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Quantification of Airborne Fungal Spores Comparing Molecular Tracers via Ion Chromatography and UV-LIF Methods

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Quantification of Airborne Fungal Spores Comparing Molecular Tracers via Ion Chromatography and UV-LIF Methods

A Thesis

Presented to

the Faculty of Natural Sciences and Mathematics

University of Denver

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Marie Ila Gosselin

August 2017

Advisor: John Alexander Huffman, PhD
ABSTRACT

Fungal spores represent an understudied subcategory of bioaerosols that can impact human health as pathogenic and allergenic particles. Fungal spores also have been shown to act as effective ice nuclei and giant cloud condensation nuclei in some cases. This has implications on the hydrological cycle on local and regional scales by impacting the formation and evolution of clouds and precipitation. The quantification of fungal spores has been limited in the past due to methods that were costly and that suffered from poor time resolution. The most commonly applied methods for airborne fungal spore analysis have traditionally included microscopy and culturing, which can undercount the atmospheric fungal concentration by an order of magnitude. New techniques utilizing molecular tracers have allowed for the estimated contribution of fungal spores to atmospheric aerosols via ion chromatography. Additionally, the development of ultraviolet- laser/light induced fluorescence (UV-LIF) instruments for bioaerosol detection has added the element of real-time, size-resolved analysis to the methods for fungal spore quantification. Here, UV-LIF and ion chromatographic techniques are explored for the estimation of atmospheric fungal spore concentrations. Results from the BEACHON-RoMBAS campaign show that the two techniques provided atmospheric fungal spore concentrations with 13% on one another. Additionally, fungal tracers
increased 3 fold during rainy periods in comparison to dry periods consistent with the increase of atmospheric fungal spores during times of increased relative humidity. Application of a thresholding scheme suggested by Savage et al. (2017) eliminated lowly fluorescent particles and reduced $R^2$ values between non correlated variables during the INIUT-BACCHUS- ACTRIS campaign.
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1.1 Primary Biological Aerosol Particles

The atmosphere is a complex mixture of gases, primarily diatomic nitrogen and diatomic oxygen, with suspended liquids and particles. An incredibly small fraction for the atmosphere is made up of particles, or aerosols. Aerosols are defined as either liquid droplets, solid particles or a heterogeneous mixture of liquid- and solid-phase particles. Primary aerosols are particles that are emitted directly into the atmosphere from sources like volcanic eruptions, biomass burning, suspension of dust, and biological organisms, along with many others. Secondary aerosol particles are formed under various transformations in the atmosphere by which gas molecules become particles. These processing methods include photo degradation, physical and chemical reaction, and heterogeneous and homogenous nucleation. Concentrations of aerosols vary locally, regionally, and globally with number concentrations ranging from $10^2$ –$10^5$ cm$^{-3}$ and mass concentrations from 1–100 µg m$^{-3}$ (Pöschl, 2005). Primary biological aerosol particles (PBAPs), or bioaerosols, are a subset of atmospheric particles that are biological in nature which consist of bacteria, fungal spores, viruses, pollen, and plant debris (Després et al., 2012; Fröhlich-Nowoisky et al., 2016). Bioaerosols range in size from a few hundred nanometers (proteins and viruses) to tens of micrometers (pollen). Bioaerosols have gained increasing attention of the scientific community in recent years,
partially because they remain understudied and under-characterized and because techniques to detect important classes of PBAP have improved markedly in the last two decades.

1.2 Size, Abundance, and Atmospheric Lifetime

Bioaerosols can span a size range of nanometers to micrometers, however, each particle type has unique size distributions and accompanying physical properties. This work primarily focuses on biological particles greater than one micrometer (1 µm) in size. Figure 1.1 illustrates the large size range of both PBAPs and other non-biological aerosols (Fröhlich-Nowoisky et al., 2016). Bacteria, fungal spores, and pollen are all typically larger than one micron in size. These PBAPs are part of what is often called the accumulation (0.01 µm – 2.5 µm) and coarse (2.5 µm – 10 µm) modes (Seinfeld and Pandis, 2016) and have atmospheric lifetimes on the order of hours to weeks, with the lifetimes directly impacted by size and deposition scheme. Bacteria typically have a longer lifetime due to their relatively small size, however, pollen, fungal spores, and bacteria are all impacted by wet and dry deposition methods (Burrows et al., 2009; Heald and Spracklen, 2009; Sofiev et al., 2006). The short lifetime reduces the long-term mixing of PBAP within the troposphere, and because PBAPs are always emitted from the ground, they are typically found in higher concentrations nearer to the ground (Crawford et al., 2016; Twohy et al., 2016). The majority of PBAPs are found within the troposphere and their short lifetime is directly linked to how readily they act as ice nuclei (IN) and cloud condensation nuclei (CCN) in wet deposition methods (Christner et al., 2008; Hoose et al., 2010; Pratt et al., 2009; Prenni et al., 2009). Typically, bacteria are on
the order of one micron in size, while they are often found agglomerated in multiples or attached to dust or plant detritus. This makes the overall size of particles that include bacteria to peak in the 2 – 4 microns range (Després et al., 2012; Fröhlich-Nowoisky et al., 2016). The small size of bacteria allows for longer transport times and slower settling (Prospero et al., 2005) when bacteria is not exposed to high humidity that results in wet deposition (Kellogg and Griffin, 2006). Bacteria have been found transported in desert sand storms on an inter-hemisphere scale (Kellogg and Griffin, 2006; Prospero et al., 2005) and around the Mediterranean (Gat 2017). Fungal spores are larger than bacteria and are often assumed to peak at approximately 2 – 4 microns with the same ability as bacteria to agglomerate and form clusters greater than 10 microns in size (Reponen et al., 1996) especially during elevated relative humidity. Fungal spores are readily act as giant cloud condensation nuclei (GCCN) due to their size as larger particles are more able to condense water onto themselves (Hassett et al., 2015; Huffman et al., 2013).

Figure 1. Examples and scale of primary biological aerosol particles. Figure taken from (Fröhlich-Nowoisky et al., 2016). Examples include (a) proteins, (b) viruses, (c) bacteria, (d) fungal spores, and (e) pollen.
1.3 Environmental and Health Implications

The fifth assessment report (AR5) from the Intergovernmental Panel on Climate Change (IPCC) found that aerosols of both anthropogenic and natural origins are understudied and poorly characterized and quantified (Flato et al., 2013). Aerosols have direct and indirect negative radiative forcing properties that have impacts on global temperature and regional hydrological cycles. One limitation of current models is the lack of wide spread data points and the high level of uncertainty when local data points are applied globally. The biological subset of aerosols can negatively impact humans, animals, and agriculture through spread of pathogens and in humans, heightened immunological response. PBAPs have been linked with both disease and allergenic responses in humans primarily due to surface proteins that lie on the exterior of the aerosol.

Fungal spores are increasingly discussed as exhibiting global importance, in part because they can exist in atmospheric concentrations of $10^2$-$10^4$ particles m$^{-3}$ (Frankland and Gregory, 1973; Gregory and Sreeramulu, 1958; Heald and Spracklen, 2009; Hummel et al., 2015; Sesartic and Dallafior, 2011) and in pristine environments can be as much as 85% of supermicron biological material mass (Pöschl et al., 2010). The number of studies detecting fungal spores is growing as techniques for their investigation and interrogation become more available and more accurate. As the potential impacts of PBAPs are explored, the methods by which they are studied have rapidly developed.
1.3 Fungal Spores

1.3.1 Fungal Spore Release

As previously mentioned, fungal spores represent a subcategory of PBAPs that are typically 2-4 microns in size. There exist four phyla of fungi: Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota, with the vast majority of fungal species in the atmosphere belonging to Ascomycota or Basidiomycota. Fungal spores are released by one of two sets of methods: dry or wet discharge. Dry discharge is typically accomplished by mechanical means such as wind or physical disruption of the fungi. Wet discharge is more complex requiring specific environmental conditions for release. The two methods predominately used in the wet discharge of spores are Buller’s drop (Buller, 1909) and the osmotic cannon (Ingold, 1971). Amongst the atmospherically relevant phyla, Ascomycota and Basidiomycota, wet discharge is the predominate method for spore release, where Ascomycota is more associate with the increase of RH and Basidiomycota is associated with precipitation. Both utilize atmospheric increases in humidity to discharge fungal spores. Due to the association with increased humidity it has been found that there is a diurnal pattern in fungal spore trends, as relative humidity is often highest overnight (Zhu et al., 2016). In the Buller’s drop found in fungi from the Basidiomycota phyla, the fungi secretes hygroscopic compounds, such as sugar alcohols and ions, near the base of the fungal spore (Noblin et al., 2009) (Figure 2). At the point
where the hilum and the spore are connected, a droplet forms. Once the droplet is too large the surface tension results in the expulsion of the fungal spore.

![Diagram](image)

*Figure 1. Depiction of Buller’s drop adapted from Noblin et al., 2009 Figure 1. The growth of a water droplet occurs from the secretion of hygroscopic compounds, mainly sugar alcohols like mannitol and arabitol, which are contained within the film. This allows for the growth of the droplet and the eventual expulsion of the fungal spore from the sterigma.*

The phyla Ascomycota contains species that rely on osmotic cannons for the active release of spores. As seen in Figure 1.3, which is an adapted figure from Trail (2007), depicts the process by which ascospores are released. Upon the production of the spore, the ascus, or simply sack, begins to take form with the walls thickening and the turgor increasing. This increase of turgor pressure is again due to the secretion of sugar alcohols like mannitol and arabitol in addition to KCl. Water is drawn into the ascus by in the influx of the hygroscopic compounds until the pressure is such that the ascus collapses and the spores are ejected.
1.3.2. Fungal Spore Quantification

Fungal spores were and are studied by culture techniques which are inexpensive however they undercount spores by more than 90% (Gonçalves et al., 2010; Pyrri and Kapsanaki-Gotsi, 2007). The nature of culturing techniques limits the enumeration process; at most 17% of atmospheric fungal spores are culturable in any growth medium and different variations of growth medium impact the percentage of fungal spores. Due to the nature of culture methods, only viable spores are cultured and enumerated. Microscopy represents an enhanced technique compared to culturing. While microscopy is costly and time consuming, it is capable of enumerating both viable and non-viable spores. One common alternative method for detection of fungal spores in the atmosphere is the use of molecular tracers that are co-emitted with the spores. Tracers have been used as a method for quantification of bioaerosols since the 1980s beginning with $\text{(1}\rightarrow\text{3)}-\beta$-D-
(1→3)-β-D-glucan is a non-specific bioaerosol tracer. Since then, several other small organic molecules have been identified as tracers for fungal spores. Ergosterol, arabinol and mannitol have come to the forefront as tracers unique to fungal spores. In a study by Burshtein et al. (2011), ergosterol, mannitol and arabinol were compared. Various other studies (Bauer et al., 2008a; Caseiro et al., 2007; Gosselin et al., 2016; Yang et al., 2012; Yttri et al., 2011; Zhang et al., 2010; Zhang et al., 2015) have quantified arabinol and mannitol as markers for fungal spore release. In a publication from Bauer et al. (2008a), the atmospheric concentration of arabinol and mannitol were compared with microscopy results from fungal spore concentration. A conversion factor was determined for interconverting between mannitol and arabinol concentrations and fungal spores. This has been applied in several studies relying on some key assumptions about the fungal size distribution and weight (Bauer et al., 2008a; Gosselin et al., 2016; Yttri et al., 2011). Gosselin et al. (2016) was the first quantitative study to compare estimated fungal spore concentration from tracers with Ultraviolet-Light/Laser Induced Fluorescent (UV-LIF) single particle instruments. Two commercially available UV-LIF instruments, the wideband integrated bioaerosol sample (WIBS) and ultraviolet aerodynamic particle sizer (UV-APS), were used in this study for interrogating real time bioaerosols. Other UV-LIF instruments, both commercial like the Bioscout (Environics Mikkeli, Finland), and instruments developed for individual research groups have been deployed in the field for use in analyzing ambient data (Hill et al., 1999). These instruments are still undergoing extensive development and characterization as field deployment continues to reveal challenges. The WIBS presents a prime example of
recent characterization. (Savage et al., 2017) conducted the most extensive laboratory campaign know to the author. This study covered more than 70 different samples including fungal spores, bacteria, pollen, pure amino acids, and other non-biological interfering compounds. This study represents the first of its kind to attempt to characterize the intended targets and possible interferences for a real-time bioaerosol sensor.

1.4 Tracers

Molecular tracers can be defined as molecules that are uniquely linked to a subset of emission sources. For example, isoterpenes are linked to biogenic emissions from pine trees and thus are used to quantify the overall impact and emissions from pine trees. Tracers have been utilized across the atmospheric community for detection of primary biological aerosols and also for subsequent source apportionment (Simoneit and Mazurek, 1989) (Table 1.1). Prior to use of tracers for direct detection of primary aerosols, formaldehyde and terpenes were investigated as links to general biogenic emissions (Zafiriou et al., 1980; Zimmerman et al., 1978). Glucose and β-1,3-glucose have often been used as general tracers for bioaerosols as they are linked to plant debris, pollen, fungal spores, and bacteria (Andreae and Crutzen, 1997; Cheng et al., 2012; Jia and Fraser, 2011; Rathnayake et al., 2017; Simoneit et al., 2004; Stone et al., 2012; Zhu et al., 2016; Zhu et al., 2015). In addition to glucose, fructose is often studied in conjunction as an indicator of soil microorganisms. Atmospheric concentrations of glucose is typically an order of magnitude greater than fructose due to its presence in biogenic emissions from trees (Jia and Fraser, 2011).
Table 1. Review of carbohydrate molecules that can be analyzed by ion chromatography and that have been used as bioaerosol tracers. Each compound along with their sources and key publications that have used those compounds for the determination of source apportionment.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Structure</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabitol</td>
<td><img src="image1" alt="Arabitol Structure" /></td>
<td>Fungal spores</td>
<td>(Bauer et al., 2008a; Bauer et al., 2008b; Burshtein et al., 2011; Gosselin et al., 2016; Lewis and Smith, 1967; Rathnayake et al., 2016; Zhang et al., 2015)</td>
</tr>
<tr>
<td>Erythritol</td>
<td><img src="image2" alt="Erythritol Structure" /></td>
<td>Fungal spores</td>
<td>(Lewis and Smith, 1967)</td>
</tr>
<tr>
<td>Fructose</td>
<td><img src="image3" alt="Fructose Structure" /></td>
<td>Soil microorganism, pollen, trees</td>
<td>(Jia, 2010)</td>
</tr>
<tr>
<td>Glucose</td>
<td><img src="image4" alt="Glucose Structure" /></td>
<td>Plant debris, pollen, fungal spores, bacteria</td>
<td>(Andreae and Crutzen, 1997; Cheng et al., 2012; Jia and Fraser, 2011; Rathnayake et al., 2017; Simoneit et al., 2004; Stone et al., 2012; Zhu et al., 2016; Zhu et al., 2015)</td>
</tr>
<tr>
<td>Levoglucosan</td>
<td><img src="image5" alt="Levoglucosan Structure" /></td>
<td>Biomass burning</td>
<td>(Andreae and Crutzen, 1997; Simoneit et al., 1999)</td>
</tr>
</tbody>
</table>
Additionally, levoglucosan has been predominately utilized as a biomass burning indicator (Andreae and Crutzen, 1997; Simoneit et al., 1999). Large disaccharides like sucrose have been found to be in excellent correlation with pollen grains (Yttri et al., 2007). Erythritol and xylitol have been found to be tracers for fungal spores, however both have limitations as fungal tracers (Lewis and Smith, 1967). Erythritol is subject to atmospheric degradation with lifetimes ~12 days (Kessler et al., 2010). Xylitol is more ubiquitous and is present in soil biota, fruits and hardwood trees and is found in relatively low abundance (Barbaro et al., 2015; Zangrando et al., 2016). Endotoxins have been atmospherically linked with gram negative bacteria and reside within the membrane of these bacteria (Andreae and Crutzen, 1997; Cheng et al., 2012; Rathnayake et al., 2017).
Fungal spores have been enumerated using the tracers, arabitol and mannitol, both sugar alcohols (Bauer et al., 2008a; Bauer et al., 2008b; Gosselin et al., 2016; Rathnayake et al., 2016; Zhang et al., 2015). Other groups have utilized the membrane component, ergosterol, to track fungal spore presence which is not always in good correlation with arabitol and mannitol (Burshtein et al., 2011). Arabitol and mannitol have come to the forefront in fungal tracers as their quantification can be performed without chemical derivatization unlike ergosterol (Gosselin et al., 2016; Rathnayake et al., 2016; Rathnayake et al., 2017; Zhang et al., 2010; Zhang et al., 2015). This analysis is done by using ion chromatography (Table 1.1).

### 1.5 General aims of thesis work

Fungal spores represent an understudied atmospheric aerosol, and they represent 85% of the supermicron biological mass in some environments (Pöschl et al., 2010). With such a sizable contribution to the biological mass, fungal spores are uniquely important to human health and precipitation cycles, especially on the regional scale. Due to the limited number of studies and the lack of active monitoring, computational inputs are severely hindered, which results in incomplete climate and allergy predication models (Spracklen and Heald, 2014). Therefore, methods that are cost effective, quick, and reliable are necessary. Here I present work regarding the quantification of fungal spores using molecular tracers and ultraviolet-laser/light induced fluorescence instruments. The main aims of the thesis were:

- To apply anion chromatography to aerosol extracts for the quantification of various carbohydrates.
• To utilize fluorescent particle mass and number concentrations from the WIBS and UV-APS as a proxy for bioaerosols.

• To correlate the atmospheric concentration of selected carbohydrates to fluorescent particle mass and number concentrations as a function of metrological conditions.

