

***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.***

**W. Elbert et al.**

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We thank C. Morris and M. Bardin for constructive comments and suggestions, which are highly appreciated and will be taken into account in the revised manuscript. Responses to individual referee comments are given below.

**R e f e r e:** The manuscript by Elbert and co-workers aims to estimate the contribution of air-borne fungi to the carbohydrate and inorganic ion content of aerosols. The authors have clearly indicated the consequences that these compounds could have for the atmosphere, and they also have revealed the paucity of information about the contribution of micro-organisms to this aspect of atmospheric chemistry. To write this

review I have engaged the help of a colleague mycologist. As microbiologists (C.E. Morris and M. Bardin, Plant Pathology Research Unit, INRA-Avignon, France) working with air-borne organisms, we have specific comments about the presentation of the biology and the taxonomy of fungi in the introduction, and about methodology for estimating the abundances of ascomycetes and basidiomycetes reported in this paper. Firstly, the authors present a description of the biology of fungi that indicates the importance of active spore release as a means of liberation of carbohydrates and ions in the atmosphere. It should be noted that active spore release is often reserved to the liberation of sexual spores.

**R e s p o n s e:** We agree and will add more information about sexual and asexual spores in the revised manuscript. However, a large number of identified, widespread, and often plant-associated basidiomycetous yeasts (BY) also discharge their asexual ballistospores/ballistoconidia actively (e.g. *Bensingtonia*, *Bullera*, *Sporobolomyces* and *Tilletiopsis* spp.). Our most recent literature search showed that more and more new species of such BY are found and known species are found at new locations (Nakase et al., 1993; Golubev, 1999; Nakase, 2000; Takashima and Nakase, 2001; Bai et al., 2002; Nakase et al., 2002; Bai et al., 2003; Zhao et al., 2003; Boekhout et al., 2006; Wang et al., 2006). In addition, we point to Boekhout (1991) who claimed that the term “ballistospores” refers to both asexual and sexual propagules, and lists a great number of BY which actively discharge asexual ballistospores/ballistoconidia. Other listings and phylogenetic trees of BY were published (Nakase et al., 1993; Nakase et al., 1995; de Azeredo et al., 1998; Fell et al., 1998; Fell et al., 2000; Nakase, 2000; Takashima and Nakase, 2001; Bai et al., 2002; Scorzetti et al., 2002; Valério et al., 2002; Bai et al., 2003; Zhao et al., 2003; Wang et al., 2006).

Nakase et al. (1993) argued “that ballistospores, budding yeast cells and stalked conidia may be produced by almost all species of BY when suitable conditions are provided, and that the production of these three kinds of conidia may be lost in all BY when suitable conditions are not provided.” Bandoni (1995) stated that ballistospores of BY may

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be repetitive, i.e. they can form new ballistospores without building a mycelium. The same was reported for *Tilletia* spp. and *Tilletiopsis washingtonensis* (Hanlin, 1994; Ingold, 1997). Buller (1934) already had pointed out that “the secondary conidium of *Tilletia tritici*, in its asymmetrical form and drop-excretion mode of discharge, exactly resembles the basidiospores of every mushroom and toadstool and of every Rust fungus.” Recently, Pringle et al. (2005) wrote that “Ballistospore discharge is a feature of 30 000 species of mushrooms, basidiomycete yeasts and pathogenic rusts and smuts”.

**R e f e r e e:** However, asexual spores are probably the most abundant contribution of fungi to the environment in terms of number and total matter released. The fungi referred to as the imperfect fungi in the introduction are the asexual forms of fungi, usually of ascomycetes, and include genera such as *Penicillium*, *Alternaria* and *Cladosporium* that are known for their abundance on plants and other surfaces and in the atmosphere.

