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Characterization of Ambient PM_{10} Bioaerosols in a California Agricultural Town

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ABSTRACT

Ambient bioaerosols in PM_{10} samples were measured at three sites in Corcoran, an agricultural town in the southern San Joaquin Valley (SJV) of California, during fall of 2000 corresponding to the cotton harvest season. Elevated bioaerosol concentrations were measured near grain elevators (GRA site) and a cotton handling facility (BAI site) as compared to levels in a residential community (COP site), ~2 km northeast of these sources. Average endotoxin levels (13 ± 17 EU/m^3) at the grain elevator site were three to eight times higher than averages at the nearby cotton-handling and residential sites. The highest level (47.6 EU/m^3) at the grain elevator site was about half of the exposure limit of 90 EU/m^3 set by the Dutch Expert Committee on Occupational Safety. Particle counts of fungal spore (66,333 particles/m^3) and pollen grain (2,600 particles/m^3) concentrations were more than double those reported in the literature. Average fungal biomarker concentrations of 170 and 131 ng/m^3 for arabitol and mannitol, respectively, were 1–2 orders of magnitude higher than those from non-agricultural areas. The low correlation ($r < 0.11$) of three fungal markers (i.e., (1→3)-β-D-glucan, arabitol, and mannitol) with fungi counts is consistent with findings by others and indicates that these are insufficient as surrogates to represent fungal exposure. Agricultural activities contributed measureable amounts to PM_{10} mass and organic carbon (OC), dominated by fungal spores (i.e., 5.4–5.8% PM_{10} mass and 11.5–14.7% OC). The sum of fungal spores, pollen grains, and plant detritus accounted for an average of 11–15% PM_{10} and 24–33% OC mass. Bioaerosols can be important contributors to PM_{10} mass in farming communities similar to Corcoran, especially during intense agricultural activities.

Keywords: Endotoxin; (1→3)-β-D-glucan; Fungal spores; Pollen grains; PM_{10} bioaerosol.

INTRODUCTION

Atmospheric bioaerosols (i.e., primary biological particles) have been associated with adverse health effects in humans and animals (Rengasamy *et al.*, 2004; Mauderly and Chow, 2008; Heederik and von Mutius, 2012; Caillaud *et al.*, 2014) and have potential roles in cloud formation and atmospheric chemistry (Sun and Ariya, 2006; Mohler *et al.*, 2007; Deguillaume *et al.*, 2008; Després *et al.*, 2012). Bioaerosols derive from natural and anthropogenic processes (Burrows *et al.*, 2009; Heald and Spracklen, 2009), and include bacteria, viruses, fungi, pollen, plant detritus, microalgae, protozoa, insect fragments, animal fur, and dander. Past studies have focused on occupational exposure in agricultural environments (Adhikari *et al.*, 2004; Liao and Chen, 2005; Lues *et al.*, 2007; Yuan *et al.*, 2010; Chien *et al.*, 2011; Pattey and Qiu, 2012), but little is known about human exposure and the effects on ambient air quality standards in population centers surrounding agricultural facilities.

This study characterizes PM_{10} (particles with aerodynamic diameters <~10 μm) bioaerosols at three neighborhood-scale sites (see Fig. 1) in the town of Corcoran on the edge of the Tulare dry lake in California's San Joaquin Valley (SJV). The SJV is a large agricultural area that produces cotton, oranges, grapes, almonds, milk, cattle, and poultry. The Corcoran area is dominated by production of Egyptian cotton and hosts major cotton processing facilities for the region. The Corcoran-Patterson (COP) site (CARB, 2015b) has measured PM_{10} compliance in the SJV airshed from 1996 to present, and has shown many 24-hour averages higher than the California state limit (50 μg/m^3) (CARB, 2015a). This study intends to: 1) assess ambient bioaerosol

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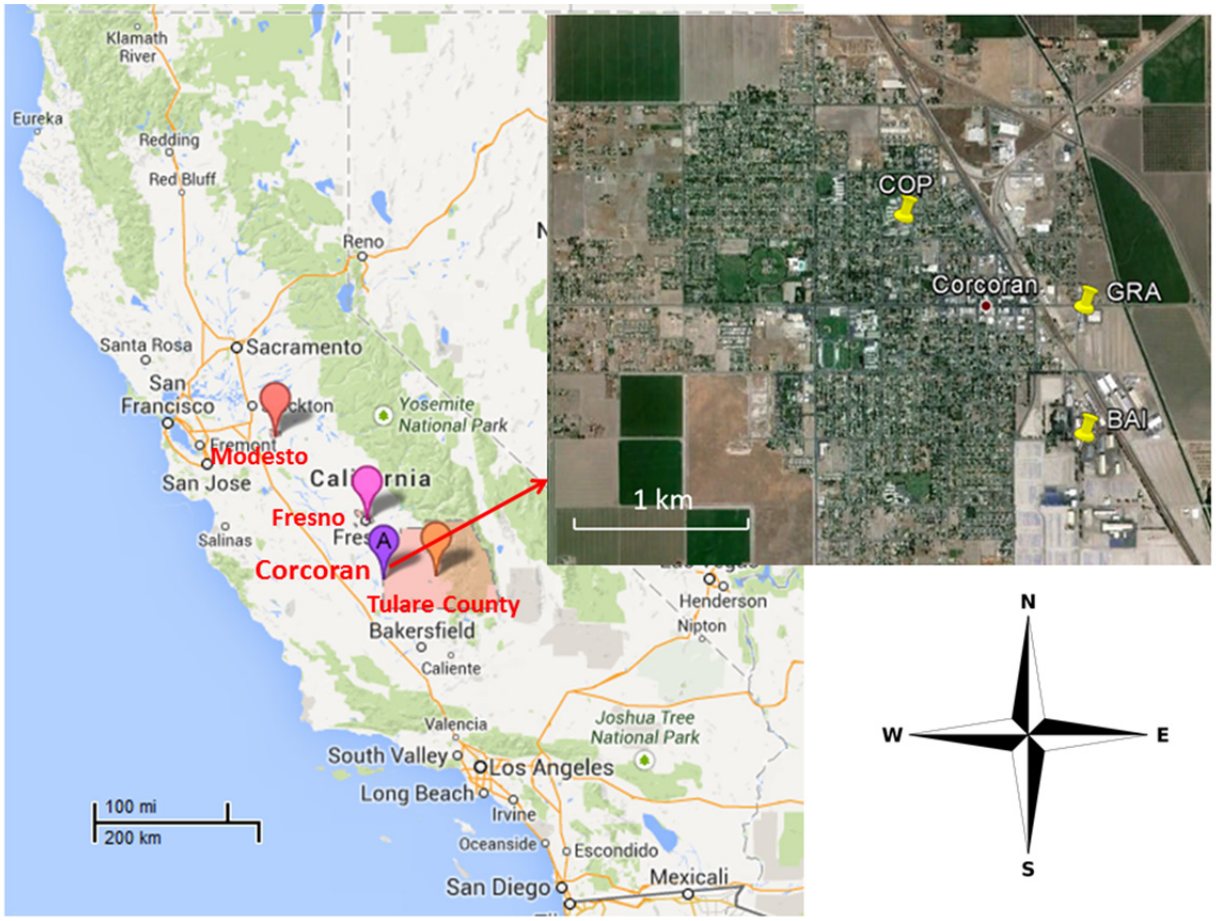

Fig. 1. Locations of Corcoran (36°05′53″N, 119°33′37″W) in Central California and the three neighborhood-scale (within 1.7 km) sampling sites (insert). The town of Corcoran lies in the central San Joaquin Valley (SJV) with a population of ~15,000. The COP site was located in a local school yard surrounded by residential communities, the BAI site was near a cotton processing facility, and the GRA site was near grain elevators. Locations for previous bioaerosol studies in the SJV are also shown, including Modesto, Fresno, and Tulare County.

concentrations on PM_{10} samples in the vicinity of agricultural activities; 2) estimate the contributions of stable bioaerosol indicators, specifically fungal spores, pollen grains, and plant detritus to PM_{10} mass and OC concentrations; and 3) examine the association between fungal spores and the three fungal biomarkers (i.e., (1→3)-β-D-glucan, arabitol, and mannitol).

