmatics seen at the Mayo clinic who had one or more episodes of respiratory arrest during the period 1980–1989 (*5*). Ten of these asthmatics were both skin test positive for *Alternaria* and had their respiratory arrest during the *Alternaria* aeroallergen season of summer and early fall. Consistent with this is data from an ecological study conducted in Chicago, Illinois, during the period May to October from 1985 to 1989 that showed the odds of death from asthma for 67 asthmatic patients ages 5–34 years were twofold higher on days when fungal spore concentrations were at or above 1,000 spores/m³ versus days below 1,000 spores/m³ (95% CI, 1.31–3.56) (*28*).

The effect estimates for the population sample in the present study were in most cases relatively small, given the range of fungal spore concentrations measured (655–6,110 spores/m³). This was particularly evident for PEFR deficits, which were in most cases not more notable than -10 l/min for a 90th percentile increase in any of the fungal types (Table 7). This change itself is unlikely to have any clinical significance for an individual asthmatic. However, given such small physiologic changes in this

cohort of 22 asthmatics, more adverse outcomes resulting from fungal exposures, such as ER visits or hospital admissions, could occur among the larger population of asthmatics (*29*). A cautionary note is appropriate here; nonexperimental studies, such as the present epidemiologic investigation, are generally viewed by epidemiologists to yield estimates of effect applicable to populations, not individuals. There are two reasons for estimating effects at a population level in epidemiologic research: 1) although the main interest is in the clinical well-being of the individual, the ultimate goal of epidemiologic research is to improve the health status of populations; and 2) a population is required in research, which aims to make causal inferences about relationships between certain factors and disease, particularly when inferences must be externally valid for the target group of interest (e.g., asthmatics) (*30*).

Despite the small population estimates of effect reported here for most of the fungal variables, it is possible that some individuals are more sensitive than others and that clinically important asthma flares from fungal exposures could be seen in other geographical areas having higher levels of outdoor fungi. The first issue is demonstrated with the individual autoregressions, which showed that four individuals (subjects 6, 8, 13, and 19) had nearly a unit increase in symptom score from a 90th percentile increase in test fungi concentration (2,156 spores/m^3, $p<0.05$; changes in Table 8 are for 1,000 spores/m^3). The second issue is demonstrated by the slope of the association between symptoms and basidiospores, which if correct for the extrapolations to 1,000 spores/m^3 shown in Tables 7 and 8, would represent a modest to severe clinical impact on an individual asthmatic (increase in score of 1.5 to a maximum of 5, which indicates a need for medical evaluation). Concentrations ≥1,000 basidiospores/m^3 have been measured in areas with more frequent rain and higher humidity during the warm seasons, such as during the late spring and fall seasons in Tulsa, Oklahoma (*25*). Other spore types less well researched had similarly large effects per 1,000 spores/m^3, with most regressions on all three outcomes being statitically significant (*Periconia*, *Botrytis*, and hyphal fragments). An analogous situation was found for fungal taxa commonly tested for in allergy assessments, namely, *Alternaria* and *Helminthosporium*, in which effects per 1,000 spores/m^3 were large and represent clinically adverse effects on both asthma symptom severity and PEFR for both fungal taxa and on inhaler use for *Helminthosporium* (Table 7). As

with basidiospores, these magnitudes of effect are extrapolations from the regression slopes and are notably larger than the 90th percentile effects. Again, if the slopes are correct, the extrapolated effects could be found in other areas, as exemplified by the Mayo study of respiratory arrests among asthmatics reported above in which measured concentrations of *Alternaria* were ≥1,000 spores/m^3 in southeastern Minnesota on a majority of days from June through October 1985 (*5*). An alternate explanation for the present findings of larger slopes for some of these fungal types is that effects for one fungal taxon could have been confounded with that of another, leading to an inflation or deflation of effects over the same spore per cubic meter change. This may explain in part why effects per 1,000 spores/m^3 for fungal taxa with otherwise low concentrations are much larger that the higher concentration spores (e.g., *Alternaria* vs. *Cladosporium*).

It is also possible that outdoor fungal spore levels in the present study did not fully capture the magnitude of effects from personal respiratory exposure to fungal spores, particularly since indoor fungi probably contribute substantially to personal fungal exposure (*31*) and short-term peaks in spores can be several magnitudes higher than those observed (*25*). Misclassification of true fungal exposure would likely have biased the estimates of effect in the present study toward the null hypothesis of no effect. A positive bias could have occurred if other allergens etiologically linked to asthma, such as house dust particles carrying various antigens, increased during times of fungal sporulation due to shared meteorological determinants.

