

# The air spora close to a compost facility in Northeast Oklahoma: Part I—spore trap sampling

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**Abstract** A compost facility in northeast Oklahoma is located relatively close to a residential area and is the focus of complaints about smell and concerns about health effects. Several species of *Aspergillus* have been known to cause health problems, and at least one of these species is dominant in compost. The atmosphere surrounding the compost facility was monitored for 1 year using Burkard spore traps to determine if there was a significant difference in *Penicillium/Aspergillus* type spores concentration between a test and control site. Samplers were situated 710 m downwind for the test site and 6,085 m upwind at the control site. There was no significant difference in mean concentration of *Penicillium/Aspergillus* type spores between the two sites ( $t = 0.576$   $P > 0.05$ ). The mean concentration of total spores was significantly higher at the upwind control site ( $t = -7.64$ ,  $P < 0.01$ ). Wind direction was examined to determine if the compost facility was a possible source for any spikes in concentration. No clear relationship was found between wind direction and mean *Penicillium/Aspergillus* concentration at the test site, but peak concentrations of *Penicillium/Aspergillus* seen at the test site were on days when it was downwind from the

composting facility. However, these concentrations were no higher than those seen at the control site on other days. If the compost was releasing large amounts of *Penicillium/Aspergillus* type spores into the atmosphere they were generally diluted to background levels by the time they reached the test site.

**Keywords** Aerobiology · *Aspergillus* · Burkard · Compost · Spore trap

## 1 Introduction

The air spora around compost has been the focus of many studies, with several concentrating on species of *Aspergillus*. *Aspergillus fumigatus* is the dominant species in compost but other species such as *Aspergillus caespitosus* and *Aspergillus niger* also show good growth (Tiscornia et al. 2005). *Aspergillus fumigatus* is able to dominate because of both its ability to use all the components of compost as a food source and its high thermotolerance of up to 50°C (Millner et al. 1977).

The dominance of *Aspergillus* species in compost is of interest because of potential health concerns. *Aspergillus fumigatus* spores are often released from compost at high concentrations (Tiscornia et al. 2005). This species is the most common mold pathogen that affects humans and has well documented health effects. It is known to cause several conditions such as aspergillosis, allergic bronchopulmonary aspergillosis,

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and aspergilloma (Vincken and Roels 1984; Kramer et al. 1989; Marsh et al. 1979). It has also been known to exacerbate pre-existing conditions such as asthma (Marsh et al. 1979). Species of *Aspergillus* utilize wind for the dispersal of their spores. The spores are carried away from the compost on a regular basis but there may be large increases in spore release when the compost is moved or turned (Millner et al. 1977, 1980; Kleyn et al. 1981; Herr et al. 2003). Studies have shown that increased proximity to compost does increase the concentration of *Aspergillus* spores in the surrounding air (Herr et al. 2003; Werf 1996). Mean daily *Penicillium/Aspergillus* type spores concentrations have also been shown to be higher on-site (3,207 spores  $m^{-3}$ ) than off-site (264 spores  $m^{-3}$ ) (Hryhorczuk et al. 2001). At a full scale composting facility, samples collected 25–40 m downwind showed increases in concentration of *A. fumigatus* up to two orders of magnitude from  $3 \times 10^2$  to  $5 \times 10^4$  cfu  $m^{-3}$  when the compost was turned, or during some other compost disruption event (Sanchez-Monedero et al. 2005). Another study found *A. fumigatus* spore concentrations close to compost piles sometimes exceeded  $10^6$  cfu  $m^{-3}$  (Clark et al. 1983). Elevated levels of *A. fumigatus* between  $57.8 \times 10^3$  and  $111.3 \times 10^3$  cfu  $m^{-3}$  were observed up to 30 m away from the compost (Taha et al. 2005). Syzdek and Haines (1995) found mean daily *A. fumigatus* concentrations were much higher 30 m from the compost facility (552 spores  $m^{-3}$ ) than at either the local community site (151 spores  $m^{-3}$ ) 540 m downwind of the facility or the control site (44 spores  $m^{-3}$ ) 10,000 m upwind of the composting facility.