Extensive efforts have been made to characterize SJV air pollution, especially for PM_{10} and $PM_{2.5}$ (particles with aerodynamic diameters <~2.5 μm; Chow *et al.*, 1993a; 1996; 2006; Chen *et al.*, 2007). The $PM_{2.5}$ fraction is dominated by ammonium nitrate and carbonaceous aerosol during fall and winter. Sulfur dioxide emissions in the SJV are low (CARB, 2012), but oxides of nitrogen and ammonia emissions are high (Mansell and Roe, 2002), so lower temperatures and higher relative humidities during late fall and winter favor ammonium nitrate formation (Stockwell *et al.*, 2000; Chow *et al.*, 2005b; Lurmann *et al.*, 2006; Chow *et al.*, 2008). $PM_{2.5}$ carbonaceous aerosol in the SJV derives from engine exhaust, biomass burning, cooking, and conversion of organic gases to particles (Chow *et al.*, 1992; Strader *et al.*, 1999; Schauer and Cass, 2000; Chow *et al.*, 2007b).

The PM_{10} coarse fraction ($PM_{10-2.5}$) contains large contributions from fugitive dust and possibly bioaerosols. During citrus harvesting in Tulare County (see Fig. 1), Lee *et al.* (2004) reported average endotoxin and total bacteria and fungi levels of 293.2 endotoxin units (EU)/m^3 and 1.9 × 10^8 organisms/m^3, respectively, based on the total suspended particles (TSP) dislodged from polycarbonate-membrane filters. They also measured 13,787 and 13,274 colony forming units (CFU)/m^3 for culturable bacteria and fungi, respectively, using Andersen two-stage bioaerosol samplers. Tager *et al.* (2010) found yearly average PM_{10} endotoxin concentrations of 0.98–1.38 EU/m^3 in Fresno (the largest city in the SJV, 80 km north of Corcoran) from 2001 to 2004, with higher concentrations during the dry season (i.e., May–October). Using the 16S rRNA clone library and Sanger sequencing, Ravva *et al.* (2011) observed phyla *Firmicutes*, *Proteobacteria*, and *Bacterioidetes* that dominated the bacterial community of TSP samples collected around two dairy farms near Modesto, CA (~140 km north of Corcoran).

None of the prior SJV studies examined contributions of bioaerosol to the mass loadings and carbonaceous fractions

in PM_{10} samples used to determine compliance with air quality standards. These prior studies focused on urban populations. Exposures in smaller communities scattered throughout the SJV may report higher concentrations owing to their proximity to crops and livestock. In this study, seven types of stable bioaerosol indicators were quantified on PM_{10} samples using a combination of microscopy (i.e., counts of fungal spores, pollen grains, and plant detritus), anion exchange chromatography (i.e., arabitol and mannitol), and *Limulus Ambebocyte Lysate* (LAL) assays (i.e., endotoxin and (1→3)-β-D-glucan).

Pollen, spores, and plant parts derive from native, agricultural, and ornamental vegetation, and are the most widely recognized allergens (Bowers *et al.*, 2013; Caillaud *et al.*, 2014). Pollen count forecasts (Intermountain Allergy and Asthma, 2014; Pollen.com, 2014) are widely followed by people with allergies. In spite of the importance of bioaerosols to human health, minimal effort has been expended in simulating their emissions, transport, and human exposure (Raynor *et al.*, 1983; Luo *et al.*, 2006). Garfin *et al.* (2013) speculate that as the regional climate warms over the next several decades, earlier and longer spring bloom for many plant species may lead to enhanced pollen production. These substances have high ligno-cellulose contents and are stable over many years, as evidenced by their use in describing long-term climate change effects on vegetation (Rhode, 2003; Louderback and Rhode, 2009).

Endotoxin is a cell wall component of gram-negative bacteria, exposure to which may cause fever, shivering, pulmonary inflammation, non-allergenic asthma, airway obstruction, and lung function deterioration (Degobbi *et al.*, 2011). High endotoxin levels have been measured near animal houses and other agricultural activities (Smid *et al.*, 1992; Reynolds *et al.*, 2002; Ko *et al.*, 2010; Yang *et al.*, 2013). Endotoxins are often associated with agricultural and house dusts, and they persist for long periods because the bacteria are no longer viable (Pearson *et al.*, 1985; Maus *et al.*, 2001).

(1→3)-β-D-glucan is a fungal cell wall component with health effects similar to endotoxins (Rylander *et al.*, 1999; Douwes *et al.*, 2000; Douwes *et al.*, 2003), and it can be used as a surrogate for fungal exposure. (1→3)-β-D-glucan also attaches to fugitive dust and persists with time (Douwes *et al.*, 1996), even being found on Asian dust aerosols that have transported over many days and long distances (He *et al.*, 2013).

Arabitol and mannitol are sugar alcohols that are used as surrogates for fungi (Bauer *et al.*, 2008a; Di Filippo *et al.*, 2013). They have no known adverse health effects, are stable owing to their low vapor pressures, and are more efficiently measured than the microscopic identification and counting needed for the large variety of fungi. They are included in this study to determine how well they might be predictors of the fungi concentrations.

MATERIALS AND METHODS

PM_{10} Sampling and Chemical Analysis

Daily, 24-hr (midnight to midnight Pacific Standard Time) PM_{10} samples were acquired during the cotton harvest season from 9 October, 2000, to 9 November, 2000, at the three sites in Fig. 1. Compliance PM_{10} levels exceeded 50 μg/m^3 on five of the six every-sixth-day hivol filter samples acquired at COP during this period. Two PM_{10} MiniVol samplers (Airmetrics Inc., Eugene, OR, USA) equipped with 47 mm-diameter Teflon-membrane (Teflo^{TM} R2PJ047, Pall Corp., Ann Arbor, MI, USA) and quartz-fiber filters (Tissuquartz^{TM} 2500 QAT-UP, Pall Corp., Ann Arbor, MI, USA) were operated at 5 L/min flow rates at each site with an inlet height of 3.3 m above ground level (Chow *et al.*, 2005a).