• To compare, for the first time, fungal molecular tracers with fluorescent particle mass and number concentrations and estimate atmospheric concentration of fungal spores at a semi-arid site in Colorado and in Cyprus.
CHAPTER TWO: METHODS

2.1 Ion Chromatography

2.1.1 Origin and Theory

Chromatography was first described by Mikhail Tsvet in 1900 and is a technique to separate complex mixtures of chemical species using the principles of attraction. Chemical species are separated between two different phases, a mobile phase and a stationary phase. The mobile phase, typically a gas or liquid is the carrier for the mixture to move through a separation column or media. The stationary phase is affixed to an inert media, typically a solid or a liquid embedded on a solid. The separation takes place based on the differential affinity of a species to reside in either the mobile or stationary phase. If the species is more prone to reside in the mobile phase it exits the column or media more quickly than species that have a greater affinity for the stationary phase. Different combinations of phases and exploitation of chemical properties allow for the separation of different mixture types. One such type of chromatography is anion exchange chromatography (AEC), modernized by Hamish Small (1975). AEC works on the principles of positive and negative charge. The stationary phase in anion exchange columns is a resin that carries a fixed charge (Weiss and Weiss, 2004). For anion-exchange columns, quaternary ammonium bases are typically used, while sulfonate groups are used in cation exchange columns. Equation 2.1 represents the reversible
equilibrium process that occurs between the analyte and the stationary phase in an anion-exchange column.

\[
\text{Resin-}X^+ M^- + A^- \leftrightarrow \text{Resin-}X^+ A^- + M^-
\]

Reaction 2.1 This equilibrium equation represents an anion exchange column where the resin contains the positively charge functional group \((X^+)\) and is balanced with the counter ion from the eluent solution \((M^-)\). When the analyte \((A^-)\) enters the column, it exchanges with the counter ion \((M^-)\) based on the analyte’s affinity towards the resin.

With a range of functionalized resins for column stationary phases and various detection techniques including conductivity, amperometric, or UV/vis detection, ion chromatography can be applied to the analysis of inorganic/organic acids, carbohydrates, amines, inorganic ions, and many more.

2.1.2 Applications

2.1.2.1 Carbohydrates

Ion chromatography, a subtype of liquid chromatography, is well-suited to the quantification of carbohydrates, because it does not require derivatization prior to sample analysis. This is the most time-consuming step in using gas chromatography and can also limit method recoveries and thus detection sensitivity (Weiss and Weiss, 2004).

Carbohydrate analysis, by liquid chromatography, is limited due to the fact that liquid chromatography systems typically operate with water and organic solvents and separation is achieved based on polarity. Ion chromatography is capable of separating and quantifying water-soluble carbohydrates. Anion exchange columns are used in carbohydrate analysis, for example, the alkyl quaternary ammonium functionalized Dionex MA1 column (Thermo Fisher Scientific, Waltham, MA) that is used throughout this thesis work. The counter ion is \(\text{OH}^-\) coming from \(\text{NaOH}\) as the eluent (mobile phase).
Low molar concentrations of NaOH, between 0.001 M and 0.1 M, create an alkaline environment that converts carbohydrates to their anion form. Similar conditions are used in the analysis of amino acids. This is possible because carbohydrate pKa's typically range between 12 and 14. An example chromatograph, Figure 2.1, illustrates the separation of 9 different carbohydrates using HPAC-PAD.

![Figure 2.1 Example chromatogram of carbohydrate separation for erythritol, xylitol, levoglucosan, arabinol, trehalose, mannitol, arabinose, glucose/xylose and fructose. This chromatograph is of a 5 parts per million (ppm) calibration standard. This represents the highest concentration calibration point.](image)

The detection of carbohydrates and amino acids requires the use of a pulsed amperometric detector. Amperometric detection utilizes the following reaction (Equation 2.2).

\[
A \leftrightarrow B + n e^- 
\]

*Reaction 2.2 The reaction represents the oxidation of an analyte, A, to B when it comes into contact with the working electrode where n electrons (e^-) are transferred to the working electrode.*

When the analyte comes in contact with the working electrode, the analyte (A) transfers electrons to the working electrode and is oxidized to B. This results in a change
in electrical current that is directly proportional to the analyte concentration. Pulsed amperometric detection, compared to using detection based on a constant potential, is required in cases where the analyte is likely to precipitate on the electrode surface. Carbohydrates and amino acids are prone to precipitation upon the working electrode, requiring the use of pulsed amperometric detection. Compared to constant potential electrodes, those that have pulsed potentials measure current on intervals or in a batched system. The intervals vary the potential applied to the electrode between positive and negative values to remove oxidizable or reducible components thus cleaning the electrode.

2.1.2.2 Cations and Anions

The separation of cations and anions, and their subsequent detection, is based on the same principles as carbohydrate analysis. Cations and anions are passed through columns with functionalized resins. In the analysis of alkali metals, ammonium and alkaline-earth metals, strong organic acids are used as eluents with polymer resin stationary phases functionalized with phosphonate and carboxyl groups. This is present in Dionex CS12A (Thermo Fisher Scientific, Waltham, MA) making it capable of separating calcium and magnesium, which is only possible using columns with phosphonate functionalization. Inorganic anions can be separated, like carbohydrates, with anion exchange chromatography using alkanol quaternary ammonium functionalized polymers. Eluent choice is dependent upon analyte and detection method. Detection of cations and anions is achieved by suppressed conductivity detection.
Conductivity detection, similar to amperometric detection, measures the resistance across a fixed distance within a conductivity cell. Due the highly conductive nature of the eluents used in cation and anion analysis, chemical suppression is required in order to measure the weaker ions. An example of suppression can be seen in equation 2.3. This can be accomplished with a resin packed column where ion exchange takes place or within a stacked membrane that uses an external regeneration compound to replenish the displaced H\(^+\) or in the case of cation analysis OH\(^-\).

\[
\begin{align*}
(1) \text{Resin-X}^- \text{H}^+ + \text{NaHCO}_3 & \leftrightarrow \text{Resin-XNa} + \text{CO}_2 + \text{H}_2\text{O} \\
(2) \text{Resin-X}^- \text{H}^+ + \text{NaCl} & \leftrightarrow \text{Resin-XNa} + \text{HCl}
\end{align*}
\]

Reactions 2.3 (1) Conversion of strongly conducting sodium bicarbonate, a commonly used eluent in anion analysis, to H\(_2\)O and CO\(_2\) from the weakly dissociated carbonic acid form. (2) Conversion of the conducting salt, NaCl, to HCl the more strongly conducting mineral acid.

Upon the removal of the strongly conductive eluent species, the analytes are the most conductive species in the effluent as seen in the case of equation 2.3 (2) where the mineral acid, HCl, is more strongly conducting than the dissociated carbonic acid.

2.1.4 Relationship to Aerosols

Ion chromatography is a powerful tool being utilized in the aerosol community. The versatility of ion chromatography, in conjunction with simple sample preparation, makes it ideal as an analysis type complimentary to online methods. As mentioned above, ion chromatography can be used to quantify carbohydrates, cations, anions, and several other atmospherically relevant species. Most notably, ion chromatography has facilitated an increase in the study of water soluble carbohydrates (Barbaro et al., 2015; Caseiro et al., 2007; Gosselin et al., 2016; Rathnayake et al., 2016; Rathnayake et al., 2017; Yang et
Additionally, ion chromatography has been used for simultaneously analysis of cation and anion is atmospheric samples (Chow and Watson, 1999; Dabek-Zlotorzynska and Dlouhy, 1993; Nesterenko, 2001) along with real-time sampling of water soluble particles using particle-into-liquid samplers (PILS) for highly time resolution measurements (Lee et al., 2003; Orsini et al., 2003). This information can be utilized in source apportionment studies (Prendes et al., 1999; Song et al., 2006; Stone et al., 2016; Watson et al., 2001) and to estimate bioaerosol concentrations and contributions (Gosselin et al., 2016; Rathnayake et al., 2016).

### 2.2 Ultraviolet – Laser/Light Induced Fluorescence

The analysis of bioaerosol particles has been limited to direct enumeration techniques such as microscopy and culturing of samples. These techniques are limited in their size and time-resolution. In recent years, instruments utilizing ultraviolet – laser/light induced fluorescence (UV-LIF) have been introduced for the detection of bioaerosols. The basis of using UV-LIF for bioaerosol detection and quantification arises from the presence of key biologically-relevant fluorophores namely, tyrosine and tryptophan as protein markers NADH, and riboflavin (Foot et al., 2008; Hairston et al., 1997; Kaye et al., 2005; Pöhlker et al., 2012). The most commonly applied commercially-available UV-LIF instruments are the ultraviolet aerodynamic particle sizer (UV-APS; TSI Inc. Model 3314, St. Paul, MN, USA) and the wideband integrated bioaerosol sensor (WIBS; University of Hertfordshire, Hertfordshire, UK, now licensed to Droplet Measurement Technologies, Boulder, CO, USA). The WIBS estimates particle diameter
using a 635 nm continuous laser and subsequent analysis of the elastic scattering by Mie theory (Foot et al., 2008; Kaye et al., 2005). Different models of the WIBS have slightly different parameters for the Xenon flash lamps and photomultiplier tubes (PMTs) that are used for excitation and fluorescence emission. The optically filtered Xenon flash lamps operate at 280 nm, for the excitation of tyrosine and tryptophan, and 370 nm, for the excitation of NADH and riboflavin. The emission from excitation at 280 nm is filtered to two PMTs, one detecting between 320 – 400 nm and the second at 410 – 650 nm. The emission from the excitation at 370 nm is measured by the second PMT between 410 – 650 nm.

The UV-APS determines particles aerodynamic diameter using time of flight between two laser beams. When the two laser system is triggered in the presence of a particle, a 355 nm laser is fired and emission is measured by a PMT between 420 and 575 nm for the excitation and emission of NADH and riboflavin (Hairston et al., 1997). Analysis techniques and schemes for utilizing UV-LIF instruments in conjunction with ion chromatography will be discussed in chapters 3 and 4.
CHAPTER THREE: FLUORESCENT BIOAEROSOL PARTICLE, MOLECULAR TRACER, AND FUNGAL SPORE CONCENTRATIONS DURING DRY AND RAINY PERIODS IN A SEMI-ARID FOREST¹

3.1 Abstract

Bioaerosols pose risks to human health and agriculture and may influence the evolution of mixed-phase clouds and the hydrological cycle on local and regional scales. The availability and reliability of methods and data on the abundance and properties of atmospheric bioaerosols, however, are rather limited. Here we analyze and compare data from different real-time ultraviolet laser/light-induced fluorescence (UV-LIF) instruments with results from a culture-based spore sampler and offline molecular tracers for airborne fungal spores in a semi-arid forest in the southern Rocky Mountains of Colorado. Commercial UV-APS (ultraviolet aerodynamic particle sizer) and WIBS-3 (wideband integrated bioaerosol sensor, version 3) instruments with different excitation and emission wavelengths were utilized to measure fluorescent aerosol particles (FAPs) during both dry weather conditions and periods heavily influenced by rain. Seven molecular tracers of bioaerosols were quantified by analysis of total suspended particle (TSP) high-volume filter samples using a high-performance anion-exchange

chromatography system with pulsed amperometric detection (HPAEC-PAD). From the same measurement campaign, Huffman et al. (2013) previously reported dramatic increases in total and fluorescent particle concentrations during and immediately after rainfall and also showed a strong relationship between the concentrations of FAPs and ice nuclei (Huffman et al., 2013; Prenni et al., 2013). Here we investigate molecular tracers and show that during rainy periods the atmospheric concentrations of arabitol (35.2 ± 10.5 ng m⁻³) and mannitol (44.9 ± 13.8 ng m⁻³) were 3–4 times higher than during dry periods. During and after rain, the correlations between FAP and tracer mass concentrations were also significantly improved. Fungal spore number concentrations on the order of 10⁴ m⁻³, accounting for 2–5 % of TSP mass during dry periods and 17–23 % during rainy periods, were obtained from scaling the tracer measurements and from multiple analysis methods applied to the UV-LIF data. Endotoxin concentrations were also enhanced during rainy periods, but showed no correlation with FAP concentrations. Average mass concentrations of erythritol, levoglucosan, glucose, and (1 → 3)-β-D-glucan in TSP samples are reported separately for dry and rainy weather conditions. Overall, the results indicate that UV-LIF measurements can be used to infer fungal spore concentrations, but substantial development of instrumental and data analysis methods appears to be required for improved quantification.

3.2 Introduction

Primary biological aerosols particles (PBAPs) are of keen interest within the scientific community, partially because methods for their quantification and characterization are advancing rapidly (Huffman and Santarpia, 2017; Sodeau and
O’Connor, 2016). The term PBAPs, or equivalently bioaerosols, generally comprises several classes of airborne biological particles including viruses, bacteria, fungal spores, pollen, and their fragments (Després et al., 2012; Fröhlich-Nowoisky et al., 2016). Fungal spores are of particular atmospheric interest because they can cause a variety of deleterious health effects in humans, animals, and agriculture, and it has been shown that they can represent a significant fraction of total organic aerosol emissions (Deguillaume et al., 2008; Gilardoni et al., 2011; Madelin, 1994), especially in tropical regions (Elbert et al., 2007; Huffman et al., 2012; Pöschl et al., 2010; Zhang et al., 2010). Current estimates of the atmospheric concentration of fungal spores range from 100 to more than $10^4$ m$^{-3}$ (Frankland and Gregory, 1973; Gregory and Sreeramulu, 1958; Heald and Spracklen, 2009; Hummel et al., 2015; Sesartic and Dallafior, 2011). Fungal spores may also impact the hydrological cycle as giant cloud condensation nuclei or as ice nuclei (Haga et al., 2013; Morris et al., 2013; Sesartic et al., 2013). Additionally, several classes of bioaerosols and their constituent components, such as (1→3)-β-D-glucan and endotoxins, have been implicated in respiratory distress and allergies (Burger, 1990; Douwes et al., 2003; Laumbach and Kipen, 2005; Linneberg, 2011; Pöschl and Shiraiwa, 2015). For example, asthma and allergies have shown notable increases during thunderstorms due to elevated bioaerosol concentrations (Taylor and Jonsson, 2004) especially when attributed to fungal spores (Allitt, 2000; Dales et al., 2003).

Molecular tracers have long been utilized as a means of aerosol source tracking (Schauer et al., 1996; Simoneit and Mazurek, 1989; Simoneit et al., 2004). In recent years, analysis of molecular tracers has been utilized for the quantification of PBAPs in
atmospheric samples and has been compared, for example, with results from microscopy (Bauer et al., 2008a) and culture samples (Chow et al., 2015b; Womiloju et al., 2003).

Three organic molecules have been predominately utilized as unique tracers of fungal spores: ergosterol, mannitol, and arabitol. The majority of atmospherically relevant fungal spores are released by active wet-discharge processes common in Ascomycota and Basidiomycota, meaning that the fungal organism actively ejects spores at a time most advantageous for the spore dispersal and germination processes, often when relative humidity (RH) is high (Ingold, 1971). While there are several mechanisms of active spore emission (e.g., Buller’s drop (Buller, 1909) and osmotic pressure canons (Ingold, 1971)), they each involve the secretion of fluid containing hygroscopic compounds, such as arabitol, mannitol, potassium and chloride ions, as well as other solutes (Elbert et al., 2007), released near the site of spore growth. When the spores are ejected, some of the fluid adheres to the spores and becomes aerosolized. Several of these secreted compounds are thought to enter the atmosphere linked uniquely with spore emission processes, and so these tracers have been used to estimate atmospheric concentrations of fungal spores. Arabitol and mannitol are both sugar alcohols (polyols) that serve as energy stores for the spore (Feofilova, 2001). Arabitol is unique to fungal spores and lichen, while mannitol is present in fungal spores, lichen, algae, and higher plants (Lewis and Smith, 1967). Ergosterol is found within the cell membranes of fungal spores (Weete, 1973) and has been used as an ambient fungal spore tracer (Di Filippo et al., 2013; Miller and Young, 1997). Comparing the seasonal trends of arabitol and mannitol with ergosterol, Burshtein et al. (2011) showed positive correlations between arabitol or
mannitol and ergosterol only in the spring and autumn, suggesting that the source of these polyols is unlikely to be solely fungal in origin or that the amount of each compound emitted varies considerably between species type and season. While ergosterol has been directly linked to fungal spores in the air, ergosterol is prone to photochemical degradation and is difficult to analyze and quantify directly. Quantification of ergosterol typically requires chemical derivatization by silylation before analysis via gas chromatography (Axelsson et al., 1995; Burshtein et al., 2011; Lau et al., 2006). In contrast, analysis of sugar alcohols by ion chromatography involves fewer steps and has been successfully applied to monitor seasonal variations of atmospheric aerosol concentration at a number of sites (Bauer et al., 2008a; Caseiro et al., 2007; Yang et al., 2012; Yttri et al., 2011a; Zhang et al., 2010, 2015) including pg m⁻³ (picograms per cubic meter) levels in the Antarctic (Barbaro et al., 2015). By measuring spore count and tracer concentration in parallel at one urban and two suburban sites in Vienna, Austria, Bauer et al. (2008a) estimated the amount of each tracer per fungal spore emitted. Potassium ions have also been linked to emission of biogenic aerosol (Pöhlker et al., 2012b) and are co-emitted with fungal spores; however, application of potassium as a fungal tracer is uncommon because it is predominantly associated with biomass burning (Andreae and Crutzen, 1997). Additionally, (1→3)-β-D-glucan (fungal spores and pollen) and endotoxins (gram- negative bacteria) have also been widely used to measure other bioaerosols (Andreae and Crutzen, 1997; Cheng et al., 2012; Rathnayake et al., 2016b; Stone and Clarke, 1992).
The direct detection of PBAPs has historically been limited to analysis techniques that require culturing or microscopy of the samples. These systems are time-consuming, costly, and often substantially under-count biological particles by an order of magnitude or more (Gonçalves et al., 2010; Pyrri and Kapsanaki-Gotsi, 2007). The sampling methods associated with these measurements also offer relatively low time resolution and low particle size resolution. Recently, techniques utilizing ultraviolet laser/light-induced fluorescence (UV-LIF) for the real-time detection of PBAPs have been developed and are being utilized by the atmospheric community for bioaerosol detection. Thus far, the most widely applied LIF instruments for ambient PBAP detection have been the ultraviolet aerodynamic particle sizer (UV-APS; TSI Inc. Model 3314, St. Paul, MN, USA) and the wideband integrated bioaerosol sensor (WIBS; University of Hertfordshire, Hertfordshire, UK, now licensed to Droplet Measurement Technologies, Boulder, CO, USA). Both of these commercially available instruments can provide information in real-time about particle size and fluorescence properties of supermicron atmospheric aerosols. Characterization and co-deployment of these instruments over the past 10 years has expanded the knowledge base regarding how to analyze and utilize the information provided from these instruments (Crawford et al., 2015; Healy et al., 2014; Hernandez et al., 2016; Huffman et al., 2013; Perring et al., 2015; Pöhlker et al., 2012a, 2013; Ruske et al., 2016), though the interpretation of UV-LIF results from individual particles is complicated by interfering material that is not biological in nature (Gabey et al., 2010; Huffman et al., 2012; Lee et al., 2010; Saari et al., 2013; Toprak and Schnaiter, 2013).
Here we present analysis of atmospheric concentrations of arabitol and mannitol in relation to results from real-time, ambient particle measurements reported by UV-APS and WIBS. We interrogate these relationships as they pertain to rain conditions (rainfall and RH) that have previously been shown to increase the concentrations of fluorescent aerosols and ice nuclei (Crawford et al., 2014; Huffman et al., 2013; Prenni et al., 2013; Schumacher et al., 2013; Yue et al., 2016). Active wet discharge of ascospores and basidiospores has frequently been reported to correspond with increased RH (Elbert et al., 2007), and fungal spore concentration has also been shown to increase after rain events (e.g., Jones and Harrison, 2004). Here we estimate airborne fungal concentrations in a semi-arid forest environment utilizing a combination of real-time fluorescence methods, molecular fungal tracer methods, and direct-to-agar sampling and culturing as parallel surrogates for spore analysis. This study of ambient aerosol represents the first quantitative comparison of real-time aerosol UV-LIF instruments with molecular tracers or culturing.