The relative differences in the present findings between the effects of the nonskin tested fungal types and the patient-specific allergic fungi should be interpreted with caution. For instance, the positive association between symptoms and basidiospores and the effect by other nonskin test fungi suggests that subjects were responding to other fungal species not commonly tested for in allergy practices. The most notable finding in this regard was that in the individual autoregression analysis, 7 of 13 pediatric asthmatics showed statistically significant and large changes in asthma symptom scores in relation to basidiospores (Table 8). The basidiospores are not typically included as part of an allergy workup of potentially allergic asthmatics in any region to our knowledge outside of research settings. Lehrer et al. (*32*) clearly demonstrated the allergenicity of basidiospores among asthmatics by finding positive SPTs to basidiospores in 30% of 339 asthmatics, as compared with only 18%

of 343 subjects without asthma ($p<0.005$). In one study, 8 asthmatics with both positive skin test reactivity and positive radioallergosorbent test to basidiospores showed a positive bronchoprovocation challenge with basidiospores, while 6 atopic asthmatics with negative basidiospore tests had negative challenges; both groups had similar levels of methacholine reactivity (*33*). Additional evidence for the importance of the basidiospores has been shown by increased asthma hospital admission and emergency room visit rates during outdoor peaks of basidiospore concentrations (*6,8,9*). Another issue of concern regarding the present study is that although many of the fungal taxa not represented in the SPT battery, such as the basidiospores, could be microscopically identified in the present study, a large proportion of the total spore concentration was comprised of unidentified taxa (mean 717 spores/m^3), which were significantly associated with PEFR and inhaler use (Table 7). Finally, allergy testing used in this study, namely epicutaneous skin punctures, can produce false negatives. Confirmatory intradermal skin tests for negative epicutaneous tests would improve sensitivity but can produce false positive results. It is possible that a battery of fungal allergy tests larger than that used in the present study could still miss etiologically important fungal allergens carried by other fungal taxa. This is consistent with a published finding that the association of asthma to allergen skin test reactivity using only 18 allergens (pollens, molds, and dust) was notably weaker than the association between serum IgE level and asthma (*1*).

Effects of air pollution and pollen. There was almost no effect of air pollution on the asthma outcomes in the present study. The effect of PM_{10} on inhaler use may have been spurious, given that both symptoms and PEFR were unrelated to PM_{10}. The lack of associations for pollen and PM_{10} were not unexpected given the low levels of these agents (Table 2). One possible source of negative bias is that semivolatile components of particulate matter <2.5 μm in aerodynamic diameter ($PM_{2.5}$) may be lost with tapered-element oscillating microbalance samplers used to measure PM_{10} (*15*). This does not preclude important effects of particulate air pollution in different settings or pollen during the peak pollen season for the study area of March and April (a topic of current investigation). Adding back the 13 high outlier observations for personal O_3 did not alter the above negative results. It is also possible, although not verifiable, that subjects were not uncapping the sampler to a degree sufficient to negatively bias estimates of effect for ozone.

Results from the previous asthma panel study discussed above contrast the present negative findings for O_3 (*11*). The previous study was conducted in an urban area of San Diego county and showed positive associations of daily asthma severity to personal O_3 exposure levels that were somewhat lower than those in the present study (*11*). For the 12 subjects followed over 6 weeks in that study, a 90th percentile increase in personal O_3 (25 ppb 12-hr average) led to a 25% increase in mean symptom scores (95% CI, 0–49%) and to a 26% increase in mean inhaler use (95% CI, 3–48%). The population for the present study was a semirural community of San Diego county located at the inversion layer base, typically having relatively high outdoor O_3 and low particulate air pollution levels. Given this, it is possible that joint exposure to modest elevations of O_3 in combination with other air pollutants common in urban environments may be etiologically important to adverse respiratory effects among asthmatics.