Prior to sampling, quartz-fiber filters were prefired at 900°C for 4 hr while Teflon-membrane filters were equilibrated in a temperature (20–23°C) and relative humidity (30–40%) controlled environment for a minimum of four weeks prior to gravimetric analysis with a Mettler Toledo XP6 microbalance (sensitivity: ± 1 μg; Mettler Toledo Inc., Columbus, OH, USA). After sampling, filters were shipped and stored in airtight containers at < 4°C. Mass by gravimetry and elements by x-ray fluorescence (XRF) were measured on the Teflon-membrane filters soon after sampling (Watson *et al.*, 1999). Portions of the quartz-fiber filters were analyzed for water-soluble ions by ion chromatography (Chow and Watson, 1999) and organic and elemental carbon (OC and EC) by thermal/optical reflectance (Chow *et al.*, 1993b). Remaining filters were stored at <4°C in sealed Petri slides until they were re-analyzed for the bioaerosols. Since only stable markers were sought, as described above, this type and length of storage is not believed to cause a major bias in the concentrations. Chow *et al.* (2007a) demonstrate that (OC) concentrations can be reproduced on similar samples stored for many years. However, the results reported here represent a lower limit to the total bioaerosol content of Corcoran PM_{10}.

Microscopic Analysis of Fungal Spores, Pollen Grains, and Plant Detritus

Microscopic analysis of particle morphology was performed with a Hitachi TM-1000 Tabletop SEM (Hitachi High-Technologies Corp., Tokyo, Japan). The Teflon-membrane filter was mounted on a metal disk specimen holder using conductive carbon tape (attached via the plastic support ring only). Carbon or gold coating was not needed due to the use of backscatter electrons and operation by charge compensation. Both standard (500–1000x) and high magnification (1000–5000x) were used to identify specific particle shapes and other characteristics. Fourteen to twenty-two images of randomly selected positions on the aerosol deposit were obtained for each filter, followed by image analysis using ImageJ software (Schneider *et al.*, 2012; Wagner and Macher, 2012). Bioaerosols were identified based on their size, shape, and texture, and classified by category before being manually counted. The minimum detection limit (MDL) was estimated to be ~200 particles/m^3. The precision of bioaerosol counts at the 90% confidence interval was ± 14% for fungal spores, ± 55% for pollen grains, and ± 22% for plant detritus. Pollen grain and plant detritus counts were low, so counting precisions were also low.

Limulus Amebocyte Lysate (LAL) Analysis of Endotoxin and (1→3)-β-D-glucan

Two 0.52 cm^2 quartz-fiber filter punches, the same size as those used for thermal/optical carbon analysis, were extracted in 5.0 mL pyrogen-free water by an orbital shaker (300 rpm) at room temperature (~23°C) for 60 min, then centrifuged at 1000 gravity (G) for 15 min. One-half mL of supernatant was submitted to a Chromo-LAL endotoxin assay (Associates of Cape Cod Inc., East Falmouth, MA, USA). For (1→3)-β-D-glucan, 0.5 mL of 3 N NaOH was added to the remaining 4.5 mL of extract (Rylander and Carvalheiro, 2006) and the mixture was agitated for 60 min with ice cooling, and then neutralized to pH = 6–8 by adding 0.75 mL of 2 N HCl. After centrifugation (1000 G for 15 min), the supernatant was assayed for (1→3)-β-D-glucan using the Glucatell® LAL kinetic time-of-onset assay (Associates of Cape Cod, Inc., East Falmouth, MA). Detailed procedures are given in the Supplemental Materials (S1). The MDLs and precisions were 0.046 EU/m^3 and ± 6.4% for endotoxin and 0.029 ng/m^3 and ± 4.2% for (1→3)-β-D-glucan, respectively.

Anion-Exchange Chromatography Analyses of Arabitol and Mannitol

Quartz-fiber filter sections with deposit areas of 4.34 to 5.36 cm^2 (the remains of the filter after other analyses) were extracted in 5.0 mL of pyrogen-free water by sonication and gentle shaking for 60 min at room temperature. The extract was analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD; Dionex ICS-3000, Sunnyvale, CA, USA) (Iinuma *et al.*, 2009), as detailed in the Supplemental Materials (S2). The MDLs and precisions were 19 ng/m^3 and ± 3.2% for arabitol and 18 ng/m^3 and ± 2.6% for mannitol, respectively.

RESULTS AND DISCUSSION

PM_{10} Mass and Chemical Composition

PM_{10} mass, major elements, ions, carbon, and bioaerosol concentrations are summarized in Table 1. Average PM_{10} mass ranged from 65.1 ± 1.0 µg/m^3 at the residential COP site to 82.1 ± 28.0 µg/m^3 at the cotton handling BAI site. The material balances in Fig. 2 show that geological material accounted for 35% (COP site) to 50% (BAI site) of measured PM_{10}. Organic mass [OM = 1.4 × OC to account for unmeasured hydrogen, nitrogen, sulfur, and oxygen, see Watson (2002) for justification] and nitrate were the next largest components, accounting for 18–22% and 15–24% of PM_{10}, respectively. Ammonium sulfate, EC, salt (available from Tulare dry lake bed, Chow *et al.*, 2003), and trace elements constituted the remaining PM_{10} mass.

Average bioaerosol concentrations were not that different among the sites. COP measured more than 80% of the concentrations at the BAI site for (1→3)-β-D-glucan, arabitol, mannitol, and pollens, and more than 60% for plant detritus. Similar large fractions were found for the comparison of COP with the GRA values, with pollen grains reduced to 63% and plant detritus to 50%. The largest differences were found for endotoxins, with COP measuring only 35% of the BAI average and 13% of the GRA average. This indicates that most of the bioaerosols are not dominated by the nearby source, but affect the broader Corcoran community. Average endotoxin concentrations of 1.7 ± 1.5 EU/m^3 at the COP site were similar to the 0.98–1.38 EU/m^3 reported by Tager *et al.* (2010) for Fresno. Dungan *et al.* (2010) measured endotoxin near an open-feedlot dairy farm in southern Idaho and found that the average endotoxin concentration decreased by ~50% and ~86% at 200 m and 1,390 m downwind of the edge of the farm, respectively. Average endotoxin concentrations at the GRA site (13.0 ± 17.0 EU/m^3) were 2.7 and 7.6 times higher than concentrations at the BAI and COP sites, respectively. The highest concentration of 47.6 EU/m^3 (23 October, 2000, at the GRA site) was about half of the of 90 EU/m^3 exposure limit in the Netherlands (Dutch Expert Committee on Occupational Safety, 2010), the only country that defines such a limit.

Average (1→3)-β-D-glucan concentrations of 8.5–10.6 ng/m^3 were higher than those reported by Chen and Hildemann (2009) for indoor (0.1–8.9 ng/m^3) and outdoor (0.3–5.4 ng/m^3) environments in urban California and by Menetrez *et al.* (2009) at a wooded rural site (0.04 ± 0.017.4 ng/m^3) in Orange County, NC. As shown in Table 2, average arabitol and mannitol concentrations of 170 and 132 ng/m^3, respectively, in Corcoran were 1–2 orders of magnitude higher than those from past studies, except for a similar site near agricultural activities in central India (Nirmalkar *et al*., 2015). Fungal spore and pollen grain concentrations vary by over 3–4 orders of magnitude among studies listed in Table 3. With intense agricultural activities, counts of fungal spores (66,333 particles/m^3) and pollen grains (2,600 particles/m^3) at Corcoran were more than twice those found elsewhere.

