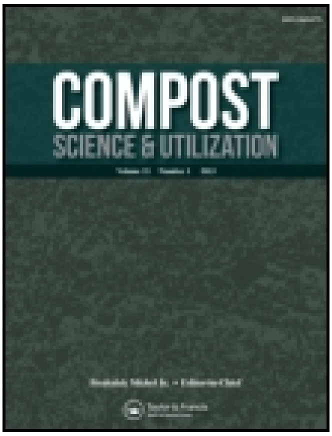


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# A Prospective Study of Health Symptoms And *Aspergillus fumigatus* Spore Counts Near a Grass and Leaf Composting Facility

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*Aspergillus fumigatus* (*A. fumigatus*), a fungus found in compost, is a respiratory allergen and can cause serious invasive disease in susceptible individuals. We conducted a study involving the collection of health symptom data and environmental monitoring data near a 40-acre grass and leaf composting facility. Analyses were based on symptom diary data from 63 individuals from the study area and 82 individuals from a reference area. Airborne *A. fumigatus* was not associated with increases in respiratory or irritative symptoms. Symptom incidence was associated with ragweed, ozone, temperature, and time since start of the study, although a tendency to report fewer symptoms as the study progressed may have confounded this result. Other features of the study design, including short-term spore count variability, lack of individual exposure data and gaps in the symptom diary data, complicated interpretation of the results. Although this study does not support an association between allergy and asthma symptom incidence and *A. fumigatus* spore levels, we could not assess the risk of unusual, but severe illnesses among very sensitive individuals.

## *Introduction*

With continuing efforts to reduce the landfilling of solid waste and increase recycling, there has been a growth in composting of leaves, brush and grass clippings. The finished compost product is used for soil conditioning and landscaping purposes. Environmental conditions inside compost piles (heat and humidity) favor the growth of certain fungi and bacteria able to tolerate high temperatures. One type of fungus found in compost, *Aspergillus fumigatus* (*A. fumigatus*), has generated concern because of potential health effects associated with exposure. *A. fumigatus* is an allergen and can cause allergy and asthma symptoms among sensitive individuals (Kwong-Chung and Bennet 1992). Other, less common, health effects associated with *A. fumigatus* include allergic bronchopulmonary aspergillosis (ABPA), aspergilloma and invasive aspergillosis (Marsh *et al.* 1979; Kwong-Chung and Bennet 1992). More generally, inhaled fungal spores, including spores of *A. fumigatus*, can elicit a variety of inflammatory responses in the lung that result from both specific immune responses and non-specific responses to cellular components such as (1,3)-β-D-glucans (Weber *et al.* 1993; Heederik and Smid 1994; Flannigan and Miller 1994; Rylander 1994) and extracellular polysaccharides (Douwes *et al.* 1999).

Facilities that compost yard trimmings (e.g., grass clippings, chipped brush and wood, leaves) are sometimes located near residential areas. Little is known, however, about the potential for these facilities to affect the health of nearby residents. Residents of a town on Long Island, New York became concerned that operations at a 40-acre grass and leaf composting facility were resulting in health symptoms, particularly respiratory symptoms and skin rashes. The New York State Department of Health, the

New York State Department of Environmental Conservation, the Suffolk County Department of Health Services, the New York State Biological Survey and the Town of Islip undertook a two-part cooperative study to address their concerns. The study involved two components: environmental monitoring and human symptom diaries.

The environmental monitoring study examined whether *A. fumigatus* spore concentrations or those of other bioaerosols in the neighborhood nearest to the facility (study neighborhood) were being influenced by composting operations. Extensive environmental data, collected by continuous air sampling over a period of 102 days (Syzdek and Haines 1995; Recer *et al.*, 2001), showed that higher *A. fumigatus* spore concentrations occurred at the study neighborhood sampling location as compared to the reference locations. The elevations in *A. fumigatus* spore levels appeared to be related to the composting facility (Syzdek and Haines 1995; Recer *et al.*, 2001).

