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EXPOSURE TO THE AIRBORNE MOULD *BOTRYTIS* AND ITS HEALTH EFFECTS

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Abstract: Most investigations into the correlation between exposure to fungi and detrimental health effects focus on the 2–4 most prevalent genera in ambient air, both outdoors and indoors. Yet over 80 genera of fungi have been shown to have allergenic potential. Also, there is no agreement about threshold values for exposure to fungi. One of the fungal genera expected to be less prevalent in ambient air and known to cause allergy is *Botrytis*. In this review, we investigate the airborne exposure level and health effect of *Botrytis,* both at general exposure and in occupational settings. The surveyed papers show that *Botrytis* is found globally with different spore seasons depending on the region investigated. The levels of *Botrytis* in the percentage of all fungi have a calculated median of around 1.1% in the different environments, confirming that it is among the less prevalent fungi. Furthermore, a substantial proportion of patients and workers are allergic to *Botrytis cinerea,* and when *B. cinerea* was included in extended test panels additional allergic patients were found. Thus, *B. cinerea* is as important as the more prevalent mould genera *Cladosporium* and *Alternaria* and we suggest that it should be included in standard allergic tests panels.

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Key words: allergy, *Botrytis*, fungi, indoor air, mould, occupational exposure, outdoor air, season, spore calendar.

INTRODUCTION

Allergy and asthma induced by fungi have been known for many years. In connection with allergy and asthma, the typical fungal genera investigated are *Cladosporium*, *Alternaria*, *Aspergillus* and *Penicillium*, probably because they are very often the most prevalent genera in ambient air. However, the diversity of species can be rather high, both within the 2–4 most prevalent genera and in less prevalent genera. Lugauskas *et al.* [60] found, for example, 100 genera containing 359 species when investigating the fungal composition indoors. Furthermore, many fungal species from at least 80 genera, have been shown to have allergenic potential. One of these fungi present in ambient air is *Botrytis cinerea* [13, 23, 56, 58]. Therefore, it is problematic to choose only to investigate the most prevalent fungal genera.

Clarifying the causality between exposure and allergy and respiratory symptoms is complicated by several factors. For example, once an allergy is confirmed it is difficult to work out where and when there has been exposure to the allergen, e.g. at work or at home. Furthermore, there does not seem to be any correlation between the ability to induce allergy and a fungus' proportional amount of the total fungal spore level [7, 31, 32, 97]. Hence, it has still not been clarified how and at what exposure level fungi cause illnesses in occupational settings, as well as at general exposure [23, 26, 56, 84]. However, for non-specific symptoms like coughing and irritation of nose and eyes, Eduard *et al.* [28] found a correlation in Norwegian farmers between these symptoms and exposure to total fungal spore levels above 2×10^4 spores/m^3, but they found no correlation with wheezing and chest tightness.

However, a correlation between occupational asthma and sensitisation to mould and flower-allergens has been found for greenhouse workers. These greenhouse workers have a higher prevalence of occupational asthma than the

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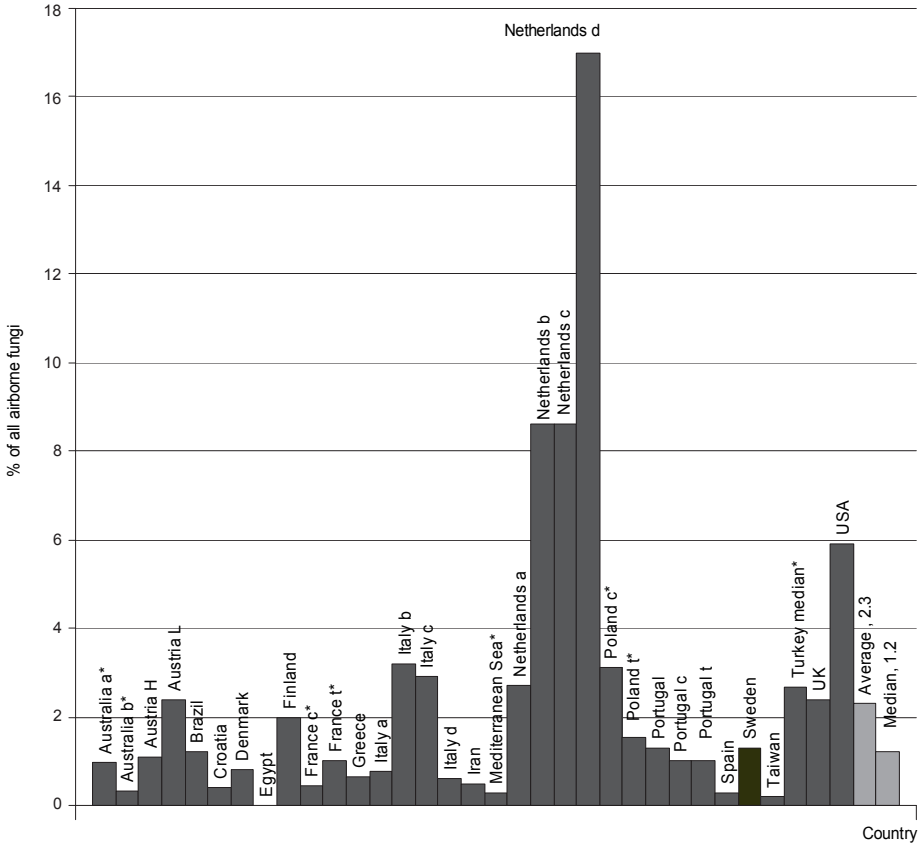

Figure 1. Prevalence of outdoor *Botrytis* in percent of all airborne fungi (black bars). More detailed information on the data can be found in Table 1. Calculated average and median of the presented data (grey bars). *Numbers calculated from paper data. References for the presented data: Australia a = [53], Australia b = [36], Austria H (high altitude) and L (low altitude) = [27], Brazil= [66], Croatia = [75], Denmark = [57], Egypt = [2], Finland = [80], France c (country) and t (city) = [92], Greece = [32], Italy a = [76], Italy b = [22], Italy c (Cagliari) and d (Perugia) = [4], Iran = [41], Eastern Mediterranean Sea = [100], The Netherlands a and b = [97], The Netherlands c = [7], The Netherlands d = [8], Poland c (country) and t (city) = [51] average of 2 years, Portugal c (country) and t (city) = [73], Spain = [43], Sweden = [70], Taiwan = [102], Turkey median* = [18], UK = [44], USA = [17].

general European population [67]. Interestingly, the prevalence of *Botrytis* ranks high in greenhouses [67, 79]. The aim of this study is to review the exposure level and health effects of airborne *Botrytis*, both at general exposure and in occupational settings. Thus, we have made an effort to compile and standardise literature data in order to obtain an overview of the current knowledge of exposure levels and health effects of *Botrytis* spp. and *B. cinerea*.