Fungal spores and pollen grains are large particles in the range of 5–10 µm in diameters as shown in Table 4. Their concentrations, as well as the concentrations of fungal biomarkers including (1→3)-β-D-glucan, arabitol, and mannitol, were high at the three neighborhood sites. Table 4 shows that fungal spores were of spherical and prolate spheroid, with prolate spheroids accounting for > 90% of spore counts. The surface area and volume of pollen grains were 4 and 8–10 times larger than those of fungal spores, respectively.

Plant detritus particle counts were variable, ranging from 9,000 to 81,000 particles/m^3 or ~1–9.2 µg/m^3 at the three sites, with diverse morphology. Identification of plant detritus is uncertain due to their variable morphologies, absence of distinct texture, and surface attachment of fungal hyphae, fiber, and film. Examples of plant detritus, diatoms, bacteria (e.g., *Streptococcus*), slime molds (e.g., *Myxomycete* spores), and insect eggs from the SEM analysis are shown in Fig. 3.

Predictability of Bioaerosol Indicators from Each Other and from Major PM_{10} Components

Correlation coefficients (r) indicate the extent to which different concentrations vary with each other, either because they are in the same particles, in different particles deriving from the same source, or affected by the same meteorology (Watson and Chow, 2015). A high correlation (r > ~0.85) indicates that one variable might be reliably predicted from

Table 1. Summary of PM_{10} mass, major elements, ions, carbon, and bioaerosol measurements.

	Residential (COP site, $N = 10$)		Cotton handling (BAI site, $N = 11$)		Grain elevator (GRA site, $N = 10$)	
	Average ± Std. dev.	Range	Average ± Std. dev.	Range	Average ± Std. dev.	Range
PM_{10} (μg/m^3)	65.1 ± 21.0	27.5–93.1	82.1 ± 28.0	46.2–123.2	77.7 ± 24.1	46.4–124.5
OC (μg/m^3)	12.0 ± 2.5	7.1–14.9	12.2 ± 3.7	6.2–18.4	14.5 ± 4.0	8.6–22.8
EC (μg/m^3)	2.3 ± 0.8	0.9–3.6	2.1 ± 0.8	1.0–3.4	2.2 ± 0.7	1.1–3.1
NO_3^- (μg/m^3)	15.4 ± 9.3	3.7–31.9	12.1 ± 9.4	3.3–31.8	13.1 ± 8.0	3.2–29.4
$SO_4^=$ (μg/m^3)	1.7 ± 0.6	0.8–2.5	2.0 ± 0.9	0.9–3.4	1.8 ± 0.9	0.8–3.7
NH_4^+ (μg/m^3)	4.3 ± 3.0	0.6–9.5	3.3 ± 3.0	0.4–9.5	3.6 ± 2.6	0.5–8.5
Geological minerals[a] (μg/m^3)	22.8 ± 12.8	10.0–52.8	41.2 ± 22.1	12.0–81.6	32.6 ± 18.7	17.8–68.3
Salt[b] (μg/m^3)	0.3 ± 0.5	0.0–1.7	0.4 ± 0.7	0.0–2.6	0.5 ± 0.8	0.0–2.6
Trace elements[c] (μg/m^3)	1.4 ± 0.6	0.9–2.8	2.1 ± 0.9	1.0–3.8	1.9 ± 0.9	1.1–4.0
Endotoxin (EU/m^3)	1.7 ± 1.5	0.1–3.7	4.9 ± 5.8	0.7–20.1	13.0 ± 17.0	0.5–47.6
(1→3)-β-D-glucan (ng/m^3)	8.5 ± 4.9	2.2–16.6	10.6 ± 6.3	2.8–19.7	9.7 ± 10.5	2.1–38.3
Arabitol (ng/m^3)	156 ± 59	87–264	181 ± 72	100–310	172 ± 89	36–371
Mannitol (ng/m^3)	120 ± 31	90–179	128 ± 37	86–202	147 ± 78	< MDL[d]–288
Fungal spores (particles/m^3)	58,000 ± 20,000	26,000–91,000	70,000 ± 16,000	45,000–92,000	71,000 ± 26,000	35,000–110,000
Fungal spores (μg/m^3)[e]	3.4 ± 1.1	1.5–5.3	4.1 ± 0.9	2.6–5.3	4.1 ± 1.5	2.0–6.4
Pollen grains (particles/m^3)	2,100 ± 1,100	800–4,000	2,500 ± 1,900	0–6,200	3,200 ± 2,000	700–6,400
Pollen grains (μg/m^3)[e]	1.2 ± 0.7	0.6–2.4	1.5 ± 1.2	0–3.7	1.9 ± 1.2	0.4–3.8
Plant detritus (particles/m^3)	21,000 ± 8000	9,000–33,000	34,000 ± 12,000	16,000–55,000	42,000 ± 15,000	32,000–81,000
Plant detritus (μg/m^3)[e]	2.4 ± 0.9	1.0–3.8	3.9 ± 1.4	1.8–6.3	4.8 ± 1.7	3.6–9.2

[a] Concentration of geological minerals is calculated as: 1.89 × [Al] + 2.14 × [Si] + 1.4 × [Ca] + 1.43 × [Fe] (assuming the oxide form of Al_2O_3, SiO_2, CaO, and Fe_2O; Chow *et al.*, 1996).

[b] Concentration of salt is calculated as: 1.65 × [Cl].

[c] Concentration of trace elements is equal to the sum of elements other than Al, Si, Ca, Fe, S, and Cl.

[d] MDL – minimum detection limit (mannitol =18 ng/m^3).

[e] Mass concentration of fungal spores, pollen grains, and plant detritus are estimated based on their average volume per particle determined by scanning electron microscopy (SEM) analysis (See Table 4), assuming a density of 1 g/cm^3. Highly variable morphologies of plant detritus particles (average volume per particle: 113.6 ± 318.7 μm^3, $N = 172$) preclude an accurate assessment.

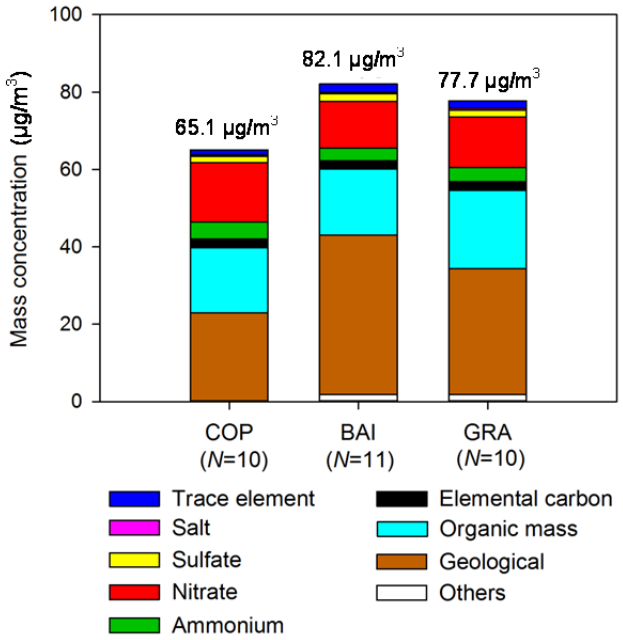

Fig. 2. PM_{10} material balance at the residential Corcoran (COP), cotton handling (BAI), and grain elevator (GRA) sites, where: Organic mass = 1.4 × [Organic carbon], to account for unmeasured H, O, S, and N; Geological material = 1.89 × [Al] + 2.14 × [Si] + 1.4 × [Ca] + 1.43 × [Fe]; Salt = 1.65 × [Cl]; and trace elements = sum of elements other than Al, Si, Ca, Fe, S, and Cl.

the other, while moderate correlations (r = ~0.5 to 0.85) indicate some predictive ability, but with high uncertainty. Low correlations (r < 0.5) are not useful for predictive purposes, even though statistical tests might show the relationships are significant.

