

The biodiversity of air spora in an Italian vineyard

Donát Magyar · Giuseppe Frenguelli ·
Emma Bricchi · Emma Tedeschini ·
Péter Csontos · De-Wei Li · János Bobvos

Received: 15 December 2008 / Accepted: 16 March 2009
© Springer Science+Business Media B.V. 2009

Abstract Biodiversity indices are frequently used to provide a numerical value of the diversity of species within an ecological community. To study fungal biodiversity in the air, a 7-day recording Hirst-type spore trap was used. The daily concentration of 124 taxa was recorded in an Italian vineyard. The predominant fungi were: *Cladosporium* spp., unknown 3-septate fusiform spore, and *Alternaria* spp. Shannon's and Simpson's biodiversity indices and evenness were calculated first for air spora. Meteorological circumstances affected the biodiversity; positive correlations were found between Simpson's biodiversity index and precipitation, but no significant correlations were found with Shannon's diversity index and evenness. To predict

Simpson's index for airborne fungi, regression analysis was performed. It was shown that the best estimator, sun hours, negatively affected the index. The biodiversity of the dominant species was low on dry days, since *Cladosporium* alone had much higher abundance on such days than other species.

Keywords Air spora · Fungi · Biodiversity · Weather

1 Introduction

The atmosphere is rich in propagula from different fungal species. Many of them may cause a range of allergic symptoms and plant diseases (Tilak and Pande 2005; Gonianakis et al. 2006; Bousquet et al. 2007; Magyar 2007). Aerobiology, alongside its roles in human and plant pathology, is becoming increasingly important in the study of biodiversity in indoor environments as well as in natural and agricultural habitats.

'Biodiversity' is a term frequently used in the scientific press. In aerobiological works, biodiversity descriptions are restricted to the list of spore types including different species. Attempts to measure airborne fungal biodiversity with biodiversity indices have not yet been made. Such indices are frequently used by ecologists to provide a numerical value of the

D. Magyar (✉) · J. Bobvos
Institute of Environmental Health, Gyáli út 2-6, 1097
Budapest, Hungary
e-mail: magyar.donat@gmail.com

G. Frenguelli · E. Bricchi · E. Tedeschini
University of Perugia, via Borgo XX Giugno 74, 06120
Perugia, Italy

P. Csontos
Research Institute for Soil Science and Agricultural
Chemistry of the Hungarian Academy of Sciences,
Herman Ottó út 15, 1022 Budapest, Hungary

D.-W. Li
The Connecticut Agricultural Experiment Station, Valley
Laboratory, 153 Cook Hill Rd, P.O. Box 248, Windsor,
CT 06095, USA

diversity of species within an ecological community (Dighton 1998). A simple biodiversity index is calculated as follows: the number of taxa in the area (numerator) divided by the total number of individuals in the area (denominator). Therefore, this index can give an acceptable result if as many taxa are identified as possible.

Whereas the spores of many fungi are difficult to identify, those of many others are highly characteristic and can be identified or distinguished even without cultivation. Spore composition was extensively studied in streams and elsewhere (e.g., in rainwater by Gönczöl and Révay 2004; in honeys by Pérez-Atanes et al. 2001; in honeydew honeys Magyar et al. 2005; on bark by Magyar 2008). Several hundreds of spore types are identified also from air samples; however, only a few works have emphasized the richness of fungal species in the atmosphere and aimed to identify rare airborne fungal taxa (Shoemaker et al. 1974; Kendrick 1990; Járαι-Komlódi and Tóth 1993; Lacey and West 2006; Bustos Delgado et al. 2008). The present study was carried out in a vineyard; from such habitat 13 fungal taxa were reported by earlier air samplings (Corbaz 1972; Picco 1992; Diaz et al. 1998), 8 of them from Italy (Picco 1992). These studies focused mostly on common allergenic or plant pathogenic fungi (*Alternaria* spp., *Botrytis cinerea*, *Cladosporium* spp., *Plasmopara viticola*, *Uncinula necator*). In Italy, the occurrence of 69 fungal species was documented on vine plants by the USDA (Farr and Rossman 2008). Apparently, the species composition of the air of vineyards has hardly been studied.

The aim of this paper is (1) to give a more detailed insight of the spore composition of a vineyard, (2) to apply biodiversity indices to the air spora, and (3) to reveal the relationship between air spora biodiversity and meteorological parameters.

2 Materials and methods

2.1 Fungal spore data

A 7-day recording air sampler (Hirst 1952; Lanzoni, Bologna, Italy, catalogue code: VPPS 2000) was used to record the daily concentration of airborne spores. The sampler was located in the middle of a traditional vineyard of Central Italy, near the city of Brufa which

is characterized by a mild Mediterranean climate. Air sampling was conducted at the blooming period of the vine plant for 3 years (from 27 May to 13 June 1994; from 5 June to 3 July 1995; and from 13 to 24 June 1996). These months were selected because they represented climatic extremes (as suggested by Troutt and Levetin 2001), having high precipitation and exceptionally windy conditions. The spore traps and meteorological instruments were placed at 12 m height on the top of a hill in Brufa (300 m a.s.l.), between the valleys of Assisi and Torgiano. The surroundings of Brufa are entirely dedicated to vine cultivation (Chardonnay, Pinot, Cabernet), its area, approximately 2,500,000 m², is kept by the Luganotti Company. Other plant species (Cupressaceae, *Olea europaea* L., *Pinus* spp., *Quercus* spp.) also occur sparsely.

The spore trap worked continuously, aspirating in air at a rate of 10 l/min. The airborne fungal spores impacted on a tape (MELINEX[®] strip) coated with a thin adhesive layer (silicone oil). The greased tape was mounted on a rotating drum within the trap, rotating 2 mm/h. The exposed tape was removed weekly and cut into 48-mm segments, thus representing 24-h periods. The segments were placed on microscope slides and stained with basic fuchsin in mounting medium (glycerine-jelly). To provide a full picture of the air spora, 100% of the areas of the samples were scanned. To count fungal content, two longitudinal transverses along the length of the slide were scanned, at 400× magnification using a DIALUX 20 microscope. For each daily recording, fungal spores were counted, classified into families, genera and sometimes into species, and expressed as number of spores trapped per cubic metre of air. Because of their high number, *Cladosporium* conidia were enumerated in four random areas of a microscope field of sight (with a diameter of 523 µm) in every 6 mm of air sample. The *Cladosporium* counts were summarized and multiplied by 16; the result of this calculation is the daily concentration of spores per cubic metre of air.