A compost facility in northeast Oklahoma is located relatively close to a residential area and is the focus of complaints about the smell and concerns about health effects. This study was undertaken to examine the air spora near this site and to determine if there was a significant difference in spore levels in air near the compost facility compared to a control site focusing on, but not limited to, *Aspergillus* spores.

## 2 Methods and materials

### 2.1 Sampling location

This study took place in northeast Oklahoma in the foothills of the Ozark Mountains. The compost

facility of interest is associated with a mushroom farm and located just outside the downtown area of a small city. There are fields located south, north, and east of the facility but southwest of the facility there is a small residential neighborhood. The mushroom farm produces its own compost on site using outdoor windrows. Front end loaders are used to move and turn the compost.

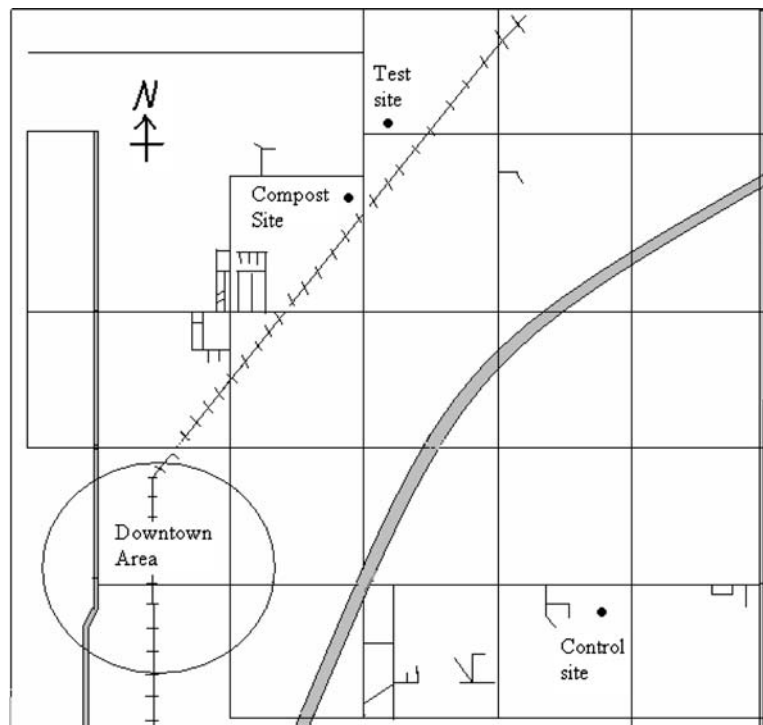
### 2.2 Air sampling

The atmosphere was monitored for one year from 9 November 2005 to 9 November 2006 with two Burkard volumetric spore traps fitted with the alternate orifice (Burkard Manufacturing, Hertfordshire, UK). The alternate orifice is  $14 \times 2$  mm reducing to  $14 \times 0.5$  mm. This feature allows it to efficiently sample spores as small as  $2.17 \mu m$  (Buttner et al. 1996). A local air-quality monitoring station approximately 710 m downwind of the facility acted as the test site. The control site was a local golf course approximately 6,085 m upwind of the compost facility (Fig. 1). Both samplers were located on the roof of small buildings approximately 3.6 m above the ground. Due to power outage problems during late April and early May 2006 the control site sampler was moved to a different location at the golf course approximately 6,135 m from the compost facility and elevated 5.5 m above the ground.

The Burkard samplers were fitted with seven-day sampling heads and particles in the air impacted on Melinex tape coated with a thin layer of Lubriseal (Thomas Scientific, USA). Following sampling the tape was removed and cut into 48 mm (24 h) segments and mounted on microscope slides using glycerin-jelly mounting media. The slides were analyzed at  $1000\times$  magnification.

