Contribution of leaf surface fungi to the air spora

Estelle Levetin* & Kip Dorsey

Faculty of Biological Science, The University of Tulsa, 600 S. College, Tulsa, OK, 74104, USA; (*Author for correspondence: E-mail: estelle-levetin@utulsa.edu; Fax +918-631-2762)

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Abstract

High concentrations of airborne fungal spores frequently occur from spring through fall in temperate areas of the world. Although it is generally assumed that fungi on leaf surfaces are contributors to the air spora, little data are available comparing the types of fungi found on leaf surfaces with those in the atmosphere. Air sampling was carried out with a Burkard Spore Trap located on the roof of a building on the University of Tulsa campus using standard methods. Leaf samples were aseptically collected from Ulmus americana and *Quercus* palustris trees on campus, placed in sterile plastic bags, and brought to the lab. For each leaf, 4 cm^2 areas of both upper and lower leaf surfaces were swabbed and plated on malt extract agar with streptomycin. Cultures were incubated at room temperature for 5-7 days and then examined microscopically. Results were expressed as colony forming units (CFU)/cm². Twenty-one fungal taxa were identified from the air samples. The most abundant taxa were *Cladosporium*, ascospores, basidiospores, and Alternaria; together these four spore types comprised over 90% of the yearly total. Yeasts were the most abundant fungi isolated from both leaf types. Among the mycelial fungi were *Phoma* species, followed by Cladosporium and Alternaria. Overall twenty genera of filamentous fungi were identified. Yeasts and Phoma are normally splash dispersed and were not identified in the Burkard air samples. However, 10 taxa isolated from leaf surfaces were registered in air samples. Crude estimates of the leaf surface area of each tree suggest that the total fungal load was approximately 5.04×10^8 CFU for Ulmus and 2.71×10^8 CFU for Ouercus. Of these levels, 19% were from fungi also detected in air samples. The data suggest that some leaf-surface fungi are major contributors to the air spora.

1. Introduction

The phylloplane, the surface of plant leaves, is a complex terrestrial habitat that is characterized by a variety of microorganisms including bacteria, filamentous fungi and yeast. Pathogens, saprobes and epiphytes occur in this habitat and numerous studies have described the phylloplane populations from various plant species (Breeze and Dix, 1981; Mishra and Dickinson, 1981; de Jager et al., 2001; Andrews et al., 2002; Inácio et al., 2002; Osono, 2002; Osono et al., 2004). The non-pathogenic fungi that inhabit the

phyllosphere depend on nutrients exuded from the leaf or those deposited from the atmosphere (Belanger and Avis, 2002; Inácio et al., 2002). In addition to nutrient levels, growth and abundance of phylloplane fungi are also influenced by environmental conditions such as water availability, UV radiation, and temperature (Breeze and Dix, 1981; Newsham et al., 1997; Sundin, 2002; Zak, 2002).

The atmosphere contains a tremendous diversity of airborne spores with high concentrations frequently occurring from spring through fall in temperate areas of the world (Gregory, 1973; Levetin, 1995). The air spora constitutes both the source of fungi that colonize the leaf surface and the sink of spores released from the leaf surface by various dispersal mechanisms (Pedgley, 1991; Kinkel, 1997; Aylor, 2002). Airborne spores impact on leaf surfaces and may adhere due to structural or chemical features of the epidermis and the spore (Andrews and Buck, 2002). Spore release from many fungi inhabiting the phylloplane is passive through the action of wind or rain splash; however, other spores are actively propelled into the atmosphere by various mechanisms (Kinkel, 1997; Aylor, 2002; Levetin, 2002). Although this connection between phylloplane fungi and airborne fungi is widely accepted, little data are available comparing the types of fungi found on leaf surfaces with those in the atmosphere over a growing season. The present study was undertaken to compare the airborne spores in Tulsa with the leaf surface fungi collected from Ulmus americana and Quercus palustris trees and to examine the effects of meteorological conditions on the airborne and leaf surface populations.