We used the Leica QWin Image Analyser Programme to study spore morphology. Fungal spores were identified on various levels. Uncertain taxa were labeled according to Magyar (2007).

2.2 Weather data

Meteorological data were obtained from a weather station (SIAP Bologna, Italy) located in the vicinity

of the sampling site. The daily records of temperature ($T_{\min}$, $T_{\max}$, T_{avg}) (°C), dew point (°C) and relative humidity were measured with a hygrothermometer. Rainfall (mm) and the duration of precipitation (min) were recorded with a rain gauge and rainfall intensity (mm/min) was calculated. A 3-cup anemometer with a standard wind vane and an electronic barometer were used to measure wind speed (m/s) and wind direction, and atmospheric pressure (Pa), respectively. Cloud cover (%), presence or absence of fog and sun hours (min) were also recorded. Evaporation (mm) was measured with the Wild evaporimeter.

Wind and temperature conditions were similar in all the three sampling periods (avg 2.7 m/s, wind gusts between 13.8 and 22.2 m/s, $T_{\max}$: 23.89°C; $T_{\min}$: 14.70°C). The period in 1996 was slightly drier (average precipitation 0.69 mm) than in the previous year (average precipitation 2.19 mm).

2.3 Data analysis

Biodiversity was expressed by the Shannon's index (HS) and Simpson's reciprocal index (D):

$$HS = - \sum_{i=1}^S P_i (\log P_i)$$

$$D = \frac{1}{\sum_{i=1}^S P_i^2}$$

where S is the total number of genus in every air sample (day) and p_i is the proportion of individuals that belong to the i th genus (Magurran 1988). Species evenness was calculated by dividing the actual diversity value with the potential maximum of diversity. The Shapiro-Wilk W test was used in testing for normality of variables (Shapiro et al. 1968). Since this statistic was significant (r 0.84, $P < 0.00001$), then the hypothesis that the respective distribution is normal was rejected. Then, the variables were logarithmically transformed to normalise data. To avoid zero values, we added 0.9 to the concentrations according to Troutt and Levetin (2001). To clarify the connection between biodiversity indices and weather variables, Pearson correlation analysis was applied using the STATISTICA programme (StatSoft, 2003, version 6.1). Linear regression was processed to predict the value of the biodiversity index using the normalized data.

We constructed rank abundance curves to assess how relative abundance and evenness depended on weather. The species were sorted with respect to their rank. The most abundant species were plotted first then the next most common and so on until the array is completed by the rarest of all. Plotting abundance against rank yielded rank abundance curve. To show the diversity dissimilarity among dry and wet (rainy) days, right tail sum (RTS) biodiversity was used (Patil and Taillie 1979)

$$RTS(i) = \sum_{j=i+1}^S p(j), 1 \leq i \leq S.$$

3 Results

3.1 Air spora composition

A total of 189 different fungal taxa were observed in our spore trap and 50 taxa appeared to be new for air samples (Table 1). The majority, 73 taxa, were classified as genus, and 67 of them were identified to species level. More than 45% of the taxa were mitosporic fungi. Ascomycetes represented also a considerable proportion (24.6%) of the total air spora. Percentages of airborne fungi belonging to Basidiomycetes, Myxomycetes, Oomycetes, Urediniomycetes, and Ustilaginomycetes were lower (1–5%).

3.2 Air spora concentration

The daily concentrations of 124 different spore types were determined (Table 1). The average of daily spore concentration for all the studied period was 2,063 spores/m³ (max 3,919 spores/m³ on 14 June 1996). The most abundant fungal particles in our air samples belonged to the genus *Cladosporium* (av 1,286.48 spores/m³, 62.33% of the total count). Unknown 3-septate fusiform spore (104.16 spores/m³, 4.65%), *Alternaria* spp. (92.03, 4.45%), unknown hyalodidymae (65.94, 3.07%) and *Ustilago tritici* (47.62, 2.08%) also made up a significant portion of airborne fungi. Hyphal fragments (mostly conidiophores belonging to different dematiaceous hyphomycetes) reached a high concentration as well (205.46). The common taxa (av above 1.00 spore/m³, 0.05%) comprised 88.52% of air spora. A wide range of other identified fungal taxa was also recorded, but

Table 1 Spore types recorded in the air samples and numerical description of concentration data (spore/m³) in Brufa, Italy