All common airborne spores were identified and counted using the single traverse method (Sterling et al. 1999). In addition, some slides were analyzed with the 12 transverse traverse hourly method. Spore counts were converted to daily or hourly concentrations and expressed as spores  $m^{-3}$  of air (Kapyla and Penttinen 1981). Spore concentrations were log transformed to normalize the data and Statistica 5.1 (StatSoft Tulsa, OK, USA) was used for data analysis. Mean concentrations at the two sites were compared using a dependent *T*-test. Wind-direction

**Fig. 1** The area surrounding the compost facility, with local roads shown as *lines* and larger roads and highways designated as *larger gray lines*. The downtown area was designated but all roads were not designated for this area. There was a small residential neighborhood southwest of the compost facility where indoor air sampling was performed. The compost site was behind the main mushroom farm building. The facility extends back into the lot. The test site was located in a small field approximately 710 m north–northeast of the compost facility. The control site was located on a local golf course 6,085 m south–southeast of the compost facility



data were obtained from the Oklahoma Mesonet meteorological station which was located 4,852 m from the test site, 3,362 m from the first control site, and 3,654 m from the second control site.

The main focus of this study was *Aspergillus* spores but *Aspergillus* and *Penicillium* spores are morphologically alike and cannot be distinguished using microscopy. For this reason these spores were counted as *Penicillium/Aspergillus* type spores. Elevated levels of *Penicillium/Aspergillus* spores were arbitrarily defined as concentrations greater than 615 spores  $m^{-3}$  which is two standard errors above the mean concentration sampled at the Aerobiology Lab in Tulsa, Oklahoma, between the years 2001–2005 and adjusted for use of the alternate orifice (Khattab and Levetin 2006).

### 3 Results

Air sampling showed both locations had the same general trend of spore concentrations and distribution. Summary statistics for all spore types identified can be seen in Table 1 for the test site and Table 2 for the control site. The yearly mean concentrations for

both sites were compared for each spore type. There was a significant difference for all spore types counted ( $P > 0.01$ ) with higher concentrations at the control site than at the test site with the exception of *Epicoccum*, *Penicillium/Aspergillus*, and *Pithomyces* spores, where there were no significant differences ( $P < 0.05$ ) (Table 3).

Of particular interest are the total spore and *Penicillium/Aspergillus* type spore concentrations. Daily total spore concentrations from both samplers show roughly the same daily trend and fluctuations; however, the concentration from the control sampler had higher levels of spores on most days (Fig. 2). Spore concentrations from both locations were low during winter and early spring and increased in late spring. There were high concentrations of total spores at the test site during late April early May but there were no data at the control site. Sampling at the control site was interrupted due to power supply problems between 30 April and 15 May 2006. The one year mean daily concentration of total spores at the test site was 4,063 spores  $m^{-3}$  and the mean at the control site was significantly higher at 4,518 spores  $m^{-3}$  ( $t = -7.64$ ,  $P < 0.01$ ) (Table 2).

**Table 1** Summary statistics of airborne spore levels at test site

Taxa	Daily mean concentration (spores m <sup>-3</sup> )	Median concentration (spores m <sup>-3</sup> )	Range spores (spores m <sup>-3</sup> )	Percent of yearly total
<i>Alternaria</i>	161	80	0–1,591	3.97
Ascospores	543	194	5–8,541	13.37
Basidiospores	388	202	5–10,239	9.56
<i>Cladosporium</i>	2,483	1,519	43–20,212	61.11
<i>Curvularia</i>	9	0	0–532	0.21
<i>Drechslera</i>	13	5	0–181	0.31
<i>Epicoccum</i>	25	16	0–192	0.62
Myxomycete spores	16	5	0–351	0.39
<i>Nigrospora</i>	7	0	0–106	0.18
<i>Penicillium/Aspergillus</i>	108	69	0–926	2.65
<i>Pithomyces</i>	6	0	0–101	0.14
Smut spores	259	191	0–2,746	6.38
Other spores	45	35	0–319	1.11

