Fungal and other spore counts as predictors of admissions for asthma in the Trent region

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Abstract

***Objectives*—The importance of airborne fungal and other spores in provoking asthma attacks is uncertain. Panel studies have generated evidence that suggests a link between outdoor spore counts and severity of asthma. There have been no population based time series studies relating outdoor exposure to spores with incidence of attacks of asthma.**

***Methods*—Outcomes were hospital admissions for asthma on 2002 days during 1987–94, for children and adults in the Trent region of England. Predictors were daily counts of 25 spore taxa from volumetric traps in Derby on the same and previous day. Admissions for asthma were adjusted for weekly, seasonal, and longer term trends by log linear autoregressive models. Spore counts on 6 days of asthma epidemics were also examined.**

***Results*—When spore counts for individual taxa were analysed as quantitative variables, two positive and two negative correlations (out of a possible 100) were significant at the 5% level. When spore counts were dichotomised at the 90th percentile, one negative and eight positive correlations (out of 100) were significant at the 5% level. All significantly positive associations related to admissions among children, but none involved the total spore count. However, total spores were above the 90th percentile on four of the six epidemic days (odds ratio (OR) 9.92, 95% confidence interval (95% CI) 1.41 to 109.84), but epidemics occurred on only four of 343 days with high total moulds.**

***Conclusions*—There was some evidence that exceptional rates of admission for asthma tend to occur on days with high total mould spore counts, but no specific taxon was consistently implicated. The predictive power was insufficient to support a public warning system.**

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Keywords: asthma; hospital admissions; moulds; fungi; spore counts; aeroallergens; log linear autoregression

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The possibility that fungal spores may cause allergic asthma has been recognised for over 30 years,[1] but epidemiological studies have generally concentrated on the relation between respiratory symptoms to indoor exposure to moulds, especially in the home.[2] The prevalence of mould allergy is difficult to determine due to difficulties in producing standardised allergen extracts,[3] but sensitisation to mould extracts occurs in a few asthmatic patients of all ages,[4–8] usually in association with reactions to other aeroallergens—such as pollen, house dust mites, and animal danders.[5]

Sensitisation to the outdoor mould *Alternaria alternata* has been implicated as a major risk factor for fatal asthma attacks in Chicago, USA.[9] This may simply reflect a common association with severe asthma, but in Chicago, deaths from asthma at ages 5–34 years occur about twice as commonly on days with a high total mould spore count (>1000 spores/m^3) as on days with lower spore counts.[10]

Panel studies of asthmatic patients report inconsistent findings relating symptom severity with concentrations of mould spores in outdoor air.[11–16] Some studies have found an association with total counts of mould spores,[11 14 16] whereas others have reported a correlation between severity indices and specific groups or species, including basidiospores[14–16]; *Cladosporium*[13]; *Alternaria*[16]; and *Didymella exitialis*.[12] The release of spores of *Didymella* and *Sporobolomyces* in response to rapid changes in humidity was suggested as a possible explanation for an outbreak of asthma attacks after a thunderstorm around Birmingham, United Kingdom, in July 1983.[17] However, subsequent work has suggested grass pollen as a more likely cofactor.[18]

No population based studies have investigated the temporal relation between non-fatal asthma attacks and counts of spores of outdoor moulds. In this paper, we examine whether, other things being equal, hospital admissions for asthma are more common on days with, or immediately after, high spore counts. In practice, other things are not equal. Admissions for asthma have an annual cycle, both in adults and (even more spectacularly) in children, when there are peaks after the end of school holidays.[19] There is also a weekly cycle, peaking on Monday. Between years, there is also a long term trend. This was detailed for the Trent region in a previous report.[20] These predictable fluctuations must be allowed for in the search for short term epidemics as might be caused by fungal spores. The importance of fungal spores can therefore be measured by defining a measure of excessive admissions for asthma, given the year and season, and finding whether higher spore counts occur with excessive admissions for asthma. This report aims to do this.