Spore type	ID no.	1994				1995				1996			
		Avg	Max	SD	Sum	Avg	Max	SD	Sum	Avg	Max	SD	Sum
<i>Agrocybe</i> spp.	2	1	4	1	18	7	28	6	182	1	4	2	12
<i>Albugo</i> spp.	3	2	10	3	28	2	15	3	43	3	6	2	29
<i>Alternaria brassicae</i> (Berk.) Sacc. ^a	5	0	0	0	0	0	4	1	8	1	4	1	7
<i>Alternaria</i> spp.	13	27	90	25	401	92	344	69	2,395	104	206	61	1142
<i>Ampulliferina persimplex</i> B. Sutton ^a	15	2	17	5	34	1	9	2	13	3	13	4	32
<i>Anthostomella/Herpotrichiella</i> spp.	16	0	0	0	0	1	5	1	29	1	5	2	15
<i>Arthriniium cuspidatum</i> (Cooke and Harkn.) Höhn ^a	17	0	2	1	2	0	0	0	0	0	0	0	0
<i>Arthriniium luzulae</i> M.B. Ellis ^a	18	0	0	0	0	0	1	0	1	0	0	0	0
<i>Arthriniium</i> spp.	19	8	50	13	116	10	51	12	250	27	68	23	293
<i>Aspergillus/Penicillium</i> spp.	22	27	125	31	410	27	193	41	701	29	87	30	319
<i>Badhamia</i> sp.	23	0	0	0	0	0	1	0	1	0	1	0	2
Basidiomycetes	24	29	103	34	442	116	310	83	3,018	44	136	41	489
<i>Belemnospora verruculosa</i> P. M. Kirk	25	0	0	0	0	0	1	0	1	1	5	1	9
<i>Beltrania rhombica</i> Penz	26	0	1	0	5	0	1	0	3	0	2	1	5
<i>Bipolaris spicifera</i> (Bainier) Subram	27	0	2	1	3	0	2	1	10	2	6	2	17
<i>Bipolaris</i> spp.	28	0	1	0	3	0	2	1	9	1	3	1	9
<i>Boletus</i> spp.	29	1	3	1	9	2	7	2	61	1	2	1	7
<i>Botrytis</i> -type	30	6	36	10	96	15	88	19	382	13	30	9	141
<i>Bovista</i> spp.	33	0	1	0	2	0	2	0	5	0	1	0	1
<i>Caloplaca</i> sp.	34	0	0	0	0	1	9	3	30	1	5	2	9
<i>Capnobotrys</i> sp. ^a	35	0	1	0	4	0	1	0	1	0	1	0	1
<i>Cercospora</i> spp.	36	1	4	1	11	1	6	1	27	4	12	4	46
<i>Chaetomium</i> spp.	37	1	2	1	9	1	5	1	14	3	9	3	33
<i>Chaetosphaerella</i> sp.	38	0	0	0	0	0	2	1	12	1	2	1	6
<i>Cheiramyces microscopica</i> (P. Karst.) S. Hughes ^a	39	0	0	0	0	0	1	0	1	0	0	0	0
<i>Cladosporium aecidiicola</i> Thüm ??	40	0	0	0	0	0	1	0	1	0	1	0	3
<i>Cladosporium echinulatum</i> (Berk.) G.A. deVries-type	41	3	14	4	41	1	8	2	23	1	4	1	10
<i>Cladosporium phlei</i> (C. T. Greg.) G.A. deVries-type	42	0	0	0	0	0	0	0	0	0	2	1	3
<i>Cladosporium</i> spp.	43	940	2,675	827	14,095	1,435	2,565	485	37,300	1,398	2,624	685	15,381
<i>Claviceps</i> sp. ^a	45	4	19	6	65	14	37	9	375	14	53	20	158
<i>Clypeosphaeria notarisii</i> Fuckel ^a	46	0	1	0	1	0	1	0	3	0	3	1	3
<i>Colletotrichum</i> -type	47	1	9	3	19	12	72	17	319	4	31	9	40
<i>Coprinus</i> spp.	48	17	90	24	258	36	112	24	943	9	24	6	94
<i>Cortinarius</i> spp.	49	0	1	0	2	1	3	1	20	1	2	1	7
<i>Coryneum</i> spp. ^a	50	0	0	0	0	0	3	1	8	0	2	1	5
<i>Cronartium/Melampsora</i> spp.	51	1	9	3	20	0	2	1	4	0	2	1	5
<i>Cucurbitaria</i> spp.	52	0	1	0	4	0	3	1	8	1	3	1	7
<i>Curvularia</i> spp.	53	0	1	0	1	0	1	0	1	0	0	0	0
<i>Delitschia</i> sp. ^a	55	0	1	0	1	0	1	0	5	0	1	0	1
<i>Dendrographium/Dendryphiella</i> sp.	56	0	0	0	0	0	1	0	2	1	3	1	6

Table 1 continued

Spore type	ID no.	1994				1995				1996			
		Avg	Max	SD	Sum	Avg	Max	SD	Sum	Avg	Max	SD	Sum
<i>Dendryphion digitatum</i> Subram.	57	0	0	0	0	0	1	0	1	0	1	0	1
<i>Diapleella clivensis</i> (Berk. and Broome) Munk.?	58	1	6	2	13	1	11	2	34	5	15	6	54
Diatrypaceae	59	3	15	5	50	12	87	19	322	32	299	89	356
<i>Dictyoarthrinium sacchari</i> (J.A. Stev.) Damon	60	1	8	2	15	1	21	4	30	7	30	12	79
<i>Didymella</i> spp.	61	1	5	2	21	43	411	83	1,123	10	51	18	111
<i>Diplocradiella scalaroides</i> G.Arnaud	62	0	0	0	0	0	1	0	1	0	0	0	0
<i>Diplodia</i> spp.	63	0	0	0	0	0	1	0	1	0	2	1	4
<i>Drechslera bisepitata</i> (Sacc. and Roum.) M.J. Rich and E.M. Fraser	64	0	0	0	0	0	1	0	4	0	1	0	3
<i>Entomophthora</i> -type	65	0	2	1	3	1	6	1	17	0	1	1	4
<i>Epicoccum nigrum</i> Link	66	5	14	4	80	15	41	11	391	22	52	13	240
<i>Excipularia fusispora</i> (Berk. and Broome) Sacc ^a	68	0	0	0	0	0	1	0	3	0	2	1	2
<i>Cercosporidium graminis</i> (Fuckel) Deighton	69	0	1	0	4	0	2	0	4	1	4	1	7
<i>Ganoderma</i> spp.	70	5	14	4	81	8	26	6	212	10	38	10	110
<i>Helicosporium</i> sp.	71	3	6	2	39	9	50	10	229	5	30	9	55
<i>Helminthosporium/Drechslera</i> spp.	72	9	41	11	141	11	39	10	278	6	15	5	68
<i>Inocybe</i> sp.	77	0	0	0	0	0	1	0	3	0	0	0	0
<i>Lasiosphaeria</i> spp. ^a	78	0	2	1	3	0	1	0	7	0	1	0	1
<i>Leptosphaeria eustomoides</i> Sacc ^a	79	0	0	0	0	0	2	0	3	0	2	1	3
<i>Leptosphaeria rubicunda</i> Rehm	80	0	3	1	6	6	52	11	152	5	20	8	51
<i>Leptosphaeria</i> spp.	81	6	77	20	85	2	22	4	49	6	32	10	69
<i>Lophiostoma vicinum</i> Sacc. ^a	82	0	1	0	4	0	2	1	12	5	39	12	51
<i>Massaria</i> ? <i>inquinans</i> (Tode) Fr.	83	0	2	1	3	0	1	0	4	0	0	0	0
<i>Massarina</i> -type	84	2	6	2	27	18	140	29	472	30	155	50	326
<i>Monilia</i> sp.	86	1	7	2	18	0	4	1	5	0	1	0	1
<i>Mucor</i> -type	87	0	3	1	3	0	0	0	0	1	7	2	7
<i>Mycosphaerella</i> spp.	91	0	1	0	2	1	6	2	36	1	4	1	9
Myxomycetes	92	4	12	4	61	8	34	9	219	10	24	7	115
<i>Neohendersonia kickxii</i> (Westend.)B.Sutton and Pollack ^a	93	0	1	0	2	0	1	0	1	0	2	1	4
<i>Nigrospora oryzae</i> (Berk. and Broome) Petch	94	0	1	0	1	0	0	0	0	0	0	0	0
<i>Oidium</i> spp.	97	35	102	34	525	8	31	8	218	10	23	7	114
<i>Oncopodiella trigonella</i> (Sacc.) Rifai ^a	98	0	0	0	0	0	0	0	0	0	1	0	2
<i>Ophiobolus acuminatus</i> (Sowerby) Duby ^a	99	2	26	7	32	2	19	5	63	8	36	11	83
<i>Ovulariopsis</i> sp.	100	0	0	0	0	0	2	0	2	0	1	0	2
<i>Paraphaeosphaeria michotii</i> (Westend.) O.E. Erikss.	101	1	5	1	8	1	5	1	30	6	22	7	69
<i>Periconia macrospinosa</i> Lefebvre and A.G. Johnson ^a	102	0	0	0	0	0	0	0	0	0	1	0	1
<i>Periconia</i> spp.	103	2	9	2	30	5	13	3	119	5	11	2	55
<i>Plasmopara</i> -type	104	1	2	1	8	1	3	1	21	0	2	1	3
<i>Pestalotiopsis</i> sp.	105	0	1	0	1	0	10	2	10	0	1	0	1
<i>Phragmidium</i> sp.	106	0	1	0	1	0	0	0	0	0	0	0	0
<i>Pithomyces chartarum</i> (Berk. and M.A.Curtis)M.B.Ellis	107	0	2	1	6	1	3	1	13	0	1	0	2

