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Airborne spores of Basidiomycetes in Mérida (SW Spain)

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Abstract

The aim of this work was to detect the presence of Basidiomycetes spores (basidiospores, teliospores, uredospores and aeciospores) in Mérida (SW Spain) and assess the influence of weather parameters. Air was sampled continuously with a volumetric seven-day Burkard spore trap for two years. Fungi spores were identified and counted at x1,000 microscope resolution. Daily and weekly meteorological data and airborne spore concentration were analysed. Twenty-three spores types were identified, including basidiospores (*Amanita*, *Agrocybe*, *Cortinarius*, *Coprinus* -2 types-, *Boletus*, *Bovista*, *Calvatia*, *Entoloma*, *Ganoderma*, *Inocybe*, *Russula*, *Scleroderma*, *Telephora*), teliospores (*Phragmidium*, *Tilletia*, *Ustillago* -4 types-), uredospores, and aeciospores (2 types), all of these types of spores included different taxa. Average concentration was of 616 spores/m^3, with maximum concentration in autumn (October), and a second concentration in spring (May-June); however, some spore types were more frequent in summer (*Bovista*, *Ganoderma*) or even in winter (*Entoloma*, *Calvatia*). The *Amanita* type was the most frequent (white-hyaline basidiospores); the second were teliospores of *Ustilago*, the third spore type was basidiospores of *Coprinus* (blackish basidiospores) and *Agrocybe* type (smoothed light to dark coloured basidiospores). Basidiospore concentration was positively correlated with temperature and negatively with relative humidity in most cases, and *Ustilago* teliospores concentration was positively correlated with wind speed. Differences in monthly rain were probably the origin between years. Airborne spores of Basidiomycetes may be separated into more than 20 types, and their seasonal concentration depended on meteorology as well as whether they were saprotrophic or parasitic.

Key words

Aeromycology, Basidiomycetes, airborne spores, basidiospores, teliospores, weather parameters

INTRODUCTION

Basidiomycetes are true fungi, mainly saprophytic and mycorrhizic, but also phytopathogenic. All of them produce abundant meiospores or basidiospores that are chiefly airborne and widespread; however, smut, rust, and other plant pathogens produce teliospores, and often uredospores and aeciospores, all of them also mainly airborne and widespread. About 30,000 species have been described [1], but the number of different spores possible to identify through aerobiological studies hardly reach 20, in spite of the fact that they show a high grate of diversity in morphology. Spores from Basidiomycetes are important from the point of view of allergy and phytopathology.

Although previously suggested in the 1950s [2], allergy to basidiospores was first demonstrated in the 1960s [3, 4], indicating that certain types of basidiospores are antigenic in humans These general studies were followed by others that were similar [4, 5, 6, 7, 8, 9, 10, 11, 12] and showed that to some extent the overall extent of mushroom allergy was not clearly known, that the prevalence of respiratory allergy to fungi was imprecisely known, but could be estimated in up to 30% of atopic individuals[13], children being one of the main groups affected [14]. Notwithstanding, Basidiomycetes spores have a great role in fungi allergy [15, 16, 17], and their allergens are release from intact basidiospores [18]. Although basidiospores are a major component of the air spore in many parts of the world, their clinical significance as triggers of respiratory allergy has not often been demonstrated. Moreover, the prevalence of fungal sensitization is not known, mainly due to the lack of standardized fungal extracts and to the overwhelming number of fungal species able to elicit IgE-mediated reactions [19]. Furthermore, in tropical environments, sensitization to airborne Basidiomycetes seems to be more prevalent than sensitization to conidia in subjects with active allergies [20].

More recently specific allergy or sensitization has been demonstrated or documented in different genera and species of Basidiomycetes: *Agrocybe* [15], *Boletus* [21], *Cantharellus cibarius* [22], *Coprinus* [21], *Coprinus comatus* [23], *Coprinus quadrifidus* [24, 25], *Fomes pectinatus* [26], *Fomes fomentarius* [26], *Ganoderma* [27, 28], *Ganoderma lucidum* [29], *Pleurotus eryngii* [11], *Pleurotus ostreatus* [25, 30], *Pleurotus pulmonalis* [16], *Psilocybe cubensis* [25], *Pisolithus tinctorius* [25], *Scleroderma* [22], *Ustilago esculenta* [31], and smuts and rusts in general [32, 33].