Sources of exposure to *Botrytis cinerea*

The mould *B. cinerea* has been isolated from numerous places around the world, but the main occurrence is in humid, temperate and subtropical regions. It is considered to be the most prevalent of the 25 species belonging to the genus *Botrytis*. The fungus grows either parasitically or saprophytically on a vast majority of plants, vegetables and soft fruits, and can cause substantial crop losses, especially in greenhouses, while other *Botrytis* species have narrower host ranges [25, 29, 59].

B. cinerea is described as a hydrophilic fungus which needs a minimum water activity of 0.9, a requirement thought to be adequately met by host tissue [29, 30]. Likewise, the conidia of *B. cinerea* require freely accessible water or a very high relative humidity to germinate [29].

The conidia of *B. cinerea* are mainly airborne and their release is regulated by a hygroscopic mechanism after a drop in humidity and air movements, such as rain splashes, that can release large numbers [25, 29]. Thus, in a greenhouse with geranium cuttings Hausbeck *et al.* [40] found that any activity that resulted in air movement, such as planting, irrigation, cleaning, fertilization and even spraying fungicides, raised conidia levels substantially. Similarly, a survey of the mycological flora of French wine cellars by Simeray *et al.* [94] showed a large amount of viable spores of *B. cinerea* after grape pressing activity in one cellar compared to the other storage cellars. Likewise, an investigation in Finland of the airborne fungi present in water-damaged buildings before and after reconstruction showed *B. cinerea* viable spores were only found during demolition activity [82]. A higher prevalence of *Botrytis* spp. indoors is also mentioned as a possible indicator of excess water indoors in the BASE study of US office buildings [63]. Thus, in conjunction with its need for available water, *Botrytis* may be thought of as an indicator fungus for water damage in buildings.

Table 1. Outdoor prevalence of airborne *Botrytis* found in different environments and countries.

Species/ Specification of environment	Exposure level CFU/m^3	% positive samples	% of all fungi	Sampling and identification method	Country, number of samples and sampling time	Ref.
B. cinerea						
The roof of an office building in Perth	10		0.97*	Andersen sampler, MEA	Australia, 1 day, November	[53]
6 different districts in Istanbul 1.5 m above ground			0 -2.673 Med. 1.5*	Passive sedimentation SMA plates, MEA, CZD and PDA	Turkey, November 2001– September 2002	[18]
Botrytis, *Botrytis* sp. and *Botrytis* spp.						
A roof 3.5 m above ground in a town park			0.3*	7-day Burkard spore trap, microscopy	Australia, 1 sample, 2002	[36]
A roof 25 m above ground in Thessaloniki	Annual av. 1118		0.64	Burkard spore trap, microscopy	Greece, 1987–2001	[32]
Different regions of Croatia 1.5 m above ground	Av. 0.026*		0.4*	Passive sedimentation SA-plates, CZ, MEA and PSA	Croatia, 1 year, 1998	[75]
City of Turku			2.0	Andersen sampler	Finland, 1982–1983	[80]
A balcony in the city of Oerebro			1.3	Passive sedimentation plates, MSA	Sweden, 18 months, 1946–1947	[70]
A roof in Copenhagen			0.8	Biap Slit-sampler, V8 agar	Denmark, 1977–1979	[57]
Not specified			2.4	Passive sedimentation plates	UK, 1951–1953	[44]
In the city of Rzeszów 12 m above ground, 2001	Peak day 418		2	Hirst type spore trap, microscopy	Poland, Spring 2001–2002	[51]
The countryside 12 m above ground, 2001	Peak day 726		3.6	Hirst type spore trap, microscopy	Poland, Spring 2001–2002	[51]
In the city of Rzeszów 12 m above ground, 2002	Peak day 213		1.1	Hirst type spore trap, microscopy	Poland, Spring 2001–2002	[51]
The countryside 12 m above ground, 2002	Peak day 475		2.5	Hirst type spore trap, microscopy	Poland, Spring 2001–2002	[51]
Based on data from different studies not specified			2-20	–	Europe	[96]
The city of Leiden	Seasonal total of daily concentration 25665		2.7	Burkard sampler	The Netherlands, 1980	[97]
The city of Groningen			8.6	Burkard sampler	The Netherlands	[97]
A roof in the city of Beatrixoord			8.6	Andersen sampler, YMA	The Netherlands, 1981–1983	[7]
Gardens of 28 asthmatic patients (17 in the city)			17	Andersen sampler, YMA	The Netherlands, 1981–1982	[8]
Low altitude on a roof 20 m above ground	Annual total 579		2.4	Jet spore sampler	Austria, 1 year, 1989–1990	[27]
High altitude 1 m above ground	Annual total 63		1.1	Jet spore sampler	Austria, 1 year, 1989–1990	[27]
The city of Besançon			0.6 and 1.5	SAS sampler, MEA, MESA and SDA	France, 1989–1990	[92]
The rural area of Emagny			0.2 and 0.7	SAS sampler, MEA, MESA and SDA	France, 1989–1990	[92]
A roof 20 m above ground level in the city of Porto			1.3	Burkard sampler, microscopy	Portugal, 1 year, 2003	[72]

Table 1. Outdoor prevalence of airborne *Botrytis* found in different environments and countries (continuation).