Table 5 demonstrates that correlations are low for most of the bioaerosols and for the more commonly measured PM_{10} components. The highest correlation (r = 0.86) is between arabitol and mannitol. Similarly high arabitol/mannitol correlations are reported in other studies (Bauer *et al.*, 2008a; Zhang *et al.*, 2010; Burshtein *et al.*, 2011; Liang *et al.*, 2013). Arabitol and mannitol are only moderately correlated with fungal spores (r = 0.51 and 0.49, respectively). Bauer et al. (2008a) found slightly stronger relationships, with a fungal spore correlation with arabitol of r=0.56 and with mannitol of r = 0.62

OC is moderately correlated with (1→3)-β-D-glucan (r = 0.7) and plant detritus (r = 0.57). Plant detritus is moderately correlated with endotoxin (r = 0.59) and (1→3)-β-D-glucan (r = 0.60). None of the markers are associated with the geological minerals, probably indicative of its derivation from multiple SJV fugitive dust emitters (Ashbaugh *et al.*, 2003; Carvacho *et al.*, 2004).

Average arabitol and mannitol concentrations per fungal spore were 2.7 ± 1.0 pg/spore and 2.1 ± 0.9 pg/spore, 125% and 24% higher than the 1.2 ± 0.5 pg/spore and 1.7 ± 0.6 pg/spore reported by Bauer *et al.* (2008a), respectively. These ratios are expected to vary owing to differences in the fungal spore types, presence of arabitol and mannitol in other bioaerosols, and artifacts from different sampling and analysis methods.

Average (1→3)-β-D-glucan content was 0.16 ± 0.10 pg/spore, within the range of 0.04–3.1 pg/spore found by Foto *et al.* (2004). The average (1→3)-β-D-glucan coating depends on the spore's surface area. The 0.0020 ± 0.0013 pg/µm^2 for Corcoran samples is at the lower end of the 0.00268–0.0598 pg/µm^2 range reported by Foto *et al.* (2004), probably due to the presence of different fungal species.

The finding that (1→3)-β-D-glucan does not correlate (r < 0.11) with fungal biomarkers (i.e., arabitol and mannitol), is indicative of more diverse sources. (1→3)-β-D-glucan has also been found in some bacteria and most higher-order plants (Rylander, 1999). Foto *et al.* (2004) reported high (1→3)-β-D-glucan content in ragweed pollens (83.0 ± 5.6 ng/mg). Arabitol and mannitol are also found in bacteria and lower-order plants. Mannitol is particularly abundant in algae (Bieleski, 1982), and biomass burning is also a source (Claeys *et al.*, 2010; Zhang *et al.*, 2013). Burshtein *et al.* (2011) compared three fungal biomarkers (i.e., ergosterol, arabitol, and mannitol) with PM_{10} samples collected in Rehovot, Israel, and found that arabitol and mannitol were poorly correlated with ergosterol, a sterol in fungal cell membranes. They concluded that these two sugar alcohols are not specific biomarkers for fungi. Di Filippo *et al.* (2013) examined size-segregated PM samples collected in urban/suburban Rome, Italy, and found different size distributions for arabitol and mannitol as compared to ergosterol. Arabitol and mannitol were correlated with levoglucosan and xylitol – two biomass burning markers – in sub-micron size fractions. Both DiFilippo *et al.* (2013) and Burshtein *et al.* (2011) recommend using ergosterol as a surrogate for fungal counts. However, Yang *et al.* (2012) observe that ergosterol is susceptible to molecular degradation and instability. Lau *et al.* (2006) found that the ergosterol content per fungal spore varied with fungal species and their growth stage. With moderate correlations (0.49–0.51) found between fungal spore counts and these two sugar alcohols, this study is consistent with other findings that arabitol and mannitol are not reliable surrogates for fungi. Although fungi appear to be the dominant bioaerosol (Elbert *et al.*, 2007; Winiwarter *et al.*, 2009), concurrent release of pollen grains, plant detritus, diatoms, bacteria, and other sugar-alcohols during agricultural activities complicate predictability among different fungal biomarkers.

Contribution of Fungal Spores, Pollen Grains, and Plant Detritus to PM_{10} mass and OC

Bioaerosol densities are difficult to measure; reported density values range from 0.9 to 1.5 g/cm^3 depending on the bioaerosol type (Burge, 1995; Cox and Wathes, 1995). An assumption of 1 g/cm^3 density (Johnson *et al.*, 1999; Matthias-Maser and Jaenicke, 2000), 20% water in fresh mass, and 50% OC in dry mass for fungal spores (Bauer *et al.*, 2008b; Wiedinmyer *et al.*, 2009) is used to estimate their contributions to PM_{10} mass and OC. As shown in Table 6, fungal spores, on average, accounted for 5.4–5.8% of PM_{10} mass and 11.5–14.7% of PM_{10} OC, with the highest ratios (9.9% of PM_{10} mass and 27.1% of PM_{10} OC)

Table 2. Comparison of arabitol and mannitol concentrations in PM samples collected at different locations and seasons.

Reference	Location (Type)	Season/month	Particle size	Arabitol[a] (ng/m^3)	Mannitol[a] (ng/m^3)
Carvalho *et al.* (2003)	Melpitz, Germany (rural)	spring	PM_{10}	4.2–35[b]	1.6–23[b]
	Hyytiälä, Finland (forest)	summer	PM_{10}	1.4–241[b]	< 0.5–88[b]
Cahill *et al.* (2006)	Blodgett Forest, CA, USA (forest)	fall	TSP	7.6	8.8
Bauer *et al.* (2008b)	Vienna, Austria (urban and suburban)	summer, fall, and winter	PM_{10}	7.0–63[b]	8.9–83[b]
Zhang *et al.* (2010)	Jianfengling, Hainan, China (tropical rainforest)	spring	$PM_{2.5}$	7.0	16.0
			PM_{10}	44.0	71.0
Burshtein *et al.* (2011)	Rehovot, Israel (urban)	spring	PM_{10}	14.0	17.7
		summer	PM_{10}	10.6	15.1
		fall	PM_{10}	18.9	49.2
		winter	PM_{10}	8.4	21.9
Yang *et al.* (2012)	Chengdu, China (urban)	spring	$PM_{2.5}$	21.5	43.9
Di Filippo *et al.* (2013)	Rome, Italy (mixture of suburban/rural)	spring	PM_{10}	10.2	19.1
		summer	PM_{10}	8.3	12.3
		fall	PM_{10}	18.0	25.8
		winter	PM_{10}	21.6	29.2
	Rome, Italy (urban)	spring	PM_{10}	24.5	15.1
		summer	PM_{10}	48.8	49.0
		fall	PM_{10}	20.8	22.4
		winter	PM_{10}	4.89	13.5
Liang *et al.* (2013)	Beijing, China (urban)	all seasons	$PM_{2.5}$	7.4	10.3
			PM_{10}	21.0	31.9
Nirmalkar *et al.* (2015)	Central India (rural)	Oct–Nov	PM_{10}	440	3300
This study	Corcoran, CA, USA (suburban-residential/agricultural)	fall	PM_{10}	170	132

[a] Average concentration unless otherwise noted.
[b] Concentration range.