From the phytopathological point of view, smuts (mainly *Ustilago* sp.) and rusts (mainly *Puccinia* sp.) are the Basidiomycetes that cause great loss in agricultural yields, chiefly cereal crops. Some aerobiological studies have aimed to assess some of the environmental factors influencing spore dispersal and infection: *Ustilago nuda* in barley [34], *Puccinia graminis* in wheat [35], *Ustilago avenae* in oat [36], *Ustilago scitaminea* in sugarcane [37], *Puccinia arachidis*

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in groundnut [38], and *Uromyces psoraleae*, *Puccinia andropogonis*, *Phragmidium speciosum* in various crops [39]. Their importance has been gerally summarized [40].

Airborne basidiospores have been aerobiologically studied in common aerobiological work sampling for general spore fungi presence in the atmosphere. More specifically, airborne basidiospores or spores from Basidiomycetes have been treated in various papers. The foremost studies were developed in 1956 in Kansas, USA, with one of the first volumetric air samplers designed [41, 42]. The presence of *Ustilago* spores was studied in four European cities during 1978–1980 [43]. In Tusla, USA, a concise work, started in 1987, dealt with a total of 18 basidiospore types [44, 45]. In México City, 7 types of airborne basidiospores were determined [46]. Airborne smuts teliospores were previously studied [47], and although many species were described, all aerobiological data provided in this work were included in a single teliospore type. *Ganoderma* airborne basidiospores were specifically studied again in Tusla (Tulsa, Oklahoma, USA) [48]. In Spain, the atmosphere of Badajoz was studied for two years – 1994 – 1995 [49], more specifically, the presence of 3 types of *Ustilago* spores [50]. Also in Seville, Spain, the airborne basidiospores were studied [51]. In the same way, a similar study was conducted in Saudi Arabia [52], and in Cracow, Poland, the diurnal periodicity of *Ganoderma* basidiospores was analysed [53].

The aim of the presented study is to show the results of airborne basidiospores from two years research in SW of Spain, and evaluate the possible influence of meteorological parameters in their temporal variation, highlighting its medical or phytopathological relevance.

MATERIAL AND METHODS

The study site was the town of Mérida, Badajoz province (SW Spain, 06° 23' W, 38° 5' N). The atmosphere was monitored for 2 years (January 1997 – December 1998) using a Hirst [54] spore trap (Burkard seven-day sampler) located 15 m above ground level on the terrace roof of the town's Clinic Hospital. White petrolatum was used as adhesive. Two longitudinal scans at x1,000 magnification were analysed for each microscope slide, both of them in the centre of the slide with a 1-mm separation to minimize errors [55]. Spore records allow hourly data of airborne spore concentration for the whole period of study.

Meteorological data were provided by the Regional Meteorological Centre located 1.7 km away. The following daily parameters were analysed: temperature, rain, relative humidity, wind speed, and wind direction. The Spearman correlation coefficient was applied to search for possible relationships between daily spore concentration and meteorological parameters, since daily and weekly data were neither normally nor log-normally distributed. To reduce random effects, only significance levels below 0.01 were taken into account. The log-transformed monthly data were normally distributed, thus allowing the use of a t-test to compare the 2 years. Statistical analyses were performed using the SPSS 15.0 programme package.

Identification of spores from Basidiomycetes (basidiospores, teliospores, uredospores and aeciospores) was performed using some general references [56, 57] and specific works [58, 59]. Nevertheless, most of them were identified from reference slides from local specimens [60]. Furthermore, as they showed a great difficulty in identifying at species or genus level, the term 'type' is used to include different taxa associated with a taxonomic name.

RESULTS

A total of 142,126 spores from Basidiomycetes were counted in the period studied. They were separated in 23 spore types, apart from the rest of basidiospores not identified (1.2%). These types included 14 types of basidiospores, 6 types of teliospores, 1 type of uredospores and 2 types of aeciospores. Table 1 briefly describes the types and a first approach of the main genera that are included in these types, in accordance with the literature mentioned.