Methods

ADMISSIONS FOR ASTHMA DATA

Acute admissions for asthma for the Trent Regional Health Authority (regional health authority) were extracted from the hospital

episodes system, as described previously.[20 21] These data contained daily counts of patients, where a day is taken as the interval from midnight to midnight on Greenwich mean time (GMT). For each of the 2435 days from 1 July 1987 to 28 February 1994, two sets of admissions for asthma were recorded, one for patients aged 0–14 years, the other for patients aged 15 years or over. These two separate series were defined because of the radically different pattern of annual cycles in the two age groups. Mean daily admissions for asthma were 10.7 a day in the 0–14 year group and 10.6 in patients aged 15 or over.

SPORE COUNT DATA

These were collected from a succession of volumetric spore traps[22] sited in Derby. Data from 1987 to 1991 were collected with a Morrow-Brown volumetric spore trap.[23] From 1987 to 1990, this was sited on a second storey roof 10 m above ground level in central Derby. In 1990, building developments caused a site change to the roof of the Mickleover site of Derby University, in a suburb about 4 km south west of central Derby, 100 m above sea level and 10 m above ground level. In 1991, the Morrow-Brown trap was replaced by a new volumetric trap (Burkard, Rickmansworth, UK). Either a daily slide or a 7 day drum trap was used, and mounting and staining techniques followed the local standard methods.[23] Spores were identified by taxon and counted over one microscope field along the length of the microscope slide, and the count was multiplied by the appropriate factor to yield the mean spore count /m^3 of air for each taxon on each day. The day corresponding to a given date is defined as the interval from 0000 GMT on that date to 0000 GMT on the next date. Two ways were used for matching the days of the spore count data with the days of the hospital episode system data on admissions for asthma. These matched the hospital admissions with the spore count on the same day (a 0 day lag) and on the previous day (a 1 day lag).

Daily counts were available for 30 elementary spore taxa, mutually exclusive on any given day (table 1). All taxa are fungal, except for *Actinomycetes* (bacteria), *Myxomycetes* (slime moulds), algae, *Pteridophytes* (fern spores), and *Pteridium* (bracken). Series of spore counts were available with varying degrees of completeness. For all taxa, there were gaps in the series in the winter months from 1987–90, but from 1990–1, when year round recording began, spore counts in these months were low. As well as the 30 elementary taxa, three summary taxa were defined by summing subsets of the elementary taxa, with a view to defining a small number of predictors of initial interest (see footnote to table 1).

STATISTICAL METHODS

These were based on the log linear autoregression model described elsewhere.[20] This was a

Table 1 Daily spore counts recorded at Derby, 1987–96

	1 July 1987 to 28 February 1994			*1 January 1991 to 31 December 1996*	
Taxon name	*Daily counts*	*Positive counts*	*High counts**	*Mean count*	*90th percentile**
Elementary taxa:					
Alternaria	2010	1321	190	65.47	160
Aspergillus or *Penicillium* type†	2010	1983	228	140.38	300
Cladosporium	2010	1994	244	2806.49	8156
Epicoccum	2010	869	297	9.12	30
Botrytis	2010	1542	271	46.83	132
Sporobolomyces	2010	2006	360	3170.21	6565
Tilletiopsis	2010	2002	392	1707.39	4095
Coloured basidiospores	2010	1887	214	264.09	692
Miscellaneous hyaline basidiospores‡	2010	1964	222	521.38	1416
Ustilago	2010	1068	226	64.66	125
Rusts	2010	1222	223	15.86	50
Didymella	2010	1649	341	376.20	700
Erysiphe	2010	1498	316	25.85	65
Leptosphaeria	2010	1921	272	116.17	312
Miscellaneous 1-septate ascospores§	2010	1649	170	49.77	125
Other ascospores	2010	1709	153	98.78	254
Torula	2010	610	297	3.14	10
Algal group	2010	1113	220	9.64	25
Hyaline indeterminates	2010	1999	506	612.29	1395
Pleospora	2010	287	111	3.63	10
Myxomycetes	2010	114	114	1.00	1
Drechslera	2010	223	223	0.58	17
Polythrinchium	2010	484	289	2.75	8
Fern spores	2010	46	46	0.07	1
Entomophthora¶	420	129	62	4.84	15
Summary taxa**:					
Total ascospores	2010	1980	307	644.53	1400
Total hyaline basidiospores or ballistospores	2010	2006	367	5398.97	11865
Total moulds	2010	2010	343	10101.00	23790