3.3 Methods

3.3.1 Sampling site

Atmospheric sampling was conducted as a part of the BEACHON-RoMBAS (Bio–hydro–atmosphere interactions of Energy, Aerosols, Carbon, H2O, Organics, and Nitrogen – Rocky Mountain Biogenic Aerosol Study) field campaign conducted at the Manitou Experimental Forest Observatory (MEFO) located 48 km northwest of Colorado Springs, Colorado (39° 06’ 0” N, 105° 5’ 03” W; 2370 m elevation) (Ortega et al., 2014). The site is located in the central Rocky Mountains and is representative of the semi-arid
montane pine-forested regions of North America. During BEACHON-RoMBAS, a large, international team of researchers conducted an intensive set of measurements from 20 July to 23 August 2011. A summary of results from the campaign are published in the BEACHON campaign special issue of Atmospheric Chemistry and Physics (http://www.atmos-chem-phys.net/special_issue247.html). All the data reported here were gathered from instruments and sensors located within a < 100 m radius (Fig. 3.1).

Figure 3.1 Aerial overview of BEACHON-RoMBAS field site at the Manitou Experimental Forest Observatory located northwest of Colorado Springs, CO. Locations of all instruments and sensors discussed here are marked and were located within a 50m radius. Figure adapted from Fig. 1a of Huffman et al. (2013).
3.3.2 Online fluorescent instruments

UV-APS and WIBS-3 (model 3; University of Hertfordshire) instruments were operated continuously as a part of the study, and particle data were integrated to 5 min averages before further analysis. The UV-APS was operated under procedures defined in previous studies (Huffman et al., 2013; Schumacher et al., 2013). A total suspended particle (TSP) inlet head ∼ 5.5 m above the ground, mounted above the roof of a climate-controlled, metal trailer, was used to sample aerosol directed towards the UV-APS. Bends and horizontal stretches in the 0.75 inch tubing were minimized to reduce losses of large particles (Huffman et al., 2013). The UV-APS detects particles between 0.5 and 20 µm and records aerodynamic particle diameter and integrated total fluorescence (420–575 nm) after pulsed excitation by a 355 nm laser (Hairston et al., 1997). Both UV-APS and WIBS instruments report information about particle number concentration, but it is instructive here to show results in particle mass for comparison between all techniques. Total particle number size distributions (irrespective of fluorescence properties) obtained from the UV-APS and WIBS were converted to mass distributions assuming spherical particles of unit particle mass density, unless otherwise stated, as a first approximation. Total particle concentration values (in µg m\(^{-3}\)) were obtained for each 5 min period by integrating over the size range 0.5–15 µm, and these mass concentration values were averaged over the length of the filter sampling periods. Uncertainty in mass concentration values reported here is influenced by assuming a single value for particle mass density and because of slight dissimilarities between size bins of the UV-APS and WIBS instruments at particle sizes above 10 µm that dominate particle mass.
A WIBS-3 was used to continuously sample air at a site ~ 50 m from the UV-APS trailer and 1.3 m above the ground. Briefly, the diameter of individual particles sampled by the WIBS is estimated by the intensity of the elastic side scatter from a continuous wave 635 nm diode laser and analyzed by a Mie scattering model (Foot et al., 2008; Kaye et al., 2005). Particles that pass through the diode laser activate two optically filtered Xenon flash lamps. The first lamp excites the particle at 280 nm and the second at 370 nm. Emission from the 280 nm excitation is filtered separately for two photomultiplier tubes (PMTs), one which detects in a band at 320–400 nm and the other in a band at 410–650 nm. These excitation and emission wavelengths result in a total of three channels of detection: \( \lambda_{\text{ex}} 280 \text{ nm}, \lambda_{\text{em}} 320–400 \text{ nm} \) (FL1 or channel A); \( \lambda_{\text{ex}} 280 \text{ nm}, \lambda_{\text{em}} 410–650 \text{ nm} \) (FL2 or channel B); and \( \lambda_{\text{ex}} 370 \text{ nm}, \lambda_{\text{em}} 410–650 \text{ nm} \) (FL3 or channel C) (Crawford et al., 2014). Individual particles are considered fluorescent here if they exceed fluorescent thresholds for any channel, as defined as the average of a “forced trigger” baseline plus 3 standard deviations (SD) of the baseline measurement (Gabey et al., 2010).

WIBS particle-type analysis is utilized to define types of particles that have specific spectral patterns. As defined by Perring et al. (2015), the three different fluorescent channels (FL1, FL2, and FL3) can be combined to produce seven unique fluorescent categories. Observed fluorescence in channel FL1 alone, but without any detectable fluorescence in channel FL2 or FL3, categorizes a particle as type A. Similarly, observed fluorescence in channels FL2 or FL3, but in no other channels, places a particle in the B or C categories, respectively. Combinations of fluorescence in these
channels, such as a particle that exhibits fluorescence in both FL1 and FL2, categorizes a particle as type AB and so on for a possible seven particle types as summarized in Fig. S1 in Appendix A.

As a separate tool for particle categorization, the University of Manchester has recently developed and applied a hierarchical agglomerative cluster analysis tool for WIBS data, which they have previously applied to the BEACHON-RoMBAS campaign (Crawford et al., 2014, 2015; Robinson et al., 2013). Here we utilize clusters derived from WIBS-3 data as described by Crawford et al. (2015). Cluster data presented here were analyzed with the open-source Python package FastCluster (Müllner, 2013). Briefly, hierarchical agglomerative cluster analysis was applied to the entire data set and each fluorescent particle was uniquely clustered into one of four groups. Cluster 1, assigned by Crawford et al. (2015) as fungal spores, displayed a 1.5–2 µm mode and a daily peak in the early morning that paralleled relative humidity (Schumacher et al., 2013). Clusters 2, 3, and 4 have strong, positive correlations with rainfall and exhibit size modes that peak at < 1.2 µm and were initially described by Crawford et al. (2014) as bacterial particles. Here we have summed clusters 2–4 to a single group referred to as ClBact, for simplicity when comparing with molecular tracers. It should be noted that assignment of name and origin (e.g., fungal spores or bacteria) to clusters is approximate and does not imply naming accuracy or particle homogeneity. Each cluster likely contains an unknown fraction of contaminating particles, but the clusters are beneficial to group particles more selectively than using fluorescent intensity alone. For more details see Robinson et al. (2013) and Crawford et al. (2015).
The WIBS-3 utilized here has since been superseded by the WIBS-4 (Univ. Hertfordshire, UK) and WIBS-4A (Droplet Measurement Technologies, Boulder, CO, USA). One important difference between the models is that the optical chamber design and filters of the WIBS-4 models were updated to enhance the overall sensitivity of the instrument (Crawford et al., 2014). Additionally, slight differences in detector gain between models and individual units can impact the relative sensitivity of the fluorescence channels. This may result in differences in fluorescent channel intensity between instrument models, as will be discussed later.

3.3.3 High-volume sampler

Total suspended particle samples were collected for molecular tracer and molecular genetic analyses using a high-volume sampler (Digitel DHA-80) drawing 1000 L min\(^{-1}\) through 15 cm glass fiber filters (Macherey-Nagel GmbH, Type MN 85/90, 406015, Düren, Germany) over a variety of sampling times ranging from 4 to 48 h (Supplement Table S1). The sampler was located < 50 m from each of the UV-LIF instruments described here, approximately between the WIBS-3 and UV-APS. Prior to sampling, all filters were baked at 500 °C for 12 h to remove DNA and organic contaminants. Samples were stored in pre-baked aluminum bags after sampling at \(\sim 20 \, ^\circ\text{C}\) for 1–30 days and then at \(-80 \, ^\circ\text{C}\) after overnight, international transport cooled on dry ice. Due to the low vapor pressure of the molecular tracers analyzed, loss due to volatilization is considered unlikely (Zhang et al., 2010). A total of 36 samples were collected during the study, in addition to handling field blanks and operational field blanks. Handling blanks were acquired by placing a filter into the sampler and
immediately removing it, without turning on the airflow control. Operational blanks were placed into the sampler and exposed to 10 s of airflow.

3.3.4 Slit sampler

A direct-to-agar slit sampler (Microbiological Air Sampler STA-203, New Brunswick Scientific Co, Inc., Edison, NJ) was used to collect culturable airborne fungal spores. The sampler was placed ~2 m above the ground on a wooden support surface with 5 cm × 5 cm holes to allow airflow both up and down through the support structure. Sampled air was drawn over the 15 cm diameter sampling plate filled with growth media at a flow rate of 28 L min⁻¹ for sampling periods of 20 to 40 min. Growth media (malt extract medium) was mixed with antibacterial agents (40 units streptomycin, Sigma Aldrich; 20 units ampicillin, Fisher Scientific) to suppress bacterial colony growth. Plates were prepared several weeks in advance and stored in a refrigerator at ca. 4 °C until used for sampling. Before each sampling period, all surfaces of the samplers were sterilized by wiping with isopropyl alcohol. Handling and operational blanks were collected to verify that no fungal colonies were being introduced by handling procedures. A total of 14 air samples were collected over 20 days and immediately moved to an incubator (Amerex Instruments, Incumax IC150R) set at 25 °C for 3 days prior to counting fungal colonies formed. Each colony, present as a growing dot on the agar surface, was assumed to have originated as 1 colony-forming unit (CFU; i.e., fungal spore) deposited onto the agar by impaction during sampling. The atmospheric concentration of CFU per air volume was calculated using the sampler airflow. Further discussion of methods and initial results from the slit sampler were published by Huffman et al. (2013).
3.3.5 Offline filter analyses

3.3.5.1. Carbohydrate analysis

Approximately one-eighth of each frozen filter was cut for carbohydrate analysis using a sterile technique, meaning that scissors were cleaned and sterilized, and cutting was performed in a positive-pressure laminar flow hood. In order to precisely determine the fractional area of the filter to be analyzed, filters were imaged from a fixed distance above using a camera and compared to a whole, intact filter. Using ImageJ software (Rasband, 1997), the area of each filter slice showing particulate matter (PM) deposit was referenced to a whole filter, and thereby the amount of each filter utilized could be determined. The total PM mass was not measured and so this technique allowed for an estimation of the fraction of each sample used for the analysis, which corresponds to the fraction of PM mass deposited. The uncertainty on the filter area fraction is estimated at 2%, determined as the percent of variation in the area of the filter edge (no PM deposit) as compared to the total filter area.

Water-soluble carbohydrates were extracted from glass fiber filter samples and analyzed following the procedure described by Rathnayake et al. (2016a). A total of 36 samples were analyzed along with field and lab blanks. All lab and field blanks fell below method detection limits. Extraction was performed by placing the filter slice into a centrifuge tube that had been pre-rinsed with Nanopure™ water (resistance > 18.2 MQ cm⁻¹; Barnstead EasyPure II, 7401). A volume of 8.0 mL of Nanopure™ water was added to the filter in the centrifuge tube to extract water-soluble carbohydrates. Samples
were then exposed to rotary shaking for 10 min at 125 rpm, sonication for 30 min at 60 Hz (Branson 5510, Danbury, CT, USA), and rotary shaking for another 10 min. After shaking, the extracted solutions were filtered through a 0.45 µm polypropylene syringe filter (GE Healthcare, UK) to remove insoluble particles, including disintegrated filter pieces. One 1.5 mL aliquot of each extracted solution was analyzed for carbohydrates within 24 h of extraction. A duplicate 1.5 mL aliquot was stored in a freezer and analyzed if necessary, due to lack of instrument response or invalid calibration check, within 7 days of extraction. Analysis of carbohydrates was done using a high-performance anion-exchange chromatography system with pulsed amperometric detection (HPAEC-PAD; Dionex ICS 5000, Thermo Fisher, Sunnyvale, CA, USA). Details of the instrument specifications and quality standards for carbohydrate determination are available in Rathnayake et al. (2016a). Calibration curves for mannitol, levoglucosan, glucose (Sigma-Aldrich), arabitol, and erythritol (Alfa Aesar) were generated with 7 points each, ranging in aqueous concentration from 0.005 to 5 ppm. The method detection limits for mannitol, levoglucosan, glucose, arabitol, and erythritol were determined to be 2.3, 2.8, 1.6, 1.0, and 0.6 ppb (parts per billion), respectively, by measuring the instrument response to filter extracts (Rathnayake et al., 2016a). One filter each was spiked with 10 ppb of the five compounds, followed by one extraction per filter from which seven aliquots were each analyzed by the instrument. The variability (3 SD) of the measured response was taken as the method detection limit. All calibration curves were checked daily using a standard solution to ensure all concentration values were within 10 % of the known value. Failure to maintain a valid curve resulted in recalibration of the instrument.
3.3.5.2 DNA analysis

Methods and initial results from DNA analysis from these high-volume filters were published by Huffman et al. (2013). Briefly, fungal diversity was determined by previously optimized methods for DNA extraction, amplification, and sequence analysis of the internal transcribed spacer regions of ribosomal genes from the high-volume filter samples (Fröhlich-Nowoisky et al., 2009, 2012). Upon sequence determination, fungal sequences were compared with known sequences using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology (NCBI) and identified to the lowest taxonomic rank common to the top BLAST hits after chimeric sequences had been removed. When sequences displayed > 97% similarity, they were grouped into operational taxonomic units (OTUs).

3.3.5.3 Endotoxin and glucan analysis

Sample preparation for quantification of endotoxin and (1→3)-β-D-glucan included extraction of five punches (0.5 cm² each) of the glass filters with 5.0 mL of pyrogen-free water (Associates of Cape Cod Inc., East Falmouth, MA, USA), utilizing an orbital shaker (300 rpm) at room temperature for 60 min, followed by centrifuging for 15 min (1000 rpm). A 0.5 mL aliquot of supernatant was submitted to a kinetic chromogenic limulus amebocyte lysate (Chromo-LAL) endotoxin assay (Associates of Cape Cod Inc., East Falmouth, MA, USA), using a ELx808IU (BioTek Instrument Inc., Winooski, VT, USA) incubating absorbance microplate reader. For (1→3)-β-D-glucan measurement, 0.5 mL of 3 N NaOH was added to the remaining 4.5 mL of extract and the mixture was agitated for 60 min. Subsequently, the solution was neutralized to pH 6–8 by
the addition of 0.75 mL of 2 N HCl. After centrifuging for 15 min (1→3)-β-D-glucan concentration was determined in the supernatant using the Glucatell® LAL kinetic assay (Associates of Cape Cod, Inc., East Falmouth, MA, USA). The minimum detection limits (MDLs) and reproducibility were 0.046 endotoxin units (EU) m\(^{-3}\) ± 6.4 % for endotoxin and 0.029 ng m\(^{-3}\) ± 4.2 % for (1→3)-β-D-glucan, respectively. Laboratory and field blank samples were analyzed as well, with lab blank values being below detection limits, while field blank values were used to subtract background levels from sample data. More details about the bioassays can be found elsewhere (Chow et al., 2015a).