The report presented here describes the symptom diary portion of the study. Daily recording of a variety of health symptoms by study participants provided data about daily changes in symptom occurrence. These data were analyzed in relation to spore concentrations observed during the same time period. Our analysis focuses on the relationship between health symptom occurrence among members of community living near the composting facility and spore concentrations measured in that community. Symptom diaries were also completed by residents of a reference community not exposed to the composting facility. Patterns of health symptom occurrence among residents of the reference community were used to provide information on seasonal trends and other variations in symptom reporting not affected by exposure to composting facility emissions.

## Methods

### *Study and Comparison Areas*

The study area was selected based on historical records of prevailing wind direction. The neighborhood northeast of the facility was determined to be the most likely one exposed to any bioaerosols transported by air from the composting facility. A comparison community more than five miles from the facility, not in the direction of the prevailing winds, and with demographic characteristics similar to the study neighborhood, was chosen.

### *Environmental Sampling*

Burkard spore-trap samples for airborne fungal spore enumeration were collected continuously from August 21 through November 30, 1992 at four sites: 1) the Islip Composting Facility; 2) a location in the study neighborhood; 3) a location in the reference neighborhood; and 4) a second reference location southwest of the facility. A detailed description of the sample collection and processing methods can be found in Syzdek and Haines 1995. Two four-minute time-weighted-average counts of fungal spore concentration were obtained from each 24-hour sample to estimate daily exposure across the entire study period. Preliminary four-minute counts indicated that substantial temporal variation could occur in spore levels during a 24-hour interval. Therefore, 24 one-hour time-weighted-average counts per day were also completed for a period of 20 consecutive days during the study period. The one-hour counts were performed for the study neighborhood sampling site only. During the 20-day period, a substantial amount of variation in daily symptom frequencies was reported among participants from the study area. However, this period had relatively little variability in average daily temperature and ozone levels.

Temperature, ozone, sulfur dioxide and nitrogen dioxide data were obtained from a New York State Department of Environmental Conservation continuous air monitoring station located about 40 kilometers (25 miles) west of the composting facility. Ragweed count data were provided by a nearby pollen counting station (Deer Park, New York).

### *Symptom Diary Study*

In order to identify candidates for inclusion in the symptom diary study, self-completion questionnaires were distributed door-to-door to all households in the study area (384) and in the comparison community (1108). Information was provided for 491 individuals in the study area and 619 in the comparison area. A large proportion of those persons reporting a physician diagnosis of allergic rhinitis and/or asthma were invited to participate in the symptom diary study. Study candidates were frequency-matched so that approximately equal numbers were selected from each community by sex and ten-year age category, with no more than two persons from a single household.

A one-page symptom diary was developed consisting of daily checklists for a one-week period. Participants were asked to check "no," "slight" or "moderate/severe" for each of ten health symptoms: eye irritation, stuffy/runny nose, sore throat, coughing, wheezing, difficulty breathing, nausea/upset stomach, skin rash, joint pain, and cold/flu. Several additional questions regarding activities and use of medications were included. Participants were asked to complete daily diaries for the period 8/9/92 through 10/31/92. Since environmental monitoring did not begin at all sample locations until August 21, diary data for 8/9/92 through 8/20/92 were excluded from the analysis.

### *Data Analysis*

For each participant, days for which diary data were missing and days for which hours at home were less than six were removed from the total number of eligible reporting days. Since an allergic response to a single exposure can persist for several days, a participant who reported a particular symptom the day before was only considered eligible to report a symptom event if there was an increase in symptom severity for that participant from one day to the next. That is, if a symptom was reported as "moderate/severe", the participant was eligible to report a symptom event if the response on the previous day was either "no" or "slight" for that symptom. After tallying individual symptom events; eye irritation, stuffy/runny nose, sore throat, coughing, nausea/upset stomach, skin rash, and joint pain were grouped together as symptoms indicative of allergic or irritative responses (hereafter allergy symptoms). Wheezing, difficulty breathing, and coughing were grouped together as asthma-related symptoms. Incidence rates were computed as the number of participants reporting incident symptom events (one or more allergy symptom events, one or more asthma symptom events) divided by the number of participants eligible to report symptoms.