Species/ Specification of environment	Exposure level CFU/m³	% positive samples	% of all fungi	Sampling and identification method	Country, number of samples and sampling time	Ref.
Botrytis, *Botrytis* sp. and *Botrytis* spp.						
A roof 20 m above ground level in the city of Porto			1	Hirst Type Spore Trap, microscopy	Portugal 3 years, 2005–2007	[73]
A farm 5 m above ground level in the rural area of Amares			1	Hirst Type Spore Trap, microscopy	Portugal 3 years, 2005–2007	[73]
A roof 8 m above ground in Madrid	Annual total 625		0.29	Hirst Type Spore Trap	Spain 2003	[43]
The city of Cagliari			2.9	Burkard type Hirst trap collector	Italy January–August 1990	[4]
The city of Perugia			0.6	Burkard type Hirst trap collector	Italy January–August 1990	[4]
Two squares in Milan 1 m above ground			0.77*	Passive sedimentation PDA plates	Italy 1 year, 1995–1996	[76]
A ship 3 m above sea level			0-1*	Burkard Spore Trap	The Eastern Mediterranean Sea 5 days in July 2005	[100]
Surface train station in Cairo in the breathing zone			0	AGI-30 sampler, MEA	Egypt May–July 1997	[2]
1-1.5 m above ground, homes of 90 asthmatic patients			0.47*	Passive sedimentation MEA plates	Iran 3 months, 2004	[41]
A roof 23 m above ground in the city of Porto Alegre			1.22	Rotorod sampler®, microscopy	Brazil 1 year, 2000–2001	[66]
1 m above ground outside homes in the city of Tampa			5.9	Air-O-Cell cassettes, microscopy	USA, 2 samples Summer 2004	[17]
Shin-Jhuang and Shi-Men		14.1	0.22*	Burkard sampler, MEA	Taiwan 2003–2004	[102]
A city park in Shin-Jhuang		24.1		Burkard sampler, MEA	Taiwan 2003–2004	[102]
The rural township of Shi-Men		5.7		Burkard sampler, MEA	Taiwan 2003–2004	[102]
The homes of 40 mould sensitive asthmatic children, Bronx		5.0		Burkard sampler, DG-18	USA, 2 samples/home 1998–1999	[74]
The homes of 48 mould sensitive asthmatic children, New York		6.3		Burkard sampler, DG-18	USA, 2 samples/home 1998–1999	[74]
The homes of 44 mould sensitive asthmatic children, Boston		4.5		Burkard sampler, DG-18	USA 2 samples/homes 1998–1999	[74]
The homes of 54 mould sensitive asthmatic children, Chicago		3.7		Burkard sampler, DG-18	USA 2 samples/home 1998–1999	[74]
The homes of 95 mould sensitive asthmatic children, Dallas		3.2		Burkard sampler, DG-18	USA 2 samples/home 1998–1999	[74]
The homes of 81 mould sensitive asthmatic children, Tucson		0.0		Burkard sample, DG-18	USA 2 samples/home 1998–1999	[74]
The homes of 52 mould sensitive asthmatic children, Seattle		25.0		Burkard sampler, DG-18	USA 2 samples/home 1998–1999	[74]

Abbreviations used in the Tables: CM: Cornmeal agar, CZ: Czapek agar, CZD: Czapek-Dox agar, DG-18: Dichloran Glycerol agar, HS: not specified, MEA: Malt Extract agar, MESA: Malt Extract Sucrose agar, MSA: Modified Sabouraud agar, PDA: Potato Dextrose agar, PSA: Potato Sucrose agar, RBA: Rose Bengal agar, SA: Sabouraud agar, SDA: Sabouraud Dextrose agar, SMA: Sabouraud Maltose agar, YMA: Yeast Morphology agar. App.: approximately, Av.: values presented as averages or means in the papers, Med.: median, Ref.: reference. *Numbers followed by an * are values calculated from the data presented in the papers or Figures.

Botrytis prevalence in outdoor air

The majority of papers on the mycological flora of ambient air investigate the airborne fungi of cities and they often only identify fungi to genus level. This also holds true for *B. cinerea,* and as a consequence we studied data from papers concerning *Botrytis*, *Botrytis* sp., *Botrytis* spp. and *B. cinerea*, hereafter referred to as *Botrytis*.

In Table 1 which shows the prevalence of *Botrytis* outdoors, the few available data do not indicate an apparent difference in the level of exposure between the city and the countryside. Even though most data come from Europe, studies show that airborne *Botrytis* can be found in many places around the world (Tab. 1).

The outdoor occurrence of *Botrytis* in the percentage of all fungi is shown in Figure 1 and ranges between 0%–17%, with a median of 1.2%, confirming it to be a fungus with a low prevalence in ambient air. The Netherlands stand out by having a relatively high prevalence of *Botrytis* ranging from 2.7%–17%.

When trying to estimate the exposure level of *Botrytis* expressed in CFU/m^3 it becomes clear that not only the methods used vary, but also the way in which data is presented. Terms like annual total, seasonal total, daily concentration, daily average, average and peak day are used, which make it difficult to compare data. However, an approximation of *Botrytis* CFU/m^3 per day lies between 0.02 CFU/m^3 and 726 CFU/m^3 (Tab. 1), and an approximation of an annual total lies between 63 CFU/m^3–39,735 CFU/m^3 (Tab. 1) [69]. In comparison, the outdoor concentration of viable fungi as reviewed by the Robert Koch Institute [84] can be around or below 100 CFU/m^3 in wintertime, and around or well above 2,000 CFU/m^3 in summertime.

Botrytis prevalence in indoor air

The indoor/outdoor ratio of fungal spores is usually less than 1 [31, 33, 56]. This seems not to be the case for *Botrytis* in non-complaint homes compared with outdoor prevalences (Tab. 1 *vs.* Tab. 2). The annual total of *Botrytis* CFU/m^3 at

Table 2. Indoor prevalence of airborne *Botrytis* in different non-complaint environments and countries.