3.3.6 Meteorology and wetness sensors

Meteorological data were recorded by a variety of sensors located at the site. Precipitation was recorded by a laser optical disdrometer (PARticle SIze and VElocity sensor – “PAR- SIVEL”; OTT Hydromet GmbH, Kempton, Germany) and separately by a tipping-bucket rain gauge. The disdrometer provides precipitation occurrence, rate, and physical state (rain or hail) by measuring the magnitude and duration of disruption to a continuous 780 nm laser that was located in a tree clearing (Fig. 1), while the tipping-bucket rain gauge measures a set amount of precipitation before tipping and triggering an electrical pulse. A leaf wetness sensor (LWS; Decagon Devices, Inc., Pullman, WA, USA) provided a measurement of condensed moisture by measuring the voltage drop across a leaf surface to determine a proportional amount of water on or near the sensor. Additional details of these measurements can be found in Huffman et al. (2013) and Ortega et al. (2014).
3.4  Results and discussion

3.4.1  Categorization and characteristic differences of Dry and Rainy periods

Increases in PBAP concentration have been frequently associated with rainfall (e.g., Bigg et al., 2015; Faulwetter, 1917; Hirst and Stedman, 1963; Jones and Harrison, 2004; Madden, 1997). Fungal polyols have also been reported to increase after rain and have been used as indicators of increased fungal spore release (Liang et al., 2013; Lin and Li, 2000; Zhu et al., 2015). Recently, it was shown that the concentration of fluorescent aerosol particles (FAPs) measured during BEACHON-RoMBAS increased dramatically during and after periods of rain (Crawford et al., 2014; Huffman et al., 2013; Schumacher et al., 2013) and that these particles were associated with high concentrations of ice nucleating particles that could influence the formation and evolution of mixed-phase clouds (Huffman et al., 2013; Prenni et al., 2013; Tobo et al., 2013). It was observed that a mode of smaller fluorescent particles (2–3 µm) appeared during rain episodes, and several hours after rain ceased a second mode of slightly larger fluorescent particles (4–6 µm) emerged, persisting for up to 12 h (Huffman et al., 2013). The first mode was hypothesized to result from mechanical ejection of particles due to rain splash on soil and vegetated surfaces, and the second mode was suggested as actively emitted fungal spores (Huffman et al., 2013). While the UV-APS and WIBS each provide data at a high enough time resolution to see subtle changes in aerosol concentration, the temporal resolution of the chemical tracer analysis was limited to 4–48 h periods defined by the collection time of the high-volume sampler. To compare the measurement results across the sampling
platforms, UV-LIF measurements were averaged to the lower time resolution of the filter sampler periods, and the periods were grouped into three broad categories: Rainy, Dry, and Other, as will be defined below.

**Figure 3.2.** Time series of key species concentrations and meteorological data over entire campaign. (a) Fluorescent particle number size distribution measured with UV-APS instrument. Color scale indicates fluorescent particle number concentration (L⁻¹). (b) Meteorological data: relative humidity (RH), disdrometer rainfall (millimeters per 15 min), leaf wetness (mV). (c) Wetness category indicated as colored bars: green, Rainy; brown, Dry; pink, Other. Bar width corresponds to filter sampling periods. Lightened colored bars extend vertically to highlight categorization. (d) Colored traces show fungal spore concentrations estimated from molecular tracers (circles) and WIBS Cl1 data (squares). (e) Stacked bars show relative fraction of fluorescent particle type corresponding to each WIBS category.

Time periods were wetness-categorized in two steps: first at 15 min resolution and then averaged for each individual filter sample. During the first stage of categorization each 15 min period was categorized into one of four groups: rain, post-rain, dry, or other. To categorize each filter period, an algorithm was established utilizing UV-APS fluorescent particle fraction and accumulated rainfall. The ratio of the integrated number of fluorescent particles to total particles was used as a proxy for the increased emission of biological particles. Figure 3.2a presents a time series of the size-resolved fluorescent
particle concentration, showing increases during rain periods in dark red. A relatively consistent diurnal cycle of increased FAP concentration in the 2–4 µm range is apparent almost every afternoon, which corresponds to near-daily afternoon rainfall during approximately the first half of the measurement period. Disdrometer and tipping-bucket rainfall measurements were each normalized to unity and summed to produce a more robust, unitless measure of rainfall rate because it was observed that often only one of the two systems would record a given light rain event. If a point was described by total rainfall accumulation greater than 0.201 it was flagged as rain. A point was flagged as post-rain if it immediately followed a rain period and also exhibited a fluorescent particle fraction greater than 0.08. The purpose of this category was to reflect the observation that sustained elevated concentrations of FAPs persisted for many hours even after the rain rate, RH, and leaf wetness returned to pre-rain values. The only measurement that adequately reflected this scenario was of the fluorescent particles measured by UV-APS and WIBS instruments. The post-rain flag was continued until the fluorescent particle fraction fell below 0.08 or if it started to rain again (with calculated rain values greater than 0.201). Points were flagged as dry periods if they exhibited rainfall accumulation and fluorescent particle fraction below the thresholds stated above. Several periods were not easily categorized by this system and were considered in a fourth category as other. This occurred when fluorescent particle fraction above the threshold value was observed with no discernable rainfall.

Once wetness categories were assigned by the algorithm at 15 min resolution, each high-volume filter sample was categorized by a similar nomenclature, but using
only three categories. These were defined as Dry, Rainy (combination of rain and post-

rain categories), or Other based on the relative time fraction in each of the four original

15 min categories. For each sample, if a given category represented more than 50 % of

the 15 min periods, the sample was assigned to that category. Despite the effort to
categorize samples systematically, several sample periods (5 of 35) appeared mis-
categorized by looking at FAP concentration, rainfall, RH, and leaf wetness in more
detail. In some circumstances, this was because light rainfall produced observable
increases in FAPs, but without exceeding the rainfall threshold. Or in other circumstances
a period of rainfall occurred at the very end or just before the beginning of a sample, and
so the many-hour period was heavily influenced by aerosol triggered by a period of rain
just outside of the sample time window. As a result, several samples were manually re-
categorized as described here. Samples 20 and 21 (Table S1) were 4 h samples that
displayed high relative humidity and rainfall; thus, samples were originally characterized
as Rainy. This period was described by an extremely heavy rain downpour (7.5 mm in 15
min); however, that seemingly placed the samples in a different regime of rain–aerosol
dynamics than the other Rainy samples and so these two samples were moved to the
Other category. Sample 23, originally Rainy, presented a FAP fraction marginally above
the 0.08 threshold, but visually displayed a trend dissimilar to other post-rain periods and
so was re-categorized as Dry. Sample 28 showed no obvious rainfall, but the
measurement team observed persistent fog in three consecutive mornings (samples 25,
27, 28), and the concentration of fluorescent particles (2–6 µm) suggested a source of
particles not influenced by rain, and so this Rainy sample was re-categorized as Other.
Sample 38 displayed a fluorescent number ratio just below the threshold value, and was first categorized as Dry; however, the measurement team observed post-rain periods at the beginning and end of the sample, and the sample was re-categorized as Other. For all samples other than these five, the categorization was determined using the majority (> 0.50) of the 15 min periods. In no cases other than the five that were re-categorized was the highest category fraction less than 0.50 of the sample time. Note that we have chosen to capitalize Rainy, Dry, and Other to highlight that we have rigorously defined the period using the characterization scheme described above and to separate the nomenclature from the general, colloquial usage of the terms. Wetness category assignment for each high-volume filter sample period is shown in Fig. 3.2 as a background color (brown for Dry samples, green for Rainy samples, and pink for Other samples) and Table S1.

To validate the qualitative differences between wetness categories described in the last section, we present observations about each of these groupings. First, we organized the WIBS data according to the particle categories introduced by Perring et al. (2015). By this method, every fluorescent particle detected by the WIBS can be defined uniquely into one of seven categories (i.e., A, AB, ABC). By plotting the relative fraction of fluorescent particles described by each particle type, temporal differences between measurement periods can be observed, as shown in Fig. 3.2e. To a first approximation, this analysis style allows for coarse discrimination of particle types. For example, a given population of particles would ideally exhibit a consistent fraction of particles present in the different particle categories as a function of time. By this reasoning, sample periods
categorized as Dry (most of the latter half of the study; brown bars in Fig. 3.2) would be expected to have a self-consistent particle-type trend, whereas sample periods categorized as Rainy (most of the first half of the study; green bars in Fig. 3.2) would have a self-consistent particle-type trend, but different from the Dry samples. This is broadly true.

During Rainy periods, as seen in Fig. 3.3a, there is a relatively high fraction (> 65 %) of ABC type particles (light blue) and a relatively low fraction (< 15 %) in BC (purple) and C (yellow) type particles, suggesting heavy influence from the FL1 channel. In contrast, during Dry periods the fraction of ABC particles (light blue) is reduced (< 25 %), whereas BC (purple) and C (yellow) type particles increase in relative fraction (> 30 and > 40 %, respectively), which suggested a diminished influence of FL1 channel.
Figure 3. 3 Characteristic differences between wetness periods (Dry, Rainy, Other). (a) Relative fraction of fluorescent particle number corresponding to each WIBS category. Bars show relative standard deviation of category fraction in each wetness group (Dry, 19 samples; Rainy, 11 samples; Other, 6 samples). (b, c) Distribution of fungal OTU (operational taxonomic unit) values. (b) Fungal community composition at phylum and class level with Agaricomycetes (dominant class with consistently 60% of diversity) removed. Relative proportion of OTUs assigned to different fungal classes and phyla for each sample category shown. (c) Venn diagram showing the number of unique (wetness category-specific) and shared OTUs (represented by numbers in overlapping areas) among the sample categories (Dry, 11 samples; Rainy, 7 samples; Other, 3 samples). OTUs classified as cluster of sequences with ≥97% similarity. Taxonomic assignments were performed using BLAST against NCBI database. In total, 3902 sequences, representing 406 fungal OTUs from 3 phyla and 12 classes were detected. Despite differences in community structure across the sample categories, phylogenetic representation appears largely similar.
It is important to note a few caveats here. First, the ability of the WIBS to discriminate distinctly between PBAP types is relatively poor and it is still unclear exactly how different particle types would appear by this analysis method. Particles of different kinds and from different sources are likely convolved into a single WIBS particle type, which could either soften or enhance the relationships with rain discussed here. Second, the assignment of particle types is heavily size dependent and sensitive to subtle instrument parameters, and so it is unclear how different instruments would present similar particle types. For example, Hernandez et al. (2016) used two WIBS instruments and found differences in relative fraction of particle categories for samples aerosolized in the lab. They reported fungal spores to be predominately A, AB, and ABC type particles, whereas Rainy sample periods, suggested to have a heavy fungal spore influence by Huffman et al. (2013), show predominantly C, BC, and ABC type particle fractions. These discrepancies may be due to the comparison of ambient particles to laboratory-grown cultures. The highly controlled environment of a laboratory may not always accurately represent the humidity conditions in which fungal spore release occurs in this forest setting (Saari et al., 2015). This could impact the fluorescence properties of fungal spore particles that have different amounts of adsorbed or associated water (Hill et al., 2009, 2013, 2015). More likely, however, is that the WIBS-3 used here exhibits differences in fluorescence sensitivity from the WIBS-4A used by Hernandez et al. (2016). Even a slight increase in sensitivity in the FL3 channel with respect to the FL1 or FL2 channels could explain the shift here towards particles with C-type fluorescence. One piece of evidence for this is the quantitative comparison of particle measurements
presented by the UV-APS and WIBS-3 instruments co-deployed here (Fig. 3.4). The number concentration of particles exhibiting fluorescence above the FL2 baseline of the WIBS-3 is approximately consistent with the number of fluorescent particles measured by the UV-APS, and significantly below the concentration of FL3 particles. The UV-APS number concentration shows the highest correlation with the WIBS-3 FL2 channel: during Rainy periods, $R^2 = 0.70$; Dry, $R^2 = 0.82$; and Other, $R^2 = 0.92$. These observations are in stark contrast to the trends reported by Healy et al. (2014) that the UV-APS fluorescent particle concentration correlated most strongly with the WIBS-4 FL3 and that the number concentration of FL3 was the lowest out of all three channels. Given that the FL3 channel of the WIBS and the UV-APS cover similar excitation and emission wavelengths, it is expected that these two channels should correlate well. Based on these data, we suggest that the WIBS-3 utilized here may present a very different particle-type breakdown than if a WIBS-4 had been used. So, while caution is recommended when comparing the relative breakdown of WIBS particle categories shown here (Fig. 3.3) with other studies, the data are internally self-consistent, and comparing qualitative differences between, e.g., Rainy and Dry periods, is expected to be robust. The main point to be highlighted here is that there is indeed a qualitative difference in particles present in the three wetness categories, as averaged and shown in Fig. 3.3a, which generally supports the effort to segregate these samples.
Further evidence that there is a qualitative difference in the three wetness categories is shown using molecular genetic analysis (Fig. 3.3b, c). The analysis of fungal DNA sequences from 21 of the high-volume samples found 406 OTUs belonging to different fungal classes and phyla. When organized by wetness type it was observed that 106 of these occurred only on Rainy samples, 148 of these occurred on Dry samples, and 37 on Other samples, with some fraction occurring in overlaps of each (Fig. 3.3c). This shows that the number of OTUs observed uniquely in either the Rainy or Dry periods is greater than the number of OTUs present in both wetness types, suggesting that the fungal communities in each grouping are relatively distinct. Further, Fig. 3.3b shows a
breakdown of fungal taxonomic groupings for each wetness group. This analysis shows that there is a qualitative difference in taxonomic breakdown between periods of Rainy and Dry. Specifically, during Dry periods there is an increased fraction of Pucciniomycetes (green bar, Fig. 3.3c), Chytridiomycota (yellow), Sordariomycetes (orange), and Eurotiomycetes (pink) when compared to the Rainy periods.

3.4.2 Atmospheric mass concentration of arabitol, mannitol, and fungal spores

To estimate fungal spore emission to the atmosphere, the concentration of arabitol and mannitol (Fig. 3.5a, b, Table 3.1) in each aerosol sample was averaged for all samples in each of the three wetness categories. The average concentration of arabitol collected on Rainy TSP samples (35.2 ± 10.5 ng m\(^{-3}\)) increased by a factor of 3.3 with respect to Dry samples, and the average mannitol concentration on Rainy samples was higher by a factor of 3.7 (44.9 ± 13.8 ng m\(^{-3}\)). Figure 3.5a, b show the concentration the variability for each wetness category, observed as the standard deviation from the distribution of individual samples. For each polyol, there is no overlap in the ranges shown, including the outliers of the Rainy and Dry category, suggesting a definitive and conceptually distinct separation between dry periods and those influenced by rain. The concentrations observed during Other periods is between those of the Dry and Rainy averages, as expected, given the difficulty in confidently assigning these uniquely to one of these categories. The observations here are roughly consistent with previous reports of polyol concentration, despite differences in local fungal communities and concentrations. For example, Rathnayake et al. (2016a) observed 30.2 ng m\(^{-3}\) arabitol
and 41.3 ng m$^{-3}$ mannitol in PM10 samples collected in rural Iowa, USA. In addition, Zhang et al. (2015) reported arabinol and mannitol concentrations in PM10 samples of 44.0 and 71.0 ng m$^{-3}$, respectively, from a study in the mountains on Hainan Island off the coast of southern China. More recently, Yue et al. (2016) studied a rain event in Beijing and observed increased polyol concentrations at the onset of the rain. The observed mannitol concentration (45 ng m$^{-3}$) was approximately consistent with observations reported here and with previous reports, while the arabinol concentration values observed were approximately an order of magnitude lower (0.3 ng m$^{-3}$).

Table 3.1: Square of correlation coefficients ($R^2$) comparing total mass concentration of molecular tracers to each other. EU: endotoxin units. Boxes colored by coefficient value (Bold Underline > 0.7; 0.7 > Bold > 0.4).

<table>
<thead>
<tr>
<th>Mass Concentration</th>
<th>Arabitol (ng m$^{-3}$)</th>
<th>Mannitol (ng m$^{-3}$)</th>
<th>(1→3)-β-D-glucan (pg m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rainy</td>
<td>Dry</td>
<td>Rainy</td>
</tr>
<tr>
<td>Mannitol (ng m$^{-3}$)</td>
<td>0.839</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>0.312</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1→3)-β-D-glucan (pg m$^{-3}$)</td>
<td>0.000</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>0.000</td>
<td>0.327</td>
<td></td>
</tr>
<tr>
<td>Endotoxins (EU m$^{-3}$)</td>
<td>0.116</td>
<td>0.126</td>
<td>0.427</td>
</tr>
<tr>
<td>Dry</td>
<td>0.012</td>
<td>0.113</td>
<td>0.103</td>
</tr>
</tbody>
</table>
The square of the correlation coefficient ($R^2$) here between concentration values of arabitol and mannitol during Rainy samples is very high (0.839; Table 3.2) suggesting that arabitol and mannitol originated primarily from the same source, likely active-discharge fungal spores. The correlation is similar to the 0.87 $R^2$ reported by Bauer et al. (2008a) and 0.93 $R^2$ reported by Graham et al. (2003). In contrast, the same correlation seen between mannitol and arabitol concentrations for Dry samples is relatively low (0.312). This is consistent with reports that arabitol can be used more specifically as a spore tracer, but that mannitol has additional atmospheric sources besides fungal spores.
Table 3.2. Square of correlation coefficients (R²) comparing fluorescent particle measurements from UV-LIF instruments to measurements from molecular tracers and direct-to-agar sampler. Columns marking tracer mass (top line) indicate correlations between time-averaged UV-LIF and tracer mass concentrations (left side), and columns marking fungal spore number indicate correlations between fungal spore number concentrations estimated from time-averaged UV-LIF and tracer or culture measurements (right side). FL1, FL2, FL3 represent individual channels from the WIBS. FL represents particles exhibiting fluorescence in any channel. Cl1, Cl2, Cl3, Cl4 are clusters that estimate particle concentrations as a mixture of various channels (Crawford et al., 2015). ClBact is a sum of the “bacteria” clusters Cl2-4. Boxes colored by coefficient value (Bold Underline > 0.7; 0.7 > Bold > 0.4).