The average concentration for these spores during the 2 years studied was 616 spores/m^3, this represented about 20% of the total fungal propagules detected. The highest daily values was 6,788 spores/m^3, reached on 22 November 1997. The most frequently occurring spore type was *Amanita*, with an average concentration of 200 spores/m^3, the second most frequent – teliospores of *Ustilago*, with 167 spores/m^3 with 3 types altogether (Fig. 1). Autumn was the season with the highest concentrations, mainly in October, and in spring there was another maximum, between May – June. March was the month with lowest concentrations (Fig. 2). The general pattern with maximum values in autumn and spring was followed mainly by the basidiospore types: *Amanita*, *Agrocybe*, *Boletus*, *Coprinus comatus*, *Coprinus micaceus*, *Cortinarius*, *Inocybe*, *Russula* and *Thelephora* (Tab. 2). On the other hand, *Calvatia*, *Entoloma*, *Scleroderma*, *Tilletia*, and *Ustilago* B types showed maximum concentrations at the end of autumn and beginning of winter. *Bovista*, *Ganoderma*, *Ustilago cynodontis*, *Puccinia* types, and both aeciospores types, showed maximum values in summer. Finally, the *Ustilago avenae* type showed a spring pattern and the *Ustilago maydis* type in autumn. The representation of *Phragmidium* type was so low that it proved impossible to discern any pattern.

Table 3 shows the results from correlation analysis between spore concentrations and meteorological parameters. It seems than temperature and relative humidity are the main meteorological factors that affect spore concentrations; nevertheless, seasonal patterns should also be taken into account. Positive correlation with temperature appeared in types with aestival higher concentrations, as in both aeciospores types, *Bovista*, *Ganoderma*, *Ustilago cynodontis* and *Puccinia* types, except for *Agrocybe, Scleroderma* and *Ustilago maydis* types. Fig. 3A shows this relationship in the *Ganoderma* type. On the other hand, a negative correlation with temperature appeared in the *Amanita*, *Cortinarius*, *Entoloma*, *Russula*, and *Telephora* types, all of them including typical autumnal mushrooms, which was probably mainly affected by the relative humidity factor, always with an opposite relationship with temperature. Fig. 3B shows this relationship in the *Cortinarius* type.

Wind is a meteorological factor not easy to interpret; wind speed appeared to be correlated only in the 4 types of the *Ustilago* type described, positively correlated in the *Ustilago avenae* and *U. cynodontis* types, and negatively correlated in the *Ustilago maydis* and *Ustilago* B types (Fig. 3C). Wind direction did not show any general pattern of influence, and seemed to affect mainly the *Cortinarius*, *Ustilago avenae* and *U. cynodontis* types (Fig. 3D). Results comparing both years

Table 1. Spore types defined from Basidiomycetes, including a first approach of genera may be included