Table 1 continued

Spore type	ID no.	1994				1995				1996			
		Avg	Max	SD	Sum	Avg	Max	SD	Sum	Avg	Max	SD	Sum
<i>Pleospora herbarum</i> (Pers.) Rabenh. ??	110	3	25	7	41	0	2	0	3	0	1	0	1
<i>Pleospora oblongata</i> Niessl. ^a	111	0	4	1	5	0	2	1	8	0	1	0	3
<i>Pleospora rubelloides</i> (Plowr. ex Cooke) J. Webster	112	0	2	1	2	0	3	1	8	1	3	1	9
<i>Pleospora</i> spp.	113	66	697	177	989	4	31	7	97	14	70	24	157
<i>Polythrincium trifolii</i> Speg	114	4	25	7	54	3	9	3	67	0	3	1	5
<i>Puccinia</i> spp.	117	47	178	51	708	19	67	18	492	60	124	38	663
<i>Rebentischia unicaudata</i> (Berk. and Broome) Sacc. ^a	118	0	1	0	2	0	1	0	6	0	1	0	3
<i>Rosellinia</i> sp.	119	0	2	1	2	0	1	0	1	0	0	0	0
<i>Schroeteria delastrina</i> (Tul. and C. Tul.) G. Winter ^a	121	1	2	1	10	1	7	1	13	2	6	2	17
<i>Scopulariopsis</i> sp.	122	0	0	0	0	1	15	3	17	0	0	0	0
<i>Septonema</i> sp.?	123	0	1	0	3	1	6	1	24	1	4	1	13
<i>Sirosporium/Acrodictys</i> sp.	124	0	3	1	6	0	1	0	4	1	5	2	15
Sordariaceae	126	0	0	0	0	0	1	0	2	0	1	0	1
<i>Sphaeropsis</i> sp.	127	0	0	0	0	0	0	0	0	0	3	1	4
<i>Spilocaea</i> spp.	128	0	2	1	4	4	24	6	104	4	7	2	43
<i>Sporidesmium</i> spp.	130	0	1	0	1	0	1	0	3	1	1	1	6
<i>Sporormiella</i> sp.	131	0	2	1	2	0	1	0	5	0	2	1	4
<i>Stachybotrys</i> sp.	132	2	11	3	23	0	3	1	6	1	2	1	7
<i>Stemphylium</i> spp.	133	5	13	5	70	7	24	5	174	10	27	10	109
<i>Stigmina ? pallida</i> (Ellis and Everh.) M.B. Ellis	134	0	2	1	4	0	1	0	2	0	1	1	4
<i>Taeniolella breviscula</i> (Berk. and M.A.Curtis) S.Hughes??	135	0	0	0	0	0	0	0	0	1	2	1	6
<i>Taeniolella</i> spp	136	11	53	14	163	8	21	6	202	11	17	3	125
Telephoraceae	137	2	12	3	28	10	42	11	249	1	3	1	14
<i>Teloschistes/Xanthoria</i> sp.	139	0	0	0	0	2	16	5	51	1	5	2	10
<i>Tetraploa aristata</i> Berk. and Broome	140	0	0	0	0	0	0	0	0	0	1	0	1
<i>Tilletia</i> sp.	141	0	0	0	0	0	0	0	0	0	1	0	2
<i>Tilletiopsis</i> -type	142	0	1	0	1	4	25	6	91	0	4	1	4
<i>Torula</i> spp.	143	13	31	10	194	8	41	9	220	13	22	5	138
<i>Tranzscheliella hypodytes</i> (Schltdl.) Ványk and McKenzie ^a	144	52	157	54	782	31	177	39	807	39	75	23	432
<i>Trichothecium roseum</i> (Pers.) Link	145	0	1	0	1	0	0	0	0	1	13	4	13
<i>Trimmatostroma salicis</i> Corda	146	0	3	1	6	0	1	0	1	0	1	0	3
<i>Trimmatostroma scutellare</i> (Berk. and Br.) M.B. Ellis ^a	147	0	3	1	5	0	2	1	6	2	10	3	18
<i>Tripospermum</i> spp. ^a	148	0	0	0	0	0	0	0	0	1	3	1	7
<i>Triposporium elegans</i> Corda	149	0	3	1	3	0	0	0	0	0	1	0	1
<i>Urocystis</i> spp.	159	0	2	1	3	0	3	1	4	0	2	1	4
<i>Ustilago bromivora</i> (Tul. and C. Tul.) A.A.Fisch.Waldh ^a	160	42	119	38	632	25	68	19	661	37	95	31	411
<i>Ustilago tritici</i> C. Bauhin/ <i>U. nuda</i> (C N. Jensen) Rostr	161	54	248	71	807	25	131	29	661	70	156	56	770
<i>Valsaria ?? insitiva</i> (Tode) Ces. and De Not	163	0	0	0	0	0	1	0	2	1	4	1	8
<i>Venturia</i> spp.	164	1	4	1	15	2	35	7	63	2	6	2	20