**Table 2** Summary statistics of airborne spore levels at control site

Taxa	Daily mean concentration (spores m <sup>-3</sup> )	Median concentration (spores m <sup>-3</sup> )	Range (spores m <sup>-3</sup> )	Percent of yearly total
<i>Alternaria</i>	195	85	0–2,917	4.31
Ascospores	599	250	0–6,126	13.25
Basidiospores	408	223	5–4,558	9.03
<i>Cladosporium</i>	2,709	1,684	27–20,300	59.94
<i>Curvularia</i>	10	0	0–388	0.21
<i>Drechslera</i>	27	11	0–1,387	0.60
<i>Epicoccum</i>	23	16	0–287	0.50
Myxomycete spores	25	5	0–255	0.54
<i>Nigrospora</i>	12	0	0–175	0.26
<i>Penicillium/Aspergillus</i>	126	82	0–1,116	2.78
<i>Pithomyces</i>	7	0	0–101	0.16
Smut spores	319	244	0–2,024	7.06
Other spores	61	48	0–1,031	1.36

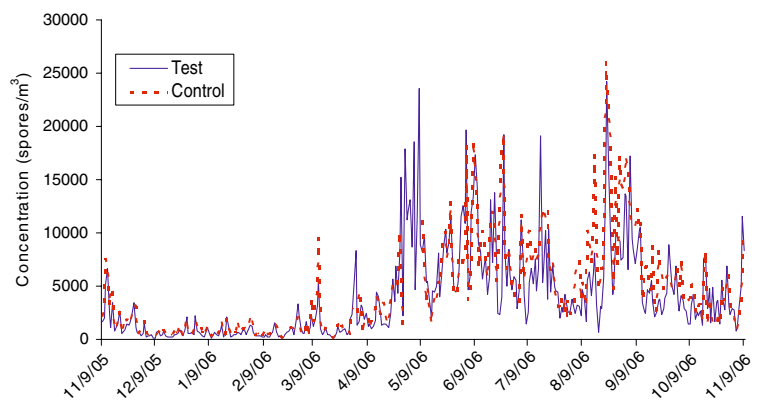
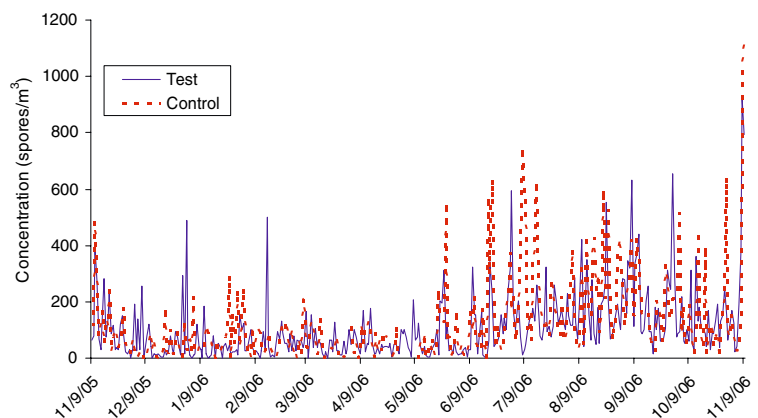
At both sampling sites there was a decrease in total spore concentrations between 23 July and 13 August 2006 (Fig. 2). During this period there were 13 days over 36°C and seven of these were over 38°C. The peak total spore concentration was in August for both sites with 23 August 2006 for the test site and 22 August 2006 for the control site.

The mean daily airborne concentration of *Penicillium/Aspergillus* type spores showed greater variability

between the two sampling sites. There were several spikes at both the test site and the control site that were not present at the other site, but for the most part they followed the same general trend (Fig. 3). There was a small difference in the yearly mean between the control site at 126 spores m<sup>-3</sup> and the test site at 108 spores m<sup>-3</sup>, but this difference was not significant ( $t = 0.576$   $P > 0.05$ ) (Table 3). As indicated, elevated levels of *Penicillium/Aspergillus* were defined as