<table>
<thead>
<tr>
<th>UVAPS</th>
<th>Mass Concentration</th>
<th>UVAPS</th>
<th>Fungal Spore Number Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arabitol (ng m⁻³)</td>
<td>Mannitol (ng m⁻³)</td>
<td>(1→3)-β-D-glucan (pg m⁻³)</td>
</tr>
<tr>
<td>Rainy</td>
<td>Dry</td>
<td>Rainy</td>
<td>Dry</td>
</tr>
<tr>
<td>FLAPS</td>
<td>0.732</td>
<td>0.127</td>
<td>0.877</td>
</tr>
<tr>
<td>FL</td>
<td>0.554</td>
<td>0.250</td>
<td>0.810</td>
</tr>
<tr>
<td>FL1</td>
<td>0.602</td>
<td>0.445</td>
<td>0.819</td>
</tr>
<tr>
<td>FL2</td>
<td>0.617</td>
<td>0.248</td>
<td>0.843</td>
</tr>
<tr>
<td>FL3</td>
<td>0.561</td>
<td>0.222</td>
<td>0.818</td>
</tr>
<tr>
<td>Cl1</td>
<td>0.824</td>
<td>0.764</td>
<td>0.799</td>
</tr>
<tr>
<td>Cl2</td>
<td>0.005</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>Cl3</td>
<td>0.267</td>
<td>0.164</td>
<td>0.261</td>
</tr>
<tr>
<td>Cl4</td>
<td>0.048</td>
<td>0.046</td>
<td>0.172</td>
</tr>
<tr>
<td>ClBact</td>
<td>0.041</td>
<td>0.081</td>
<td></td>
</tr>
</tbody>
</table>
The same correlation was also performed between arabitol or mannitol and other molecular tracers (endotoxins and (1→3)-β-D-glucan), but all $R^2$ value were less than 0.43, suggesting that the endotoxins and glucans analyzed were not emitted uniquely from the same sources as arabitol and mannitol.

Results from the two UV-LIF instruments were averaged over high-volume sample periods, and a correlation analysis was performed between tracer mass and fluorescent particle mass showing positive correlations in all cases. The FAP mass from the UV-APS shows high correlation with the fungal polyols during Rainy periods, with $R^2$ of 0.732 and 0.877 for arabitol and mannitol, respectively (Table 3.3; Fig. 3.5c, d). The same tracers correlate poorly with the UV-APS during Dry conditions. This is expected, because Ascomycota and Basidiomycota spores emitted by wet-discharge methods are the only fungal spores reported to be associated with arabitol and mannitol (Elbert et al., 2007; Feofilova, 2001; Lewis and Smith, 1967). This high correlation suggests that the UV-APS does a good job of detecting these wet-discharge spores, and corroborates previous statements that particles detected in ambient air by the UV-APS are often predominately fungal spores (Healy et al., 2014; Huffman et al., 2012, 2013). In contrast, the low slope value and the poor correlation during Dry periods suggest that the UV-APS is also sensitive to other kinds of particles, as designed. The small positive x offset (FAP mass; Table A2, Fig. 3.5c, d) during Rainy periods is likely due to particles that are too weakly fluorescent to be detected and counted by the UV-APS, which is consistent with observations made in Brazil (Huffman et al., 2012).
Table 3.3. Campaign-average concentrations of molecular tracers (top) and their respective mass contributions (bottom). Values are mean ± standard deviation; n shows number of samples used for averaging. Total particulate matter mass calculated from UV-APS number concentration (m⁻³), converted to mass over aerodynamic particle diameter range 0.5 – 15 µm using 1.5 g cm⁻³ density.

<table>
<thead>
<tr>
<th></th>
<th>Mass Concentration</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Arabitol (ng m⁻³)</td>
<td>Mannitol (ng m⁻³)</td>
<td>Erythritol (ng m⁻³)</td>
<td>Levoglucosan (ng m⁻³)</td>
<td>Glucose (ng m⁻³)</td>
<td>Endotoxins (EU m⁻³)</td>
<td>(1→3)-β-D-glucan (pg m⁻³)</td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>10.6 ± 2.5 n=18</td>
<td>11.9 ± 3.2 n=18</td>
<td>0.840 ± 0.610 n=16</td>
<td>14.2 ± 10.7 n=15</td>
<td>38.7 ± 21.3 n=18</td>
<td>0.192 ± 0.0970 n=18</td>
<td>8.85 ± 7.68 n=18</td>
<td></td>
</tr>
<tr>
<td>Rainy</td>
<td>35.2 ± 10.5 n=11</td>
<td>44.9 ± 13.8 n=11</td>
<td>1.12 ± 0.38 n=3</td>
<td>12.4 ± 19.1 n=8</td>
<td>73.2 ± 50.5 n=11</td>
<td>1.43 ± 1.22 n=10</td>
<td>10.6 ± 8.2 n=11</td>
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<tr>
<td>Other</td>
<td>20.2 ± 8.9 n=6</td>
<td>22.7 ± 8.3 n=6</td>
<td>0.664 ± 0.515 n=6</td>
<td>9.21 ± 1.66 n=5</td>
<td>56.5 ± 39.2 n=6</td>
<td>0.311 ± 0.159 n=6</td>
<td>6.08 ± 6.08 n=6</td>
<td></td>
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</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>Mass Contribution (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>0.18 % ± 0.05 n=18</td>
<td>0.20 % ± 0.073 n=18</td>
<td>0.014 % ± 0.011 n=16</td>
<td>0.21 % ± 0.17 n=15</td>
<td>0.67 % ± 0.49 n=18</td>
<td>0.16 % ± 0.16 n=18</td>
<td></td>
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</tr>
<tr>
<td>Rainy</td>
<td>0.83 % ± 0.32 n=11</td>
<td>1.07 % ± 0.44 n=11</td>
<td>0.032 % ± 0.009 n=3</td>
<td>0.27 % ± 0.41 n=8</td>
<td>1.60 % ± 1.09 n=11</td>
<td>0.25 % ± 0.21 n=11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.25 % ± 0.28 n=6</td>
<td>0.37 % ± 0.29 n=6</td>
<td>0.013 % ± 0.015 n=6</td>
<td>0.15 % ± 0.11 n=5</td>
<td>0.83 % ± 0.64 n=6</td>
<td>0.12 % ± 0.19 n=6</td>
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</tr>
</tbody>
</table>

Particle mass from WIBS C11, assigned to fungal spores (Crawford et al., 2015), also correlates strongly with the same two molecular tracers. Both Rainy periods (R² 0.824) and Dry periods (R² 0.764) correlate well with arabitol (Fig. 3.5e), while mannitol (Fig. 3.5f) only shows a strong correlation during the Rainy periods (R² 0.799). Mannitol is a common polyol in higher plants while arabitol is only found in fungal spores and lichen (Lewis and Smith, 1967). So the strong correlation of each polyol with UV-LIF mass during Rainy periods when actively discharged spores are expected to dominate and the similarly strong correlations associated with arabitol suggest that the C11 cluster does a reasonably good job of selecting fungal spore particles. The poor correlation between
mannitol and Cl1 during dry periods illustrates that the background mannitol concentration is likely not due to fungal spores alone, but has contributions from other higher plants that contain mannitol. Particle concentrations detected by individual WIBS channels and in the other clusters were also compared with polyol concentrations, but each correlation is relatively poor compared to that with respect to Cl1. As seen in Table 3.3 and Figs. A2–A3, correlations in FL1, 2, and 3 with arabitol are poor (< 0.4) in the Dry category and good (0.4 < $R^2 < 0.7$) in the Rainy category. For mannitol, all the UV-LIF instruments show high correlation (> 0.7) in all cases. This is likely due to mannitol being a non-specific tracer and suggests that the majority of UV-LIF particles observed during all periods was dominated by PBAPs.

### 3.4.3 Estimated number concentration of fungal spore aerosol

Bauer et al. (2008a) reported measurements of fungal spore number concentration in Vienna, Austria, using epifluorescence microscopy, and also measured fungal tracer mass collected onto filters in order to estimate the mass of arabitol (1.2 to 2.4 pg spore$^{-1}$) and mannitol (0.8 to 1.8 pg spore$^{-1}$) associated with each emitted spore. Bauer et al. (2008a) and Yttri et al. (2011b) reported ratios of mannitol to arabitol of ca. 1.5 (± standard deviation of 26 %) and 1.4 ± 0.3, respectively. Our measurements show slightly lower ratios of mannitol to arabitol, but that the ratio is dependent on wetness category: Rainy, 1.29 ± 0.17; Dry, 1.12 ± 0.23; and Other, 1.24 ± 0.54. The mannitol to arabitol ratio would be expected to vary as a function of fungal population present in the aerosol,
whether between different wetness periods at a given location or between different physical localities.

Using the approximate mid-point of the Bauer et al. (2008a) reported ranges, 1.7 pg mannitol per spore and 1.2 pg arabitol per spore, atmospheric number concentrations of spores collected onto the high-volume filters were calculated from the polyol mass concentrations measured here. Based on these values, and assuming all polyol mass originated with spore release, the mass concentration averages (Fig. 3.5) were converted to fungal spore number concentrations (Fig. 3.6). The trends of spore concentration averages are the same as with the polyol mass, because the numbers were each multiplied by the same scalar value. After doing so, the analysis reveals an estimated spore concentration during Dry periods of $0.89 \times 10^4$ (± 0.21) spores m$^{-3}$ using the arabitol concentration and $0.70 \times 10^4$ (± 0.19) spores m$^{-3}$ using the mannitol concentration (Table 3.4). The estimated concentration of spores increased approximately 3-fold during Rainy periods to $2.9 \times 10^4$ (± 0.8) spores m$^{-3}$ (arabitol estimate) and $2.6 \times 10^4$ (± 0.8) spores m$^{-3}$ (mannitol estimate) (Fig. 3.6a, b). These estimates match reasonably well with estimates reported by Spracklen and Heald (2014), who modeled the concentration of airborne fungal spores across the globe as an average of $2.5 \times 10^4$ spores m$^{-3}$, with ca. $0.5 \times 10^4$ spores m$^{-3}$ over Colorado.
Table 3.4. Campaign-average fungal spore concentration and mass contribution estimated from arabitol and mannitol mass measurements. Values are mean ± standard deviation; n shows number of samples used for averaging. Fungal spore mass assumption of 33 pg spore⁻¹ (Bauer et al., 2008b). Total particulate matter mass calculated from UV-APS number concentration (m⁻³), converted to mass over aerodynamic particle diameter range 0.5 – 15 µm using 1.5 g cm⁻³ density.

<table>
<thead>
<tr>
<th>Fungal Spore Number Concentration (m⁻³)</th>
<th>Arabitol</th>
<th>Mannitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>8900 ± 2100 n=18</td>
<td>6900 ± 1900 n=18</td>
</tr>
<tr>
<td>Rainy</td>
<td>29300 ± 8700 n=11</td>
<td>26400 ± 8100 n=11</td>
</tr>
<tr>
<td>Other</td>
<td>16900 ± 7400 n=6</td>
<td>13400 ± 4900 n=6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungal Spore Mass Contribution (%)</th>
<th>Arabitol</th>
<th>Mannitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>4.8 % ± 1.43 n=18</td>
<td>3.7 % ± 1.1 n=18</td>
</tr>
<tr>
<td>Rainy</td>
<td>22.9 % ±8.8 n=11</td>
<td>20.7 % ±8.5 n=11</td>
</tr>
<tr>
<td>Other</td>
<td>9.8 % ± 7.7 n=6</td>
<td>7.3 % ± 5.6 n=6</td>
</tr>
</tbody>
</table>
Figure 3.6. Estimated fungal spore number concentration, calculated using mass of arabitol and mannitol per spore reported by Bauer et al. (2008a). Estimates from arabitol (top row) and mannitol (bottom row). Average fungal spore concentration, calculated using arabitol mass (a), mannitol mass (b), and colony-forming units (c) in each wetness category. Central marker shows mean value of individual filter concentration values, bars represent standard deviation (SD) range of filter values, and individual points show outliers beyond mean SD. Correlation of fungal spore number calculated from arabitol (d), mannitol (e), and colony-forming units (f) concentrations with estimated fluorescent particle mass from UV-APS. Correlation of fungal spore number calculated from arabitol (g), mannitol (h), and colony-forming unit (i) concentrations with fluorescent particle concentrations from WIBS cluster 1. R² value shown for each fit (right two columns). Linear fit parameters are shown in Table S3.

The UV-LIF instruments discussed here are number-counting techniques and in this instance have been applied as spore counters. As a first approximation, each particle detected by the UV-APS was assumed to be a fungal spore with the same properties used in the assumptions by Bauer et al. (2008a). Figure 3.6d, e, g, h show correlations of fungal spore number concentration estimated from polyol mass on the y axes and from
UV-LIF measurements on the x axes. The first, and most important observation is that the estimated fungal spore concentration from each technique is on the same order of magnitude, $10^4 \text{ m}^{-3}$. Looking at individual correlations reveals a finer layer of detail. These results show that the number concentration of fungal spores estimated by the UV-APS is greater than the number of fungal spores estimated by the tracers, as evidenced by slope values of ca. 0.2 and 0.35 for Rainy and Dry conditions, respectively (Table A3, Fig. 3.6, e). Again, this suggests that the UV-APS detects fungal spores as well as other types of fluorescent particles. The $R^2$ values ($\sim 0.5$) during Rainy periods indicate that the additional source of particles detected by the UV-APS is likely to have a similar source, such as PBAPs mechanically ejected from soil and vegetative surfaces with rain splash (Huffman et al., 2013). The magnitude of the over-estimation is higher during Dry periods, which would be expected because Rainy periods exhibited much higher particle number fractions associated with polyol-containing spores.

The Cl1 cluster from WIBS data shows correlations with estimated fungal spores from arabitol and mannitol that have slopes much closer to 1.0 than correlations with UV-APS number (Fig. 3.6g, h, Table A3). For example, the slope of the Cl1 correlations with each polyol during Rainy periods is ca. 0.87. This suggests only a 13% difference between the spore concentration estimates from the two techniques during Rainy periods. The average number concentration of Cl1 during Rainy periods is $1.6 \times 10^4 \pm 0.8$ spores m$^{-3}$. In both cases the slopes with respect to Cl1 are greater than 1.0 during Dry periods, suggesting that the cluster method may be missing some fraction of weakly fluorescent particles. Huffman et al. (2012) similarly suggests that particles that are weakly
fluorescent may be below the detection limit of the instrument and Healy et al. (2014) suggested that both UV-APS and WIBS-4 instruments significantly under-count the ubiquitous Cladosporium spores that are most common during dry weather and often peak in the afternoon when RH is low (De Groot, 1968; Oliveira et al., 2009).

Fundamentally, however, the results from the UV-APS, and even more so the numbers reported by the clustering analysis by Crawford et al. (2015), reveal broadly similar trends with the numbers estimated from polyol-to-spore values reported by Bauer et al. (2008a).

The fungal culture samples show similar division during Rainy and Dry periods as arabitol and mannitol concentrations (Fig. 3.6c), with an increase of ca. 1.6 × during Rainy periods. The trend of a positive slope with respect to the UV-LIF measurements is also similar between the tracer and culturing methods. In general, however, the R² value correlating CFU to fungal spore number calculated from the UV-LIF number is lower than between tracers and UV-LIF numbers (Table 3.3, Fig. A4). This is not unexpected for several reasons. First, the short sampling time of the culture samples (20 min) leads to poor-counting statistics and high number concentration variability, whereas each data point from the high-volume air samples represents a period of 4–48 h. Second, culture samplers, by their nature, only account for culturable fungal spores. It has been estimated that as low as 17% of aerosolized fungal species are culturable, and so it is expected that the CFU concentration observed is significantly less than the total airborne concentration of spores (Bridge and Spooner, 2001; Després et al., 2012). Nonetheless, the culturing analysis here supports the tracer and UV-LIF analyses and the most important trends are
consistent between all analysis methods. The concentration of fungal spores is higher during the Rainy periods, and there is a positive correlation between both tracer and CFU concentration and UV-LIF number.

In a pristine environment, such as the Amazon, supermicron particle mass has been found to consist of up to 85% biological material (Pöschl et al., 2010). Total particulate matter mass was calculated here from the UV-APS number concentrations (m$^{-3}$) and converted to mass for particles of aerodynamic diameter 0.5–10 µm. In only this case a density of 1.5 g cm$^{-3}$ was utilized to calculate a first approximation of total particle mass to which all other mass measurements were compared. An average TSP mass density of 1.5 g cm$^{-3}$ was utilized, because organic aerosol is typically estimated with density < 1.0 g cm$^{-3}$, biological particles are often assumed to have ca. 1.0 g cm$^{-3}$ density, and mineral dust particles have densities of up to ca. 3.5 g cm$^{-3}$ (Dexter, 2004; Tegen and Fung, 1994). Fungal spore mass was estimated here using the fungal spore concentrations calculated from arabitol and mannitol mass (Fig. 3.6) and then using an estimated 33 pg reported by Bauer et al. (2008b) as an average mass per spore. Dividing the resultant fungal spore mass by total particulate mass provides a relative mass fraction for each high-volume sample period. These calculations suggest that fungal spores represent ca. 23 ± 9% (using arabitol) or 21 ± 8% (using mannitol) of total particulate mass during Rainy periods (Table 3.4, Fig. 3.7). This represents a nearly 6-fold increase in percentage compared to Dry periods (4.8% ± 1.4 and 3.7% ± 1.1, respectively). A similar increase during Rainy periods was also seen in the mass fraction of fungal cluster
Cl1, which represented 17% ± 10 of the particle mass during Rainy and 2% ± 1 during Dry periods (Table A4).

Figure 3.7. Estimated fraction of total aerosol mass contributed by fungal spores. Fungal spore mass concentration (μg m⁻³) calculated separately from mannitol and arabitol concentration and using average mass per spore reported by Bauer et al. (2008b). Total particulate matter mass calculated from UV-APS number concentration (m⁻³) and converted to mass over aerodynamic particle diameter range 0.5–15 μm using density of 1.5 g cm⁻³. Central marker shows mean value of individual filter concentration values, bars represent standard deviation (SD) range of filter values, and individual points show outliers beyond mean SD.