Species/Specification of environment	Exposure level CFU/m^3	% positive samples	% of all fungi	Sampling and identification method	Country, number of samples and sampling time	Ref.
B. cinerea						
1.5 m above ground, 6 different districts in Istanbul			0 -2.083 Med. 1.3*	Passive sedimentation SMA plates, MEA, CZD and PDA	Turkey, November 2001–September 2002	[18]
1.5 m above ground, 49 non-complaint urban and suburban homes			0.28	Standard RCS centrifugal sampler, MEA	Argentina, 1 day in 2002 and 2003	[6]
Auditoria app. 1 m above ground			<1.0	RCS centrifugal Sampler, RBA	Canada, 11 samples	[88]
Auditoria app. 1 m above ground			0	Andersen N6 sampler, RBA	Canada, 11 samples	[88]
Botrytis						
39 schools 1 m above ground	Av. 1-21			Andersen N6 sampler, MEA	Canada, 1996–1997	[5]
Two underground stations 1 m above floor level			0.9*	Passive sedimentation PDA plates	Italy, 1 year, 1995–1996	[76]
In the breathing zone at a Cairo underground station			2.44	AGI-30 sampler, MEA	Egypt, May–June 1997	[2]
1 m above ground low altitude lounge	Annual total 490		3.4	Jet spore sampler	Austria, 1 year, 1989–1990	[27]
1.5 m above ground low altitude kitchen	Annual total 609		4.8	Jet spore sampler	Austria, 1 year, 1989–1990	[27]
1.5 m above ground high altitude station	Annual total 166		1.3	Jet spore sampler	Austria, 1 year, 1989–1990	[27]
1 m above ground in 18 non-complaint homes, rainy season			0	Air-O-Cell cassettes, microscopy	USA, 3 samples, 2004	[17]
9 non-complaint homes		11*		Electrostatic dust sampler (ALF-75), MEA	Sweden	[71]

For abbreviations see Table 1.

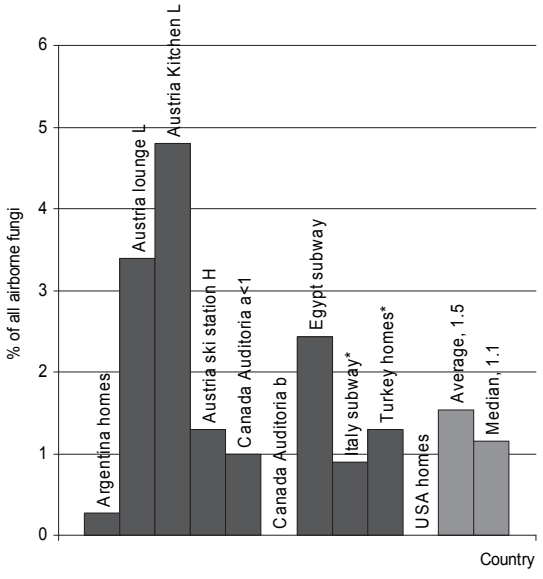

Figure 2. Prevalence of *Botrytis* in percent of all airborne fungi in indoor non-complaint environments (black bars), i.e. neither occupants nor investigators have reported health concerns. More detailed information on the data can be found in Table 2. Calculated average and median of the presented data (grey bars). *Numbers calculated from paper data. References for the presented data: Argentina = [6], Austria lounge L and kitchen L (low altitude) and ski station H (high altitude) = [27], Canada auditoria a and b = [88], Egypt subway = [2], Italy subway = [76], Turkey homes = [18], USA homes = [17].

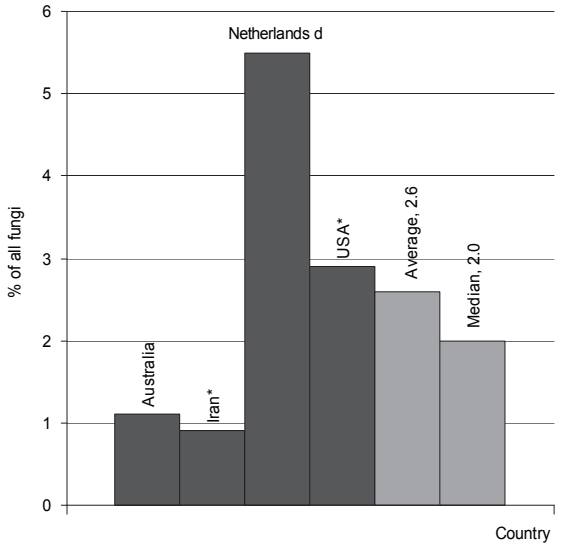

Figure 3. Prevalence of *Botrytis* in percent of all fungi in indoor homes of patients and complaint homes (black bars). More detailed description of the data can be found in Table 3. Calculated average and median of the presented data (grey bars). *Numbers calculated from paper data. References for the presented data: Australia = [31], Iran = [41], The Netherlands d = [8], USA = [85].

low alpine altitude lies between 490 CFU/m^3–609 CFU/m^3 indoors, and 579 CFU/m^3 outdoors [27]. The corresponding annual total at high alpine altitude in indoor air is 166 CFU/m^3 while the outdoor value is 63 CFU/m^3. At high altitude, the indoor/outdoor ratio was also more than 1 for the total fungi counts [27].

The CFU/mg dust for *Botrytis* could be up to 40 in non-complaint homes and up to 600 in complaint homes [71]. The indoor prevalence of *Botrytis* in percent in non-complaint environments and homes ranges between 0%–4.8%, with a calculated median of 1.1% (Fig. 2).

The indoor prevalence of *Botrytis* in complaint environments and homes ranges from 0.9%–5.5%, with a calculated median of 2.0% (Tab. 3 and Fig. 3). This is higher than the medians for both indoor non-complaint environments and homes, as well as for outdoor air. Furthermore, Sharma *et al.* [90] found an average of 112 CFU/m^3 *Botrytis* sp. per measurement in the homes of allergic patients in India, and that the level of indoor *Botrytis* exposure was substantially higher than outdoor exposure. Even though there are few data for complaint environments and the methods used to obtain data greatly differ, it is notable that there is a tendency for a higher *Botrytis* level in complaint homes than in non-complaint environments.