*High counts defined as >90th percentile for the period of continuous recording (1 January 1991 to 31 December 1996)
†Comprising *Aspergillus*, *Penicillium* and other mutually indistinguishable genera.
‡Excluding *Sporobolomyces* and *Tilletiopsis*.
§Excluding *Didymella*.
¶Recorded from 1 January 1993 (all other taxa started 31 March 1987).
**The three summary taxa are: total ascospores (*Didymella* +*Leptosphaeria* + *Pleospora* + miscellaneous 1-septate ascospores + other ascospores); total hyaline basidiospores and ballistospores (*Sporobolomyces* + *Tilletiopsis* + miscellaneous hyaline basidiospores); total moulds (the total of all spore counts except algal group, *Myxomycetes*, *Actinomycetes*, fern spores and *Pteridium* (non-fungal), and *Humicola*, *Entomophthora* and miscellaneous smuts (not available from 1987)).

statistical model used to forecast the number of admissions for asthma expected in a particular regional health authority in each age group on each day, before spore counts were considered. A separate model was fitted to the admissions data for each age group in the Trent regional health authority, taking into account weekly and annual cycles, longer term trends, and the short term (autoregressive) tendency of numbers of admissions for asthma to be similar to those for the previous 8 days. For each day, a standardised residual was defined, roughly equal to the relative excess of observed admissions for asthma compared with those expected (or forecast), expressed in units of the coefficient of variation (CV).

The standardised residuals were included in analyses in two ways. Firstly, the ability of high spore counts to predict high residuals was measured with Somers' *D*.[24] This variable compares two conditional probabilities: given that 2 days have unequal spore counts, Somers' *D* is the probability that the day with the higher spore count has the higher standardised residual for admissions for asthma, minus the probability that the day with the higher spore count has the lower standardised residual. It has the advantage that it can be applied both to continuous and to dichotomous exposure variables. For a dichotomous exposure, the statistical significance of Somers' *D* is equivalent to that of the Mann-Whitney *U* test. For continuous data it is equivalent to a test of rank correlation. In these analyses, spore counts were entered in two ways, directly as quantitative variables and after conversion to binary variables, indicating whether or not the count was high in the sense of attaining (or exceeding) the value of a threshold. For each taxon, the threshold was either one or the 90th percentile of counts recorded for that taxon in years from 1991 onwards, whichever was greater (table 1).

Secondly, attention was focused on 6 days of spectacular epidemics, identified elsewhere[20] as days on which the standardised residual, for at least one age group, was over 4 CV units. These epidemics occurred on 30 April 1988 and 15 September 1990 in the 0–14 age group, and on 23 August 1987, 14 June 1989, 23 July 1989, and 4 August 1991 in the age group 15 or more. Odds ratios were calculated (with exact 95% confidence intervals (95% CIs)) to estimate the relative risks of epidemics between days of high and low spore counts for each taxon, together with p values for Fisher's exact test. Also, for each epidemic and taxon, the triplet of three spore counts corresponding to the epidemic day, and the days 7 days before and after it, were compared.

All analyses were carried out with the SAS system[25] or Stata software.[26]

The number of significance tests was large, because of the number of available taxa and the multiple age groups, lag periods, and methods of comparison. The number of alternative statistical procedures for dealing with multiple significance tests is also large, but nearly all use minimum p values.[27] Tables 2 and 3 therefore show the minimum p values for all available elementary and summary taxa in each analysis.