3.4.4 Variations in endotoxin and glucan concentrations

Endotoxins measured in the atmosphere are uniquely associated with gram-negative bacteria (Andreae and Crutzen, 1997). Here, we show correlations between total endotoxin mass and WIBS ClBact, which was assigned by Crawford et al. (2015) to be bacteria due to the small particle size (< 1 μm) and high correlation with rain. This assignment of particle type to this set of clusters is quite uncertain, however, and should be treated loosely. The correlation between endotoxin mass and UV-APS and the WIBS
clusters was very poor, in most cases $R^2 < 0.1$ (Table 3.3, Fig. 3.8), suggesting no apparent relationship. Analysis of bacteria by both UV-LIF techniques is hampered by the fact that bacteria can be < 1 µm in size and because both instruments detect particles with decreased efficiency at sizes below 0.8 µm. So weak correlations may not have been apparent due to reduced overlap in particle size. Despite the lack of apparent correlation between the techniques, the relatively variable endotoxin concentrations were elevated during Rainy periods, consistent with Jones and Harrison (2004), who showed that bacteria concentrations were elevated after rainy periods. Glucans, such as $(1\rightarrow3)$-$\beta$-D-glucan, are components of the cell walls of pollen, fungal spores, plant detritus, and bacteria (Chow et al., 2015b; Lee et al., 2006; Stone and Clarke, 1992). In contrast to the observed difference in endotoxin concentration during the different wetness periods, $(1\rightarrow3)$-$\beta$-D-glucan showed no correlations with UV-LIF concentrations (Table 3.3) and no differentiation during the different wetness periods.
3.5 Conclusions

Increased concentrations of fluorescent aerosol particles and ice nuclei attributed to having a biological origin were observed during and immediately after rain events throughout the BEACHON-RoMBAS study in 2011 (Huffman et al., 2013; Prenni et al., 2013; Schumacher et al., 2013). Here we expand upon the previous reports by utilizing measurements from two commercially available UV-LIF instruments, of several molecular tracers extracted from high-volume filter samples, and from a culture-based sampler in order to compare three very different methods of atmospheric fungal spore analysis. This study represents the first reported correlation of UV-LIF and molecular tracer measurements and provides an opportunity to understand how an important class of PBAPs might be influenced by periods of rainy and dry weather. We found clear patterns in the fungal molecular tracers, arabitol and mannitol, associated with Rainy conditions that are consistent with previous findings (Bauer et al., 2008a; Elbert et al., 2007;
Feofilova, 2001). Fungal polyols increased 3-fold over Dry conditions during Rainy weather samples, with arabitol concentration of 35.2 ± 10.5 ng m$^{-3}$ and mannitol concentration of 44.9 ± 13.8 ng m$^{-3}$. Additionally, the very high correlation of the fungal tracers with WIBS Cl1 ($R^2 > 0.8$ in many cases) provides support for its assignment by Crawford et al. (2015) to fungal spores. Similarly, the UV-APS correlates well with fungal tracers, however over-counts the number concentration estimated from the tracers, confirming that the UV-APS is sensitive also to other types of particles beyond fungal spores, as expected. The estimated spore count from the WIBS Cl1 concentration was within ~13% of the spore count estimated by the tracer method, with concentrations ranging from 1.6 to $2.9 \times 10^4$ spores m$^{-3}$. These values are broadly consistent with concentrations modeled by, e.g., Spracklen and Heald (2014), Hoose et al. (2010), and Hummel et al. (2015). These spore counts represent 17–23% of the total particle mass during Rainy conditions and 2–5% during Dry conditions. Culture-based sampling also shows a similar relationship between CFU and UV-LIF concentrations and an increase of ~1.6 × between Dry and Rainy conditions. Despite the fact that the tracer and UV-LIF approaches to estimating atmospheric fungal spore concentration are fundamentally different, they provide remarkably similar estimates and temporal trends. With further improvements in instrumentation and analysis methods (e.g., advanced clustering algorithms applied to UV-LIF data), the ability to reliably discriminate between PBAP types is improving. As we have shown here, this technology represents a potential for monitoring approximate fungal spore mass and for contributing improved information on
fungal spore concentration to global and regional models that to this point has been lacking (Spracklen and Heald, 2014).
CHAPTER FOUR: CYPRUS

4.1 Introduction

4.1.1 Motivation

Cyprus is a location that represents a unique opportunity to measure air masses coming from three different continents, Europe, Africa, and Asia, with different aerosol influences. Europe and the Middle East bring pollution, the Mediterranean Sea contributes sea salt and natural particles, and dust arrives from the Arabian Peninsula and North Africa. The long range transport out of Africa across the North Atlantic and into the Mediterranean experiences seasonal trends (Prospero, 1996). The greatest dust events occur in the late winter to early spring, typically January to May. These dust events are known to dramatically increase the concentration of PM$_{10}$ and respirable PM$_{2.5}$ (Krasnov et al., 2014; Middleton et al., 2008; Querol et al., 2009). Additionally, long-range transport of dust is often accompanied with biogenic aerosols including bacteria and fungal spores (Gat et al., 2017; Prospero et al., 2005). Both dust and bioaerosols are known to act as cloud condensation nuclei and ice nuclei (Haga et al., 2013; Kristensen et al., 2016; Morris et al., 2013; Rosenfeld et al., 2001; Sesartic et al., 2013). To investigate
the impact of these dust events and other air mass on aerosols and ice nuclei the field campaign INIUT-BACCHUS- ACTRIS\textsuperscript{2} was undertaken.

4. 1. 2. Sampling Site

During April of 2016 (01 April - 26 April), numerous instruments and samplers were deployed at Agia Marina Xyliatou Atmospheric Station, Nicosia, Cyprus (35°02′18″N, 33°03′38″E, 550m a.s.l.). During the course of the month-long field campaign, researchers noted little rain fall and visibly observed dust events. Among the instruments deployed were those for ice nuclei enumeration and composition and aerosol enumeration, composition, and sizing. This chapter will focus on results from the wideband integrated aerosol sensor and chemical analysis following collection onto high volume air filter samples.

4. 1. 3. Sampling Instruments

The WIBS used here was a WIBS-4A owned by the University of Denver. Recent publications have shown that there is a need for calibration and standardization amongst WIBS instruments and thus it is important to note the instrument used (Hernandez et al., 2016(Savage et al., 2017)). The WIBS-4A used here has the excitation and emission wavelength ranges of $\lambda_{\text{ex}}$ 280 nm, $\lambda_{\text{em}}$ 310–400 nm (FL1 or channel A); $\lambda_{\text{ex}}$ 280 nm, $\lambda_{\text{em}}$ 420–650 nm (FL2 or channel B); and $\lambda_{\text{ex}}$ 370 nm, $\lambda_{\text{em}}$ 420–650 nm (FL3 or channel C).

\textsuperscript{2} Ice Nuclei research Unit-Biogenic versus Anthropogenic emissions on Clouds and Climate: towards a Holistic Understanding - Aerosols, Clouds, Trace gases Research infrastructure
This differs slightly from the WIBS-3 used during the BEACHON-RoMBAS campaign, as discussed in chapter 3.

Additionally, a high volume air filter sampler with a total suspended particle inlet was operated at 1000 L min\(^{-1}\) on the roof of a trailer. Samples were collected for 24 hr time periods. The inlet for the WIBS was extended above the roof housing the WIBS and had a PM\(_{10}\) inlet at ~4.5 m above the ground. Data for the WIBS is on a single particle basis and then time averaged into 5 minutes intervals.

### 4.2 Methods

#### 4.2.1 Filter analysis

An overview of filter analysis will be presented briefly here. For more details, refer to Section 3.5.5.1. Filters were stored at 4 °C prior to analysis. The compounds of interest are of low volatility and are not expected to degrade at refrigerated temperatures (Zhang et al., 2015). Water extraction was performed on a portion of a filter such that the filter is submerged in 5 mL Nanopure\(^{TM}\) water within a centrifuge tube. Filters were then shaken for 10 minutes at 24 rpm, sonicated at 60 Hz for 30 minutes, and then shaken again for 10 minutes at 24 rpm. Extracts are then filtered through PETF (GE Healthcare, UK) filters at 0.45\(\mu\)m to remove dirt particles and any disintegrated filters pieces.

Instrument detection limits were determined as 3 standard deviations (\(\sigma\)) of the lowest calibration point on a standard linear curve. Method detection limits were determined as 3 \(\sigma\) of the lowest level of analyte recovered from a spiked filter. Recovery was between 95% ± 10 for all nine carbohydrates over 5 spike samples. The method for chromatographic separation is detailed in Section 3.3.5.1. Briefly, the extracts were
analyzed on Dionex ICS 5000+ (Thermo Fisher, Sunnyvale, CA, USA) with pulsed amperometric detection. Separation was achieved on a Dionex MA1 column utilizing 480 mM NaOH and sample injection volumes of 10 µL.

4. 2. 2. WIBS Categories and Sigma Values

Particle analysis for the WIBS data was performed in accordance with established methods by Gabey et al. (2010) and Perring et al. (2015). The threshold for consideration of fluorescent particles was first established by (Gabey et al., 2010) as baseline plus 3\(\sigma\) where the baseline is recorded as the forced triggering of the Xenon flash lamps when no particles are present. The WIBS outputs three fluorescent intensity values for each particle it detects: FL1, FL2, and FL3. One method for determining a particle type is by categorization based on fluorescence properties. Perring et al. (2015) defined 7 categories based on the three excitation and emissions channels. These categories are summarized in section 3.2.2 and in the supplemental table Figure A1. Recent work has been done by Savage et al. (2017) exploring fluorescent thresholds defined at baseline plus 3\(\sigma\), 6\(\sigma\), or 9\(\sigma\). This work concluded that threshold values of baseline plus 9\(\sigma\) removes most non-biological interfering particles such as dust and brown carbon without greatly impacting total particle counts for fluorescent particles. Additionally, Savage et al. (2017) extensively cataloged the category composition of more than 70 different particles ranging from fungal spore and bacteria to dust and brown carbon. Fungal spores are dominated by fluorescent particles that are type A, AB, and ABC. This thresholding scheme will be explored in correlations with various atmospheric carbohydrates. As in
section 3.2.2., WIBS number concentrations were converted to mass concentrations assuming spherical particles that have unity density.

4.3. Hypotheses

Based on previous work, several correlations analyses will be performed between atmospheric carbohydrate concentrations and various WIBS particle categories. We would expect the following:

- Carbohydrate atmospheric concentrations to be comparable to other dry environments.
- Approximate fungal number concentration can be determined from arabitol and mannitol concentration.
- Correlations will exist between known fungal tracers: mannitol, arabitol, erythritol and trehalose.
- Good correlations with WIBS categories A, AB, ABC with fungal tracers based on (Savage et al., 2017) and (Gosselin et al., 2016).
- Correlations will be enhanced by increasing threshold values from baseline plus $3\sigma$, $6\sigma$ and $9\sigma$ for A, AB, ABC categories.

4.4 Results

4.4.1 Atmospheric Carbohydrate Mass Concentrations

In total, the atmospheric concentration of ten water-soluble carbohydrates were determined for 26 filter samples (Table 4.1). Glucose and xylose coelute using the column and elution program utilized and are represented here as a single concentration. Average concentration values for all carbohydrates are similar to those reported during
the BEACHON campaign during dry periods. Reported alongside the mean of each carbohydrate is the variability within the sample population, represented as the standard deviation of values from individual filters. The estimation of error for each sample was estimated to be less than 15%, with error arising from the extraction method, analysis method and sampling techniques.

The magnitude of the carbohydrate concentration values observed in Cyprus fits well alongside BEACHON concentrations, which is to be expected given the dry conditions observed in both locations. The average humidity during the INIUT campaign was 33.5% ± 15.7 and was 58.7% ± 16.2 during the BEACHON campaign. Weather conditions observed in Cyprus were similar to the dry periods of the BEACHON campaign. The glucose concentration was much lower in Cyprus than observed during BEACHON, which is likely due to a generally lower concentration of biological aerosols, as glucose is associated with various vegetative emissions (Rathnayake et al., 2016).

Trehalose, a tracer that is associated with fungal spores, but also found in higher plants and algae, can represent as much as 90% of the dry biomass for dormant fungal spores (Feofilova, 2001). As a result of this, it is expected that trehalose mass would be much greater than arabitol and mannitol mass. In contrast to expectation, however, the mass of trehalose observed (6.51 ng m⁻³) was much lower than the concentration of arabitol and mannitol. Comparably based on the findings of Bauer et al. (2008a; 2008b), arabitol and mannitol each represent 3% of the total spore mass. While arabitol and mannitol are known to be thermally stable (Zhang et al., 2015), there has been little done to study the atmospheric lifetime and stability of trehalose. Arabinose and levoglucosan are not
present in large quantities and in a limited number of samples. Both have been shown to be biomass burning tracers and are greatly increased after forest fires (Medeiros et al., 2006). Biomass burning was not noted to have influenced the measurements during theINIUT campaign, so high concentrations of biomass burning tracers were not expected.

Table 4.1 Atmospheric mass concentrations (ng m\(^{-3}\)) of 10 carbohydrates with mean, standard deviation for sample variance and the number of samples in which the analyte was above the limit of quantification. ND is used to represent not detected.

<table>
<thead>
<tr>
<th>Atmospheric Concentration (ng m(^{-3}))</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>No. of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabitol</td>
<td>10.59</td>
<td>6.64</td>
<td>26</td>
</tr>
<tr>
<td>Mannitol</td>
<td>10.70</td>
<td>6.55</td>
<td>26</td>
</tr>
<tr>
<td>Erythritol</td>
<td>2.56</td>
<td>1.49</td>
<td>20</td>
</tr>
<tr>
<td>Levoglucosan</td>
<td>5.26</td>
<td>3.93</td>
<td>23</td>
</tr>
<tr>
<td>Arabinose</td>
<td>1.34</td>
<td>0.71</td>
<td>6</td>
</tr>
<tr>
<td>Trehalose</td>
<td>6.51</td>
<td>4.29</td>
<td>25</td>
</tr>
<tr>
<td>Glucose &amp; Xylose</td>
<td>13.91</td>
<td>10.00</td>
<td>26</td>
</tr>
<tr>
<td>Xylitol</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Fructose</td>
<td>20.04</td>
<td>11.33</td>
<td>17</td>
</tr>
</tbody>
</table>

The concentrations of arabitol and mannitol are remarkably similar to those determined during the dry periods of the BEACHON campaign (Table 4.2). Additionally, the ratio of mannitol to arabitol (1.01) is similar to previous studies in semi urban environments (Bauer et al., 2008a; Gosselin et al., 2016; Rathnayake et al., 2016; Yttri et al., 2007). Mannitol and arabitol are strongly correlated with one another here, indicating
a common source (Figure 4.3). The fungal tracer erythritol was also found to be correlated with mannitol ($R^2=0.41$) and arabitol ($R^2 = 0.61$), indicating similar sources. Erythritol is known to also be present in algae and lichen while arabitol is not (Lewis and Smith, 1967).

As in BEACHON, an estimate of the number of fungal spores present in the air sampled in Cyprus can be calculated from the arabitol and mannitol concentrations. Using the conversion factor of 1.2 pg spore$^{-1}$ and 1.7 pg spore$^{-1}$ for arabitol and mannitol, respectively, the estimates for atmospheric fungal spores are $8800 \pm 5500$ spores m$^{-3}$ and $6300 \pm 3600$ spores m$^{-3}$ from arabitol and mannitol calculations.

Table 4.2 Comparison of atmospheric concentrations of five carbohydrates that were quantified during the BEACHON and INIUT campaigns with mean, standard deviation, and number of samples.

<table>
<thead>
<tr>
<th>Atmospheric Concentration (ng m$^{-3}$)</th>
<th>CO Rainy</th>
<th>CO Dry</th>
<th>Cyprus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabitol</td>
<td>35.2 ± 10.5 (n=11)</td>
<td>10.6 ± 2.5 (n=18)</td>
<td>10.6 ± 6.6 (n=26)</td>
</tr>
<tr>
<td>Mannitol</td>
<td>44.9± 13.8 (n=11)</td>
<td>11.9 ± 3.2 (n=16)</td>
<td>10.7 ± 6.6 (n=26)</td>
</tr>
<tr>
<td>Erythritol</td>
<td>1.12 ± 0.38 (n=3)</td>
<td>0.84 ± 0.610 (n=18)</td>
<td>2.56 ± 1.49 (n=20)</td>
</tr>
<tr>
<td>Levoglucosan</td>
<td>12.4 ± 19.1 (n=8)</td>
<td>14.2 ± 10.7 (n=15)</td>
<td>5.26 ± 3.93 (n=23)</td>
</tr>
<tr>
<td>Glucose</td>
<td>73.2 ± 50.5 (n=11)</td>
<td>38.7 ± 21.3 (n=18)</td>
<td>13.9 ± 10.0 (n=26)</td>
</tr>
</tbody>
</table>
4.4.1 Carbohydrates and Fluorescent Particles

Correlations were made between the observed mass concentrations of each carbohydrate detected and WIBS fluorescent particle mass (µg m⁻¹) for each of the seven fluorescent particle types (A, B, C, AB, AC, ABC). For WIBS particle type analysis a threshold of baseline plus 3σ was utilized. Arabitol and mannitol show the best correlation with B and C type particles when a 3σ threshold is applied (Figure 4.4). This is inconsistent with the findings reported by Savage et al. (2017) which showed that fungal spores are typically characterized as type A, AB, and ABC particles. This trend is also seen with the other fungal tracers, erythritol, and trehalose (Appendix B). Upon comparing arabitol and mannitol with the fluorescent particle data analyzed using a threshold definition of baseline plus 6σ, correlations with A and AB particles were improved and those with B
and C particles were decreased (Figure 4.5). This trend of less correlation with B and C particles while correlations with A and AB particles improves can be seen in both WIBS particles analyzed with a threshold of baseline plus 9σ and baseline plus 12σ (Figures 4.6, 4.7). The decrease of correlation for B and C type particles between 9σ and 12σ is marginal and 9σ is sufficient to remove interfering particles.