Table 3. Comparison of fungal spore and pollen grain concentrations in PM samples collected at different locations and seasons.

Reference	Location (Type)	Season/month	Particle size	Fungal spores[b] (particles/m^3)	Pollen grains[c] (particles/m^3)
Wu *et al.* (2004)	Tainan, Taiwan (urban)	without sandstorm	TSP[a]	28,684	n/a[d]
		with sandstorm	TSP	29,038	n/a
Adhikari *et al.* (2006)	Cincinnati, OH, USA (urban)	spring, summer, and fall	Inhalable	4,229	224
Lee *et al.* (2006a)	Cincinnati, OH, USA (urban)	fall	Inhalable	2,456–28,700[b]	1–44[b]
Lee *et al.* (2006b)	Cincinnati, OH, USA (urban)	spring	Inhalable	106–7,704[b]	1–1,234[b]
		fall	Inhalable	39–3,187[b]	5–22[b]
		winter	Inhalable	24–3,608[b]	1–5[b]
Bauer *et al.* (2008a)	Vienna, Austria (urban and suburban)	summer, fall, and winter	PM_{10}	3,000–42,000[b]	n/a
Bauer *et al.* (2008b)	Vienna, Austria (urban)	April	PM_{10}	15,000	n/a
		May		3,600	n/a
		June/July		26,000	n/a
	Vienna, Austria (suburban)	April	PM_{10}	27,000	n/a
		May		7,800	n/a
		June/July		29,000	n/a
Crawford *et al.* (2009)	Cincinnati, OH, USA (urban)	fall	Inhalable	5,286	0.49
This study	Corcoran, CA, USA (suburban-residential/agricultural)	fall	PM_{10}	66,333	2,600

[a] Total suspended particles.
[b] Average concentration unless otherwise noted.
[c] Concentration range.
[d] Data not available.

Table 4. Characteristics of fungal spores, pollen grains and plant detritus acquired from the PM_{10} Teflon-membrane filter samples.

Bioaerosols	Morphology	N[a]	Radius (μm)	Volume (V, μm^3)	Surface area (S, μm^2)
Fungal spores	prolate	120	$a = 1.8 \pm 0.4$	58.0 ± 39.5	76.2 ± 37.9
	spheroid[b]		$b = 3.8 \pm 1.0$	n/a	n/a
	spherical[c]	30	$r = 2.5 \pm 0.4$	71.0 ± 41.4	80.4 ± 29.7
Pollen grains	spherical[c]	50	$r = 5.0 \pm 1.0$	594.1 ± 403.0	328.9 ± 137.7
Plant detritus	diverse[d]	172	n/a[e]	113.6 ± 318.7	140.1 ± 231.0

[a] N refers to the number of particles selected for size measurement by scanning electron microscopy (SEM).
[b] The volume of prolate spheroid particles is calculated as: $V = 4/3\pi a^2 b$, where a is the minor axis and b is the major axis of a prolate spheroid; the surface area is calculated as: $S = 2\pi a^2 \left(1 + \frac{b}{ae}(1/\text{sine})\right)$, where $e^2 = 1 - \frac{a^2}{b^2}$.
[c] The volume of spherical particles is calculated as: $V = 4/3\pi r^3 b$, where r is the radius of the sphere; the surface area is calculated as: $S = 4\pi r^2$.
[d] The morphologies of plant detritus particles are highly diverse. Therefore, each individual particle was approximated by one or a few cylinders. The volume of a cylinder is calculated as: $V = \pi r^2 h$, where r and h are the radius and length of the cylinder, respectively; the surface area is calculated as: $S = 2\pi r(r + h)$.
[e] Data not available.

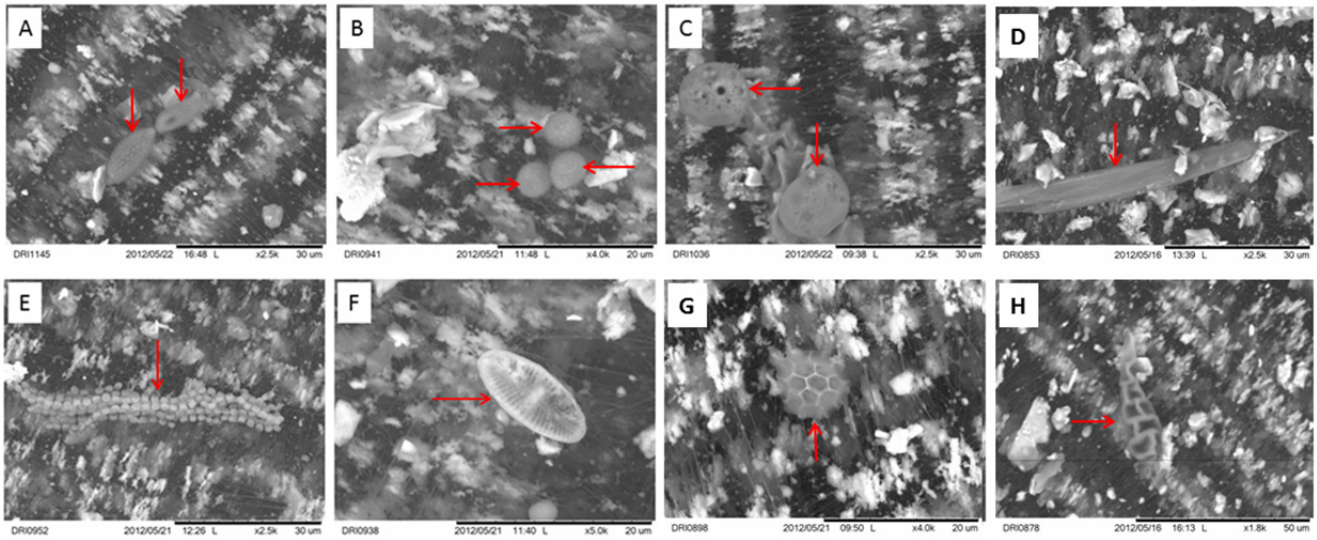

Fig. 3. Example images of ambient bioaerosols detected from PM_{10} Teflon-membrane filter samples collected in Corcoran, California, by scanning electron microscopy (SEM; see magnification on individual Figures) for: A – fungal spores (Type 1: prolate spheroid), B – fungal spores (Type 2: spherical), C – pollen grains, D – plant detritus, E – bacteria (*Streptococcus*), F – a diatom, G – a slime mold, and H – an insect egg. Pores on the pollen surface were caused by high-energy electron beams during SEM analysis (The opaque background is a result of Teflon-membrane filters; high magnification [1000–5000x] was used to identify specific particle shapes and other characteristics).

found at the cotton handling BAI site on 24 October, 2000. This is consistent with fungal spore fractions of 0.4–64% and 4–22% in PM_{10} and $PM_{2.5}$ OC, respectively, reported in previous studies (see Table 7). Using the conversion factor of 13 pg C/spore by Bauer *et al.* (2002b) would underestimate the fungal spore OC to total OC ratio by 46%. This is expected since fungal spore OC content should be size dependent, and larger fungal spores (58 μm^3/spore) were found in this study (Table 4) compared to 34 μm^3/spore by Bauer *et al.* (2002b).