Results

SPORE COUNTS

Table 1 summarises the spore counts for July 1987 to February 1994 inclusive, corresponding to the time series of hospital admissions, and the thresholds for high counts, derived from the period January 1991 to December 1996, the period of recording all year round at Derby. Note that some taxa have a lot more or less than 10% of their available counts in the high category. The excesses exist because, from 1987 to 1990, winter counts, which usually would have been low, were not available. The deficiencies exist because, for some taxa, the 90th percentile is zero, so a threshold of one was used instead.

RELATION BETWEEN ADMISSIONS FOR ASTHMA AND HIGH AND LOW SPORE COUNTS

Table 2 summarises the analyses of standardised residuals from the time series of admissions for asthma relative to spore counts for each taxon, expressed as Somers' *D* (indicating the direction of the association) and the corresponding p value (indicating its significance). Results are shown for spore counts on the same day, and on the previous day, dichotomised into low and high categories on the left, and with spore counts treated as continuous variables on the right. The number of days contributing to these statistics is 2002 (2010 minus the first 8 days which had no forecast counts) for all taxa except *Entomophthora*, which started to be recorded late (419 days).

When spore counts for individual taxa were analysed as quantitative variables, two positive and two negative correlations (out of a possible 100) were significant at the 5% level: close to what would be expected by chance alone. The significant positive associations were both with residual categories in the younger age group: miscellaneous hyaline basidiospores (age 0–14, lag 0, p=0.014) and other ascospores (age 0–14, lag 0, p=0.043). Significant inverse associations were found with coloured basidiospores (age ⩾15, lag 0, p=0.019) and *Drechslera* (age ⩾15, lag 1, p=0.023).

When spore counts were dichotomised at the 90th percentile, there were more positive associations than expected by chance alone: one negative and eight positive correlations (out of 100) were significant at the 5% level. All the significant positive associations were in the younger age group, but none were significant at the 1% level. These were: miscellaneous hyaline basidiospores (age 0–14, lag 0, p=0.015); *Didymella* (age 0–14, lag 0, p=0.028); *Leptosphaeria* (age 0–14, lag 0, p=0.014); other ascospores (age 0–14, lag 0, p=0.043); total ascospores (age 0–14, lag 0, p=0.027); *Botrytis* (age 0–14, lag 1, p=0.012); other ascospores (age 0–14, lag 1, p=0.048); and total ascospores (age 0–14, lag 1, p=0.017). The only significant negative association was for *Drechslera* (age ⩾15, lag 1, p=0.023).

The total mould spore count was not significantly associated with daily admissions for asthma in either age group in any of these analyses (minimum p=0.083).

Table 2 Somers' D of asthma excess with respect to spore counts on the same day and lagged by 1 day