**Table 3** Comparison of mean concentration of spore types at sampling locations

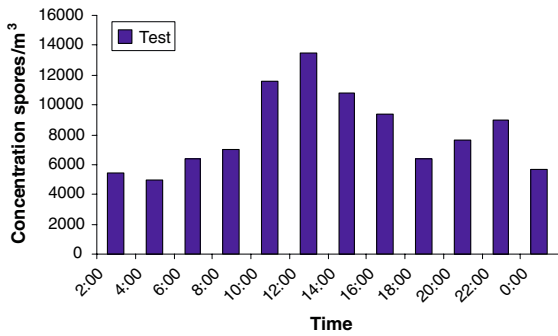
Taxa	Log transformed mean		
	Test site	Control site	<i>T</i> (352)
<i>Alternaria</i>	1.748	1.820	-3.352*
Ascospores	2.270	2.384	-6.688*
Basidiospores	2.256	2.331	-4.877*
<i>Cladosporium</i>	3.039	3.124	-7.013*
<i>Curvularia</i>	0.406	0.481	-2.630*
<i>Drechslera</i>	0.650	0.880	-7.784*
<i>Epicoccum</i>	1.047	1.015	1.048
Myxomycete spores	0.733	0.849	-3.650*
<i>Nigrospora</i>	0.455	0.550	-3.354*
<i>Penicillium/Aspergillus</i>	1.771	1.790	-0.576
<i>Pithomyces</i>	0.416	0.463	-1.662
Smut spores	2.222	2.325	-6.388*
Total spores	3.322	3.407	-7.647*

\*  $P < 0.01$ **Fig. 2** Mean daily total spore concentrations for the entire one-year sampling period starting on 9 Nov 2005 and ending on 9 Nov 2006**Fig. 3** Mean daily *Penicillium/Aspergillus* spore concentration for the entire one-year sampling period starting on 9 Nov 2005 and ending on 9 Nov 2006

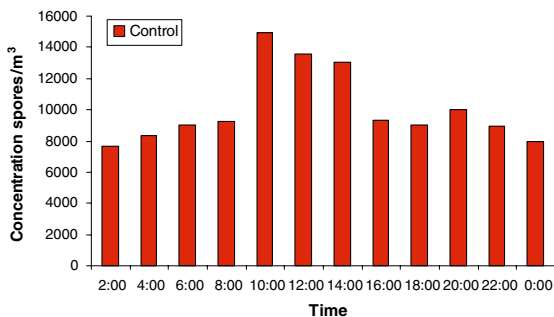
concentrations over 615 spores  $m^{-3}$ . There were four days at the test site with elevated spore concentrations (7 Sep, 30 Sep, 8 Nov, and 9 Nov 2006) and six days at the control site with elevated spore concentrations (21 Jun, 8 Jul, 16 Jul, 30 Oct, 8 Nov, and 9 Nov 2006).

In addition to average daily concentration, some slides were also analyzed to determine hourly spore concentration levels. Eleven slides were counted for hourly concentration from the control site and ten slides from the test site. All of the slides from the control site had daily *Penicillium/Aspergillus* concentrations over 500 spores  $m^{-3}$ , but due to the lower concentrations at the test site some of the days counted were lower than 500 spores  $m^{-3}$ .

The highest hourly concentration of total spores at the test site was at 12:00 h with 13,496 spores  $m^{-3}$  with elevated levels seen between 10:00 and 14:00 h (Fig. 4). The highest hourly total spore concentration



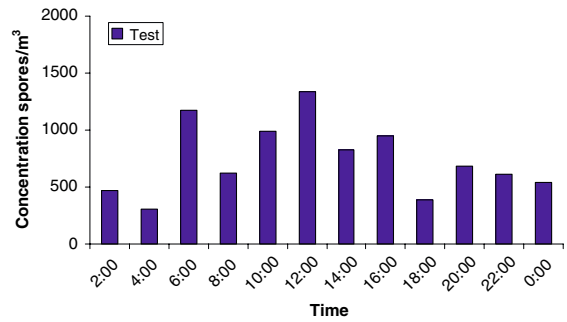
**Fig. 4** Mean hourly concentrations of total spores at the test site on ten *Penicillium/Aspergillus* peak days