PM_{10} contributions from pollen grains were lower than those from fungal spores, accounting for an average of 1.9–2.7% of PM_{10} mass (4.2–5.4% OC) with a maximum of 6.3% PM_{10} (11.6% OC) at the GRA site on 9 October, 2000. Plant detritus particles accounted for an average of 3.8–6.3% PM_{10} mass (8.1–13.5% OC) with the maximum contribution of 11.5% PM_{10} (24.1% OC) found at the cotton handling BAI site on 22 October, 2000. The sum of fungal spores, pollen grains, and plant detritus accounted for averages of 11.2–14.8% PM_{10} mass (23.8–32.8% OC), ranging from 5.3% PM_{10} mass (19.1% OC) at the BAI site to 24.1% PM_{10} mass (30.1% OC) at the GRA site.

CONCLUSIONS

As abundances of bioaerosols in PM_{10} near agricultural

Table 5. Correlation coefficients (r) among bioaerosol indicators and major PM_{10} components for all sites. r > 0.5 are in bold to indicate which components might be predicted to some extent by measuring another component.

	Endotoxin (EU/m^3)	(1→3)-β-D-glucan (ng/m^3)	Arabitol (ng/m^3)	Mannitol (ng/m^3)	Fungal spores (particles/m^3)	Pollen grains (particles/m^3)	Plant detritus (particles/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Geological material (μg/m^3)
Endotoxin (EU/m^3)										
(1→3)-β-D-glucan (ng/m^3)	0.43									
Arabitol (ng/m^3)	0.28	0.11								
Mannitol (ng/m^3)	0.27	0.00	**0.86**							
Fungal spores (ng/m^3)	0.38	0.36	**0.51**	0.49						
Pollen grains (particles/m^3)	0.01	0.09	–0.14	–0.14	–0.09					
Plant detritus (particles/m^3)	**0.59**	**0.60**	0.32	0.31	**0.64**	0.01				
OC (μg/m^3)	0.41	**0.70**	0.17	0.19	0.29	0.39	**0.57**			
Nitrate (μg/m^3)	–0.30	0.20	0.01	0.09	0.11	0.46	0.00	0.42		
Geological material (μg/m^3)	0.20	0.31	–0.02	–0.13	0.10	–0.22	0.32	0.16	–0.41	

Table 6. Contributions[a] of fungal spores, pollen grains, and plant detritus to PM_{10} mass and organic carbon (OC).

		Fungal spores (% contribution)		Pollen grains (% contribution)		Plant detritus (% contribution)	
		Average ± Std. dev.	Range	Average ± Std. dev.	Range	Average ± Std. dev.	Range
PM_{10} mass	COP (N = 10)	5.4 ± 1.7	3.2–8.8	2.0 ± 0.9	0.6–3.5	3.8 ± 1.3	2.4–6.8
	BAI (N = 11)	5.8 ± 3.1	2.5–9.9	1.9 ± 1.4	0–4.1	5.4 ± 2.8	1.6–11.5
	GRA (N = 10)	5.8 ± 2.5	2.2–9.4	2.7 ± 2.0	0.3–6.3	6.3 ± 1.5	3.9–8.4
	All sites combined (N = 31)	5.7 ± 2.4	2.2–9.9	2.2 ± 1.5	0–6.3	5.2 ± 2.2	1.6–11.5
PM_{10} OC	COP (N = 10)	11.5 ± 3.6	4.5–16.7	4.2 ± 1.9	1.7–6.4	8.1 ± 3.0	3.7–14.5
	BAI (N = 11)	14.7 ± 6.1	7.8–27.1	4.6 ± 2.9	0–10.0	13.5 ± 5.7	5.7–24.1
	GRA (N = 10)	11.9 ± 4.6	5.7–20.4	5.4 ± 3.5	0.7–11.6	13.3 ± 2.3	10.5–17.4
	All sites combined (N = 31)	12.8 ± 5.0	4.5–27.1	4.7 ± 2.8	0–11.6	11.7 ± 4.7	3.7–24.1

[a] Fungal spores, pollen grains, and plant detritus were assumed to have a density of 1 g/cm^3 (Johnson *et al.*, 1999; Matthias-Maser and Jaenicke, 2000) and contain 20% of water in fresh mass and 50% of carbon content in dry mass (Bauer *et al.*, 2008b; Wiedinmyer *et al.*, 2009). The contributions of fungal spores, pollen grains, and plant detritus to PM_{10} mass can be calculated as: $N \times \bar{V} \times \rho/PM_{10} \times 100\%$, where N is the number concentration of the bioaerosol, $\bar{V}$ is the average volume of bioaerosol particles (See Table 4), ρ is the particle density, and PM_{10} is the PM_{10} mass concentration. Their contributions to PM_{10} *OC* can be calculated as: $[N \times \bar{V} \times \rho \times (1 - H_2O\%) \times C\%]/OC \times 100\%$, where $H_2O\%$ is the water content in the bioaerosol's fresh mass, $C\%$ is the carbon content in its dry mass, and *OC* is the organic carbon concentration.

Table 7. Contribution of fungal spores to organic carbon in PM samples collected at different locations and seasons.

Reference	Location (Type)	Season	PM size	Contribution (%) to OC[a]
Bauer *et al.* (2002a)	Mt. Rax, Austria (forest)	winter	$PM_{10-2.1}$	5.8
Womiloju *et al.* (2003)	Toronto, Canada (urban, suburban and rural)	spring and summer	$PM_{2.5}$	12–22[b]
Bauer e*t al.* (2002b)	Schafberg, Vienna, Austria (suburban)	spring and summer	PM_{10}	10
	Rinnböckstrasse, Vienna, Austria (urban)	spring and summer	PM_{10}	4.3
Kourtchev *et al.* (2008)	Jülich, Germany (rural)	summer	$PM_{2.5}$	3.6
Cheng *et al.* (2009)	Hong Kong, China (urban)	spring	PM_{10}	2.4–7.1[b]
Wiedinmyer *et al.* (2009)	Mt. Werner, CO, USA (forest)	spring	PM_{10}	16–64[b]
Zhang *et al.* (2010)	Hainan, China (rural)	spring	PM_{10}	4.6–26.1[b]
Di Filippo *et al.* (2013)	Rome, Italy (suburban/rural)	all four seasons	PM_{10}	0.5–2.4[b]
	Rome, Italy (urban)	All four seasons	PM_{10}	0.4–0.8[b]
This study	Corcoran, CA, USA (rural)	fall	PM_{10}	12.8

[a] Average concentration unless otherwise noted.
[b] Concentration range.