There is another factor that may have contributed to differences in results: the inland area of the present study generally experiences daily maximum temperatures that are 10–20°F higher than the coastal communities of San Diego county represented by the former study. The use of air conditioners is therefore considerably more frequent in Alpine than in the coastal area, as evidenced in the activity diaries of subjects (*12*) and reports by the Alpine school teachers of the pediatric subjects. None of the subjects in the previous study used air conditioning in their homes, as compared to 86% in the Alpine study. We also found no effects of temperature in the previous study, but in the present study, we found unusually strong protective effects of higher temperature on symptoms, PEFR, and as-needed inhaler use. Rather than a direct protective effect of temperature, it is more likely that higher temperatures were driving a decrease in personal allergen exposure due to filtration of indoor allergen particles (including particles carrying dust mite antigens) from refrigerative air-conditioning through precipitation in removed water (*34*) and the inhibitory effects of cool dry air on indoor fungal growth and sporulation (*35*). Short-term temporal changes in indoor airborne particulates related to the onset of hot, dry weather could also occur as a result of changes in air exchange rates, physical disturbance from increased indoor residence time, and changes in the aerodynamic properties of particles. Although cool, dry air resulting from refrigerative air-conditioning is expected to inhibit indoor dust mite growth (*36*), this

would likely occur over longer seasonal time periods. For instance, the warm, dry period after 26 May in the present study would be expected to gradually diminish indoor dust mite numbers over the remainder of the study. Antigenic particles from dust mites, on the other hand, could still persist in household reservoirs such as carpeting and furniture. However, the literature on day-to-day changes in airborne dust mite antigen exposure is incomplete, and we are not aware of any published research that has examined the relationship of asthma severity to daily personal exposure to airborne allergens. The approach to measuring personal dust mite antigen exposures has been recently presented in the literature, although the methods are not well defined (*37*).

Because O_3 is maximally generated by sunlight and is strongly and positively correlated to temperature, the indirectly protective effect of hot dry weather on allergen-induced asthma could have overwhelmed any of the suspected effects of O_3 on airway caliber or inflammation among asthmatics (*38*). Such an effect should be kept in mind in studies examining the effect of air pollutants on asthma morbidity, particularly when meteorological factors are used as control variables. Also, small but significant intercorrelations between aeroallergens and air pollutants were found in the present study (Table 3), which provides evidence that outdoor allergens should not be ignored in studies of asthma and outdoor air pollution. Compared to the southwestern United States, epidemiological research findings in areas of the United States where heat and humidity often increase together are likely to be different, particularly since fungal spore and dust mite levels would be more likely to be positively correlated to temporal trends in photochemical air pollutants.

Strengths and limitations. Exposure misclassification is a major limitation of environmental epidemiologic research clearly applicable to the present study. For instance, we do not know the degree to which the outdoor fungal concentrations we measured represented personal exposures. However, personal sampling technology for fungal spores does not exist and is not an active area of research, to our knowledge. It also is unclear how to weight outdoor exposure to fungi by the number of hours spent outdoors, given their diverse particle size distributions and expectation of variable degrees of penetration into indoor environments, which may also have significant indoor point sources (e.g., damp subfloors). One limitation of the present research that prohibited such modeling was

a lack of indoor air samples. We believe that it is important for future epidemiologic research on the respiratory effects of airborne fungi to develop approaches to model personal exposure to specific fungal taxa. A currently feasible approach would be to examine indoor and outdoor microenvironmental concentrations for each subject. A further advancement would be to estimate internal thoracic dose based upon minute ventilation from activity diaries and particle size distributions of the fungal taxa.

Outcome misclassification in diary studies is an additional source of error due to a lack of truly verifiable patient data. This can occur through a lack of subject compliance (invalid PEFR maneuvers, falsified records, and distant recall), an issue that has plagued asthma diary studies to the present (*39,40*). Data quality could be greatly improved by the use of subnotebook diaries, nebulizer chronologs, and forced expiratory volume in 1 sec (FEV_1)/PEFR monitors that are electronic and time-date stamped, but these alternatives are costly.

The alternative to the above-mentioned approaches of personal exposure measurements and verifiable response data that is often used in epidemiologic studies is to enhance statistical power by increasing sample size, which can overwhelm methodological inaccuracy and interindividual variability. However, a considerable amount of biological information is lost in the bargain. Although much of this mechanistic information can be provided by experimental studies, it is difficult to extrapolate the experimental conditions to conditions in natural settings. The present repeated-measures design actually enhances power because the sample size was not simply 22 subjects, but was the person-days of observation, which for symptom scores and fungi was 1,100.