	Dichotomised (high v low) spore counts Same day (lag 0)				*Quantitative (higher v lower) spore counts Same day (lag 0)*				*Dichotomised (high v low) spore counts Previous day (lag 1)*				*Quantitative (higher v lower) spore counts Previous day (lag 1)*			
	Ages 0–14		*Ages ⩾15*		*Ages 0–14*		*Ages ⩾15*		*Ages 0–14*		*Ages ⩾15*		*Ages 0–14*		*Ages ⩾15*	
Taxon name	*Somers' D*	*p Value*	*Somers' D*	*p Value*	*Somers' D*	*p Value*	*Somers' D*	*p Value*	*Somers' D*	*p Value*	*Somers' D*	*p Value*	*Somers' D*	*p Value*	*Somers' D*	*p Value*
Elementary taxa:																
Alternaria	0.01	0.8991	−0.06	0.2207	0.02	0.3738	0.00	0.8883	0.01	0.7995	0.02	0.6664	0.00	0.8674	0.01	0.5271
Aspergillus or *Penicillium* type	−0.00	0.9995	0.03	0.4555	0.01	0.3538	0.01	0.5445	0.03	0.4134	−0.02	0.6460	0.01	0.3376	−0.01	0.5196
Cladosporium	0.02	0.6105	0.02	0.6292	0.01	0.3460	−0.02	0.2436	0.05	0.2503	0.01	0.8690	0.00	0.8751	−0.01	0.3846
Epicoccum	−0.04	0.2919	−0.02	0.5342	0.01	0.5302	−0.03	0.0865	0.05	0.2030	−0.02	0.6680	0.00	0.9060	−0.00	0.8959
Botrytis	0.05	0.2033	0.02	0.6631	0.03	0.1013	−0.02	0.3103	0.10	0.0122	0.05	0.1962	0.03	0.0549	0.02	0.2810
Sporobolomyces	0.06	0.0655	0.02	0.5150	0.02	0.0966	−0.02	0.2670	0.04	0.2389	−0.00	0.9154	0.01	0.3321	−0.02	0.1477
Tilletiopsis	0.06	0.0890	−0.01	0.6638	0.02	0.0973	−0.02	0.1180	0.07	0.0417	0.00	0.9040	0.02	0.1586	−0.01	0.4845
Coloured basidiospores	0.05	0.2213	0.01	0.9016	0.02	0.1467	−0.04	0.0192	0.04	0.3458	0.00	0.9470	0.02	0.2263	−0.03	0.0849
Miscellaneous hyaline basidiospores	0.10	0.0145	0.04	0.3338	0.04	0.0135	−0.02	0.1908	0.05	0.2431	0.01	0.8786	0.02	0.1674	−0.02	0.1232
Ustilago	0.01	0.7433	−0.01	0.8426	0.02	0.4102	−0.02	0.2585	0.00	0.9493	−0.02	0.6632	0.01	0.7536	−0.00	0.8912
Rusts	0.04	0.3378	0.00	0.9789	0.03	0.1100	0.00	0.8339	−0.00	0.9154	0.04	0.2836	−0.00	0.9073	0.01	0.6299
Didymella	0.08	0.0277	0.04	0.2492	0.02	0.2526	−0.02	0.2904	0.07	0.0606	0.01	0.6973	0.02	0.2596	−0.01	0.4304