Botrytis prevalence in occupational settings

The indoor prevalence of *Botrytis* in different working environments ranges between 0%–11.6%, with a calculated median of 1.0% for indoor measurements and 0.4% for outdoor measurements (Fig. 4). As expected, *Botrytis* exposure is relatively high in environments such as greenhouses, and grain mills, and for one measurement, in a wine cellar during grape pressing, although the outdoor value for the grain mill was also rather high (Tab. 4). Surprisingly, the prevalence of *Botrytis* was low at an Indian fruit market. Most interestingly, for Egypt, it is notable that for both a subway station (Fig. 2) and a flourmill (Fig. 4), *Botrytis* was found only indoors. This could be due to its requirement for high water availability, implying that it in Egypt could be defined as an indoor fungus. The approximate exposure to *Botrytis* indoors in occupational settings ranges from below the detection level to 125 CFU/m^3 (Tab. 4).

Factors affecting the inflammatory and allergenic potential of *Botrytis*

B. cinerea is generally not described or shown as a mycotoxin-producing fungus [1, 61, 62, 89, 99], although one study found that it had ciliostatic activity in a chicken trachea bio-assay [77]. Like other fungi, *B. cinerea* contains (1→3)-β-D-glucan and chitin in its cell wall. Studies have shown that fungal (1→3)-β-D-glucan can elicit respiratory inflammation [11, 87, 91, 98]. Moreover, a recent study indicates that chitin may also be involved in allergic reactions upon frequent exposure [14].

Denning *et al.* [23] reviewed proteins approved as fungal allergens, and many of them seem to be involved in spore

Table 3. Indoor prevalence of airborne *Botrytis* in complaint and patients' homes.

Species	Specification of environment	Exposure level CFU/m^3	% positive samples	% of all fungi	Sampling and identification method	Country, number of samples and sampling time	Ref.
Botrytis spp.	Homes of asthmatic patients	Total for 21 months 104283♦		13.5♦	Andersen sampler, YMA	The Netherlands 21 months 1981–1982	[8]
Botrytis spp.	Homes of 28 asthmatic patients (17 in the city)		82	5.5	Andersen sampler, YMA	The Netherlands 1981–1982	[8]
Botrytis	Homes of 90 asthmatic patients, city of Sari		100	0.9*	Passive sedimentation plates, MEA	Iran 2004	[41]
Botrytis	80 homes, most of them damp, city of Latrobe Valley			1.1	Andersen sampler, microscopy	Australia 1994–1995	[31]
Botrytis	44 homes of asthmatics, area and city of East Moline	Av. 25		2.9*	Andersen sampler	USA	[85]
B. cinerea	Homes of 130 asthmatic patients[a]		27.7		Rodac Contact plates and swabs, MEA	Belgium 1981–1992	[9]
B. cinerea	Homes of 130 asthmatic patients	<100	68.46		Reuter Centrifugal air sampler, HS-RBA	Belgium 1981–1992	[9]
B. cinerea	83% homes with mould damage, Vilnius		8.0		Slit sampler Krotov 818, MEA, SDA, CZ and CM	Lithuania 1994–2000	[60]
B. cinerea	83% homes with mould damage, Vilnius		1.7		Passive sedimentation plates, MEA, SDA, CZ and CM	Lithuania 1994–2000	[60]
Botrytis	Patients' homes[b]		4		–	Denmark	[34]
Botrytis	Patients' homes[b]		8		–	Denmark	[34]
B. cinerea	Homes of 175 allergic and control children, Stockholm		2		Floor dust collected in vacuum cleaners, V8 agar	Sweden 1988	[101]
Botrytis	9 damp homes		44*		Electrostatic dust sampler (ALF-75), MEA	Sweden 7 days	[71]
Botrytis sp.	Homes of 90 allergic patients	Av. 112			Andersen sampler, RBA	India 6 samplings 2002–2003	[90]
Botrytis	Homes of 44 mould sensitive asthmatic children, Boston		4.5		Burkard sample, DG-18	USA 2 samples, 1998-1999	[74]
Botrytis	Homes of 40 mould sensitive asthmatic children, Bronx		5.0		Burkard sampler, DG-18	USA 2 samples, 1998–1999	[74]
Botrytis	Homes of 54 mould sensitive asthmatic children, Chicago		3.7		Burkard sampler, DG-18	USA 2 samples, 1998–1999	[74]
Botrytis	Homes of 95 mould sensitive asthmatic children, Dallas		0.0		Burkard sampler, DG-18	USA 2 samples, 1998–1999	[74]
Botrytis	Homes of 48 mould sensitive asthmatic children, New York		4.2		Burkard sampler, DG-18	USA 2 samples, 1998–1999	[74]
Botrytis	Homes of 52 mould sensitive asthmatic children, Seattle		21.2		Burkard sampler, DG-18	USA 2 samples, 1998–1999	[74]
Botrytis	Homes of 81 mould sensitive asthmatic children, Tucson		0.0		Burkard sampler, DG-18	USA 2 samples, 1998–1999	[74]

For abbreviations used in the table see Table 1. ♦Value is for both indoor and outdoor occurrence combined. [a]Samples collected from walls and horizontal surfaces. [b]Unspecified dust.

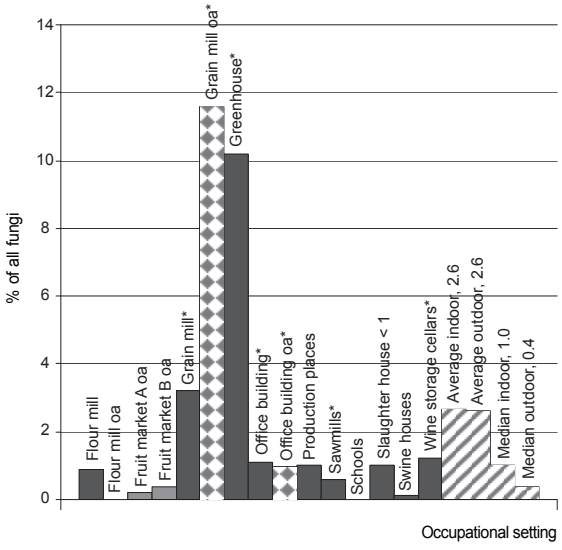

Figure 4. Indoor and outdoor prevalence of *Botrytis* in percent of all fungi found for different occupational settings. More detailed description of the data can be found in Tables 1–4. Indoor prevalence (black bars), outdoor prevalence (oa) of an indoor – outdoor pair (bars with squares in a line), outdoor prevalence (grey bars) and the calculated average and median from the presented data (bars with grey stripes). *Numbers calculated from paper data. References for the presented data: Flour mill and Flour mill oa = [3], Fruit market A and B = [48], Grain mill and Grain mill oa = [22], Greenhouse = [68], Office building and Office building oa = [53], Production places = [95], Sawmills = [93], Schools = [45], Slaughter house = [39], Swine houses = [15], Wine storage cellars = [15].