2. Materials and methods

Air sampling was carried out using a Burkard Spore Trap (Burkard Manufacturing Co., Rickmansworth, Hertfordshire, England) set for 7 day sampling onto Melinex tape that was coated with a thin film of Lubriseal (Thomas Scientific, Swedesboro, NJ, USA). The sampler was stationed at 12 m above ground on the roof of the Biology building on The University of Tulsa campus. The university is located in an older residential neighborhood about 5 km from the city center. Sampler drums were changed weekly and the tapes cut into 48 mm segments representing the previous 7 days. Tapes were mounted onto microscope slides, stained with glycerin jelly, and examined at $1000 \times$ magnification using the single longitudinal transverse method as previously described (Sterling et al., 1999). Concentrations were expressed as spores per cubic meter of air. Bioaerosol data was log transformed to normalize the data for statistical analyses.

Leaf samples were aseptically collected from Ulmus americana (American elm) and Quercus palustris (pin oak) trees on campus, placed in sterile plastic bags, and immediately brought to the laboratory. Each week three healthy-looking leaves were collected from the lower canopy of each tree. Leaf collection began in mid-April when leaves first appeared on the trees and continued until leaf fall in late November. Both species are common on campus and within the city and were selected for this reason.

For each leaf, 4 cm^2 areas of both adaxial and abaxial leaf surfaces were separately wiped with a sterile cotton swab that had been slightly dampened with sterile distilled water. A sterile stainless steel template with a 4 cm^2 opening was used to insure accurate and consistent sample area and care was taken to avoid the central mid-rib vein of the leaf. Each swab was then rinsed in 1 ml of sterile distilled water by vortexing for 30 seconds. A 0.5 ml aliquot of each spore suspension was plated on malt extract agar with streptomycin. Cultures were incubated at room temperature for 5-7 days, colonies were counted and then examined microscopically. Standard keys were used for colony identification (Ellis, 1971, 1976; Barnett and Hunter, 1998) Results were expressed as colony forming units $(CFU)/cm^2$ of leaf area.

For each tree estimates of leaf area were based on measurements of 10 randomly selected leaves collected in late summer. Total numbers of leaves per tree were also estimated by the following steps. The average number of leaves per twig was determined from counting the leaves on 10 twigs. The number of twigs per small branch, the number of small branches on each main branch, and the number of main branches on the tree were also determined for calculating total leaf number. Total leaf surface area was also determined.

Meteorological data were obtained from the National Weather Service station in Tulsa, located approximately 8 km from the University. Statistica 5.1 (StatSoft, Inc., Tulsa, OK) software was used for data analysis.

3. Results

Airborne spores were registered every day during 2002. Concentrations ranged from 53 spores/m³ on 18 January to a yearly peak of 48,188 spores/m³ on 22 September (Figure 1). There were 21

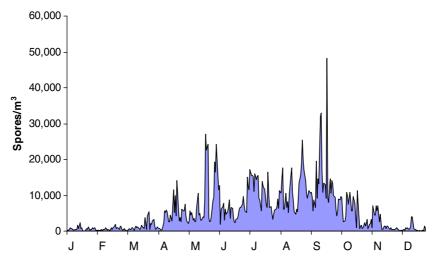
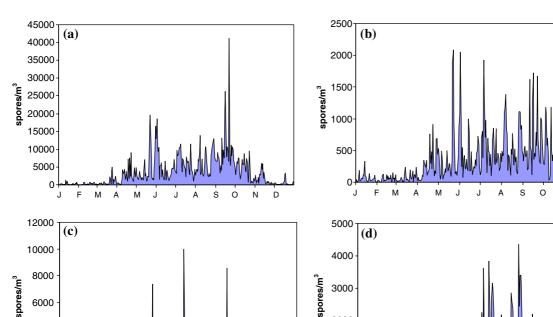


Figure 1. Average daily concentration of total spores in the Tulsa atmosphere during 2002.

taxa that were routinely counted on the air samples; however, many other taxa were identified. For example, basidiospores were counted as a single category even though *Coprinus*, *Ganoderma*, *Stropharia*, *Conocybe*, and *Agaricus* basidiospores were frequently seen. In a similar manner, ascospores were grouped as a single category although *Leptosphaeria*, *Pleospora*, and Diatrypaceae ascospores were often abundant. The summary data of all taxa identified are shown in Table 1. Four taxa, *Cladosporium*, *Alternaria*, total ascospores, and total basidiospores accounted for over 90% of the air spora. The average daily concentrations of these four