Table 1 continued

Spore type	ID no.	1994				1995				1996			
		Avg	Max	SD	Sum	Avg	Max	SD	Sum	Avg	Max	SD	Sum
Xylariaceae	165	3	10	3	44	10	31	8	269	15	36	10	165
<i>Zygophiala jamaicensis</i> E.W. Mason	167	0	0	0	0	0	0	0	0	0	1	0	1
Unknown hyalodidymae	75	8	25	8	123	78	457	100	2,021	105	505	181	1,153
Unknown filiform spores	155	4	11	4	53	3	26	5	84	3	15	5	29
Unknown hyaline 3-septate fusiform spores	156	7	20	6	99	183	947	222	4,748	14	55	18	153

ID no. = identity number for spore types

Taxa found outside the counting area: *Acrodictys* sp., *Aglaospora profusa* (Fr.) De Not., *Ampelomyces* sp.^a, *Amphisphaerella deceptiva* M.E. Barr^a, *Ascobolus* sp., *Asterosporium asterospermum* (Pers.) S. Hughes, *Bactrodesmium spilomeum* (Berk. and Broome) E.W. Mason and S. Hughes^a, *Chaetomium? murorum* Corda^a, *Coniosporium* sp.^a, *Coremiella* sp.^a, *Dendryphion nanum* (Nees) S. Hughes^a, *Drechslera bromi* (Died.) Shoemaker^a, *Drechslera tritici-repentis* (Died.) Shoemaker^a, *Endophragmiella fallacia* P.M. Kirk^a, *Endophragmiella* sp., *Gilmaniella* sp., *Gliomastix* sp., *Helicoon* sp., *Heptameria uncinata* (Niessl.) Rehm, *Hysterographium* sp., *Lepteutypa* sp., *Leptosphaeria dumetorum* Feltgen^a, *Leptosphaeria graminis* (Fuckel) Sacc.^a, *Leptosphaeria Niessliana* Rabenh.^a, *Leptosphaeria thurgoviensis* E. Müll.^a, *Melanographium citri* (Gonz. Frag. and Cif.) M.B. Ellis^a, *Microbotryum reticulatum* (Liro) R. Bauer and Oberw.^a, *Monodictys castaneae* (Wallr.) Hughes^a, *Navicella pileata* (Tode) Fabre, *Nodulosphaeria ulnispora* (Cooke) L. Holm^a, *Podospora* sp.^a, *Pollaccia* sp.^a, *Pseudovalsa umbonata* (Tul.) Sacc.^a, *Spegazzinia* sp., *Splanchnonema quercicola* M.E. Barr^a, *Sporidesmium folliculatum* (Corda) E.W. Mason and S. Hughes^a, *Sporidesmium leptosporum* (Sacc. and Roum.) S. Hughes^a, *Sporidesmium valdivianum* (Speg.) M. B. Ellis, *Taeniolella scripta* (P. Karst.) S. Hughes^a, *Trematosphaeria pertusa* (Pers.) Fuckel^a, *Ustilago hordei* Bref., *Ustilago striiformis* (Westend.) Niessl^a, and 23 unknown taxa

^a First observation of the taxa in air

they made up fewer than 11% of the total fungal spores.

3.3 Biodiversity

The daily average of the identified morphological spore types calculated for the total studied period was 66; its highest number (112 taxa) was recorded in a rainy day (22 June 1996). Simpson's diversity index of airborne fungal genera ranged between 1.08 and 8.56, its average was 2.75 (Table 2; Fig. 1).

Daily biodiversity value of the Simpson's index correlated positively with precipitation (Table 3). No significant correlations were found with Shannon's diversity index and evenness.

The rank abundance curves for the wet days were much shallower than those for dry ones, because the relative abundances of genera were more evenly distributed than on dry days. Rank abundance curves of dry days were more steep than those of wet ones (Fig. 1).

The regression analysis performed enabled the development of a model to predict Simpson's index for airborne fungal genera showing the best estimator of data variability to be sun hours ($D = 4.405 -$

$0.002 \times \text{sun hours} + 0.49$; 0.49 should be added when wind speed > 0.5 m/s). This model was statistically significant (F test, $P < 0.001$); and accounted for 21% of the variance ($R^2 = 0.49$, $P < 0.002$).