germination and responses to oxidative stress thought to be a consequence of contact with the human immune response. Furthermore, Green *et al.* [35] compared the allergenic properties of spores and germinated spores of 11 allergenic fungi including *B. cinerea*. The quantity of allergens released increased significantly after germination of *B. cinerea* spores. In contrast, the quantity did not increase for *Cladosporium herbarum*.

Likewise, Kauffman *et al.* [52] looked at the allergen expression of *B. cinerea* during different phases of growth in Sabouraud-2% glucose medium at 20°C. They found that the binding of IgE and IgG by culture filtrate was maximal in the early phase of growth. Thus, the allergenic potential of *Botrytis* seems to be affected by its state of growth and germination ability.

Prevalence of allergy towards *B. cinerea*

A survey of the standard skin prick tests from 29 allergy centres in Europe shows that routine testing is mainly carried out for one to two fungi. The fungi in question are *Alternaria* and *Cladosporium*, sometimes combined with each other, or *Aspergillus* and *Candida*, and at 2 centres a mould-mix is tested. Some of the centres in southern Europe test for more than 2 fungal species simultaneously including *Fusarium* and *Penicillium,* in addition to the genera mentioned above. *B. cinerea* is only tested routinely at the centres in Kraków and Montpellier [42]. Hence, finding papers investigating allergy towards *Botrytis* is difficult: those we found are presented in Table 5.

Allergy towards *B. cinerea* ranges between 1.3%–52%, with a calculated median of 18.8%, compared to a calculated median of 40.5% of allergy to at least one fungal species (Fig. 5 and Tab. 5).

Moreover, Immonen *et al.* [45] and Korhonen *et al.* [55] found that allergy towards *B. cinerea* is the most prevalent allergy, or is just as prevalent as allergy towards the typically investigated *Alternaria*, *Cladosporium* and *Aspergillus* in young Finnish children newly diagnosed with asthma and schoolchildren suspected of asthma (Tab. 5). This is surprising considering that no airborne *B. cinerea* was identified at the children's school [45], and that *B. cinerea* seems to have a low prevalence in ambient air in Finland (Tab. 1).

In addition, Karlsson-Borgå *et al.* [49] and Koivikko *et al.* [54], using Phadebas RAST, compared a standard mould test panel with an extended mould panel in patients. The extended mould panel included *B. cinerea*. In Sweden and Denmark, Karlsson-Borgå *et al.* [49] found 18% additional mould allergic patients, and in the USA 75%. *B. cinerea* allergy was the second most prevalent mould allergy in Sweden and Denmark and the most prevalent in the USA. Koivikko *et al.* [54] found an additional 3.3% of mould allergic patients with the extended panel, and *B. cinerea* was the fourth most prevalent fungal allergy in the test groups. Altogether, it seems that a substantial proportion of test persons do have allergy towards *B. cinerea* (Tab. 5) even though exposure to this mould in ambient air in most places seems to be low.

Prevalence of allergy towards *B. cinerea* in occupational settings

In certain occupational settings, such as in the production of raspberries, wine and certain dessert wines, where infestation with *B. cinerea* is necessary [19, 46, 83], or greenhouses [79], exposure to *B. cinerea* can reach high levels. Thus two cases from Austria of hypersensitivity pneumonitis/allergic alveolitis caused by *B. cinerea* have been reported in two farm workers working with noble rot grapes [78]. In grape farm workers in South Africa only 1% had allergy towards *B. cinerea* (Tab. 5) though a relatively high exposure could be expected. In the same group, 1.6% suffered from allergy towards at least one fungus in a mould mix containing *Altanaria alternata*, *C. herbarum* and *Fusarium*. Hence, even though the level of allergy towards *Botrytis* was low it made up a high proportion of the fungal allergies.

The prevalences of allergy towards *B. cinerea* in greenhouse workers in the Netherlands are 4% for chrysanthemum workers and 13.8% for bell pepper workers (Tab. 5). The difference between the two types of greenhouse is interesting since the data were obtained by the same research group and using the same test extract. The greenhouse

Table 4. Prevalence of airborne *Botrytis* in different working environments and countries.