Table 1. Summary statistics of airborne spores from Tulsa, Oklahoma

Taxa	Daily mean concentration spores/m ³	Median concentration spores/m ³	Peak concentration spores/m ³	Cumulative season total	Percent of yearly total
Cladosporium	3543.1	2156	41,206	1,293,240	64.86
Ascospores	577.0	292	10,052	210,595	10.56
Basidiospores	526.0	223	4348	191,941	9.63
Alternaria	294.2	159	2087	107,379	5.39
Penicillium/Aspergillus	173.0	95	2208	63,279	3.17
Other/unidentified	84.0	42	690	30,501	1.53
Smut spores	57.0	32	743	21,962	1.10
Myxomymycete spores	47.0	16	483	17,008	0.85
Periconia	32.0	5	323	11,783	0.59
Epicoccum	27.2	11	308	9940	0.50
Drechslera-type spores	21.0	5	212	7795	0.39
Nigrospora	18.0	5	175	6436	0.32
Pithomyces	16.0	0	180	5995	0.30
Curvularia	14.0	0	223	5214	0.26
Rust spores	8.0	0	165	2936	0.15
Stemphyllium	8.0	0	239	2851	0.14
Torula	5.0	0	85	1811	0.09
Fusarium	4.0	0	90	1604	0.08
Myrothecium	2.7	0	440	993	0.05
Spegazzinia	2.0	0	85	552	0.03
Tetraploa	0.2	0	21	80	0.004



F М Α М s 0 Ν D Ν D F М 0 Figure 2. Average daily concentrations of (a) Cladosporium conidia, (b) Alternaria conidia, (c) all ascospores, and (d) all basidiospores in the Tulsa atmosphere during 2002. Note the difference in scale for the four taxa.

2000

1000

C

taxa are shown in Figure 2. Concentrations of all airborne spores began increasing in April as the seasonal temperatures increased. April is also the time that leaf expansion occurs. By the end of April all the trees in the area are fully leafedout.

Leaf collection began on 18 April during the week when leaves first appeared on the trees. The final leaf collection was made on 23 November when over 95% of the leaves had abscised. Filamentous fungi and yeast were isolated from all leaf surfaces throughout the study period (Figure 3). A total of 21,624 colonies were isolated and 23 taxa were identified. In fact 15 taxa were identified during the first week of sampling. Filamentous fungi were identified to the genus level whenever possible. The summary statistics for all taxa are shown in Table 2. Although many genera were represented by more than one species, no consistent efforts were made to identify colonies to the species level. Yeasts were placed in two categories: (1) black yeasts which included Aureobasidium as well as unidentified black yeasts and (2) other yeasts which included Rhodotorula, Sporobolomyces and others. For data analysis the two groups were combined.

Ν D

Overall yeasts (black yeasts and other yeasts) were the most abundant fungi isolated from both leaf types. Among the mycelial fungi, Phoma species were the most common followed by Cladosporium and Alternaria. These four types accounted for over 90% of the leaf surface fungi isolated and occurred on 100% of all the leaf samples. The weekly concentrations of these four fungal types are shown in Figure 4. Another 5.5% of all leaf surface fungi were non-sporulating colonies. The remaining 17 taxa represented less than 5% of the total colonies.

Generally there were a greater number of colonies isolated from Ulmus leaves (Table 3) but there was no significant difference between mean concentrations on *Quercus* and *Ulmus* (t = -1.39, p > 0.05). Also, there were a greater number of colonies isolated from the abaxial surface but no significant difference between the means of the two surfaces (t = -1.42, p > 0.05).

6000

4000

2000

0

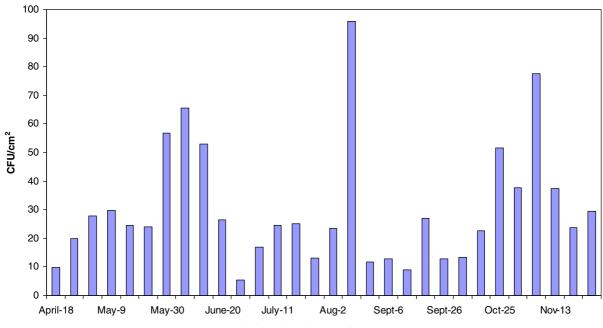


Figure 3. Average weekly concentration of all leaf surface fungi isolated from Ulmus americana and Quercus palustris leaves.