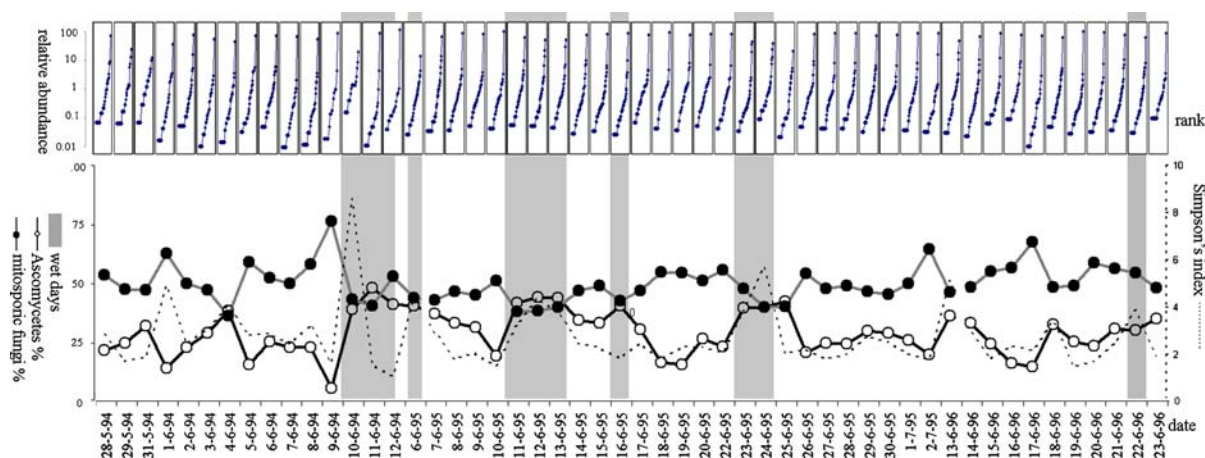
Finally, RTS diversity ordering showed that wet days had higher biodiversity concerning its dominant taxa, than dry days (Fig. 2).

4 Discussion

The present study contributed to the knowledge of the species composition of the air, adding several new taxa, which appeared to be new to the air of vineyards. From such a habitat only the following fungi have been found by earlier air samplings: *Agrocybe*, *Alternaria*, *Botrytis*, *Cladosporium*, *Coeleosporium senecionis*, *Epicoccum*, *Fusarium*, *Helminthosporium*-type, *Leptosphaeria*, *Penicillium*, *Plasmopara viticola*, *Polythrincium trifolii*, *Puccinia*, *Rhizopus*, *Stemphylium*, *Torula*, and *Uncinula* (Corbaz 1972; Picco 1992; Diaz et al. 1998). Most of them were present on our slides, but *Botrytis*- and *Plasmopara*-type spores (possibly belonging to the fungal pathogens of grapevine) did not reached high concentrations in the air.

Table 2 Numerical description of air spora composition and biodiversity indices (Brufa, Italy)

		Wet days	Dry days	All days
Shannon's biodiversity index	Avg	1.80	1.65	1.68
	Max	2.56	2.47	2.56
	Min	0.27	1.03	0.27
	SD	0.68	0.35	0.44
Simpson's biodiversity index	Avg	3.85	2.45	2.75
	Max	8.56	5.13	8.56
	Min	1.08	1.48	1.08
	SD	2.08	0.80	1.30
Species richness (number of species)	Avg	61.36	66.90	65.73
	Max	112	96	112
	Min	20	23	20
	SD	26.34	13.55	16.89
Relative abundance of genera of Ascomycetes	Avg	43.24	26.77	30.25
	Max	60.23	53.90	60.23
	Min	13.22	1.30	1.30
Relative abundance of genera of mitosporic fungi	Avg	43.89	51.71	50.06
	Max	54.43	76.47	76.47
	Min	38.18	36.36	36.36

**Fig. 1** The temporal patterns of rank abundance curves, the percentage of the number of genera of mitosporic fungi and Ascomycetes, and Simpson's biodiversity index (Brufa, Italy). Grey columns represent wet days

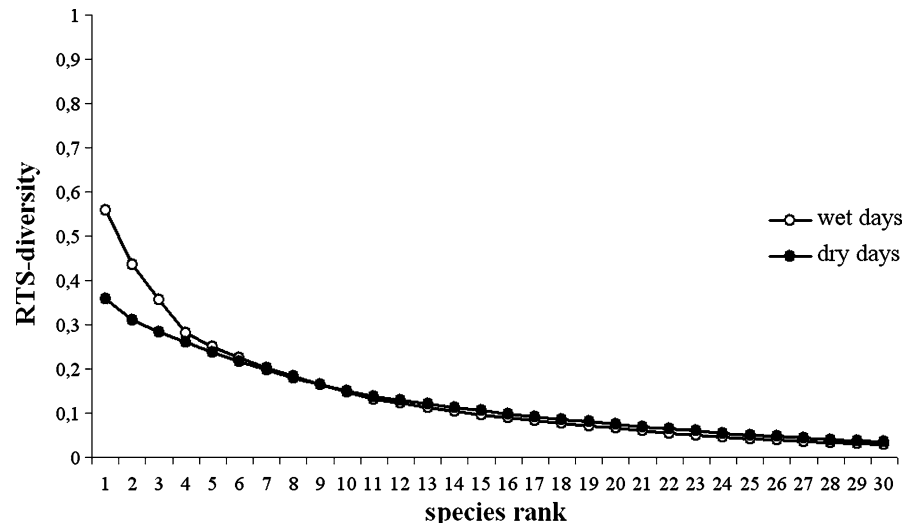
In our study, non-viable air sampling method was applied, which ignored the morphologically indistinguishable species. Including all morphological spore types (identified *plus* unknown) a total of 189 taxa were recognized. Using a viable (cultural) air sampling method, a similar number of the taxa was reported from the air of Turin by Airaudi and Marchisio (1996), where 165 species of mesophilic

fungi and 26 thermotolerant species were isolated. More than 30% of these airborne species belonged to the genera of *Aspergillus*, *Penicillium*, and yeasts. Our method is not suitable for the identification of such taxa, because their spores cannot be distinguished and may be easily overlooked. It is preferred, however, to identify many other taxa not growing on agar media, e.g., obligate biotrophic fungi (Pyrri and

Table 3 Correlation between meteorological variables and biodiversity indices of airborne fungi (Brufa, Italy)

Meteorological factors	Shannon's biodiversity index	Simpson's biodiversity index	Evenness
$T_{\max}$	0.16	-0.12	-0.27
$T_{\min}$	0.09	-0.11	-0.17
T_{avg}	0.14	-0.12	-0.24
Sun hours	0.02	-0.19	-0.06
Evaporation	0.03	-0.12	-0.05
Cloud cover	-0.02	0.16	0.10
Relative humidity	0.02	0.20	0.04
Precipitation	0.06	0.34*	0.06
Rain hours	-0.04	0.29*	0.21
Rain intensity	0.15	0.34*	-0.11
Wind gust	0.14	0.12	-0.15
Wind speed	-0.22	-0.21	0.18
Air pressure	-0.09	-0.14	0.06

$T_{\max}$, temperature maximum;
 $T_{\min}$, temperature minimum;
 T_{av} , temperature average
 * Significant correlations
 $P < 0.05$

Fig. 2 RTS-diversity patterns comparing the air spora diversity of dry and wet days (Brufa, Italy)

Kapsanaki-Gotsi 2007). Therefore, it is not possible to identify and enumerate the entire community with a single method. To obtain the total number of fungal taxa in an air sample, a combination of a non-viable method and a wide variety of selective isolation methods would be needed.