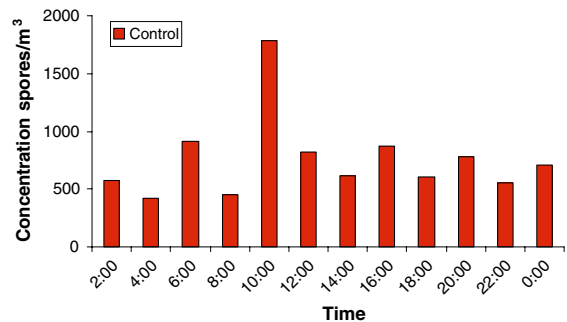
**Fig. 5** Mean hourly concentrations of total spores at the control site on eleven *Penicillium/Aspergillus* peak days

at the control site was at 10:00 h with a mean concentration of 14,920 spores m<sup>-3</sup> and high levels until 14:00 h (Fig. 5). At both sampling sites the mean concentration decreased after 14:00 h and remained lower for the rest of the day. Mean concentrations of total spores were also lower during the morning hours between 2:00 and 8:00 h at both sampling sites.

The mean hourly concentration of airborne *Penicillium/Aspergillus* type spores followed the same pattern as the total spores. The highest hourly concentration of *Penicillium/Aspergillus* type spores at the test site was 1,336 spores m<sup>-3</sup> at 12:00 h (Fig. 6). This was in part due to a spike at 12:00 h of 4,731 spore m<sup>-3</sup> on 8 Nov 2006. It should be noted that the wind was from the south on this day. At the control site the highest mean hourly concentration of airborne *Penicillium/Aspergillus* type spores was 1,789 spores m<sup>-3</sup> at 10:00 h (Fig. 7). This 10:00 peak of airborne *Penicillium/Aspergillus* type spores at the control site was very distinct and all other times