Name of type	Type of spores	Description	Main taxa included
Aeciospores A	Aeciospores	Elliptical to sub-spherical, 16–22 mm, orange to yellowish, echinulate very thick wall, 3–6 wide pores or inconspicuous pores.	*Puccinia*
Aeciospores B	Aeciospores	Elliptical to pyriform, 18–26 mm, pinkish to greyish, echinate thick wall, apparently with minute pores.	*Puccinia*
Agrocybe	Basidiospores	Elliptical, 8–16 mm, light to dark coloured, thick wall, prominent germ pore.	*Agaricus, Agrocybe, Coltricia, Hygrocybe, Hypholoma, Lentinus, Paxilus, Pholiota, Pleurotus, Psilocybe, Volvariella*
Amanita	Basidiospores	Ovoid, rectangular to elliptical, 10–16 mm, hyaline to grey, often guttulated, inconspicuous germ pore.	*Amanita, Auricularia, Cantharellus, Clitocybe,Collybia, Cratarellus, Hygrophorus, Lepiota, Lepista, Marasmius, Melanoleuca, Mycena, Omphalotus, Tremella, Tricholoma*
Boletus	Basidiospores	Fusiform to oblong, 12–20 mm, grey to light coloured, often guttulated, light wall, small or inconspicuous germ pore.	*Boletus, Chroogomphus, Gomphidius, Leccinum, Suillus, Xerocomus*
Bovista	Basidiospores	Sub-spherical, 4–6 mm, yellow-brown, thick wall, prominent germ pore generally with a peduncle- hilar appendage – up to 10 mm length.	*Bovista*
Calvatia	Basidiospores	Spherical, 4–6 mm, grey to olivaceus, verrucated or warted thick wall, inconspicuous germ pore.	*Calvatia, Lycoperdon, Geastrum, Tulostoma, Vascellum*
Coprinus comatus	Basidiospores	Elliptical, 12–20 mm, dark grey to blackish, thick wall, prominent and broad germ pore up to 2 mm in diameter.	*Coprinus sp.*
Coprinus micaceus	Basidiospores	Ovoid to triangular, mitriform, with conspicuous distal peak, 8–10 mm, dark reddish to blackish, thick wall, prominent germ pore	*Coprinus micaceus, Coprinus angulatus*
Cortinarius	Basidiospores	Elliptical to fusiform, asymmetric, 12–16 mm, light brown coloured, verrucated or warted thick wall, conspicuous germ pore.	*Alnicola, Corntinarius, Hebeloma, Gymnopilus*
Entoloma	Basidiospores	Elliptical to spherical, angular o polyhydric, with prominent angles, 8–12 mm, greyish, light wall, inconspicuous germ pore.	*Entoloma*
Ganoderma	Basidiospores	Elliptical, 8–10 mm, golden brown, verrucated thick wall, prominent germ pore with truncate apex or neck	*Ganoderma*
Inocybe	Basidiospores	Elliptical to ovoide, nodulose, 8–10 mm, wall with blunt projections, inconspicuous germ pore.	*Inocybe*
Phragmidium	Teliospores	Oblong, 3–4 celled, stalked, 20–35 mm, blackish, very thick wall, hyaline clavate stalk.	*Phragmidium*
Scleroderma	Basidiospores	Spherical, 8–12 mm, brownish to yellowish, verrucated to equinated thick wall, inconspicuous germ pore.	*Astraeus, Battarrea, Scleroderma, Pisolithus*
Puccinia	Uredospores	Spherical, 18–26 mm, brownish to reddish, granulate very thick wall, with 4–8 wide pores.	*Puccinia*
Russula	Basidiospores	Ovoid to elliptical, 7–10 mm, hyaline to pinkish, reticulated to verrucated light wall, inconspicuous germ pore.	*Russula, Lactarius*
Thelephora	Basidiospores	Spherical to ovoid, nodulose, 6–10 mm, greyish to brownish, echinulated light wall, inconspicuous germ pore.	*Hydnellum, Sarcodon, Thelephora*
Tilletia	Teliospores	Elliptical to spherical, 10–16 mm, greyish to pinkish, reticulated thick wall, with wide lumen, inconspicuous term pore.	*Tilletia*
Ustilago avenae	Teliospores	Spherical to elliptical, 6–8 mm, verrucated thick wall, with a lighter zone, inconspicuous germ pore.	*Ustilago avenae*
Ustilago cynodontis	Teliospores	Spherical to sub-elliptical, 6–8 mm, greyish to brownish, smooth thick wall, inconspicuous germ pore.	*Ustilago cynodontis*
Ustilago maydis	Teliospores	Spherical to sub-elliptical, 10–14 mm, brownish, verrucated thick wall, inconspicuous germ pore.	*Ustilago maydis*
Ustilago B	Teliospores	Spherical, 14–18 mm, brownish to reddish, verrucated thick wall, inconspicuous germ pore.	*Tilletia, Ustilago*

showed no statistical difference for total spores (t=1.413, p=0.172), nor for any of the spores types, except for *Bovista* type (t=2.531, p=0.019).

DISCUSSION

Rarely airborne sexual spores from Basidiomycetes are the most abundant fungal propagule, probably due to tropical conditions [61], because the first place is often occupied by conidia. The importance of spores of Basidiomycetes in the air is variable: 11–17% in Saudi Arabia [52], 16% in Tulsa, USA [62], 20.7% in Kerala, India [63], 30% in Sevilla, Spain [51], and 32% in Mexico DF, Mexico [46]. The representation found in the presented study (20%) occupies an intermediate position between the works cited.

The number of different spores types identified from Basidiomycetes in aerobiological studies varied, sometimes there was only a differentiation between basidiospores and spores from smuts [61]; in other cases, more types are described, 3 [52, 63], 7 [46], 11 [64], 15 [65], and 18 [44, 45, 51]. Some of the types identified are clearly defined and appear throughout the literature: *Agrocybe*, *Boletus*, *Bovista*, *Calvatia*, *Cortinarius*, *Entoloma*, *Ganoderma*, *Inocybe*,

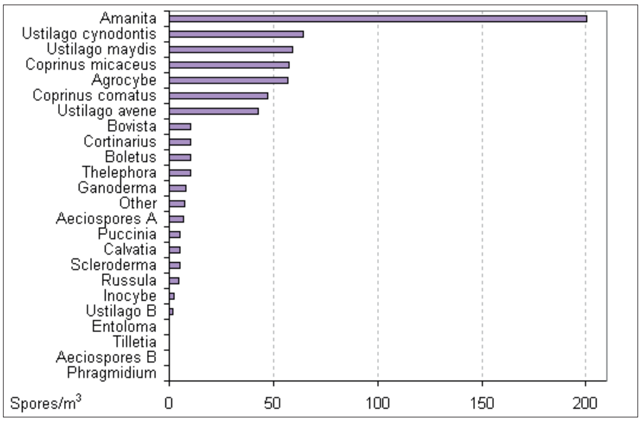

Figure 1. Annual average concentration of spores per cubic metre.