Table 2.	Summary	statistics	of leaf	surface	fungi	isolated	from	Ulmus	americana	and	Quercus	palustris	
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	Mean seasonal concentration CFU/cm ²	Peak leaf concentration CFU/m ²	Percent of tota
Filamentous fungi			
Phoma	3.731	68.5	12.30
Cladosporium	3.269	71.0	10.77
Non-sporulating	1.682	72.3	5.54
Alternaria	1.424	7.5	4.69
Epicoccum	0.309	3.5	1.01
Drechslera	0.225	3.0	0.74
Penicillium	0.192	5.5	0.63
Nigrospora	0.131	1.5	0.43
Curvularia	0.126	2.5	0.41
Pithomyces	0.115	4.0	0.38
Acremonium	0.092	9.0	0.30
Hyalodendron	0.033	1.5	0.11
Arthrinium	0.032	0.5	0.11
Trichoderma	0.021	2.5	0.07
Aspergillus	0.019	4.0	0.06
Fusarium	0.011	1.5	0.03
Sporothrix	0.007	2.5	0.02
Seimatosporium	0.006	2.0	0.02
Rhizopus	0.003	0.5	< 0.01
Choanephora	0.001	0.5	< 0.01
Geotrichum	0.001	0.5	< 0.01
<i>easts</i>			
Black yeasts/Aureobasidium	9.950	500	32.80
All other yeasts	8.963	160	29.54

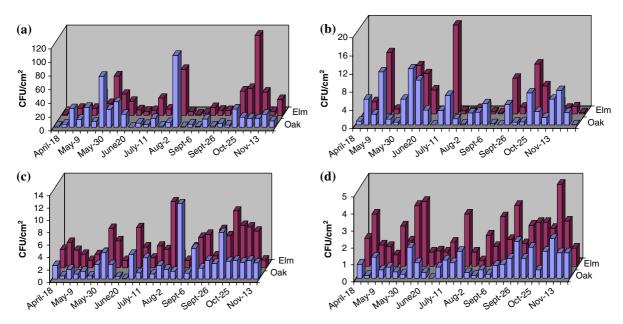


Figure 4. Average weekly concentrations of (a) yeast, (b) Phoma, (c) Cladosporium, and (d) Alternaria isolated from Ulmus Americana (Elm) and Quercus palustris (Oak) leaves.

Table 3. Mean concentration of total leaf surface fungi fromUlmus americana and Quercus palustris

Plant species	Adaxial leaf surface CFU/cm ²	Abaxial leaf surface CFU/cm ²
Ulmus americana	32.7	34.6
Quercus palustris	22.3	31.7

Temperature was the most important meteorological factor for airborne spore levels (Table 4). Airborne concentrations of total spores and major airborne taxa were significantly related to the average daily temperature (p < 0.001). Rainfall was the most important fac-

Table 4. Correlation of airborne fungal spore concentrations with meteorological variables

Taxa	Pearson correlation coefficients (r)					
	Average daily temperature		Average daily windspeed			
Total spores	0.781***	0.039	-0.117*			
Cladosporium	0.745***	-0.003	-0.102			
Alternaria	0.726***	-0.002	-0.068			
All ascospores	0.676***	0.146**	-0.122*			
All basidiospores	0.789***	0.026	-0.119*			

p < 0.05, p < 0.01, p < 0.01, p < 0.001

tor for leaf surface fungal concentrations; several taxa showed significant correlations with weekly rainfall totals for the 7 days prior to leaf collection (Figure 5, Table 5).

Ten fungal taxa were identified on both leaf surface cultures and the air samples; these included Cladosporium, Alternaria, Epicoccum, Curvularia, Pithomyces, Drechslera, Fusarium, Nigrospora, Penicillium, and Aspergillus. Since Penicillium and Aspergillus spores are counted as a single category on the slides from the air samples, the leaf surface concentrations of these two genera were added together for analysis. When these taxa were examined individually there was no significant correlation between leaf surface concentrations and airborne concentrations during the growing season. However, when the seasonal mean concentrations for the nine taxa were compared using a Spearman correlation, there was a significant correlation between leaf surface fungi and airborne fungi (r = 0.74, p < 0.05), showing a similar ranking of these taxa in the atmosphere and on leaf surfaces. In addition, leaf surface concentration of Phoma showed a significant correlation (Spearman r = 0.41, p < 0.05) with airborne ascospore levels (Figure 6).