To account for possible delays in symptom occurrence and recognition from one day to the next, a lagged incidence (Ponka 1991; Pope *et al.* 1991) was computed. All symptom incidence analyses were performed using both the "unlagged" and "lagged" symptom incidence rates, producing similar results. Only results using the unlagged rates are presented in this report.

Since symptom incidence is a count of symptom events per day, Poisson regression analysis was used (Frome 1983). *A. fumigatus* spore concentration was analyzed as an independent variable. Temperature, ozone, sulfur dioxide, nitrogen dioxide and ragweed pollen have all been associated with respiratory symptoms and were considered as potential predictors of symptom occurrence. In addition, time since start of the study was considered due to its representation of seasonality and because of "fatigue effect"(Abramson 1990) (a tendency to report fewer symptoms over the course of a diary study). A number of these factors would be expected to be correlated. For example, as the study period progressed from August through October (i.e., time since start of the study increased), temperature and ragweed would have been expected to decrease. To control for a number of intercorrelated factors, factor analysis was used to compute a "combined factor" to represent the levels of all the component variables for each day. A number of the variables evaluated in this study had values with non-normal distributions (spore counts, ragweed counts, ozone levels). When analyzed as continuous variables, the natural logarithm was used.

The range of observed values for the independent variables was divided into quartiles to permit calculation of rate ratios. Rate ratios, with 95% confidence intervals, were calculated as the ratio of symptom incidence for the second, third or fourth quartile of the independent variable of interest to the symptom incidence for the first quartile (the referent). A rate ratio significantly different from one indicates a significant difference in symptom incidence compared to the referent quartile.

Analyses based on the entire study period (using 4-minute counts) and the 20-day period (using 1-hour counts) are presented separately. In analyzing data for the 20-day interval, factors shown to predict symptom incidence during the entire study period were considered as potential predictors for the 20-day interval. Also, a paired t-test was used to compare symptom prevalence on selected days having either low or high *A. fumigatus* concentrations. Only participants for whom data were available for each of the selected days were included in this analysis. The "low *A. fumigatus* days" had to be preceded by at least one other day with a relatively low *A. fumigatus* concentration. This was done in order to reduce the influence of delayed responses that might carry over from exposure on the previous day.

## Results

A total of 237 candidates, 116 from the study area and 121 from the reference area, were invited to participate in the symptom diary study. At initial telephone follow-up, eight refusals were identified, five of whom were no longer eligible due to a change in residence. During the course of the study, 53 persons from the study area and 38 from the reference area either refused participation or provided sparse data. One additional subject from the reference area was excluded from the analysis due to serious chronic disease. A total of 63 participants (54% of original candidates) from the study area and 82 participants (68% of original candidates) from the reference area were included in the analysis.

### *Entire Study Period*

Daily allergic and irritant symptom incidence averaged 21.4% among study area participants and 16.5% among reference area participants. Asthma-related symptom incidence average 7.8% and 5.0% for study area and reference area participants, respectively. Among both study and reference area participants, incidence rates for both allergy and asthma symptoms declined over the course of the study period.

TABLE 1.
Summary statistics for ragweed pollen, temperature and chemical air contaminants for the entire study period (72 days). Ragweed values are 24-hour time-weighted averages. Temperature and chemical values are daily maximum observations.

| | Mean | Range | Standard Deviation |
|---|---|---|---|
| Maximum temperature (°F) | 71 | 45-95 | 11 |
| Maximum ozone level (ppm)* | 0.044 | 0.016-0.126 | 0.021 |
| Maximum nitrogen dioxide (ppm)** | 0.046 | 0.007-0.113 | 0.02 |
| Maximum sulfur dioxide (ppm) | 0.013 | 0.001-0.037 | 0.008 |
| Ragweed pollen (grains/m$^3$) | 7.7 | 0-67 | 12.2 |

* ppm = parts per million.
**Nitrogen dioxide data were available for 62 days.