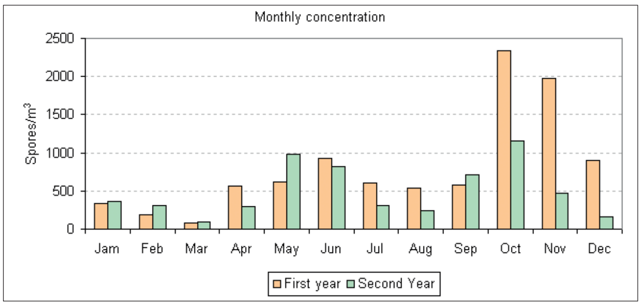

Figure 2. Monthly average spore concentration in spores per cubic meters for the 2 years studied.

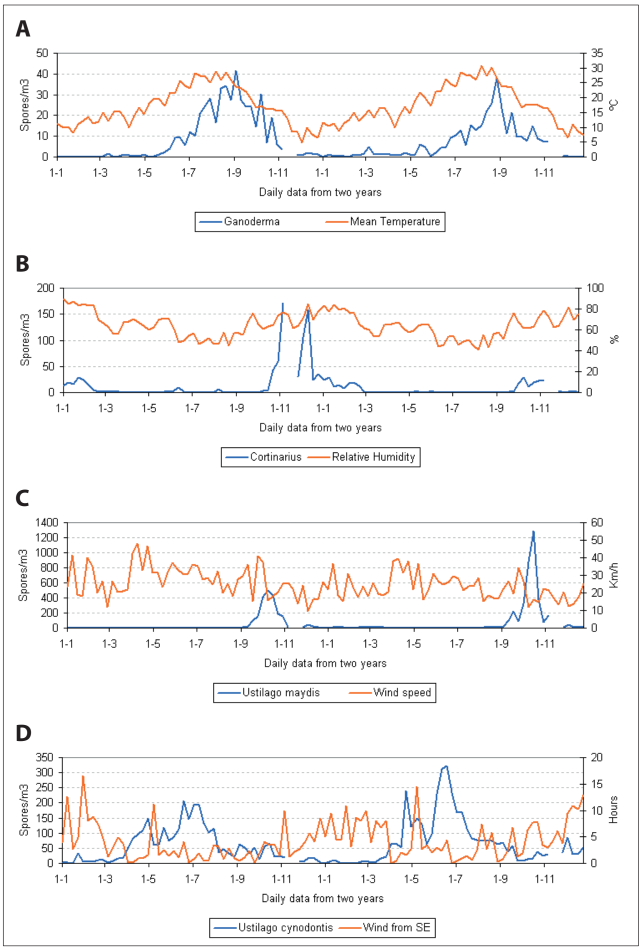

Figure 3. Weekly spore concentration and weather parameters in two years for some of the spore types studied.

Table 2. Average monthly concentration in spores per cubic metre. * p<0.05, ** p<0.01

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Aeciospores A	1	3	8	7	13	29	12	3	1	1	0	0
Aeciospores B	0	0	0	0	0	0	1	0	0	0	0	0
Agrocybe	28	35	6	8	26	82	104	75	45	132	145	56
Amanita	153	85	7	44	362	158	13	100	316	640	534	224
Boletus	1	1	0	1	2	55	1	0	1	23	50	9
Bovista	4	2	4	3	18	5	14	22	21	16	14	7
Calvatia	8	2	1	1	1	3	3	5	5	10	10	14
Coprinus micaceus	18	25	8	24	74	143	51	19	29	173	143	27
Coprinus comatus	61	44	14	20	60	74	31	18	29	120	82	34
Cortinarius	19	11	1	0	1	2	0	0	0	20	58	38
Entoloma	1	2	0	0	0	1	0	0	0	0	3	4
Ganoderma	0	0	1	1	2	5	14	26	24	13	5	1
Inocybe	0	2	1	1	3	10	0	0	0	1	5	3
Phragmidium	0	0	0	0	0	0	0	0	0	0	0	0
Scleroderma	2	0	1	1	7	4	5	8	8	10	18	4
Puccinia	0	0	1	4	6	17	12	10	6	4	2	0
Russula	9	4	0	0	1	4	0	0	0	3	27	18
Thelephora	22	18	0	2	5	17	2	1	1	11	36	20
Tilletia	0	0	0	0	0	0	0	0	1	2	1	0
Ustilago avenae	5	1	14	212	114	63	39	21	11	9	2	7
Ustilago cynodontis	6	5	11	98	100	197	140	62	46	30	27	30
Ustilago maydis	5	5	6	3	2	1	2	5	84	521	49	20
Ustilago B	0	0	0	0	1	1	1	1	4	7	3	2
Other basidio-spores	9	6	2	3	4	5	11	11	12	8	7	10

