

Interactive comment on “Contribution of fungi to primary biogenic aerosols in the atmosphere: active discharge of spores, carbohydrates, and inorganic ions by Asco- and Basidiomycota” by W. Elbert et al.

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In response to concerns raised by the referee Cindy Morris with regards to fungal spore identification, other researchers and I regularly differentiate between pollen grains and fungal spores collected from the atmosphere with the use of Calberla's stain which specifically dyes the pollen wall to a pink or red color. For detailed protocol see Smith (2000). The individual fungal types were then classified. For the Amazon analysis, I used a range of spore identification resources, including Smith (1990, 2000), Colinvaux et al., (1999) and information available to certified pollen and spore counters at the US

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National Allergy Bureau (<http://www.aaaai.org/nab/index.cfm?p=nabresources>).

Ascospores are single to multiseptate, generally with a well-formed outer wall, and often fusoid, curved, or filiform. If there is a constriction, it occurs at both ends. They are readily differentiated from the hyaline conidia of yeasts which are unicellular, ovate-oblong or spherical, and thin-walled. The characteristic morphology of other small fungal spores, such as the dark, thick-walled, spheroidal smuts with their roughened surface, chains of smooth, spheroidal *Aspergillus*/*Penicillium*, *Cladosporium* (with a single dark hilum) and basidiospores (subglobose to broadly ellipsoid) can also be differentiated with a high level of certainty, and taxonomic keys have been produced in the past. Classifying the individual ascospore types is more difficult, relies on an extensive data base including number of septa, shape, size, and color, and was not attempted at the species level for this study.

We focused on characterizing the atmospheric contribution from ascospores, spores from the Basidiomycota, and their spore-propulsion fluids. We believe there are chemical markers that can be used to detect these emissions, and these are present in much lesser quantities in the asexual conidia passively released into the air by many Deuteromycota and Ascomycota.

In air samples from the Amazon, ascospores were identified as: *Xylaria*, *Caloplaca*, *Diatrypaceae*, *Amphisphaeria*/*Massaria*, *Massaria*, *Leptosphaeria*, and about 20% undifferentiated ascospores. Asexual spores of Ascomycotina, such as *Aspergillus*/*Penicillium*, *Alternaria longissima*, *Cladosporium* and *Fusarium* were also identified. Laboratory experiments show these asexual spores are released into the air by wind currents in excess of 2.5 m/s (Glovsky et al., 2003, Gorny, 2004).

A recent paper by Zoppas et al., (2006) described the morphological identification of the following ascospores in air samples from a southern city in Brazil: *Leptosphaeria*, *Daldinia*/*Xylaria*, *Paraphaeosphaeria*, *Diatrypaceae*, *Pleospora*, and undifferentiated ascospores. The following basidiospores were also identified: *Coprinus*, *Gano-*

derma, Agaricus, Agrocybe, and undifferentiated basidiospores. Ascospores and basidiospores made up about 50% of the spore count, over 2 years.

Many stations at the US National Allergy Bureau are reporting detailed spore counts for 2006 (<http://www.aaaai.org/nab/index.cfm?p=pollen>). This involves an ascospore count, which includes Leptosphaeria, Venturia, Ascobolus, Diatrypaceae, Pleospora, Xylaria, Chaelomium, Sporomiella, Claviceps, and undifferentiated ascospores. Basidiospore classification includes Coprinus, Agrocybe, Agaricus, Inocybe, Laccaria, Ganoderma, and undifferentiated basidiospores. In November 2006, the average counts, per cubic meter of air per 24 hours, across the US were: 1,064 basidiospores: (range 17 to 4,890) from 16 counting stations; 1,268 ascospores (range 12 to 10,395) from 19 stations; and 116 rust urediniospores (range 2 to 624) from 8 stations. This new data supports our assumption regarding the wide-spread occurrence of asco- and basidiospores.

Within the Basidiomycota, the Hymenomycetes have ballistospores. Basidiomycetes that do not employ a ballistospore method of discharge are usually either aquatic species or the Gastromycetes, such as puffballs and underground truffles. Since the rapid release of ballistospores is driven by the physiology of the fungus, disruptions to normal development, such as early harvesting of basidiocarps by humans, can inhibit the rapid discharge method and result in passive release from the basidiocarp upon mechanical disruption or vibrations. Otherwise, it is a common method of spore discharge, and an early evolutionary adaptation within the Basidiomycota. We did not determine the species of basidiospores present in the Amazon study, although this would have been useful.

Rust fungi are Urediniomycetes (Teliomycetes), in the Basidiomycota, and they release urediniospores, approx 8 x 12 micron, throughout most of their distribution. When there are alternate hosts, such as Oxalis plants, basidiospores/ballistospores develop. In our paper, rust urediniospores were included with the basidiospores in the comparative analysis of ABS sugars since rust urediniospores are rich in mannitol (Voegelé et al.,

2005). We recognize that rust basidiospores can be confused for Botrytis ascospores. Most smuts are Ustilaginomycetes, in the Basidiomycota. The teliospores (ustilospores) are included in the smut count, and any basidiospores/ballistospores that were emitted would be included in the basidiospore count. We also included rust teliospores in the analysis, since, although there is little information on their chemistry, smut extracts contains mannitol (Gaunt and Manners, 1973). For the yeasts, some may be basidiomycetous yeasts, and these are also reported to possess ballistospores and ballistoconidia. We did not include the unicellular hyaline conidia counts in this analysis since, in previous reports, the majority of airborne yeasts were not Basidiomycetes.

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