Statistical analysis showed that precipitation correlated strongly with the fungal diversity of the air. Wet and dry periods had somewhat different spore compositions. It was proved that Simpson's biodiversity index was increased significantly by precipitation. Rain caused species turnover, removing dry spores (mostly mitosporic fungi) and

aerosolizing wet ones (mostly Ascomycetes; Fig. 1). The relative abundance of mitosporic fungi in wet days was higher than that of Ascomycetes on dry days (Table 2). On wet days, dry spores are also present when the wash-out effect of precipitation is incomplete, or their take-off occurs before and after rains. On dry days, some Ascomycetes appear in the night-time, when sufficient saturation provokes spore discharge (Jones and Harrison 2004). However, lower species number and small relative abundance was observed for ascospores on dry days. In the analysis of rank abundance curves, steep gradients of the dry days indicated low

evenness, as some high-ranking genera had much higher abundance than the low ranking ones.

It should be mentioned that the correlation and regression analyses of the biodiversity index did not take into account temporal changes in species composition, such as species turnover. Hence, these methods overlook the difference between the species composition of wet and dry days. To resolve this problem, we analyzed the RTS curves. It was demonstrated that biodiversity of rainy days, *concerning their common species*, was higher than those of dry days. The biodiversity of the dominant species on dry days was reduced by the high concentration of *Cladosporium* spp. On wet days, however, *Cladosporium* spp. were not so prevalent as on dry days, possibly because rainfall washed out its spores, and the dominance of this genus decreased. In consequence of this, rain correlated positively with Simpson's biodiversity index. According to the regression analysis, sun hours became the most important factor. Simpson's biodiversity index was decreased by this factor, possibly because it has a positive effect on *Cladosporium* counts ($P < 0.001$; authors, unpublished results). The effect of wind is positive on the biodiversity index, possibly by aerosolization of spores of different taxa.

It is well established that inhalation of fungal spores induces respiratory allergy symptoms in sensitized individuals. Among all patients suffering from such diseases, 3–40% of them are sensitized to fungal spores (Kurup et al. 2000; Bavbek et al. 2006; Gonianakis et al. 2006). In Italy, it has been observed that in patients with inhalation allergy the frequency of positive skin test with mold extracts is about 10%. Fungal spores involved in allergic diseases belong to 80 genera, the most significant of which belong primarily to Deuteromycetes and Ascomycetes and, secondly, to Basidiomycetes (Gioulekas et al. 2004). Positive skin tests in asthmatic patients were found to *Alternaria*, *Botrytis*, *Helminthosporium*, *Epicoccum*, *Aspergillus*, *Cladosporium*, and *Mucor* (in descending order of their frequency; Wilken-Jensen and Gravesen 1984). Some of them had high abundance in our air samples, and had higher RTS rank on dry days than on rainy ones. There were also several plant pathogenic components in our air samples, e.g., *Alternaria*, *Puccinia*, *Stemphylium*, *Helminthosporium*, and *Ustilago* species, which had also markedly higher rank in the RTS curves of dry days.

5 Conclusions

Biodiversity indices could be applied for air spora. Such indices show temporal dynamics, which are strongly affected by meteorological factors, mostly by sun hours. On dry days, the total biodiversity is less than on wet ones, since the dominance of *Cladosporium* reduces the biodiversity of the common species and the evenness of air spora.

Acknowledgments The authors are grateful to Maureen Lacey (Rothamsted, UK), Sándor Tóth (Szt. István University, Hungary) and Kálmán Vánky (Herbarium Ustilaginales Vánky, Germany) for their precious help in the identification of spores. The first author wish to acknowledge the Hungarian Scientific Research Fund (OTKA, grant n. F67908) for financial support.

References

- Airaudi, D., & Marchisio, V. F. (1996). Fungal biodiversity in the air of Turin. *Mycopathologia*, 136, 95–102. doi: [10.1007/BF00437502](https://doi.org/10.1007/BF00437502).
- Bavbek, S., Erkeköl, F. O., Ceter, T., Mungan, D., Ozer, F., Pinar, M., et al. (2006). Sensitization to *Alternaria* and *Cladosporium* in patients with respiratory allergy and outdoor counts of mold spores in Ankara atmosphere, Turkey. *The Journal of Asthma*, 43, 421–426. doi: [10.1080/02770900600710706](https://doi.org/10.1080/02770900600710706).
- Bousquet, P. J., Chinn, S., Janson, C., Kogevinas, M., Burney, P., & Jarvis, D. (2007). Geographical variation in the prevalence of positive skin tests to environmental aero-allergens in the European community respiratory health survey. *Allergy*, 62, 301–309. doi: [10.1111/j.1398-9995.2006.01293.x](https://doi.org/10.1111/j.1398-9995.2006.01293.x).
- Bustos Delgado, I., Angulo Romero, J., Dominguez Vilches, E., & Galan Soldevilla, C. (2008). Preliminary survey of fungal spore biodiversity in the nature reserve of “Sierra de Hornachuelos” in Andalucía (Spain). *Aspects of Applied Biology* 89, 59–60.
- Corbaz, R. (1972). Etudes des spores fongiques captées dans l'air I. Dans un vignoble. *Phytopathologische Zeitschrift*, 74, 318–328. doi: [10.1111/j.1439-0434.1972.tb02587.x](https://doi.org/10.1111/j.1439-0434.1972.tb02587.x).
- Diaz, M. R., Iglesias, I., & Jato, M. V. (1998). Seasonal variation of airborne fungal spore concentrations in a vineyard of North–West Spain. *Aerobiologia*, 14, 221–227. doi: [10.1007/BF02694210](https://doi.org/10.1007/BF02694210).
- Dighton, J. (1998). Analysis of micromycete communities in soil: A critique of methods. *Mycological Research*, 7, 796–798.
- Farr, D. F., & Rossman, A. Y. (2008). *Fungal Databases*. Systematic Mycology and Microbiology Laboratory, ARS, USDA. <http://nt.ars-grin.gov/fungaldatabases/>.
- Gioulekas, D., Damialis, A., Papakosta, D., Spieksma, F., Giouleka, P., & Patakas, D. (2004). Allergenic fungi spore records (15 years) and sensitization in patients with respiratory allergy in Thessaloniki-Greece. *Journal of*