**R e s p o n s e:** According to a large number of earlier and recent scientific studies, the atmospheric concentrations of dry discharged spores (DDS) from “imperfect” and other fungi are in the same range as those of AAS and ABS (Gregory and Hirst, 1957; Gregory and Sreeramulu, 1958; Adams et al., 1968; Chatterjee and Hargreave, 1974; Kramer and Eversmeyer, 1984; Li and Kendrick, 1995; Newson et al., 2000; Troutt and Levetin, 2001; Levetin, 2004; Wu et al., 2004; Fang et al., 2005; Ho et al., 2005; Zoppas et al., 2006). In the revised manuscript we will present a global average emission estimate of total fungal spores including AAS, ABS, and DDS.

**R e f e r e e:** Furthermore, surfaces of both sexual and asexual spores have sugars that would be released into liquid used in the protocols of chemical analysis. How would these be differentiated from other origins of carbohydrates in their samples?

**R e s p o n s e:** In our study we do not consider the potential release of additional chemical compounds from inside the fungal spores, as their walls are not easily ruptured under the conditions usually applied for the extraction of aerosol filter samples for

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chemical analysis (extraction with pure water or aqueous buffers at room temperature; Graham et al., (2002)). The extraction of spore contents and spore wall components usually requires more severe conditions (e.g. high pressure disintegration, acid or base hydrolysis etc.) (Weijman, 1979; Suzuki and Nakase, 1988; Weijman and De Miranda, 1988; Davoli and Weber, 2002; Solomon et al., 2006).

Cell walls of ascomycetes and basidiomycetes mainly consist of chitin microfibrils which are bundled in linear N-acetylglucosamine chains. These fibers are cross-linked by highly branched glucose polymers, the so-called glucans (Freytag and Mendgen, 1991a, b; Carlile et al., 2001; Selbmann et al., 2003; Webster and Weber, 2007), which are thought to have antitumor and antimetastatic activities (Bao et al., 2001; Kimura et al., 2006). Several studies have shown that cell walls of spores of ascomycetes, basidiomycetes, and asco- and basidiomycetous yeasts contain mainly (in variable amounts) the hexoses glucose, mannose, galactose, xylose, arabinose, fucose, rhamnose, ribose, and as a minor component some hexitols like inositol and maybe mannitol (Weijman and de Hoog, 1975; Briza et al., 1988; Dörfler, 1990; Prillinger et al., 1993; Nakase, 2000). Usually, however, these carbohydrates are firmly bound within the spore walls, and none of the above studies provided information about their release (except during invasion of host plants). These aspects may merit further investigation in future studies aimed at fully unraveling the primary biogenic components of air particulate matter but go beyond the scope of our study.

**R e f e r e:** Secondly, active spore release is not a universal property of ascomycetes and basidiomycetes. In fact, many fungi - and basidiomycetes in particular - can release spores via passive dropping from the fruiting body from which the spores are suspended. Given that this property is not universal among ascomycete and basidiomycete fungi, it would be useful if the authors clearly stated what genera or species of fungi they have taken into account in this work. We bring up this point again below for other reasons.

**R e s p o n s e:** A large number of studies (Ingold, 1971, 1999; Carlile et al., 2001;

Ingold, 2001b; Pringle et al., 2005; Webster and Weber, 2007), indicate that most of the known species of Ascomycota and Basidiomycota actively discharge their spores. Ingold (1971), for example, pointed out that “in the great majority of Ascomycetes, the largest group of the Fungi, the ascus is a turgid cell that finally bursts in a regular manner violently liberating its contained ascospores”. Buller (1909) stated that “in the majority of the Ascomycetes, the ascus is an explosive mechanism of considerable power, and it often shoots out its spores to a distance of one or several centimeters”, and Gregory (1973) pointed out that the ‘squirt-gun mechanism’ is found in many Ascomycetes.