Analyses were performed to determine whether air quality parameters were associated with incident symptoms on a daily basis. Ozone, sulfur dioxide, nitrogen dioxide, temperature and ragweed pollen results for the entire study period were examined (Table 1). Sulfur dioxide and nitrogen dioxide levels were not associated with increased symptom incidence. Ozone, ragweed, temperature and time since start of the study were each significantly ($p<0.05$) associated with allergy and asthma incidence in separate Poisson models. These four variables were intercorrelated ($r=0.66$ to 0.83), prompting the use of factor analysis to compute a single "combined factor" to account for the influence of all four variables in a single Poisson regression model. Among study area participants, the combined factor was significantly associated with the frequency of incident allergy and asthma symptoms. The rate ratios tended to increase for the higher quartiles of the combined factor in a dose-response fashion. For the reference area, a significant positive relationship was observed between the combined factor and the incidence of allergy symptoms; however, the relationship was not significant for the incidence of asthma symptoms (Table 2).

Daily mean and maximum *A. fumigatus* concentrations (four-minute time-weighted averages; Table 3) were each evaluated by Poisson regression analysis for

TABLE 2.
Symptom incidence rate ratios for quartiles of a "combined factor" obtained from factor analysis of ragweed, ozone, temperature and time since start of study using data for the entire study period.

| | Study Area | | | Reference Area | | |
|---|---|---|---|---|---|---|
| Symptoms | # Events/ # Eligible | Quartile | Rate ratio (95% Confidence Interval) | # Events/ # Eligible | Quartile | Rate Ratio (95% Confidence Interval) |
| Allergy | 793/2879 | 4 | 1.85 (1.47-2.34)* | 746/3764 | 4 | 1.58 (1.26-2.00)* |
| | | 3 | 1.71 (1.36-2.17)* | | 3 | 1.42 (1.12-1.79)* |
| | | 2 | 1.35 (1.06-1.73)* | | 2 | 1.31 (1.04-1.66)* |
| | | 1 | 1.0 | | 1 | 1.0 |
| Asthma | 279/3271 | 4 | 1.61 (1.14-2.27)* | 226/4257 | 4 | 1.16 (0.79-1.71) |
| | | 3 | 1.17 (0.81-1.67) | | 3 | 1.18 (0.80-1.74) |
| | | 2 | 0.97 (0.66-1.43) | | 2 | 1.10 (0.74-1.62) |
| | | 1 | 1.0 | | 1 | 1.0 |

* p-value < 0.05, statistically significant.

TABLE 3.
Summary statistics for daily *A. fumigatus* for the entire study period (72 days) and for the 20-day period. *A. fumigatus* spore concentrations for the entire study period are mean or maximum 4 minute time-weighted averages (2 or 3 per day). *A. fumigatus* spore concentrations for the 20-day period are mean or maximum one-hour time-weighted averages (24 per day) for the study area only.

| | *A. fumigatus* spore concentration (spores/m$^3$) | | | |
|---|---|---|---|---|
| | Mean | Range | Standard Deviation | N |
| 72-day study period | | | | |
| Daily average | | | | |
| Reference area | 44 | 0 - 230 | 60 | 72 |
| Study area | 180 | 0 - 4710 | 566 | 72 |
| Study area (highest value censored) | 120 | 0 - 830 | 160 | 72 |
| Daily maximum | | | | |
| Reference area | 87 | 0 - 452 | 120 | 72 |
| Study area | 470 | 0 - 14,000 | 1700 | 72 |
| Study area (highest value censored) | 270 | 0 - 2000 | 400 | 72 |
| 20-day period – Study area only | | | | |
| Daily average | 130 | 2 - 830 | 210 | 20 |
| Daily maximum | 2300 | 58 - 19,000 | 4600 | 20 |
| "High" days | | | | |
| Daily average | 350 | 160 - 830 | 250 | 6 |
| Daily maximum | 6400 | 2100 - 19,000 | 6200 | 6 |
| "Low" days | | | | |
| Daily average | 20 | 2 - 44 | 16 | 6 |
| Daily maximum | 300 | 58 - 680 | 260 | 6 |