- Investigational Allergology and Clinical Immunology*, 14, 225–231.
- Gönczöl, J., & Révay, Á. (2004). Fungal spores in rainwater: stemflow, throughfall and gutter conidial assemblages. *Fungal Diversity*, 16, 67–86.
- Gonianakis, M. I., Neonakis, I. K., Gonianakis, I. M., Baritaki, M. A., Bouros, D., Potamias, G., et al. (2006). Mold allergy in the Mediterranean island Crete, Greece: A 10-year volumetric, aerobiological study with dermal sensitization correlations. *Allergy and Asthma Proceedings*, 27, 354–362. doi:[10.2500/aap.2006.27.2918](https://doi.org/10.2500/aap.2006.27.2918).
- Hirst, J. M. (1952). An automatic volumetric spore trap. *The Annals of Applied Biology*, 39, 257–265. doi:[10.1111/j.1744-7348.1952.tb00904.x](https://doi.org/10.1111/j.1744-7348.1952.tb00904.x).
- Járai-Komlódi, M., & Tóth, S. (1993). Studies of rare airborne fungal spores and conidia in Hungary. *Acta Botanica Hungarica*, 38, 383–399.
- Jones, A. M., & Harrison, R. M. (2004). The effects of meteorological factors on atmospheric bioaerosol concentrations—a review. *The Science of the Total Environment*, 326, 151–180. doi:[10.1016/j.scitotenv.2003.11.021](https://doi.org/10.1016/j.scitotenv.2003.11.021).
- Kendrick, B. (1990). Fungal allergens. In E. G. Smith (Ed.), *Sampling and identifying allergenic pollens and moulds* (pp. 134–164). San Antonio: Blewstone Press.
- Kurup, V., Shen, H., & Banerjee, B. (2000). Respiratory fungal allergy. *Microbes and infection*, 2, 1101–1110. doi:[10.1016/S1286-4579\(00\)01264-8](https://doi.org/10.1016/S1286-4579(00)01264-8).
- Lacey, M., & West, J. (2006). *The air spora*. Dordrecht: Springer.
- Magurran, A. E. (1988). *Ecological diversity and its measurement*. New South Wales: Croom Helm.
- Magyar, D. (2007). Aeromycological aspects of mycotechnology. In M. K. Rai (Ed.), *Mycotechnology: Current trends and future prospects* (pp. 226–263). New Delhi: I.K. International Publishing House.
- Magyar, D. (2008). The tree bark: A natural spore trap. *Aspects of Applied Biology* 89, 7–16.
- Magyar, D., Gönczöl, J., Révay, Á., Grillenzoni, F., & Seijo-Coello, M. D. C. (2005). Stauro- and scolecoconidia in floral and honeydew honeys. *Fungal Diversity*, 20, 103–120.
- Patil, G. P., & Taillie, C. (1979). An overview of diversity. In J. F. Grassle, G. P. Patil, W. Smith, & C. Taillie (Eds.), *Ecological diversity in theory and practice* (pp. 3–27). Maryland: International Publishing House.
- Pérez-Atanes, S., Seijo-Coello, M. D. C., & Méndez-Álvarez, J. (2001). Contribution to the study of fungal spores in honeys of Galicia (NW Spain). *Grana*, 40, 217–222. doi:[10.1080/001731301317223240](https://doi.org/10.1080/001731301317223240).
- Picco, A. M. (1992). Presence in the atmosphere of vine and tomato pathogens. *Aerobiologia*, 8, 459–463. doi:[10.1007/BF02272916](https://doi.org/10.1007/BF02272916).
- Pyrri, I., & Kapsanaki-Gotsi, E. (2007). A comparative study on the airborne fungi in Athens, Greece, by viable and non-viable sampling methods. *Aerobiologia*, 23, 3–15. doi:[10.1007/s10453-006-9039-6](https://doi.org/10.1007/s10453-006-9039-6).
- Shapiro, S. S., Wilk, M. B., & Chen, H. J. (1968). Comparative study of various tests for normality. *Journal of the American Statistical Association*, 63, 1343–1372. doi:[10.2307/2285889](https://doi.org/10.2307/2285889).
- Shoemaker, R. A., LeClair, P. M., & Smith, J. D. (1974). Some parasites of *Bromus inermis* and airborne spores trapped over brome grass crops. *Canadian Journal of Botany*, 52, 2415–2421. doi:[10.1139/b74-313](https://doi.org/10.1139/b74-313).
- Tilak, S. T., & Pande, B. N. (2005). Current trends in aeromycological research. In M. K. Rai & S. K. Deshmukh (Eds.), *Fungi: Diversity and biotechnology* (pp. 281–510). Jogpur: Scientific Publishers.
- Troutt, C., & Levetin, E. (2001). Correlation of spring spore concentrations and meteorological conditions in Tulsa, Oklahoma. *International Journal of Biometeorology*, 45, 64–74. doi:[10.1007/s004840100087](https://doi.org/10.1007/s004840100087).
- Wilken-Jensen, K., & Gravesen, J. (1984). *Atlas of moulds in Europe causing respiratory allergy*. Copenhagen: ASK Publishing, Foundation for Allergy Research in Europe.