Erysiphe	−0.06	0.0911	0.00	0.9055	−0.01	0.6871	−0.03	0.0811	0.02	0.6000	−0.02	0.6437	0.00	0.8535	−0.01	0.5294
Leptosphaeria	0.10	0.0137	0.04	0.2927	0.01	0.4747	−0.01	0.5456	0.08	0.0382	0.04	0.3258	0.02	0.2086	−0.00	0.8987
Miscellaneous 1-septate ascospores	0.08	0.1153	0.02	0.6426	0.02	0.2918	−0.02	0.2803	0.04	0.3680	0.07	0.1072	0.01	0.4924	0.00	0.8004
Other ascospores	0.10	0.0428	0.09	0.0918	0.03	0.0432	−0.01	0.5020	0.10	0.0480	0.06	0.1966	0.02	0.1295	−0.01	0.4641
Torula	−0.00	0.9830	−0.01	0.6813	0.00	0.9427	−0.01	0.6726	0.05	0.1479	0.01	0.7971	0.01	0.7910	0.01	0.6340
Algal group	−0.01	0.8166	−0.05	0.2361	0.01	0.5727	−0.01	0.6519	0.01	0.8316	0.08	0.0547	−0.00	0.8836	0.02	0.3961
Hyaline indeterminates	0.03	0.3515	−0.02	0.5568	0.01	0.3474	−0.02	0.1563	0.04	0.1812	−0.02	0.4251	0.01	0.5717	−0.03	0.0886
Pleospora	0.01	0.9206	−0.01	0.8624	−0.03	0.3941	−0.02	0.4894	−0.08	0.1466	0.01	0.7761	−0.01	0.8474	−0.01	0.8422
Myxomycetes	0.02	0.6729	−0.00	0.9937	0.03	0.6420	−0.00	0.9625	0.05	0.4324	−0.09	0.1250	0.04	0.4346	−0.08	0.1220
Drechslera	0.04	0.3515	0.02	0.6328	0.04	0.3555	0.02	0.6554	0.01	0.7499	−0.09	0.0227	0.01	0.7792	−0.09	0.0233
Polythrinchium	0.02	0.5037	−0.03	0.3567	0.01	0.7716	−0.03	0.2199	−0.01	0.8759	−0.05	0.1327	−0.01	0.8020	−0.01	0.5842
Fern spores	0.01	0.8981	−0.07	0.4405	0.01	0.8941	−0.07	0.4434	0.09	0.3322	−0.04	0.6362	0.09	0.3343	−0.04	0.6404
Entomophthora	0.05	0.5493	0.00	0.9928	−0.01	0.7946	−0.04	0.4489	−0.02	0.7915	−0.05	0.5519	−0.00	0.9294	−0.06	0.2830
Minimum p value:		0.0137		0.0918		0.0135		0.0192		0.0122		0.0227		0.0549		0.0233
Summary taxa:																
Total ascospores	0.08	0.0273	0.07	0.0441	0.02	0.1552	−0.01	0.3519	0.09	0.0169	0.06	0.1150	0.02	0.1710	−0.01	0.3626
Total hyaline basidiospores or ballistospores	0.05	0.1067	0.00	0.9364	0.03	0.0604	−0.02	0.2475	0.04	0.2406	0.00	0.9775	0.02	0.2026	−0.02	0.2196
Total moulds	0.02	0.4828	0.06	0.0830	0.02	0.1315	−0.02	0.2199	0.04	0.2026	0.02	0.4824	0.02	0.1536	−0.01	0.3551
Minimum p value:		0.0273		0.0441		0.0604		0.2199		0.0169		0.1150		0.1536		0.2196