Fungal spores are typically less than 10 µm in diameter, with most being between 2 -4 µm (Reponen et al., 1996). It is instructive to analyze the correlations of the fungal tracers, arabitol and mannitol, with WIBS fluorescent particles smaller than 10 µm in size. Upon analysis, it was found that there was no correlation between WIBS A and AB type particles below 10 µm and the fungal tracers, arabitol and mannitol (Figure 4.8 a,b). When the same fungal tracers are compared for particles great than 10 µm, the correlations seen in the total particle mass correlations become apparent. While there very few particles that make up the larger particle fraction, the correlation is driven by these large particles. Additionally, the particle mass below 10 µm is less than the arabitol and mannitol atmospheric mass concentration.
Figure 4. 2 Atmospheric arabitol and mannitol concentration (ng m\(^{-3}\)) correlated with WIBS fluorescent particle mass (µg m\(^{-3}\)) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles, (f) BC type particles, and (g) ABC type particles. R\(^2\) value shown for each fit in a-g. Threshold values are baseline plus 3\(\sigma\).

Figure 4. 3 Atmospheric arabitol and mannitol concentration (ng m\(^{-3}\)) correlated with WIBS fluorescent particle mass (µg m\(^{-3}\)) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles, (f) BC type particles, and (g) ABC type particles. R\(^2\) value shown for each fit in a-g. Threshold values are baseline plus 6\(\sigma\).
Figure 4.4 Atmospheric arabitol and mannitol concentration (ng m$^{-3}$) correlated with WIBS fluorescent particle mass (µg m$^{-3}$) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles, (f) BC type particles, and (g) ABC type particles. $R^2$ value shown for each fit in a-g. Threshold values are baseline plus 9σ.

Figure 4.5 Atmospheric arabitol and mannitol concentration (ng m$^{-3}$) correlated with WIBS fluorescent particle mass (µg m$^{-3}$) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles, (f) BC type particles, and (g) ABC type particles. $R^2$ value shown for each fit in a-g. Threshold values are baseline plus 12σ.
**Figure 4.** Atmospheric arabitol and mannitol concentration (ng m⁻³) correlated with WIBS fluorescent particle mass (µg m⁻³) (a) A type particles smaller than PM₁₀, (b) AB type particles smaller than PM₁₀; (c) A type particles greater than PM₁₀; and (d) AB type particles greater than PM₁₀. R² value shown for each fit in a-d. Threshold values are baseline plus 9σ.

4.5 Conclusions

The carbohydrate analysis in Cyprus revealed that there was likely very little contribution of biogenic particles to the overall aerosol mass concentration in the area. This was confirmed by the low concentration of fluorescent particles. The similarities between dry conditions during the BEACHON campaign and the INIUT campaign indicate the presence of very few wet discharge fungal spores. This represents the first use of increased threshold values during a field campaign and showed that the increased thresholding removed non-biological fluorescent particles, thereby improving correlations between fluorescent particles and fungal tracers. Additionally, estimates of fungal spores calculated from arabitol and mannitol in an arid environment may indicate
a global background level of arabitol and mannitol. Wind direction and back trajectory modeling was conducted, but not analyzed in conjunction with the carbohydrate analysis.
CHAPTER FIVE: CONCLUSION

Over the course of two separate field campaigns, UV-LIF instruments and high volume air samples were collected at two different sites, Manitou Experimental Forest outside of Colorado Springs, CO and Agia Marina Xyliatou Atmospheric Station, near Nicosia, Cyprus. The UV-LIF instruments, namely the WIBS, was utilized to characterize fluorescent particles in real time with the application of two separate analysis types, particle clustering and threshold variation. Additionally, ion chromatography was used to quantify atmospheric molecular tracers. These two campaigns were the first to compare fluorescent particles with water soluble carbohydrates and to subsequently estimate atmospheric fungal spore concentrations.

The BEACHON-RoMBAS campaign was the first to quantify fungal tracers and directly compare them with fluorescent particle mass from the WIBS and UV-APS. The clustering analysis done by collaborators at the University of Manchester showed excellent correlation between particles identified as fungal spores and the fungal tracers, arabitol and mannitol. These correlations were enhanced when sampling days were separated into rainy and dry. When the fungal tracers were converted into fungal spores, based on conversion factors established by Bauer et al. (2008a), and compared with the WIBS fungal cluster resulting in estimates that were only 13% different.
The campaign in Cyprus was the first to utilize different thresholding values for the analysis of WIBS fluorescent particle data. Again, water soluble carbohydrates were quantified. A total of nine carbohydrates were quantified and when compared to other regions and field campaigns were similar to arid environments. Specifically, arabitol and mannitol were nearly identical to the concentrations found in dry samples during the BEACHON campaign. When thresholding values were applied to the fluorescent particles detected by the WIBS, correlations were improved with the fungal tracers, arabitol, mannitol, trehalose, and erythritol, for fluorescent particle types associated with fungal spores, such as A, AB, and ABC. These thresholding values also decreased the number of interfering non-biological fluorescent particles. Particles greater than 10 µm were responsible for the correlations of fluorescent particles with various tracers.

In general, improvements in the chemical and physical characterization of bioaerosols have allowed for greater time- and size- resolution of atmospheric particles. Additionally, the transition away from physical means of enumerating fungal spores such as culture and microscopy samples has allowed for faster samples analysis and greater accuracy in quantifying fungal spores. This ease of measurement and high accuracy is ideal for contributing to global climate models that are still lacking fungal data.

Future work specifically on these projects include:

1. Anion and cation quantification using ion chromatography for the Cyprus samples.
2. Application of clustering algorithms to the WIBS data from the Cyprus campaign.
3. Exploration of wind speed and direction in conjunction with cation and anion concentrations.

4. Determination of sources using crustal tracer $\text{Ca}^{2+}$ and marine tracer $\text{Na}^{+}$.

5. Investigation of genomic sampling and microscopy fungal samples.

The field of molecular tracers and the use of ion chromatography for their determination would profit from extensive study in the field of mycology. Bauer et al. (2008) represents the only study with suggested conversion factors for molecular tracers to spores. As such:

1. Lab characterization of fungal spores and water soluble carbohydrates after the aerosolization for fungal spores and subsequent air filter sampling.

2. This study will need to span species along with growing conditions.

The increasing number of campaigns utilizing the WIBS and other new technologies leads to the need for investigation of:

1. Standardization of fluorescent calibration as currently there is no method to ensure standard detection of fluorescent properties.

2. Possible improvements in WIBS and other technologies that can increase spectral resolution thus requiring new ways to process and handle the dramatic increase in fluorescence information.
REFERENCES


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Morris, C., Sands, D., Glaux, C., Samsatly, J., Asaad, S., Moukahel, A., Goncalves, F. L. T., and Bigg, E.: Urediospores of rust fungi are ice nucleation active at $>\text{10} \degree\text{C}$ and harbor ice nucleation active bacteria, Atmospheric Chemistry and Physics, 13, 4223-4233, 2013.


Spracklen, D. and Heald, C. L.: The contribution of fungal spores and bacteria to regional and global aerosol number and ice nucleation immersion freezing rates, Atmospheric Chemistry and Physics, 14, 9051-9059, 2014.


**APPENDIX A: SUPPLEMENT FOR CHAPTER 3**

Table A.1. Summary information for each hi-volume filter sample including: start and stop times (local time; U.S. date format m/dd/yyyy), average air temperature, relative humidity, rain amount (normalized to 2.0) leaf wetness, number ratio of fluorescent particles from the UV-APS, and wetness category determined as described in Section 3.1. Star symbol (*, first column) indicates samples used in fungal DNA determination. Cross symbol (†, last column) indicates that category assignment was manually changed from the algorithm determination (original category in parentheses). Nf represents the number of fluorescent particles, Ntot represents the number of total particles as measured by the UV-APS.

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<th>Rain Amount (Normalized)</th>
<th>Leaf Wetness (mV)</th>
<th>FAP Number Ratio (Nf/Ntot)</th>
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Table A. 2 Linear equation fit parameters for Rainy and Dry conditions for Figure 3.5c-f. Each equation represents the linear trend line for correlations of arabitol (3.5c,e) or mannitol (3.5d,f) with UV-APS FAP mass (3.5c,d) or WIBS Cl 1 FAP mass (3.5e,f).

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Table A. 3. Linear equation fit parameters for Rainy and Dry conditions for Figure 3.6d-i. Each equation represents the linear trend line for correlations of estimated fungal spores (m-3) from (3.6d,g) arabitol, (3.6e,h) mannitol or (3.6f,i) colony forming units (CFU) with (3.6d,e,f) UV-APS FAPs or (3.6g,h,i) WIBS Cl 1 FAPs.

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Table A. 4 Percentage of particle mass in various UV-LIF instrument categories and total particle mass. Each mass value compared to total particle mass, determined using UV-APS number size distributions, converted to a mass for particles of aerodynamic diameter 0.5 – 10 µm and using particle mass density of 1.5 g cm⁻³. WIBS particles were integrated into total number over the same size range in optical diameter and using unity density. Ranges shown are standard deviation of 5-minute time averages.

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<th>Rainy</th>
<th>Other</th>
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<td>2.15 ± 1.38</td>
<td>16.98 ± 10.14</td>
<td>4.03 ± 3.42</td>
</tr>
<tr>
<td>Cl2</td>
<td>4.72 ± 1.43</td>
<td>6.01 ± 1.57</td>
<td>6.68 ± 2.38</td>
</tr>
<tr>
<td>Cl3</td>
<td>19.92 ± 5.81</td>
<td>13.22 ± 5.78</td>
<td>23.79 ± 10.60</td>
</tr>
<tr>
<td>Cl4</td>
<td>4.44 ± 1.64</td>
<td>8.83 ± 3.73</td>
<td>6.53 ± 3.45</td>
</tr>
<tr>
<td>FL 1</td>
<td>8.42 ± 3.37</td>
<td>62.05 ± 35.10</td>
<td>24.70 ± 23.61</td>
</tr>
<tr>
<td>FL 2</td>
<td>18.51 ± 4.02</td>
<td>71.55 ± 31.34</td>
<td>38.26 ± 24.77</td>
</tr>
<tr>
<td>FL 3</td>
<td>36.79 ± 6.26</td>
<td>85.95 ± 28.23</td>
<td>61.77 ± 28.29</td>
</tr>
<tr>
<td>FL</td>
<td>38.01 ± 6.34</td>
<td>87.99 ± 28.53</td>
<td>64.92 ± 30.66</td>
</tr>
<tr>
<td>UV-APS FAP</td>
<td>25.53 ± 2.99</td>
<td>51.50 ± 14.83</td>
<td>32.87 ± 9.45</td>
</tr>
</tbody>
</table>

| Total Particle Mass (µg m⁻³) | UV-APS Total | 3.70 ± 1.11 | 2.70 ± 0.58 | 4.85 ± 2.56 |

Figure A. 1. Particle type assignment strategy for WIBS data. Particle category type defined as fluorescent in a given channel when the fluorescence intensity (I) in channel FL1, FL2, or FL3 is greater than the threshold value, defined as blank + 3SD. Colors correspond to particle type used also in Figures 2-3.
Figure A. 2 Atmospheric arabitol concentration (ng m$^{-3}$) correlated with WIBS fluorescent particle mass (µg m$^{-3}$) (a) any fluorescent particle, FL; (b) particles fluorescent in channel 1, FL1; (c) particles fluorescent in channel 2, FL2; (d) particles fluorescent in channel 3, FL3. $R^2$ value shown for each fit in a-d. Linear fit parameter are shown in the table below.

<table>
<thead>
<tr>
<th>Figure</th>
<th>Linear Fit Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rainy</td>
</tr>
<tr>
<td>A2.a</td>
<td>$y=7.1x+2.4$</td>
</tr>
<tr>
<td>A2.b</td>
<td>$y=5.4x+16.7$</td>
</tr>
<tr>
<td>A2.c</td>
<td>$y=6.6x+9.2$</td>
</tr>
<tr>
<td>A2.d</td>
<td>$y=7.2x+2.4$</td>
</tr>
</tbody>
</table>

Linear equation fit parameters for Rainy and Dry conditions for Figure S2a-d. Each equation represents the linear trend line for correlations of arabitol (ng m$^{-3}$) with WIBS fluorescent channel particle mass (µg m$^{-3}$). (a) any fluorescent particle, FL; (b) particles fluorescent in channel 1, FL1; (c) particles fluorescent in channel 2, FL2; (d) particles fluorescent in channel 3, FL3.
Figure A. 3 Atmospheric mannitol concentration (ng m$^{-3}$) correlated with WIBS fluorescent particle mass (µg m$^{-3}$) (a) any fluorescent particle, FL; (b) particles fluorescent in channel 1, FL1; (c) particles fluorescent in channel 2, FL2; (d) particles fluorescent in channel 3, FL3. R$^2$ value shown for each fit in a-d. Linear fit parameter are shown in the table below.

<table>
<thead>
<tr>
<th>Figure</th>
<th>Linear Fit Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rainy</td>
</tr>
<tr>
<td>A3.a</td>
<td>y=11.3x-7.5</td>
</tr>
<tr>
<td>A3.b</td>
<td>y=8.3x+16.4</td>
</tr>
<tr>
<td>A3.c</td>
<td>y=10.3x+4.9</td>
</tr>
<tr>
<td>A3.d</td>
<td>y=11.5x-7.4</td>
</tr>
</tbody>
</table>

Linear equation fit parameters for Rainy and Dry conditions for Figure S3a-d. Each equation represents the linear trend line for correlations of mannitol (ng m$^{-3}$) with WIBS fluorescent channel particle mass (µg m$^{-3}$). (a) any fluorescent particle, FL; (b) particles fluorescent in channel 1, FL1; (c) particles fluorescent in channel 2, FL2; (d) particles fluorescent in channel 3, FL3.
Figure A. 4 Atmospheric colony forming unit (CFU) concentration (CFU m⁻³) correlated with WIBS fluorescent particle (m⁻³) (a) any fluorescent particle, FL; (b) particles fluorescent in channel 1, FL₁; (c) particles fluorescent in channel 2, FL₂; (d) particles fluorescent in channel 3, FL₃. $R²$ value shown for each fit in a-d. Linear fit parameter are shown in the table below.

<table>
<thead>
<tr>
<th>Figure</th>
<th>Rainy Linear Parameters</th>
<th>Dry Linear Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>S4.a</td>
<td>$y=0.003x+124$</td>
<td>$y=0.003x+86$</td>
</tr>
<tr>
<td>S4.b</td>
<td>$y=0.005x+138$</td>
<td>$y=0.009x+189$</td>
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<tr>
<td>S4.c</td>
<td>$y=0.004x+113$</td>
<td>$y=0.006x+122$</td>
</tr>
<tr>
<td>S4.d</td>
<td>$y=0.003x+118$</td>
<td>$y=0.003x+84$</td>
</tr>
</tbody>
</table>

Linear equation parameters for Rainy and Dry conditions for Figure S4a-d. Each equation represents the linear trend line for correlations of colony forming units (CFU m⁻³) with WIBS fluorescent channel particles (m⁻³). (a) any fluorescent particle, FL; (b) particles fluorescent in channel 1, FL₁; (c) particles fluorescent in channel 2, FL₂; (d) particles fluorescent in channel 3, FL₃.
APPENDIX B: SUPPLEMENTAL FIGURES FOR CHAPTER 4

Figure B. 1 Atmospheric erythritol concentration (ng m⁻³) correlated with WIBS fluorescent particle mass (µg m⁻³) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles, (f) BC type particles, and (g) ABC type particles. $R^2$ value shown for each fit in a-g. Threshold values are baseline plus 3 $\sigma$.

Figure B. 2 Atmospheric levoglucosan (ng m⁻³) correlated with WIBS fluorescent particle mass (µg m⁻³) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles, (f) BC type particles, and (g) ABC type particles. $R^2$ value shown for each fit in a-g. Threshold values are baseline plus 3 $\sigma$. 

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Figure B. 3 Atmospheric fructose concentration (ng m\(^{-3}\)) correlated with WIBS fluorescent particle mass (µg m\(^{-3}\)) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles,(f) BC type particles, and (g) ABC type particles. \(R^2\) value shown for each fit in a-g. Threshold values are baseline plus 3 \(\sigma\).

Figure B. 4 Atmospheric glucose concentration (ng m\(^{-3}\)) correlated with WIBS fluorescent particle mass (µg m\(^{-3}\)) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles,(f) BC type particles, and (g) ABC type particles. \(R^2\) value shown for each fit in a-g. Threshold values are baseline plus 3 \(\sigma\).
Figure B. 5 Atmospheric trehalose concentration (ng m$^{-3}$) correlated with WIBS fluorescent particle mass (µg m$^{-3}$) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles, (f) BC type particles, and (g) ABC type particles. $R^2$ value shown for each fit in a-g. Threshold values are baseline plus 3 $\sigma$.

Figure B. 6 Atmospheric arabinose concentration (ng m$^{-3}$) correlated with WIBS fluorescent particle mass (µg m$^{-3}$) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles, (f) BC type particles, and (g) ABC type particles. $R^2$ value shown for each fit in a-g. Threshold values are baseline plus 3 $\sigma$. 
Figure B. 7 Atmospheric erythritol concentration (ng m⁻³) correlated with WIBS fluorescent particle mass (µg m⁻³) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles,(f) BC type particles, and (g) ABC type particles. R² value shown for each fit in a-g. Threshold values are baseline plus 6 σ.

Figure B. 8 Atmospheric trehalose concentration (ng m⁻³) correlated with WIBS fluorescent particle mass (µg m⁻³) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles,(f) BC type particles, and (g) ABC type particles. R² value shown for each fit in a-g. Threshold values are baseline plus 6 σ.
Figure B. 9 Atmospheric erythritol concentration (ng m$^{-3}$) correlated with WIBS fluorescent particle mass (µg m$^{-3}$) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles,(f) BC type particles, and (g) ABC type particles. $R^2$ value shown for each fit in a-g. Threshold values are baseline plus 9 $\sigma$.

Figure B. 10 Atmospheric trehalose concentration (ng m$^{-3}$) correlated with WIBS fluorescent particle mass (µg m$^{-3}$) (a) A type particles (b) B type particles; (c) C type particles; (d) AB type particles, (e) AC type particles,(f) BC type particles, and (g) ABC type particles. $R^2$ value shown for each fit in a-g. Threshold values are baseline plus 9 $\sigma$. 