The repeated measurements in each subject allowed analyses to be performed on each individual. The present panel design shares similar features to the cross-over clinical trial in that both study designs are statistically efficient (enhanced signal-to-noise ratio) because multiple treatment conditions (or exposures) are studied in each subject and variability in exposure–response relationships due to between-subject characteristics is controlled for by design (*41*). The last advantage is due to a reduction in the variability of the response variable without reductions in the magnitude of the exposure–response relationship, thereby enhancing power and precision (*42*). Nevertheless, intra-individual temporal variation due to measurement error is a major limitation. Although many subjects showed statistically significant effects from fungi, particularly for

symptoms among the pediatric subjects and basidiospores, it is probably not justified at this point to call them responders and the others nonresponders. Presenting dose–response information on each individual is an important task for an experimental study or clinical trial. However, this is a nonexperimental epidemiologic study, which has limited power to examine effects in individual subjects. The primary limitation relates to the fundamental difference between experimental versus nonexperimental epidemiologic (observational) studies: the investigator in a nonexperimental study does not have control over the conditions of exposure, including reduction in the variation of extraneous factors in comparison with the factor of interest (*43*). One major consequence of this is the difficulty of accurately assessing the conditions of exposure, which leads to exposure misclassification and inflation of random error. This in turn diminishes the statistical power to detect an effect. In the present study there is, by the nature of its design, insufficient accuracy in the assessment of the conditions of exposure to declare that any individual regression analysis is valid for the subject. Nevertheless, we have presented individual autoregressions and, while marginal or significant effects ($p<0.1$ or $p<0.05$) are seen for certain individuals, this should not be interpreted as more indicative of an adverse effect among those particular individuals. The expected lack of clear heterogeneity of effects from individual regressions as compared with population averaged effects was previously shown in similar studies by Neas et al. (*44*) who described a panel study of daily respiratory effects of air pollutants; Hoek et al. (*45*) who used four repeated pulmonary function tests in children; and a reanalysis of three summer camp studies of air pollution and pulmonary function (*46*). Brunekreef et al. (*46*) provided evidence that the variability of individual regression slopes may be largely due to within-subject variability rather than between-subject variability. One general solution to this issue is to advance the field of air pollution epidemiology by improving methods of repeated measurement studies in a manner that provides a view of temporal effects in individual subjects with a minimum of exposure and outcome misclassification. This paper, its companion paper by Liu et al. (*12*) described above, and our previous asthma panel study (*11*), represent an advancement in this direction because they are among the first to use daily personal ozone measurements over long periods.

Another issue that emerges from the present results is that effects of fungal spores were not entirely consistent across the three asthma outcomes. Given the low

to modest intercorrelations between the response variables reported above, it is expected that exposures associated with one outcome may not be associated with the other outcomes. Differences in estimates of effect across the outcomes are probably a reflection of both the utility of multiple asthma outcomes and how interindividual variability contributes to such differences in results. For instance, PEFR provides information about physiological changes in response to agents that may not be detected with symptom reports, particularly since some asthmatics appear to be poor perceivers of bronchoconstriction (*47*). On the other hand, the symptom scoring system we use allows the asthmatic to gauge his or her daily quality of life resulting from asthma, whereas lung function measurements such as PEFR represent a snapshot of one physiological parameter, which may not be representative of the daily severity of asthma. This underestimate of asthma severity by PEFR is particularly likely if the patient has been using as-needed β-agonist inhalers throughout the day or prior evening. The use of inhalers, in turn, is expected to vary between individuals based upon their perception of asthma severity and knowledge of, and compliance with, appropriate inhaler use.

Implications. The findings from this study suggest that elevations in outdoor fungal spores may adversely affect symptom severity, medication use, and lung function of asthmatics. This is the second in a series of longitudinal studies to show that outdoor levels of particular fungal spore types are associated with daily asthma status to different degrees depending upon the genus-specific fungal allergy of the individual. The effects of fungal spore concentrations were generally greater among individuals having skin prick sensitivity to common fungal spore allergens. However, many exceptions were found that suggested a more complex and diverse pattern of fungal allergy among asthmatics. If skin prick allergy testing was expanded to a wider variety of fungal taxa, these simple and well-tolerated tests could be used to stratify large numbers of individuals in epidemiological studies in order to examine the effect of relevant aeroallergen levels and their interactive influence with other cofactors such as air pollutants. The present findings suggest that further longitudinal investigations of asthma determinants are warranted involving both allergy testing and aerometric measurements for a wide range of fungi, pollen, and indoor allergens. Advancements in the methods of exposure assessment for airborne allergens is urgently needed for such investigations to be carried out. Available tools and method-

ologies from air pollution research can and should now be applied to this task.

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