**Fig. 6** Mean hourly concentration of *Penicillium/Aspergillus* spores on ten peak days at the test site

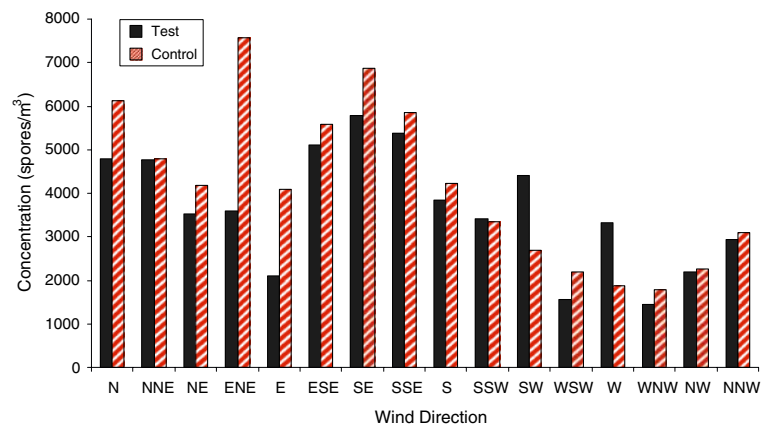


**Fig. 7** Mean hourly concentration of *Penicillium/Aspergillus* spores on eleven peak days at the control site

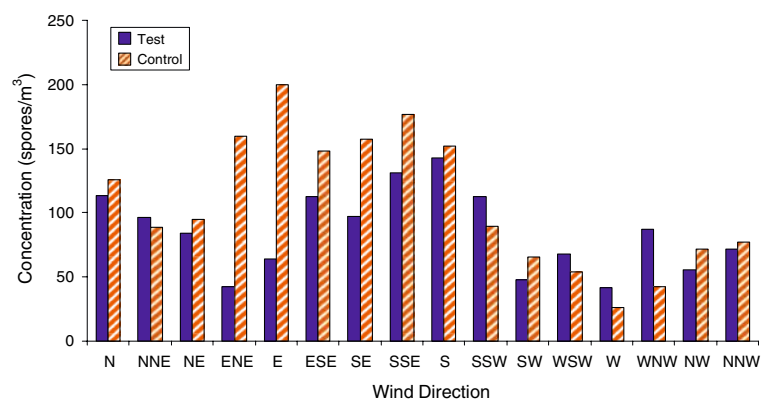
showed reduced mean airborne spore concentrations (Fig. 7). On 9 Aug 2006 and 9 Nov 2006 there were spikes at 10:00 h that contributed to the clear peak at 10:00 h at the control site. These spikes had very high concentrations of *Penicillium/Aspergillus* with 8,043 spore m<sup>-3</sup> on 9 Aug 2006 and 6,151 spore m<sup>-3</sup> on 9 Nov 2006. If these two days were removed then the peak at 10:00 h would be much less distinct. The peak at the test site was less distinct than that seen at the control site and there was a smaller secondary peak of 1,178 spores m<sup>-3</sup> at 6:00 h. This secondary peak was due in large part to a spike of 4,732 spores m<sup>-3</sup> at 6:00 h on 9 Nov 2006.

There was no clear relationship between daily wind direction and either total spore concentrations or *Penicillium/Aspergillus* type spore concentrations (Figs. 8, 9). The total spore concentrations were slightly higher at the control site for most wind directions with only the west and southwest showing higher spore concentrations at the test site (Figs. 8, 9). The test site did not show a significant difference in *Penicillium/Aspergillus* concentrations between

**Fig. 8** Mean concentration of total spores by wind direction. Some wind directions have more data points than others due to the different number of days when the prevailing winds were from that direction



**Fig. 9** Mean concentration of *Penicillium/Aspergillus* spores by wind direction. Some wind directions have more data points than others due to the difference in days when the prevailing winds were from that direction



the two sites on days when the prevailing wind was from the south or south–southwest ( $P > 0.05$ ; Fig. 9). In fact, there were no significant differences in *Penicillium/Aspergillus* spore concentration between the two sampling sites for any of the wind directions.

#### 4 Discussion

The air spora around the compost facility, as measured at the test site, was similar to that seen at the control site. Both sites had the same basic airborne spore distribution pattern with only a few minor differences. The distribution pattern is in agreement with other studies that have also shown *Cladosporium*, ascospores, and basidiospores to be the most common spore types in the atmosphere in this region (Troutt and Levetin 2001; Burch and Levetin 2002). Although both sites did have the same general distribution pattern, both total spores and most of the individual spore types showed higher concentrations at the control site. *Epicoccum*,

*Pithomyces*, and *Penicillium/Aspergillus* spore types showed no significant difference between the two sites. Spore concentrations from two Burkard samplers at close proximity in similar landscapes would be expected to be similar with no significant differences. So this difference in concentration between the control site and the test site for most spore types was unexpected. It is interesting to note that there was no significant difference for *Penicillium/Aspergillus* type spores.

This difference in airborne spore levels seen between the sites could be due to several factors related to the location of the sampling stations. Care was taken to standardize the two sampling locations as much as possible such as placing the samplers at the same height in areas without trees close to the sampler, but possible locations were limited and some differences did exist. The sampler at the control site was moved halfway through the study, after multiple power outages, to another small storage shed at the golf course. This second site was 540 m away and elevated 1.9 m over the previous location. Because

the control site sampler was located in the middle of a golf course rather than a field there was more traffic and activity present around it on a daily basis. This was especially true during the summer months when spore levels are at their highest. This activity came mainly in the form of golfers and golf carts. The golf carts when driven over the grass can disrupt resting fungal spores causing them to become airborne, increasing the airborne concentration at this site. Frequent lawn mowing at the control site that was not seen at the test site was also a possible source of this higher airborne spore concentration. Lacey (1975) found that both *Cladosporium* and *Epicoccum* spore concentrations increased after mowing. Another possible source of the difference in mean spore concentration between the two sites may be a difference in relative humidity. There are several small ponds located on the golf course and the control site sampler was located close to two of these ponds for the first half of the study. These ponds and watering of the greens resulted in more moisture at the control site possibly leading to greater fungal growth on leaf surfaces. There were no ponds in close proximity to the test site sampler.