Estimates of the leaf surface area of each tree suggest that the total fungal load was

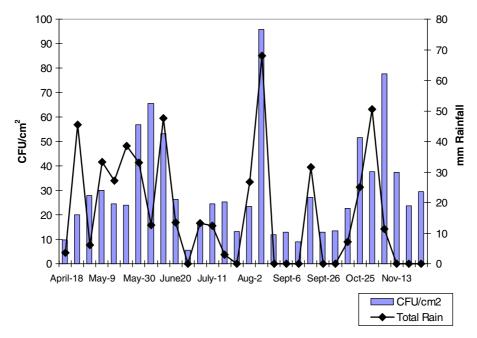


Figure 5. Comparison of total weekly rainfall with average concentration of leaf surface fungi isolated from Ulmus americana and Quercus palustris

 5.04×10^8 CFU for *Ulmus* and 2.71×10^8 CFU for *Quercus* (Table 6). These numbers are based on the averages of all leaf surface fungi isolated; however, only 10 of the leaf surface taxa were registered in the air samples. Table 7 presents the average number of CFU for these taxa.

4. Discussion

Airborne spore concentrations recorded during this study were consistent with previous data from Tulsa in addition to data from other areas (Gregory, 1973; Sterling et al., 1999; Trout and Levetin, 2001; Newhouse and Levetin, 2004).

Table 5. Correlation of major leaf surface fungi with total weekly rainfall

Taxa	Spearman correlation coefficients (r)
Total colonies	0.557**
Yeast	0.554**
Phoma	0.481*
Cladosporium	0.131
Alternaria	-0.232

p < 0.01, p < 0.001.

Leaf surface fungi identified also parallel those found in other studies (Breeze and Dix, 1981; Mishra and Dickinson, 1981; McCormack et al., 1994; Andrews et al., 2002; Inácio et al., 2002) as well as unpublished data from this lab. The leaves used for analysis were collected from the lower portion of the tree canopy. It is possible that higher in the canopy difference may exist and future studies will address canopy position.

It was notable that 15 taxa were isolated from leaf surfaces on the first day of sampling when the leaves had not fully expanded. The presence of these fungi supports the idea that the air spora constitutes the source of many fungi that can potentially colonize the leaf surface (Pedgley, 1991; Kinkel, 1997; Aylor 2002). Some researchers investigating leaf surface fungi wash the leaves in sterile distilled water prior to culturing (Inácio et al., 2002; Osono, 2002). This step prevents harvesting spores impacted on the leaf blade and limits the recovery to fungi growing on the phyllosphere. However, deposited spores can germinate and colonize the leaf surface or become re-entrained into the atmosphere. The method of fungal isolation used in this study was specifically selected to include impacted spores as well as fungi that colonized the leaf surfaces.

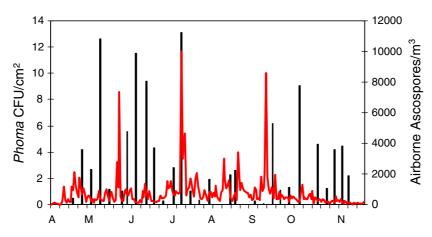


Figure 6. Comparison of average daily airborne ascospore concentration (solid line) with weekly concentrations of *Phoma* isolated from *Ulmus americana* and *Quercus palustris* Leaves (bars).