Environment category	Specification of environment	Exposure level CFU/m³	% positive samples	% of all fungi	Sampling and identification method	Country, number of samples and sampling time	Ref.
Botrytis							
Outdoor	1.5 m above ground, outside a grain mill	Av. 60		11.6*	Orthogonal impact Microflow active sampler, SDA	Italy 8 samples	[22]
Indoor	1.5 m above ground, different areas in a grain mill	Av. 5–75		3.2*	Orthogonal impact Microflow active sampler, SDA	Italy 8 samples	[22]
Indoor	Greenhouses, flower and ornamental plant growers			10.2*	Personal sampler, microbiology laboratory	Spain	[68]
Indoor	1.5 m above ground, flour mill store			0.9	AGI-30 sampler, MEA	Egypt, 8 samplings, 2004–2005	[3]
Outdoor	1.5 m above ground, flour mill store			0	AGI-30 sampler, MEA	Egypt, 8 samplings, 2004–2005	[3]
Indoor	1.2 m above ground, twelve sawmills	0–46		Av. 1.3 0.5–5.5* Med. 0.6	SAS sampler, MEA and MESA	France 8 samples	[93]
Indoor	1,5 m above floor, swine breeding houses			0.12	Andersen sampler, MEA	Taiwan 2 days, 1995	[15]
Outdoor	1 m above ground, fruit market			0.2	Rotorod sampler, microscopy	India, 8 days interval 1 year, 1993–1994	[48]
B. cinerea							
Outdoor	0.5–1 m above ground, fruit market			0.4	Passive sedimentation plates, PDA, CZ and RBA	India 15 days interval 1 year, 1993–1994	[48]
Indoor	0.8–1 m above ground, office building in Perth	0–5 Av. 2.1*		1.1*	Andersen sampler, MEA	Australia 2 samples	[53]
Indoor	1.2 m above floor level, in nine wine storage cellars	8–125		Av. 7.6 0.8–61* Med. 1.2	One-stage volumetric sieve sampler (SAS Compact), MESA	France 2 samples, 1997	[94]
Indoor	Production places (meat, flour, sweets and dairy)			1.01	Passive sedimentation plates, MEA	Turkey, 16 samples, 1995–1996	[95]
Indoor	Four schools in the city of Turku			0	Andersen sampler, 2% MEA and DG18	Finland, 15–19 samples, 1999	[45]
Botrytis sp./spp.							
Indoor	1.7 m above ground, slaughterhouse			< 1%	Andersen impactor and SKC Biosampler impinger, MEA or saline solution	Austria 48 samples June–November 2002	[39]
Indoor	1.5 m above ground, greenhouses		32.4		Polypropylene air monitoring cassettes, DG-18 and MEA	Spain	[79]
B. cinerea							
Indoor	Greenhouses		48		PDA surface contact plates	Italy 1987–1988	[21]
Outdoor	40 cm above the beds, strawberry farm, until the first day of harvest	0–2.0 × 10³			Burkard sampler, microscopy	Spain, October 2001–13 February 2002	[10]
Outdoor	40 cm above the beds, strawberry farm, until the first day of harvest	0–2.8 × 10³			Burkard sampler, microscopy	Spain, October 2002–6 February 2003	[10]
Outdoor	40 cm above the beds, strawberry farm, during harvest	0–2.6 × 10⁴			Burkard sampler, microscopy	Spain, 13 February–20 May 2002	[10]
Outdoor	40 cm above the beds, strawberry farm, during harvest	0–1.3 × 10⁵			Burkard sampler, microscopy	Spain, 6 February–19 May 2003	[10]
Outdoor	1.5 m above ground, 5 ha vineyard	Av./day 100–1000*			Hirst type spore trap, microscopy	Spain June–September 1994	[24]

For abbreviations used in the table see Table 1.

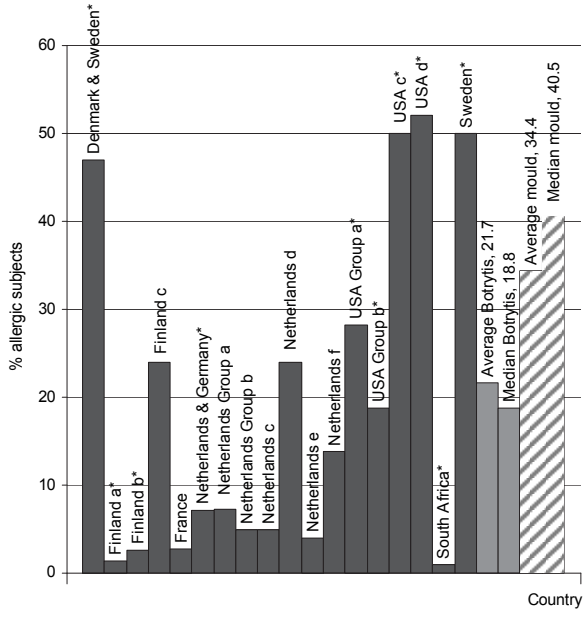

Figure 5. Prevalence of allergy towards *B. cinerea*# in test persons (black bars). More detailed information on the data can be found in Table 5. Average and median of allergy towards at least one fungus in test persons calculated from the paper data presented in Table 5 (bars with grey stripes), and the calculated average and median of the presented data of allergy towards *B. cinerea* (grey bars). # In references (Ref.) [70], [42], [12] and [20] only *Botrytis* was investigated and in ref. [8] *Botrytis* spp. Ref. for the presented data: Denmark and Sweden = [49], Finland a = [45], Finland b = [55], France = [42] and [12], The Netherlands and Germany = [8], The Netherlands Group a and b = [7], The Netherlands c and d = [97], The Netherlands e = [38], The Netherlands f = [37], USA Group a and b = [20], USA c = [16], USA d = [49], South Africa = [47], Sweden = [70].

workers prevalences of allergy to *Botrytis* are comparable to the values found in atopic and suspected atopic test persons in the Netherlands. However, patients suspected of mould allergy in the Netherlands had a higher prevalence of allergy to *Botrytis* (Tab. 5).

Combining the information that a substantial part of patients and workers are allergic to *B. cinerea,* and that extending the Phadebas RAST test panel detected new patients, makes it desirable to include *B. cinerea* in a standard allergy test. Furthermore, taking into account that test persons in the surveyed papers are rarely allergic to only one fungal species, using an extended fungi test panel as standard might assist in identifying a higher proportion of mould allergic patients.

Seasonal variations in *Botrytis* exposure

Diagnosing and treating allergy in patients is dependent on both diagnostic tools and patient history. For that reason, knowledge of allergen exposure is important to doctors and patients [64]. Even though the amount of spores present does not seem to influence a fungus' ability to cause allergy, the amount does seem important when an allergy is established. Hence, Malling [64] reviewed a study of asthma patients that showed a correlation between asthma symptoms and *Cladosporium* spore counts. Furthermore, a study into autumnal asthma showed a better correlation between symptoms and fungal spore counts than between symptoms and ragweed pollen [64].

A comparison of the few papers that look into the distribution of *Botrytis* throughout the year shows a mixed picture, but indicate an all-year presence for the temperate climate in Europe with an approximate season starting in April and lasting until November, peaking in August–September [8, 27, 44, 50, 51, 69, 96]. In contrast, a 15-year study in Thessaloniki (Greece) showed a low nearly even distribution over the year with a slight decrease in December and January [32]. In Porto Alegre (Brazil), *Botrytis* was only found in autumn and winter [66] and in Porto (Portugal) a 1-year study found a seasonal distribution from April–December, where *Botrytis* peaked from July–December, with a low in November [72]. In Canada, Bartlett *et al.* [5] found low exposure to *Botrytis* at schools in winter (November–February) and spring (April–June), with a major increase in the autumn season (September–November).