Concerning basidiomycetes, Gregory (1973) stated that active basidiospore discharge is found “almost throughout the Basidiomycetes”. Ingold (1971) pointed out that “a ballistospore is a spore discharged after the manner of the basidiospores in Hymenomycetes, Tremellales, and Uredinales (rusts)” and that “the ‘secondary conidia’ of *Tilletia* and the aerial spores of the mirror-picture yeasts (*Sporobolomycetaceae*) are also ballistospores.” Previously, Buller (1934) had already reported that “the secondary conidium of *Tilletia tritici*, in its asymmetrical form and drop-excretion mode of discharge, exactly resembles the basidiospores of every mushroom and toadstool and of every Rust fungus.” That rust and smut fungi actively discharge basidiospores, at least during one stage of their life cycle, was often reported by others as well (Buller, 1909-1950; Deml, 1986; Ingold, 1997; Piepenbring et al., 1998; Piepenbring, 1999, 2001). Recently, Pringle et al. (2005) stated: “ballistospore discharge is a feature of 30 000 species of mushrooms, basidiomycete yeasts and pathogenic rusts and smuts”. In fact, all basidiomycota except a few subgroups (e.g., gasteromycetes and some basidiomycetous yeasts) appear to discharge their spores actively (Gregory, 1973; Carlile et al., 2001; Ingold, 2001b; Pringle et al., 2005; Webster and Weber, 2007) (<http://www.anbg.gov.au/fungi/spore-discharge-mushrooms.html>).

**R e f e r e e:** Finally, in the introduction, the authors present four categories of fungi. As “mitosporic” fungi they include Chytridomycota (pg. 11321, lines 5-6). This statement

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is erroneous. The Chytrids are part of a class of more primitive fungi very different from the imperfect fungi. In the paper cited as a reference (Ribes et al, 2000), we did not find mention of the chytrids as part of the mitosporic fungi.

**R e s p o n s e:** We agree and will correct this mistake in the revised manuscript. We had misinterpreted the term “catch-all category” in which Ribes et al. (2000) had placed the Deuteromycetes.

**R e f e r e e:** To identify fungi in air samples, the authors made direct observations with a light microscope at up to 1500x magnification and identified spores by morphology. But they have not indicated how spores were categorized as AAS and ABS. What morphological criteria were used? Although some spores of ascomycetes and basidiomycetes have distinctive morphologies (such as *Alternaria* conidia), fruiting bodies are generally necessary for definitive identification as members of these groups. For example, morphological differentiation of *Botrytis* conidia (deuteromycete - ascomycete) from *Ustilago* teliospores (basidiomycete) has proven to be difficult based on the observation with a light microscope even with the help of image analysis (see Benyon et al. 1999. *Aerobiologia* 15:211-223). Furthermore, some pollen particles may resemble fungal spores.

**R e s p o n s e:** See Taylor (2006).

**R e f e r e e:** Moreover, the authors pointed out that quantified fungal spores were in the 1-10 micrometer size range. However, some fungal spores can reach lengths greater than 10 micrometer.

**R e s p o n s e:** We agree. Nevertheless, the majority of airborne fungal spores has been found in the aerodynamic diameter range of 1-10 micrometer (Buller, 1909; Kapooria, 1971; Boekhout, 1991; Lin and Li, 1996; Fell et al., 1998; Golubev, 1999; Fonseca et al., 2000; Ingold, 2001a; Takashima and Nakase, 2001; Bai et al., 2002; Burge, 2002; Nakase et al., 2002; Bai et al., 2003; Zhao et al., 2003; Wu et al., 2004; Wang et al., 2006). Note that the aerodynamic diameter of aspheric spores is generally

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smaller than the length of the major axis.

**R e f e r e e:** Based on our observations of air samples, we have found it very difficult to accurately distinguish particles, even as experienced mycologists. We believe that methods for quantifying AAS and ABS need to be clarified. Images showing fields of view from their microscopic observations may be useful to substantiate the morphological criteria used. Information concerning the fungal genera investigated and categorized as AAM or ABM fungi would clearly be useful for the reader.