Table 6. Estimates of total leaf surface fungi on Ulmus americana and Quercus palustris

	<i>Ulmus</i> adaxial	<i>Ulmus</i> abaxial	<i>Quercus</i> adaxial	<i>Quercus</i> abaxial
Mean concentration of all leaf surface fungi (CFU/cm ²)	32.7	34.6	22.3	31.7
Estimated leaf size (cm ²)	23	23	50	50
Estimate number of leaves on tree	325,000	325,000	100,000	100,000
Leaf surface area estimate for tree (cm ²)	7.5×10 ⁶	7.5×10 ⁶	5.0×10 ⁶	5.0×10 ⁶
Estimated CFU Total per tree (CFU)	2.45×10^{8} 5.04×10^{8}	2.59×10 ⁸	1.12×10^{8} 2.71×10^{8}	1.59×10 ⁸

Table 7. Estimates of the average number of colony forming units per tree for taxa detected in both leaf surface isolates and air samples

Taxa	Mean concentration CFU/cm ²	Estimate of average CFU on <i>Ulmus</i>	Estimate of average CFU on <i>Quercus</i>
Cladosporium	3.269	3.27×10^7	4.90×10^{7}
Alternaria	1.424	1.42×10^{7}	2.14×10^{7}
Epicoccum	0.309	3.09×10^6	4.64×10^{6}
Drechslera	0.225	2.25×10^{6}	3.38×10^{6}
Penicillium	0.192	1.92×10^{6}	2.88×10^6
Nigrospora	0.131	1.31×10^{6}	1.97×10^{6}
Curvularia	0.126	1.26×10^{6}	1.89×10^{6}
Pithomyces	0.115	1.15×10^{6}	1.73×10^{6}
Aspergillus	0.019	1.90×10^{5}	2.85×10^{5}
Fusarium	0.011	1.10×10^{5}	1.65×10^{5}

Yeasts and *Phoma* were the most abundant taxa isolated from the surface of *Ulmus americana* and *Quercus palustris* leaves. *Yeast* is a general term to describe unicellular fungi that reproduce by budding. Several hundred species of yeast occur in three fungal divisions (Kendrick, 2000). During this study no consistent effort was made to identify yeasts to genus level; however, *Aureobasidium* was a major component of the *black yeasts* category. In the *other yeasts* category, multiple species were present based on pigmentation and cell shape. This included *Rhodotorula* and *Sporobolomyces*. The abundance of yeast isolated from the leaf surfaces is consistent with other studies (Breeze and Dix, 1981; Mishra and Dickinson,

1981; McCormack et al., 1994; Andrews et al., 2002; Inácio et al., 2002). Yeasts are generally dispersed by rain splash; however, Taylor et al. (2004) recently reported that *Aureobasidium pullulans* was the most abundant taxon identified on Burkard spore trap samples from a southern California location. In addition they found that this species forms an aerosol in controlled emission experiments.

Phoma has also been frequently recorded from leaf surfaces (Mishra and Dickinson, 1981; Osono, 2002; Osono et al., 2004). *Phoma* is a large genus of anamorphic fungi in the form class Coelomycetes that is characterized by conidia formation in a pycnidium. Conidia ooze out of the pycnidium and are dispersed by rain splash. There are approximately 223 species in the genus which includes both saprobes and plant pathogens. Many common ascomycetes have a Phoma anamorph including species of Leptosphaeria, Didymella, Mycosphaerella, Pleospora, Phaeosphaeria, and Diaporthe (Hanlin, 1990). The ascospores from these genera are common in the air samples. It is possible that some of the leaf surface colonies of *Phoma* develop sexual stages and produced some of the airborne ascospores. It is also possible that some of the Phoma isolates in the leaf surface cultures developed from Leptosphaeria or other ascospores produced on the leaf or even impacted on the leaf. Both of these possibilities would explain the significant correlation between concentrations of leaf surface Phoma isolates and airborne ascospores.

Estimates of the leaf surface area of each tree suggest that the total fungal load was 5.04×10^8 CFU for *Ulmus* and 2.71×10^8 CFU for *Quercus*. Although yeasts and *Phoma* are not readily airborne, 10 taxa of leaf-surface fungi were identified in the air samples. The 10 taxa represent 19% of the leaf surface fungi and the average concentration of these fungi on the leaves represent a potential for dispersal under suitable environmental conditions.

In summary, this study showed that fungi isolated from *Ulmus* and *Quercus* leaves include taxa with airborne dispersal and those with rain splash dispersal. Those taxa with an airborne dispersal can be major contributors to the air spora based on their estimated concentration on the two trees. Questions remain about the contribution of *Phoma* and yeast to the air spora which are assumed to be splashed dispersed. More work should be done to determine their potential significance.

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