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APPENDIX C: BEACHON-ROMBAS SIZE DISTRIBUTION PROJECT

The following appendix lists initial work toward analysis of data that was intended for comparison with results presented in Chapter 3. We determined that these results never gelled into an obviously coherent story, and so they were not included in previous work. A summary of plots and analysis are presented here as a record of my work.

From the BEACHON-ROMBAS campaign there were also Andersen samplers and slit samplers. Andersen samplers operate similar to the slit samplers what were discussed in Section 3.2.4 Slit sampler. Andersen samplers have various size cuts that allow for size resolution that is not possible with the slit samplers. Both methods are direct-to-agar methods. As such, they are limited in their ability to quantify all fungal or bacterial particles. Typically direct-to-agar methods under count the number concentration of atmospheric bacteria or fungal spores by an order of magnitude (Gonçalves et al., 2010; Pyrri and Kapsanaki-Gotsi, 2007). The investigation of a subset of the samples collected during the BEACHON campaign revealed a diurnal pattern that is consistent with fungal spores and patterns of PBAP at the BEACHON site (Huffman et al., 2013; Schumacher et al., 2013). There are two mechanisms/ reasons for the diurnal pattern of fungal spores. First, fungal spores are susceptible to radiation and in periods of intense sunlight, like during a summer day, their survivability decreases. Second, fungal spores are typically dispersed during periods of rain or high relative humidity. The formation of dew overnight and elevated levels of relative humidity after the sun goes
down results in the active dispersal of fungal spores during the night. The following figures represent a subset of the samples that were taken during the BEACHON campaign. These samples are highlighted as they were taken every four hours over the course of 24 hours for a total of 6 samples. There were only two day in which sampling every four hours occurred. As seen in Figures C. 1, 2,3,4,5, and 6, there is a diurnal pattern that emerges. Figure C.1 is an Andersen sample taking for 20 minutes at 8 am. This sample has the second lowest concentration of colony forming units (CFU) during the day of intensive sampling. The lowest CFU concentration occurred at noon when solar radiation is typically the greatest. Concentrations of fungal spore begin to recover as the sunsets (Figures C.3, C.4) and during the overnight samples (Figure C.5, C.6), CFU concentrations are the greatest.

![Figure C. 1 Size distribution for Andersen sample 6 at 8 am, July 31, 2011. Colony forming units are report along the left y-axis, pigmented and non-pigmented fungi along with bacteria. UV-APS and WIBS fluorescent particles on the right y-axis. The WIBS fluorescent particles are all assigned to Cluster 1.](image-url)

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Figure C. 2 Size distribution for Andersen sample 7 at 12 pm, July 31, 2011. Colony forming units are report along the left y-axis, pigmented and non-pigmented fungi along with bacteria. UV-APS and WIBS fluorescent particles on the right y-axis. The WIBS fluorescent particles are all assigned to Cluster 1.

Figure C. 3 Size distribution for Andersen sample 8 at 4 pm, July 31, 2011. Colony forming units are report along the left y-axis, pigmented and non-pigmented fungi along with bacteria. UV-APS and WIBS fluorescent particles on the right y-axis. The WIBS fluorescent particles are all assigned to Cluster 1.
Figure C. 4 Size distribution for Andersen sample 9 at 8 pm, July 31, 2011. Colony forming units are reported along the left y-axis, pigmented and non-pigmented fungi along with bacteria. UV-APS and WIBS fluorescent particles on the right y-axis. The WIBS fluorescent particles are all assigned to Cluster 1.

Figure C. 5 Size distribution for Andersen sample 10 at 12 am, August 1, 2011. Colony forming units are reported along the left y-axis, pigmented and non-pigmented fungi along with bacteria. UV-APS and WIBS fluorescent particles on the right y-axis. The WIBS fluorescent particles are all assigned to Cluster 1.
To illustrate the observed link with fungal spores and radiation, spore watch data was compared with IR data. A spore watch is a rotating impactor that collects fungal spores, either viable or not, on a sticky film. This can then be time resolved and analyzed to determine fungal spore taxa and atmospheric concentration. The use of a spore watch or spore trap require a highly skilled technician for analysis post collection in the identification process and similar to the direct-to-agar samplers undercount the true atmospheric fungal concentration. Figures C.7 and C.8 illustrate how fungal respond to increasing IR. It is known the Cladosporium produces a fungal spore that is dark in color. This adaptation is proposed to have occurred to increase the spores’ survivability during exposure to sunlight. Figure C.7 shows the concentrations of various fungal taxa and the
total of fungal spores without Cladosporium. There is a clear relationship (Figure C.7) between the decrease in IR and the rebounding of the fungal spore concentration.

If however the concentration of Cladosporium is added back to the figure (Figure C.8), the rebound that occurs after the IR decreases is not as great as the majority of fungal spores are Cladosporium and they are able to survive the increases in IR that occur during the day.
Figure C. 8 Spore Concentration during July 31 at 9am through August 1 at 5 am, 2011 at the BEACHON-ROMBAS site. Spore concentration is represented as spores m$^{-3}$ from spore trap measurements (left axis) and colony forming units as CFU m$^{-3}$ (right axis). Additionally, radiation intensity (RI) is plotted as W m$^{-2}$ (left axis). This graph shows spore data from the spore trap with Cladosporium factored into the total spores m$^{-3}$. 
APPENDIX D: PROTEIN NITRATION AND HPLC

The goal of this project was to replicate the results achieved by Yang et al. (2010) for a method that used high performance liquid chromatography (HPLC) with ultraviolet-visible (UV-vis) light detection to determine how nitrated a protein. In the paper by Yang et al. (2010), the proteins Bovine Albumin Serum and Ovium Albumin Serum were utilized. These proteins were then nitrated with various molar ratios of tetranitromethane (TNM)/tyrosine (Tyr). Total protein concentration was determined using a bicinchoninic acid assay (BCA) (Sigma Aldrich, St. Louis, MO) as this does not rely on the light properties of native tyrosine to quantify proteins. Quantification of proteins using the BCA assay is done at 562 nm. Nitrated tyrosine can be detected in a basic solution (pH 10) at 452 nm (Figure 1). The average fraction of tyrosine in the protein that have been nitrated can then be determined. This is referred to as nitration degree (ND). In a complex mixture of proteins, such as a tissue sample or atmospheric sample, proteins cannot be separated from each other and thus only a bulk measure of ND can be determined for all of the proteins in the sample with using a basic UV-Vis technique. Employing a HP-LC-UV-Vis allows for separation of protein and then subsequent detection of the nitrated tyrosine residues. This technique was utilized and proof of concept was achieved.

Separation was performed using Discovery® BIO Wide Pore C18 HPLC Column 5 μm particle size, L × I.D. 25 cm × 2.1 mm (Supelco, Bellefonte, Pennsylvania). Figure 2 shows the first attempt to build a calibration curve for A357/A280 with respect to the ND of the BSA molecule. This attempt did not span the full range of ND for BSA and another calibration curve was produced (Figure 3). The degree to which BSA can be nitrated is
limited to 14 out of the 21 Tyr residues or 60%. The determined ND for the calibration standards that were produced exceeded 60% and were thus considered invalid. The production of calibration standards with varying degrees of nitration is a process that must be done in the lab as there are no suppliers that report the number of nitrated tyrosine residues on their nitrated BSA products nor do they offer a selection of varying degrees of nitrated BSA. The project was not continued due to lack of availability to the HPLC-UV-vis.

Figure D. 1 UV-Vis absorbance spectra of the amino acid tyrosine and its nitrated variants at pH 3.5 and 10. Native tyrosine in an acid buffer has an absorbance maximum at 280 nm. Nitrated tyrosine at acid pH has an absorbance maximum at 350 nm and at basic pH a maximum absorbance at 425 nm. Figure from (Yang et al., 2010).

![UV-Vis absorbance spectra of tyrosine and nitrated variants](image-url)
Figure D. 2 Calibration curve with Bovine Albumin Serum nitrated to various degrees (x axis) with corresponding ratio of absorbance (357 nm/280 nm) from HPLC-UV-VIS (y axis).

Figure D. 3 Calibration curve with Bovine Albumin Serum nitrated to various degrees (x axis) with corresponding ratio of absorbance (357 nm/280 nm) from HPLC-UV-VIS (y axis). This was attempt two that showed nitration degrees outside of the applicable range.
APPENDIX E: IGOR TOOLS

The following represent a compilation of the various general tools and functions I wrote in Igor Pro (Wavemetrics, 1999, Portland, OR). Prior to each function is a description of the purpose of the function.

**ConvertNumberMxToMass** was designed to convert a number matrix or two dimensional array into a mass matrix. This data is typically from WIBS or UV-APS, potentially other single particle instruments. The code calculates the particle mass by finding the volume of the particles under the assumption that the particle is spherical. The particle is then converted to volume to mass. The InputDensityVar variable allows the user to define the density. The output is picograms/air volume.

```
//******************************************************************************
Function ConvertNumberMxToMass(InputNumberMx, InputDiamWv, InputDensityVar)
  // Written 2015.07.31 by JAH
  Wave InputNumberMx // Must be: time = columns, size = rows
  Wave InputDiamWv
  Variable InputDensityVar
  Variable rowVar, colVar

  Duplicate/o InputNumberMx NewMassMx

  For (colVar = 0 ; colVar < dimsiz e(InputNumberMx, 1) ; colVar += 1)
    For (rowVar = 0 ; rowVar < numpnts(InputDiamWv) ; rowVar += 1)
      variable probe1 = inputdensityvar
      variable probe2 = inputdiamwv[rowvar]
      variable probe3 = inputnumbermx[rowvar][colVar]
      NewMassMx[rowVar][colVar] = (4*pi*InputDensityVar/3) * (InputDiamWv[rowVar]/2)^3 * (InputNumberMx[rowVar][colVar])
    Endfor
  Endfor

End Function
//******************************************************************************
```
**BatchAverage** was designed to calculate whisker plot values including mean, median and percentiles for multiple data ranges.

```plaintext
//******************************************************************************
Function BatchAverage(WaveToAverage, FromPtWv, ToPtWv, SampleNameWv)
   // Written 2015.07.31 by JAH
   // Purpose: To average a given wave (e.g. time series) into discreet values, but do some many times automatically
   Wave WaveToAverage, FromPtWv, ToPtWv
   Wave/t SampleNameWv
   Variable n
   For (n = 0 ; n < numpnts(FromPtWv) ; n += 1)
      CalcWhiskerValuesRange(WaveToAverage, SampleNameWv[n], FromPtWv[n], ToPtWv[n])
   Endfor
End Function
//******************************************************************************
```

**MarieFunction** performs BatchAverage and compiles the mean, median, and standard deviation into a single array.

```plaintext
//******************************************************************************
Function MarieFunction(WaveToAverage, FromPtWv, ToPtWv, SampleNameWv)
   // Written 2015.07.31 by JAH
   // Purpose: To collect data run from BatchAverage(..) into one wave
   Wave WaveToAverage, FromPtWv, ToPtWv
   Wave/t SampleNameWv
   BatchAverage(WaveToAverage, FromPtWv, ToPtWv, SampleNameWv)
   Duplicate/o FromPtWv MeanWv, MedianWv, StDevWv
   For (n = 0 ; n < numpnts(FromPtWv) ; n += 1)
      String GoGetStr = SampleNameWv[n] + "Wv"
      Wave GotGetWv = $GoGetStr
      MeanWv[n] = GotGetWv[0]
      MedianWv[n] = GotGetWv[1]
   Endfor
End Function
//******************************************************************************
```
**CalcCalCurveandUK** utilizes x and y values for a calibration for known and signal values. Values are fit to a linear trend line. Samples values are also plotted and the x value is determine. Additionally, a graph is created with the calibration curve and the samples.

```plaintext
//******************************************************************************
Function CalcCalCurveandUK(CalYValues, CalXValues, UnknownValues, UnknownNameWv)
    // Written 2015.12.4 MIG
    // Purpose: find and plot unknown values along a calibration curve
    // Primarily for UV-Vis or Fluorescence work
    Wave CalYValues, CalXValues // y values and x values from the calibration curve
    Wave UnknownValues // unknown y values
    Wave t UnknownNameWv // labels for the unknown values. needs to be letters
    Variable n=numpnts(UnknownValues)
    CurveFit/M=2/W=0/Q line, CalYValues/X=CalXValues/D
    Wave w_coef
    Make/o/n=(n) UnknownConc = 0
    UnknownConc = unknownvalues - w_coef[0]
    UnknownConc /= w_coef[1]
    String name = "fit_ " + NameOfWave(CalYValues)
    string y = NameOfWave(CalYValues)
    string uk = NameOfWave(UnknownValues)
    Display /W=(582,317.75,1041.75,680 ) CalYValues vs CalXValues
    AppendToGraph $name
    AppendToGraph UnknownValues vs UnknownConc
    ModifyGraph mode($y)=3, mode($uk)=3
    ModifyGraph marker($y)=19
    ModifyGraph rgb($y)=(0,0,0), rgb($uk)=(0,15872,65280)
    ModifyGraph textMarker($uk)={UnknownNameWv,"default",1,0,5,0.00,0.00}
    Label left "Absorbance or Fluorescence "
    Label bottom "Concentration"
    Legend/X=2.06/Y=80.80
End Function

//**********************************************************************

**NumberRatioCatThresholds** is an algorithm developed to determine if a sample time period should be considered wet, dry, dew or other. This is the algorithm that is used to determine sample category in chapter three and the Gosselin et al., 2016 publication.

```
// Added threshold values for rain, leafwetness and UV-APS number ratio

Wave RainWv, LeafwetnessWv // normalized rainwv from tipping bucket and disdrometer, leafwetness in mv
Wave NumberRatioWv
Variable LWThresholdVar // need to determine prior in mv
Variable RThresholdVar // rain threshold variable to eliminate areas of rain with no corresponding increase in fluorescence or RH
Variable NThresholdVar // to account for post rain periods of high fluorescence activity
Wave StartWv, StopWv, SampleNameWv // sample information for HiVol
Variable n
Variable m=numpnts(rainWv)
Make/o/n=(m) DryCat = 0
Make/o/n=(m) RainCat = 0
Make/o/n=(m) PostCat = 0
Make/o/n=(m) DewCat = 0

For (n=0; n <= numpnts(rainWv); n+=1)
    if (rainwv[n] > RThresholdVar) // if it is raining flag the rain cat wv with a one
        raincat[n]=1
    else
        if (NumberRatioWv[n] >= NThresholdVar)
            if (rainwv[n-1] > 0) // if the leafwetness variable is above the set threshold and it has been raining flag the post cat
                do
                    postcat[n]=1
                    n += 1
                while (NumberRatioWv[n] >= NThresholdVar && rainwv[n] == 0) // this will flag the post cat until the leafwetness has returned to threshold or it starts to rain
                if (rainwv[n] > 0 && rainwv[n] != 0) // this if applies to the n value that breaks the do-while loop (otherwise it does not get flagged)
                    raincat[n] = 1
                else
                    drycat[n] = 1
                endif
                else
                    dewcat[n]=1 // if the mv is above threshold and not raining then flag dewcat
                endif
            else
                DryCat[n]=1 // if it is not raining and mv is below the threshold flag the dry cat
            endif
        endif
    endif
Endfor

Variable j, k
j = numpnts(SampleNameWv)
Make/o/n=(j) drycat_frac
Make/o/n=(j) dewcat_frac
Make/o/n=(j) raincat_frac
Make/o/n=(j) postcat_frac

For (k = 0; k <= numpnts(SampleNameWv); k += 1) // this loop takes the flagged waves and sums and normalizes to get a fraction for each sample defined by the SampleNameWv
    Drycat_frac[k] = sum(drycat, StartWv[k], StopWv[k])/(StopWv[k]-StartWv[k]+1)
    Dewcat_frac[k] = sum(dewcat, StartWv[k], StopWv[k])/(StopWv[k]-StartWv[k]+1)
    Raincat_frac[k] = sum(raincat, StartWv[k], StopWv[k])/(StopWv[k]-StartWv[k]+1)
    Postcat_frac[k] = sum(postcat, StartWv[k], StopWv[k])/(StopWv[k]-StartWv[k]+1)
Endfor

End Function

//**********************************************************************
APPENDIX F: DIONEX ICS 5000+ PARTS

This appendix includes information regarding parts that have been removed and replaced in the DIONEX ICS 500+. Additionally, explanations for the cause for removal and potentiality of the part being able to operate again is included.

(1) pH probe: Removed 10/2016

Reason for replacement: pH under basic conditions of the NaOH eluent were being read as acidic (<7). When calibration was attempted, error of unable to calibrate was reported. Unsure of reason for the malfunctioning part.

(2) Electrochemical detector block: Removed 03/2017

Reason for replacement: corroded pH inlet. This is likely due to operation of the EC at low flow rates. The system should be run at analysis flow rates. If there is no analysis for more than a week, the system should be shut down and put into long term storage.

(3) Conductivity Detector: Removed 04/2017

Reason for replacement: detector was leaking. After the part was replaced, casing was opened and revealed that internal tubing was broken. The unit would likely be functional if the tubing was replaced.

(4) Quaternary Analytical Pump: Removed 04/2017
Reason for replacement: pump stopped working. Upon inspection by field tech, rear pump seals were not clear and salt had precipitated out and crystalized. Rear seals must be manually washed once a month to prevent crystallization of salt solutions especially NaOH. The quaternary pump that was removed would likely be functional if the pump heads were thoroughly cleaned and the crystalized salt was removed.

(5) Vacuum pump and mini vacuum degasser: Removed 04/2017
Reason for replacement: was whistling and reported a degasser malfunction. Field tech could not determine the problem and elected to replace the vacuum pump and degasser. Upon inspection, the field tech believed that it was incorrectly reinstalled after the quaternary pump was replaced.