Of particular interest were the total and *Penicillium/Aspergillus* type spore concentrations. There was no significant difference in the mean concentration of airborne *Penicillium/Aspergillus* type spores between the two sampling sites. There were however, several spikes in airborne spore concentration at one site and not the other. These spikes could be due to a disruption event such as the turning of the compost, which has been shown to release large plumes of spores into the surrounding atmosphere (Millner et al. 1977, 1980; Kleyn et al. 1981; Herr et al. 2003).

One way to determine if the *Penicillium/Aspergillus* type spores were likely to originate at the compost facility was to examine the wind direction data on days with elevated concentrations. There were four days with airborne *Penicillium/Aspergillus* type spore concentrations of 615 spores  $\text{m}^{-3}$  or higher at the test site. On two of these days the prevailing wind was from the south with the test site downwind of the compost facility and on one day the wind was from the south-southeast. There were no wind direction data for the remaining day. Since the test site was downwind of the compost facility on some days it was a possible source of these spores. There were six days with elevated *Penicillium/Aspergillus* concentrations at the control

site. If the control site was detecting spores from the compost facility they would most likely be seen on days when the wind was from the northeast or north-northeast. None of the spikes occurred on days when the wind was from the northeast or north-northeast. The wind was from the south on four of the days, from the south-southeast on one of the days, and there were no wind direction data for the remaining day.

If spores were originating from the compost facility the mean spore concentration from the south and south-southwest wind direction would be expected to be highest at the test site. While this is true when the prevailing winds were from the south-southwest it is not true when they were from the south and there was no significant difference between the two sites for these wind directions.

Hourly concentrations were determined on days with the highest concentrations of *Penicillium/Aspergillus* type spores to see if the concentrations were due to a single spike possibly arising from the compost. If large concentrations of airborne *Penicillium/Aspergillus* type spores were being released into the surrounding air on these days it would be expected to show up on the hourly counts when the release occurred. Several spikes of *Penicillium/Aspergillus* were observed at both the test site and the control site during the study. On 8 Nov 2006 there was a spike at 12:00 h that was not seen at the control site. The prevailing winds on this day were from south. So it is possible that the spores originated from the compost facility. On 9 Nov 2006 there were spikes at both the test and control sites. At the test site the spike was at 6:00 h but at the control site the spikes were at 10:00 h and 16:00 h. The wind was from the south-southeast, which indicates that the spikes at the test site could have originated at the compost facility. The time of the spikes also may be of importance. *Penicillium* and *Aspergillus* are both members of the dry air spora and typically have peaks later in the day during periods of lower relative humidity. This spike at 6:00 could be due to a disruption event of the compost such as turning by front-end loaders (Millner et al. 1977, 1980; Kleyn et al. 1981; Herr et al. 2003; Fischer et al. 1994). However, this fails to explain the large spikes seen at the control site. The cause and potential source of these spikes remains unclear.

It is possible that the compost facility is releasing large amounts of airborne *Penicillium/Aspergillus* type spores but the test site sampler is located too far



away from the compost and by the time the spores reach the sampler the concentration is diluted to background levels. In another simultaneous study at this same facility using culture-based methods it was shown that there were elevated levels of *A. fumigatus* at the sampling site closest to the facility, 150 m, but concentrations decreased with distance to background levels by 700 m (Gillum and Levetin 2007). The Burkard sampler at the test site was located downwind of the facility and closer than the nearest residential neighborhood which is upwind of the facility. Spores emanating from the compost would not be expected to produce elevated levels in the residential neighborhood if they were not seen at the test site. The days when airborne *Penicillium/Aspergillus* type spores were higher than 615 spores m<sup>-3</sup> at the test site were days when the prevailing winds were from the south or south-southeast. This is consistent with the compost facility being a possible source of the spores, but mean concentrations throughout the year were higher at the control site. At 700 m downwind of the compost facility any elevated levels of *Penicillium/Aspergillus* type spores do not appear to any greater than one might experience playing golf several kilometers away.

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