communities may contribute to excessive PM_{10} levels, this study demonstrated the feasibility of using archived (< 4°C) filter samples to evaluate the contribution of the seven stable bioaerosol indicators. Except for the uncertainties of SEM analyses on Teflon-membrane filter samples, adequate minimum detection limits and reproducibility (~3–6%) were found. Daily bioaerosol concentrations varied over tenfold during the study period and were over twofold those reported in the literature for fungal spores (66,333 particles/m^3) and pollen grains (2600 particles/m^3) and among the highest for arabitol (170 ng/m^3) and mannitol (132 ng/m^3). Zones of influence from sources within the three-site network are ~2 km, except for endotoxin, demonstrating the neighborhood-scale influence of agricultural bioaerosols. Fungal spores were the dominant bioaerosol, accounting for 11.5–14.7% of PM_{10} OC, followed by plant detritus (8.1–13.5% OC) and pollen grains (4.2–5.4% OC). Correlations between fungal spore counts and the three most commonly used biomarkers for fungi (i.e., (1→3)-β-D-glucan, arabitol, and mannitol) were low to moderate ($0.36 < r < 0.51$), casting doubt on the generality of their use as surrogates. Arabitol and mannitol were not correlated with (1→3)-β-D-glucan ($r < 0.11$), probably due to their variable sources. The specificity and applicability of these species as biomarkers for fungal exposure and their applications for estimating the count or mass of fungal is limited. Bioaerosols from different sources may differ in particle density, water content, and carbon content. To achieve a more accurate estimate of bioaerosol contributions to PM_{10}, efforts are needed to determine the density and composition of dominant bioaerosol species.

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SUPPLEMENTARY MATERIALS

Supplementary data associated with this article can be found in the online version at http://www.aaqr.org.

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Supplemental Material

Characterization of Ambient PM_{10} Bioaerosols in a California Agricultural Town

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S1. Endotoxin and (1→3)-β-D-glucan Analyses

Endotoxin and (1→3)-β-D-glucan analyses used kinetic chromogenic limulus amebocyte lysate (LAL) assays with different activating factors: factor C for endotoxin (in Chromo-LAL assay) and factor G (in Glucatell assay) for (1→3)-β-D-glucan. The assays consist of a colorless substrate and a proenzyme extracted from amoebocyte cells in the blood of the horseshoe crab, *Limulus polyphemus*. The proenzyme is converted to an active enzyme with the presence of endotoxin or (1→3)-β-D-glucan. The enzyme then catalyzes the dissociation of the colorless substrate into a short peptide segment and a yellow organic compound (e.g., p-nitroaniline) that can be photometrically quantified. The speed of color development, measured by the time needed to attain a pre-specified optical density (i.e., onset time), is proportional to the concentration of endotoxin or (1→3)-β-D-glucan.

A Chromo-LAL endotoxin assay (Associates of Cape Cod Inc., East Falmouth, MA, USA) was run in duplicate on an incubating microplate reader (ELx808IU, BioTek Instrument Inc., Winooski, VT, USA) at 37 °C and absorbance wavelength (λ) of 405 nm, with an onset optical density (OD, i.e., absorbance) of 0.1 selected. A control endotoxin standard (*Escherichia coli* O113:H10; potency: 1 ng = 24 EU; Associates of Cape Cod Inc., East Falmouth, MA, USA) was diluted in series to 50, 5, 0.5, 0.05, and 0.005 EU mL^{-1}, three times each to establish a standard calibration curve. To eliminate the interference of (1→3)-β-D-glucan, the Chromo-LAL reagent was reconstituted with the Glucashield glucan-blocking buffer solution (Associates of Cape Cod Inc., East Falmouth, MA, USA). The possible inhibition or enhancement was tested by spiking a test sample with 10 μL of 5 EU mL^{-1} endotoxin standard, and a further dilution was conducted when the percent recovery of the spiked sample was > 200% or <50%. A sample was re-analyzed when the coefficient of variation (CV) between duplicates exceeded ±10%.

Glucatell (1→3)-β-D-glucan assay (Associates of Cape Cod Inc., East Falmouth, MA, USA) was run in duplicate on the incubating microplate reader at 37 °C and λ=405 nm, with an onset OD of 0.03 selected. A standard calibration curve was developed by diluting the stock standard (included in the Glucatell assay) to 100, 50, 25, 12.5, 6.25 and 3.125 pg mL^{-1} three times each. A similar quality assurance procedure, as that adopted for endotoxin analysis, was applied.

Both assays require the analyte concentration in filter extracts within the concentration range of calibration standards. Accordingly, the assays' minimum detection limits (MDLs) were calculated based on the lowest-concentration standard to be 0.046 EU m^{-3} and 0.029 ng m^{-3} for endotoxin and (1→3)-β-D-glucan, respectively. The precision of the assays was calculated by running replicates (>3 per microplate), following the method of Watson *et al.* (2001). The accuracy of the assays was primarily limited by the uncertainty in prepared calibration standards. Due to lack of a quality control standard, this uncertainty could not be reliably assessed. Thus, no estimation of the assays' accuracy was conducted.

Glassware and metal tools (e.g., tweezers and filter punchers) were baked at 250 °C for >4 hr prior to the experiment, and they were sterilized repeatedly on a micro-incinerator during the experiment to prevent cross-contamination among samples. Pipette tips, microplates and centrifuge tubes were certified by the suppliers to be pyrogen-free and were tested in the laboratory by running negative control samples. Laboratory and field blank samples were also tested, and they, as well as the negative control samples, contained endotoxin and (1→3)-β-D-glucan levels below the MDLs. Thus, no blank subtraction was conducted and all values reported were as-measured.

S2. Arabitol and Mannitol Analyses

Arabitol and mannitol in filter extracts were quantified using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The system (Dionex ICS-3000, Sunnyvale, CA, USA) was equipped with a Dionex CarboPac MA1 column/guard column and a sample loop of 30 μL. The separation was run with an aqueous sodium hydroxide (NaOH) eluent (354 mM, in ultrapure water) at a flow rate of 0.4 mL min^{-1}. To prepare the calibration curve, arabitol (99% purity, Fluka) and mannitol (>98% purity, Acros Organics) were dissolved in ultrapure water and diluted in series to 5.0, 2.5, 1.0, 0.5, 0.2, 0.1, 0.05 μg mL^{-1}. A mixture solution of carbohydrates (including arabitol and mannitol, all diluted at 1.0 μg mL^{-1}; Absolute Standards, Inc., Hamden, CT, USA) was used as the quality control standard.

For both compounds, the MDL was calculated as three times the standard deviation of the lowest-concentration standard (0.05 μg mL^{-1}). The test of field blank samples showed that their arabitol and mannitol concentrations were below the MDLs (19 ng m^{-3} for arabitol and 18 ng m^{-3} for mannitol). Thus, all values reported were as-measured and were not adjusted for field blanks. The precision of the analyses was calculated by running replicates (>3 per batch), following the method of Watson *et al.* (2001). It was estimated to be <±3.2% for arabitol and <±2.6% for mannitol. The accuracy of the analyses (<±1.5%) was assessed by differences between measured and actual concentrations of the quality control standard.

Similar to endotoxin and (1→3)-β-D-glucan analyses, glassware and metal tools were baked at 250 °C for >4 hr prior to the experiment. Pipette tips and vials were tested by running negative control samples and no arabitol or mannitol was detected.

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