Table 3 Odds ratios for asthma epidemics in relation to high and low spore counts

	Spore counts on the same day (lag 0)							*Spore counts on the previous day (lag 1)*						
	High count days		*Low count days*					*High count days*		*Low count days*				
Taxon name	*Epidemic*	*Other*	*Epidemic*	*Other*	*Odds ratio*	*(95% CI)*	*p Value*	*Epidemic*	*Other*	*Epidemic*	*Other*	*Odds ratio*	*(95% CI)*	*p Value*
Elementary taxa:														
Alternaria	1	189	5	1807	1.91	(0.04 to 17.21)	0.4507	3	187	3	1809	9.67	(1.28 to 72.57)	0.0136
Aspergillus or *Penicillium* type	1	227	5	1769	1.56	(0.03 to 14.01)	0.5164	1	227	5	1769	1.56	(0.03 to 14.01)	0.5164
Cladosporium	3	241	3	1755	7.28	(0.97 to 54.58)	0.0270	3	241	3	1755	7.28	(0.97 to 54.58)	0.0270
Epicoccum	1	293	5	1703	1.16	(0.02 to 10.44)	1.0000	2	292	4	1704	2.92	(0.26 to 20.45)	0.2160
Botrytis	2	265	4	1731	3.27	(0.29 to 22.90)	0.1850	2	265	4	1731	3.27	(0.29 to 22.90)	0.1850
Sporobolomyces	3	352	3	1644	4.67	(0.62 to 34.98)	0.0727	2	353	4	1643	2.33	(0.21 to 16.30)	0.2890
Tilletiopsis	3	384	3	1612	4.20	(0.56 to 31.43)	0.0905	2	385	4	1611	2.09	(0.19 to 14.65)	0.3282
Coloured basidiospores	2	212	4	1784	4.21	(0.38 to 29.52)	0.1278	1	213	5	1783	1.67	(0.04 to 15.06)	0.4930
Miscelaneous hyaline basidiospores	1	221	5	1775	1.61	(0.03 to 14.44)	0.5065	0	222	6	1774	0.00	(0.00 to 6.83)	1.0000
Ustilago	0	219	6	1777	0.00	(0.00 to 6.94)	1.0000	1	219	5	1777	1.62	(0.03 to 14.59)	0.5031
Rusts	1	220	5	1776	1.61	(0.03 to 14.52)	0.5048	2	219	4	1777	4.06	(0.36 to 28.46)	0.1350
Didymella	3	338	3	1658	4.91	(0.65 to 36.74)	0.0656	2	339	4	1657	2.44	(0.22 to 17.12)	0.2720
Erysiphe	2	310	4	1686	2.72	(0.24 to 19.05)	0.2372	2	310	4	1686	2.72	(0.24 to 19.05)	0.2372
Leptosphaeria	1	271	5	1725	1.27	(0.03 to 11.44)	0.5841	1	271	5	1725	1.27	(0.03 to 11.44)	0.5841
Miscelaneous 1-septate ascospores	1	169	5	1827	2.16	(0.05 to 19.47)	0.4132	0	170	6	1826	0.00	(0.00 to 9.20)	1.0000
Other ascospores	2	151	4	1845	6.11	(0.55 to 42.94)	0.0710	3	150	3	1846	12.31	(1.63 to 92.40)	0.0074
Torula	1	294	5	1702	1.16	(0.02 to 10.40)	1.0000	2	293	4	1703	2.91	(0.26 to 20.37)	0.2172
Algal group	0	218	6	1778	0.00	(0.00 to 6.97)	1.0000	1	218	5	1778	1.63	(0.03 to 14.67)	0.5014
Hyaline indeterminates	3	497	3	1499	3.02	(0.40 to 22.58)	0.1687	2	498	4	1498	1.50	(0.14 to 10.53)	0.6435
Pleospora	0	111	6	1885	0.00	(0.00 to 14.59)	1.0000	0	111	6	1885	0.00	(0.00 to 14.59)	1.0000
Myxomycetes	0	114	6	1882	0.00	(0.00 to 14.18)	1.0000	0	114	6	1882	0.00	(0.00 to 14.18)	1.0000
Drechslera	3	217	3	1779	8.20	(1.09 to 61.47)	0.0204	3	217	3	1779	8.20	(1.09 to 61.47)	0.0204
Polythrinchium	1	287	5	1709	1.19	(0.03 to 10.70)	1.0000	0	288	6	1708	0.00	(0.00 to 5.06)	0.6023
Fern spores	0	46	6	1950	0.00	(0.00 to 36.97)	1.0000	1	45	5	1951	8.67	(0.18 to 79.53)	0.1303
Minimum p value							0.0204							0.0074
Summary taxa:														
Total ascospores	3	304	3	1692	5.57	(0.74 to 41.69)	0.0499	2	305	4	1691	2.77	(0.25 to 19.43)	0.2313
Total hyaline basidiospores or ballistospores	3	359	3	1637	4.56	(0.61 to 34.15)	0.0764	2	360	4	1636	2.27	(0.20 to 15.92)	0.2975
Total moulds	4	335	2	1661	9.92	(1.41 to 109.84)	0.0091	2	337	4	1659	2.46	(0.22 to 17.24)	0.2696
Minimum p value							0.0091							0.2313

ODDS RATIOS FOR EPIDEMICS

There were six epidemics, all of which occurred on days with counts on the same and the previous day for all elementary taxa, except *Entomophthora*. Table 3 shows the relation between the epidemic days and high spore counts for each taxon, with 0 day and 1 day lags. Asthma epidemics were about 10 times more likely on a day with a high total mould spore count ($p<0.01$), but with wide 95% CIs for the odds ratio (1.4 to 109.8). Although the association of epidemics with total spore count on the previous day was weaker, there were significant associations (at the 5% level) with counts of *Alternaria*, *Drechslera*, and other ascospores, when analysed with a 1 day lag.

COUNT TRIPLET ANALYSIS

A more quantitative test of the relation between epidemics of asthma and spore counts, which also controls for seasonal variation, was to compare spore counts on the epidemic day with those 7 days before and 7 days afterwards. In this case we have only six epidemics, and therefore no more than six triplets of counts for such an analysis. For three out of these six epidemics, the total spore count on the epidemic day was higher than either of the total spore counts 7 days before and after.

Discussion

These analyses are an attempt to assess the predictive power of spore count data, the availability of which was sometimes fragmentary, to predict admissions for asthma, the background variation of which was complicated. This is why the approach of residual analysis was chosen. This predictive power was measured in four different ways. Three of these have produced no more significant results than we might expect by chance, given the number of 95% CIs that have been calculated. All four methods suggest that spore counts alone cannot be the basis of a public warning system without generating an extremely high rate of false alarms.