Puccinia (uredospores, aeciospores) *Russula*, *Scleroderma*, *Thelephora*, *Tilletia* (teliospores), *Ustilago* (teliospores).

Nevertheless, one especially difficult group is the hyaline or colourless basidiospores that although recognized in some works, they were not counted [45]. In the presented study, they have been ascribed under *Amanita* which includes a wide range of related taxa, and are probably the *Clitocybe* type described previously [45]; also from the work of that author it is difficult to separate the types *Paenolina*, *Stropharia*, *Agaricus*, *Chlorophyllum*, and *Conocybe*, which have been included in the *Agrocybe* type. Other inclusions from that work are *Panaeolina* in *Cortinarius* type, and *Psatirella* in *Coprinus* type. In all cases, the authors of the presented study consider that there are insufficient differences to separate them; furthermore, from another work [51], the authors matched the *Bankeraceae* type to the *Scleroderma* type,and the *Rhodophyllus* type to the *Entoloma* type, and both the *Phylacteria* and *Tomentella* types to the *Thelephora* type. The authors consider the addition of more names to be unnecessary.

There is great consensus that spores from Basidiomycetes, at least basidiospores, reach the maximum in autumn, often with a second peak in spring or early summer, as was found

Table 3. Correlation coefficients between spore concentration and weather parameters. * p<0.05; ** p<0.01.

	Rain	T max	T min	T mean	Wind Sp	Calms	Quad 1	Quad 2	Quad 3	Quad 4	Rel Humid
Aeciospores A	–0.205*	**0.712****	**0.656****	**0.710****	0.208*	**–0.378****	**–0.287****	**–0.397****	**0.351****	**0.436****	**–0.657****
Aeciospores B	–0.113	**0.388****	**0.331****	**0.377****	–0.063	–0.018	–0.113	–0.089	0.178	–0.007	**–0.448****
Agrocybe	–0.185	**0.309****	**0.371****	**0.338****	–0.125	0.057	–0.009	–0.158	0.113	0.119	–0.120
Amanita	**0.435****	**–0.300****	–0.111	–0.217*	0.057	–0.023	–0.023	0.088	0.079	–0.135	**0.531****
Boletus	–0.156	0.057	0.030	0.046	–0.072	0.105	0.037	–0.034	–0.026	0.130	–0.005
Bovista	–0.056	**0.297****	**0.300****	**0.297****	–0.072	–0.005	–0.192	–0.126	0.138	**0.261****	–0.141
Calvatia	0.034	–0.203*	–0.147	–0.196	–0.225*	0.218*	0.095	0.138	–0.061	–0.056	**0.284****
Coprinus comatus	0.211*	–0.153	–0.008	–0.078	0.019	–0.017	0.036	0.031	0.034	–0.034	**0.388****
Coprinus micaceus	0.040	0.148	0.223*	0.190	0.064	–0.093	–0.078	–0.210*	0.119	0.248*	0.061
Cortinarius	0.145	**–0.583****	**–0.550****	**–0.585****	–0.234*	**0.353****	**0.480****	**0.525****	**–0.453****	**–0.462****	**0.612****
Entoloma	0.162	**–0.376****	**–0.273****	**–0.337****	–0.121	0.170	**0.274****	**0.265****	–0.182	**–0.318****	**0.369****
Ganoderma	**–0.406****	**0.776****	**0.757****	**0.783****	–0.043	–0.082	–0.244*	**–0.455****	**0.320****	**0.410****	**–0.626****
Inocybe	0.106	–0.119	–0.129	–0.119	0.016	0.087	0.141	0.042	–0.106	0.086	0.072
Phragmidium	0.146	–0.021	0.033	0.016	0.138	–0.169	–0.124	–0.089	0.134	0.057	0.078
Scleroderma	–0.064	**0.304****	**0.330****	**0.308****	–0.068	–0.039	–0.252*	–0.235*	**0.267****	0.219*	–0.090
Puccinia	–0.092	**0.517****	**0.619****	**0.581****	0.125	–0.216*	–0.148	**–0.355****	0.241*	**0.293****	**–0.324****
Russula	0.143	**–0.488****	**–0.385****	**–0.457****	–0.004	0.040	**0.276****	0.250*	–0.214*	–0.195	**0.516****
Thelephora	0.210*	**–0.371****	–0.243*	**–0.312****	–0.013	0.061	**0.277****	0.238*	–0.168	–0.231*	**0.458****
Tilletia	–0.125	0.059	0.003	0.032	–0.063	0.126	–0.118	–0.053	0.073	0.011	–0.046
Ustilago avene	0.028	**0.477****	**0.456****	**0.484****	**0.399****	**–0.423****	**–0.353****	**–0.456****	**0.411****	**0.474****	**–0.473****
Ustilago cynodontis	–0.174	**0.630****	**0.573****	**0.617****	**0.281****	**–0.415****	**–0.380****	**–0.493****	**0.421****	**0.499****	**–0.610****
Ustilago maydis	–0.137	–0.103	–0.145	–0.141	**–0.428****	**0.450****	0.172	0.222*	–0.247*	–0.200*	0.201*
Ustilago B	–0.182	0.194	0.174	0.174	**–0.318****	**0.259****	–0.066	–0.007	0.079	–0.089	–0.045
Other basidiospores	–0.044	0.055	0.079	0.063	0.182	–0.167	–0.004	–0.213*	–0.019	**0.357****	0.032