**R e s p o n s e:** See Taylor (2006).

**R e f e r e e:** We wonder: If the authors simply evaluated the contribution of fungal spores (without using the AAS and ABS categories) to carbohydrate and ion concentration in aerosols, would it change the overall conclusion of the paper?

**R e s p o n s e:** We are not sure about the exact meaning of this question. As detailed above, it is difficult to quantify the internal composition and release/detection of the internal contents of fungal spores in atmospheric aerosol samples. We are confident that the conclusions of our paper with regard to ABS and AAS are valid, and we hope that this is further clarified by this interactive comment and in the revised manuscript. In the revised manuscript, we will also include more information about the total abundance of fungal spores in the atmosphere (including AAS, ABS and dry discharged spores) and a global average emission estimate of total fungal spores. An account of all potential contributions of fungi to all sorts of carbohydrates and ions in atmospheric aerosols, however, would go beyond the scope of our study.

**R e f e r e e:** We understand that they put their results into context and make extrapolation based on information about the abundances of these two types of fungi and about the mechanisms by which spore release generates aerosols of sugars and ions. However, their extrapolations are already likely to be overestimates given that they derive global emissions from observations conducted in the Amazon.

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**R e s p o n s e:** Please note that our global emission estimates do neither include nor depend on a simple extrapolation of results from tropical rainforests. They have been derived from a comprehensive survey of studies from all over the world reporting on emissions and atmospheric concentrations of fungal spores and of the sugar alcohol mannitol. In fact, the global estimate calculations are based on (i) the mannitol concentration averaged from results of seven measurement campaigns at extratropical locations (Norway and Belgium; see Table 3 in Elbert et al. (2006)), and (ii) on the literature-derived amount of mannitol emitted per ABS (see Table 8 in Elbert et al. (2006)). In doing so, we have made some fairly conservative assumptions and explicitly pointed out that in tropical regions the abundance of fungal spores and related chemical compounds are typically higher than in extratropical regions. Therefore, our global estimates are more likely to be underestimates rather than overestimates. Nevertheless, we have already pointed out in the discussion paper that further studies on these subjects are required to corroborate the first estimates and elucidate the actual abundance and effects of fungal spores, carbohydrates and other primary biogenic aerosol particles and components in the atmosphere.

**R e f e r e e:** Much of this emission would be from plant surfaces. There are about  $10^{10}$  km<sup>2</sup> of leaf surfaces on Earth. Tropical forests contribute to about 10-15% of this. Boreal and temperate forests, savanna and cultivated land also constitute a large proportion of the plant surfaces on Earth. These biomes could contribute fungi to the atmosphere, but most likely at intensities different from that of the Amazon. (For details about the different ecosystems contributing to total planetary leaf surfaces see: Morris C.E., Kinkel L.L. 2002. Fifty years of phyllosphere microbiology: Significant contributions to research in related fields. pp. 353-363 In: Lindow S.E., Poinar E., Elliot V. (eds.) Phyllosphere Microbiology, APS Press, Minneapolis.) We propose that they clearly state that these extrapolations are based on certain assumptions, and that they give an indication of maximum values.

**R e s p o n s e:** We agree that the plant surface area is likely to be of high importance

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for the local and regional abundance of fungi and emission of fungal spores. However, as already pointed out above, our global emission estimates were and are not based on an extrapolation from the Amazon to the globe. They have been derived from a comprehensive survey of studies from all over the world reporting on emissions and atmospheric concentrations of fungal spores and of the sugar alcohol mannitol. In our global estimate calculations we have used average values, which lie in the middle of the range of concentrations observed across the world and as such do not depend on assumptions about regional differences. The development of regional emission inventories or parameterizations, which would have to take into account surface properties such as local climate and vegetation, is beyond the scope of our study.

#### References:

See subsequent Author Comment in the interactiv discussion.

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Interactive comment on Atmos. Chem. Phys. Discuss., 6, 11317, 2006.

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