The first approach (table 2) dichotomised spore counts into high and low categories. If high spore counts precipitated asthma, then warnings might be issued to the public in the event of spore counts exceeding a danger threshold. The thresholds used here do not seem to be very powerful predictors, as when 1 day has a high spore count and 1 day has a low spore count, the high count day is not especially more likely to have a higher asthma excess than the low count day.

The second method (also shown in table 2) sought a correlation between admissions for asthma and spore counts across their full range, without giving an undue influence to tiny minorities of outlying counts. Somers' *D* can be used to sense such a gradient without committing ourselves to a particular functional form for the exposure-response relation, the estimation of which would be highly affected by small numbers of extremely high spore counts.

The third approach (table 3) concentrates attention on spectacular epidemics, of which there were only six in the Trent regional health authority between July 1987 and February 1994. High total moulds on the same day predicted four of those six epidemics, but only four of 343 high spore days were associated with asthma epidemics. If we use a more stringent definition of high spore count (above the 99th percentile), the predictive power is one epidemic (in 1987) among 34 very high spore days.

The fourth way compares epidemic days with the days 1 week before and after, by analogy with similar methods used elsewhere on 55 epidemic episodes.[20] This avoids the problem of seasonal confounding, asthma epidemics being more common in the summer when spore counts are generally higher, but is statistically much less powerful than comparisons that include all non-epidemic days (table 3).

A general problem with all these analyses is that of multiple comparisons. With over 20 taxa being tested, at least one will be significant at the 5% level, on average, just by chance, even if all null hypotheses are true and none of the taxa have any predictive value. Also, we tested each taxon up to eight times (two lags multiplied by four different statistical measures of predictive power), and split some analyses into two age groups. The overall pattern of results lacked consistency, except that all the significant positive associations occurred in the 0–14 age group. Nevertheless, this comprehensive analysis of admissions for asthma relative to outdoor spore counts showed several positive associations which might form a basis for future work.

Causal relations were not convincingly found, but cannot be ruled out. For instance, it may be that mould spores are patchily distributed, so that a high count at Derby correlates poorly with aeroallergen concentrations over the rest of the Trent region, and asthma epidemics take place only when spores are abundant in all parts of the region. This possibility could be rectified by analysing data from a smaller and more densely populated area. However, we can conclude that outdoor spore counts measured at Derby do not seem to predict daily rates of admission for asthma in the Trent region as a whole sufficiently well to be the basis for a public warning system.

Another possibility is that spores cause asthma attacks only in combination with other factors, such as thunderstorms.[28] This seems to be the case for grass pollen,[21] but, to demonstrate this synergism convincingly, we had to combine data from several regions, many of which lack data on mould spores. Only 1 of the 6 epidemic days in Trent (23 August 1987) was associated with substantial local thunderstorm activity (on the previous day), so this interaction cannot be the explanation for most well defined epidemics.[20] However, this thunderstorm on 22 August 1987 was associated with exceptionally high counts of *Didymella* spores,[29] which may have played a part in that specific epidemic, as suggested for an earlier asthma epidemic in Birmingham.[17]

Measurements of air pollution were not available for the whole Trent region, but our results suggest that it is unlikely that mould

spores are a major unmeasured confounding influence in other time series analyses relating outdoor air pollutants with frequency of asthma attacks. However, specific interactions between mould exposure and outdoor air pollutants were not considered in our analysis. Experimental chamber studies have raised the possibility that earlier exposure to gaseous air pollutants may decrease the threshold of response to grass pollen or house mite allergens,[30–32] and the same might apply to fungal allergens. However, no evidence emerged from time series in London to support a synergistic interaction of pollens and pollutants in assessing rates of admission for asthma.[33]

The London analyses are currently being extended to include fungal aeroallergens and will seek to test specifically the significant relations which have emerged in Trent, as well as possible interactions between pollutants and aeroallergens.

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