in the presented study work. Thus, the months with highest concentration in the works studied were June and September [45], September [66], between July-September, a and a second peak in April [61], November [49, 51], or mainly in the wet season [46].

The average concentration ranges in spores per cubic metre found in the papers consulted were between 388 [62], 1,222–1,307 [66], and 3,433 [61]. Comparatively, the results of the presented study did not reach very high values (616 spores/m^3). The highest concentration in the papers consulted for airborne basidiospores reached more than 10,000 spores/m^3 [61]; nevertheless, highest value found in the presented study (6,788 spores/m^3) was higher than that found in other works: 2,000 spores/m^3 [46], 3,600 spores/m^3 [45], 4,746 spores/m^3 [51], 4,852 spores/m^3 [49], and 6,000 spores/m^3 [52].

The type most frequently recorded in the literature was *Coprinus* [46,47,52] and *Ganoderma* [64], the second *Agaricus* [45, 64] and *Ustilago* [51]. Although the *Amanita* type was to be the first, it is possible that this spore type, due to their difficulty in identification, could be underestimated in the papers consulted.

It is widely accepted that concentrations of spores of Basidiomycetes are directly relate to rain [46, 61, 67] or humidity [46, 49]. However, dew point results are not always the same [66, 67]; it seems therefore that the availability of water is necessary to develop not only the mycelium but the fructification and spore release.

In the presented study, annual rain was lower than the mean value for this station (509.8 mm) in both years studied (279.5 and 272.9 mm respectively); nevertheless, the rain in May was higher than normal values in both years, and September was also higher in the second year. No relationship was found between total spores of Basidiomycetes and rain or relative humidity, but in 2 cases this relationship did appear with rain (*Amanita* and *Ganoderma* types), and in 8 cases with relative humidity (*Amanita*, *Cortinarius*, *Coprinus comatus*, *Calvatia*, *Entoloma*, *Ganoderma*, *Russula*, *Thelephora* types). Many of these types showed a second peak in spring, probably due to the rains in May of both years studied.

Coprinus type was considered to be the most important spore type in some papers [45, 46, 51], detected mainly from the end of August until December: up to 2,387 spores/m^3 in Seville [51] from June – October [46]. In the presented study, two types of *Coprinus* spores were separated, and altogether represented the third place, after *Amanita* and *Ustilago* types, with a peak of concentration of 1,584 spore/m^3 in October. Only in the *Coprinus comatus* type a relationship with relative humidity was found [41, 42].

The *Ganoderma* type has been one of the airborne basidiospores most widely studied, perhaps because the allergy it causes. Maximum concentration has been recorded in summer: July – September (Singh et al., 1995), June – October [48], and August [68]. The highest concentrations were 336 spores/m^3 [68] and 90 spores/m^3 [61]. Their concentration were correlated positively with temperature [48] and precipitation [48, 53], and relative humidity [53]. The presented results agree partially with these papers. Maximum concentration found was 88 spores/m^3 and appeared at the end of summer, but correlation with rain was negative. It can be supposed that temperature is the main factor and rain and humidity depend on the climate, as in Mediterranean countries rain and relative humidity increase mainly in winter.