TABLE 4.
Symptom incidence rate ratios for quartiles of maximum daily *A. fumigatus* spore level based on 4 minute counts using data for the entire study period. Rate ratios were adjusted for a "combined factor" obtained from factor analysis of ragweed, ozone, temperature and time since start of the study.

| | Study Area | | | Reference Area | | |
|---|---|---|---|---|---|---|
| Symptoms | # Events/ # Eligible | Quartile | Rate Ratio (95% Confidence Interval) | #Events/ # Eligible | Quartile* | Rate Ratio (95% Confidence Interval) |
| Allergy | 793/2879 | 4 | 1.08 (0.86-1.35) | 746/3764 | 4 | 0.87 (0.71-1.06) |
| | | 3 | 1.09 (0.87-1.37) | | 3 | 1.05 (0.87-1.27) |
| | | 2 | 1.02 (0.82-1.29) | | 1 | 1.0 |
| | | 1 | 1.0 | | | |
| Asthma | 279/3271 | 4 | 0.83 (0.58-1.18) | 226/4257 | 4 | 0.82 (0.58-1.15) |
| | | 3 | 1.08 (0.77-1.52) | | 3 | 0.88 (0.63-1.22) |
| | | 2 | 0.95 (0.67-1.34) | | 1 | 1.0 |
| | | 1 | 1.0 | | | |

* For the reference area, 47.2% of daily *A. fumigatus* values were 0, therefore quartiles 1 and 2 were combined.

their relationship to allergy and asthma symptom incidence. For both daily mean and daily maximum *A. fumigatus* concentrations, neither the unadjusted model nor the model adjusting for the combined factor showed a positive association. The adjusted symptom rate ratios for quartiles of maximum daily *A. fumigatus* spore levels are presented in Table 4.

*Twenty Days with 1-hour A. fumigatus Spore Counts*

One-hour time-weighted average *A. fumigatus* spore counts (24/day) were performed for the study neighborhood sampling site only. For this reason, the relationships between 1-hour *A. fumigatus* spore concentrations and health symptoms are examined for study area participants only. Daily mean and maximum 1-hour *A. fumigatus* spore concentrations (Table 3) were each evaluated and were not associated with increases in the incidence of allergy or asthma symptoms. The results of the Poisson regression analysis using maximum daily *A. fumigatus* spore counts adjusted for ragweed and time since start of the study are shown in Table 5. Ozone and temperature varied little over the 20-day study period and therefore did not require adjustment. Each of the individual symptom incidence rates (eye irritation, runny nose, skin rash, etc.) were also analyzed using Poisson regression analysis and no significant positive associations between *A. fumigatus* counts and symptom incidence resulted.

Within the 20-day period, symptom frequencies on six days with the highest *A. fumigatus* counts were compared to symptom frequencies on six days with relatively low *A. fumigatus* levels using a paired t-test. Daily maximum 1-hour *A. fumigatus* counts ranged from 58 - 680 spores/$m^3$ on "low" days to 2,100 - 19,000 spores/$m^3$ on "high" days. High *A. fumigatus* spore counts were not associated with increased reporting of allergy or asthma symptoms. The mean (intra-individual) difference in the number of days reporting an allergy symptom for the 6 high *A. fumigatus* days versus the 6 low *A. fumigatus* days was 0.04 (standard error=0.15). For asthma symptoms, the mean difference in symptom frequency was -0.29 (standard error=0.18) for the 6 high *A. fumigatus* days versus the 6 low *A. fumigatus* days. Ragweed pollen and time since start of the study were not controlled for in these analyses due to the small number of days being analyzed.

TABLE 5.
Symptom incidence rate ratios for quartiles of maximum *A. fumigatus* counts for the 20 days for which 1-hour *A. fumigatus* counts were available (study area only). Rate ratios are adjusted for daily ragweed count and time since start of the study.

| | # Events/ # Eligible | Quartile | Rate Ratio (95% Confidence Interval) |
|---|---|---|---|
| Allergy | 271/847 | 4 | 1.11 (0.76-1.62) |
| | | 3 | 0.59 (0.38-0.92) |
| | | 2 | 0.90 (0.58-1.39) |
| | | 1 | 1.0 |
| Asthma | 93/983 | 4 | 0.48 (0.26-0.89)* |
| | | 3 | 0.47 (0.24-0.93)* |
| | | 2 | 0.48 (0.25-0.92)* |
| | | 1 | 1.0 |

* $p < 0.05$, statistically significant.