Airborne smut spores (*Ustilago* types) has been widely studied mainly because of their phytopathogenic character but also for their allergy relevance. In Europe the concentration has ranged from 29 to 113 spores/m^3, with maximum concentration between May and July, and mainly in June [43]. In some cases it has been the spore from Basidiomycetes more important, representing up to 1/3 of total basidiospores and with maximum peak of 4000 spores/m^3 [52], but in most cases their maximum concentration is lower: 259 spores/m^3 [62], 379–454 spores/m^3 [66]. They have appeared in the atmosphere in October-November and January [46], March and October [32]. Some studies have found correlation with meteorology, finding that shedding of spores was generally encouraged by dry and reduced by wet weather, and was affected by the intensity and duration of rainfall [37]. It has been showed that the prevailing wind velocity, especially its gustiness, influences the rate of smut dissemination [34]. Moreover, it has been pointed out that the increase in wind velocity accompanying rain was an important factor in dispersal [36]. Correlation with temperature has also been found [66]. An abundant collection of smut spores images have been showed but they were counted altogether in their aerobiological study as only one type [47]. We have differentiated four smut spore types and altogether they represent the second place in importance. They appear in different seasons, *Ustilago avenae* type in spring, *Ustilago cynodontis* type in summer, *Ustilago maydis* type in autumn, and the fourth type, *Ustilago* B type, that possibly being teliospores of *Tilletia*, because some of them do not have a reticulate pattern in the wall [69]. The behaviour of airborne smut spores is close related with the biology of the plant parasited, so it depends mainly on the host phenology. It seems that temperature increase concentration and humidity decrease it, at least for the most abundant smut spore types.

Airborne rust spores (*Puccinia* type) are not frequently included in aerobiologic studies. These are big spores easy to identify by their size, thick wall and colour, being brown uredospores (uredinospores) and aeciospores generally being orange, yellow or pink. Their relationship with meteorology has been studied mainly from the phytopathological point of view. It was found that airborne uredospores showed maximum concentration when temperatures were 29–31°C and relative humidity 75–85%, and that their numbers decreased with increasing height above ground level, with a sharp reduction above 2 m [38]. It has been pointed out that aeciospores are expelled with a discharge mechanism depending directly on humidity [39, 41, 42]. Wind also was taken into account, indicating that increased spore production was associated with increased wind speed [35]. Inthe presented study, uredospores and aeciospores were found mainly in summer, whereas other authors have recorded this type also in autumn [32, 46], and with a positive relationship with temperature and negative relationship with relative humidity.

CONCLUSIONS

Airborne spores from Basidiomycetes normally occupy the second place after conidia with respect to total fungal propagules in the air, only in a particular ambient, as in the tropics, they could be the most abundant. In the presented study, Hyaline basidiospores (*Amanita* type) were the most abundant basidiospore type, but in the literature this type is rarely considered, and supposedly could be underestimated because the difficulty in their identification. Elliptical basidiospores, light to dark coloured, with smooth wall and clear germ pore show a great variability in size and colour, all of them have been included in only one type (*Agrocybe* type). Nevertheless, it is the belief of the authors that it would be necessary to standardize names and type description to compare appropriately aerobiological results. It seems clear that in western countries autumn is the season with the highest concentration of basidiospores; however, spring also offers a second peak for basidiospores, probably depending on rain distribution. On average, throughout the whole period of study, more than 600 spores/m^3 were recorded, but with a daily peak 10 times higher. Water supply seems to be a major factor for the development and shedding of spores from Basidiomycetes in the air, but relative humidity is the most important meteorological factor.

The most important 3 spore types from Basidiomycetes, in the following order: the *Amanita*, *Ustilago* and *Coprinus*. *Ganoderma* types, did not show correlation with rain or relative humidity as shown in other studies, but did correlate with temperature, It may therefore be supposed that the correlation with rains depends mainly on seasonal distribution of precipitation, which differs in each country.

In the presented study, three types of *Ustilago* spores were clearly separated, with 3 different seasonal patterns: in spring – *U. avenae* type, summer – *U. cynodontis* type, and autumn – *U. maydis* type), and their seasonal behaviour may depend mainly on host phenology, rather than meteorological parameters. Uredospores and aeciospores appeared mainly in summer and showed relationships with temperature and relative humidity, positive and negative, respectively.

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