### *Discussion*

A symptom diary approach was used to evaluate the relationship between bioaerosol concentrations, in particular *A. fumigatus* spore concentrations, and respiratory symptoms associated with allergic, irritative or general inflammatory responses. In a diary study, individual symptom patterns can be related to exposure measures, allowing the population of interest to serve as its own control. With this method, factors which could be difficult to control for in a cross-sectional comparison study would not confound the relationship under examination.

During the 20-day period for which we obtained 1-hour counts, a fairly wide range in spore counts was observed. However, increases in symptom incidence were not associated with the elevations in these counts among participants in the study area. Thus, although the counts of *A. fumigatus* spores measured in air in the community near the composting facility were somewhat higher than those for the reference community (Syzdek and Haines 1995; Recer *et al.*, 2001), increased rates of health symptoms in response to elevations in *A. fumigatus* counts were not demonstrated in this study.

An important limitation of the study is that data providing reliable estimates of average daily *A. fumigatus* spore counts were only available for approximately one-quarter of the original study period. The sharp short-term fluctuations observed in spore levels were not anticipated. Modifying the spore-count method to average over these short-term fluctuations reduced the sensitivity of the original approach for relating daily spore exposure to symptom incidence. Also, fungal spore counts at the sampling locations are probably only an approximate measure of individual exposure to fungal spores, which may have further limited our ability to detect associations. Indoor spore counts may differ significantly from those measured outdoors, and many people spend most of their time indoors. In addition, other indoor and outdoor environments away from home may have an effect on symptom occurrence.

It was our intent to have each subject act as his or her own control by using a method of data analysis which produces a summary estimate based on separate logistic regression of data for each subject in the study (Korn 1979). Losses due to dropouts from the study and gaps in the data due to incomplete or missing diaries did not permit the use of that method of data analysis. For Poisson regression analysis of incidence rates, participants do not serve as their own controls. Therefore, there is the potential for differences in individual susceptibility to confound the relationship being evaluated. While participants do act as their own controls in the paired t-test of differences in symptom prevalence, data sparseness limited that analysis to 12 days and 48 participants. The t-test analysis had a 63% to 91% probability of correctly identifying increases of 10% to 15%, respectively, in the frequency of allergy or asthma symptom reporting on high *A. fumigatus* days versus low *A. fumigatus* days. Thus, while it is likely that a true increase in symptom frequency of 15% or more would have been detected, a 10% or smaller increase could have been missed.

Despite the limitations described above, it is interesting to note that symptom incidence was associated with ragweed, ozone, temperature and time since start of the study. This was true for participants living near the composting facility and, to a lesser degree, participants from the reference community. The presence of a dose-response trend suggests that changes in one or more of these variables are having an effect on symptom incidence. If ragweed, ozone and temperature are independently related to symptom incidence (and not just in association with time since start of the study), our ability to assess the effect of environmental influences on allergy and asthma symptom occurrence would be supported. However, time since start of the study was strongly correlated with ragweed, ozone and temperature and could provide an alternative explanation for the decrease in symptom incidence observed over the course of the study. A general tendency to report fewer symptoms as a study progresses, termed a "fatigue effect," has been noted in other studies using diary data (Abramson 1990). Separating the relative importance of the environmental factors and time since start of the study was not possible due to their strong intercorrelations.

The results of this study suggest that if elevated concentrations of *A. fumigatus* spores due to operations at the composting facility are causing increases in allergy and asthma symptom prevalence, the increase was too small for us to detect given the study limitations. We propose that future studies employ more objective measures of immune response and respiratory function. Also, less labor-intensive exposure measures that can reliably assess individual daily bioaerosol exposures would be desirable.

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