The distribution of *B. cinerea* during the day was investigated by Blanco *et al.* [10] during two seasons in a strawberry field in Spain. They found a diurnal pattern where the conidial concentration increased from 08:00 and decreased towards 15:00 while peaking between 09:00–11:00 in the first season. In the second season, conidial concentration increased from 06:00 and decreased towards 17:00, while peaking between 12:00–14:00. Similarly, Jarvis [46] studied the dispersal of *B. cinerea* conidia in a raspberry field in Scotland and found a diurnal pattern for *B. cinerea* spore dispersal in response to changes in relative humidity. Thus, *Botrytis* increased from 07:00 and decreased towards 18:00, while peaking between 09:00–13:00 and 15:00–17:00. However, this pattern was not followed during rainfall at night and days with otherwise unsuitable conditions for spore dispersal, at those times *Botrytis* was also dispersed. In a vineyard in Switzerland Corbaz [19] similarly showed a diurnal dispersal pattern for *Botrytis* spores. Thus, spore dispersal also increased between 06:00–09:00, but in contrast peaked between 09:00–20:00, while decreasing towards 24:00. Consequently, *Botrytis* allergic patients should ventilate their rooms in the very early morning hours and in the late evening hours, while avoiding ventilation during rainfall and windy weather.

DISCUSSION

The quality of fungal test extracts for detecting fungal allergy varies greatly, and the range of available test extracts is low compared to the number of fungi with allergenic potential [13, 56, 84]. Hence, Malling *et al.* [65] investigated test extracts of *C. herbarum* from three different manufacturers and at different concentrations. They found that within the same test group the percentage of positive skin prick test reactions ranged from 10%–60%. Hence, the level of allergy towards fungi in general and among them *B. cinerea* may well be underestimated. Thus, with the limitations of

Table 5. Levels of allergy towards *Botrytis cinerea* found in the surveyed papers.

Specifications of test persons	Number of persons	% positive SPT *Botrytis*	% positive RAST *Botrytis*	% positive allergy fungi[b]	Allergy test extract	Country and Year	Ref.
Suspected asthmatic, allergic or rhinitis patients	404		2.7		Stallergènes SA, Fresnes	France	[12, 42]
Suspected and asthmatic school children	144	1.3*		4	ALK panel, Denmark	Finland	[45]
Newly diagnosed asthmatic children	114	2.6*		< 5	Possibly ALK panel	Finland	[55]
Suspected mould allergic children with asthma	121		24	40.5	Extended Phadebas RAST, Pharmacia	Turku, Finland	[54]
Subgroup of allergic rhinitis patients with suspected mould allergy	39		28.2*	44	ImmunoCap (modified RAST), Pharmacia	Chicago, USA	[20]
Randomly selected patients suspected of allergic rhinitis	32		18.8*	44	ImmunoCap (modified RAST), Pharmacia	Chicago, USA	[20]
Allergic fungal sinusitis patients	10	50*		100	MMP, Bayer	USA	[16]
Suspected mould allergic patients	21		52*	66*	Extended Phadebas RAST, Pharmacia	USA	[49]
Suspected mould allergic patients	34		47*	77*	Extended Phadebas RAST, Pharmacia	Denmark and Sweden	[49]
Patients	150		50*		Mould extract after Feinberg	Sweden	[70]
Suspected allergic patients	692	4.9			Extract from cultivated mould mycelium	Groningen, The Netherlands	[97]
Suspected mould allergic patients	180	24			Extract from cultivated mould mycelium	Leiden, The Netherlands	[97]
Suspected asthmatic and/or allergic patients	68	7.3[a]			Diephuis Laboratories Groningen, Netherlands	Beatrixoord, The Netherlands, 1981–1983	[7]
Suspected asthmatic and/or allergic patients	692 (833)	4.9[a]		(4.6)	Diephuis Laboratories Groningen, Netherlands	Beatrixoord, The Netherlands, 1981–1983	[7]
Mould allergic asthmatic patients	28	7.1*		100	Diephuis Laboratories Groningen, Netherlands	The Netherlands and Germany, 1981–1982	[8]
Chrysanthemum greenhouse workers	104	4		NI	ALK Abelló, Netherlands	The Netherlands, 2000	[38]
Predatory mite allergic workers in Bell pepper greenhouses	109	13.8		NI	ALK Abelló, Netherlands	The Netherlands, 1999–2000	[37]
Table grape farm workers	190	1*		NI	ALK	South Africa	[47]

Abbreviations used in the table: NF = not found, NI = not investigated, SPT = Skin-prick test, RAST = radioallergosorbent test. [a] results from an intracutaneous skin test, [b] positive skin prick test or radioallergosorbent test to at least one of the fungi of a test-panel in the entire test group. In ref. [70], [42], [12] and [20] only *Botrytis* was investigated and in ref. [8] *Botrytis* spp.

current knowledge, about 20%–30% of atopic individuals are estimated to suffer from respiratory allergy towards fungi, while the same is true for about 6% of the general population [56, 84]. In that respect, a substantial amount of test persons are allergic towards *B. cinerea*, especially when considering that the surveyed papers show that *Botrytis* in most instances is a genus with a low airborne prevalence (Tables 1–4) compared to e.g. *Alternaria*, *Cladosporium*, *Aspergillus*, yeasts, basidiospores and sterile mycelia.

This study shows that *Botrytis* spp. can be sampled using different methods (Tables 1–4). However, we have described a relatively low airborne prevalence of *Botrytis*. This may be due to the sampling methods applied [84] because the viability of fungal spores during sampling might be influenced by e.g. mechanical forces and/or transient declines in water activity of the sampling media. For example, Saldanha *et al.* [88] recovered *B. cinerea* with a Reuter Centrifugal Sampler (RCS) but not with an Andersen sampler. The first sampler is significantly better at sampling fungi defined as having a high water activity requirement than fungi defined as xerotolerant compared to the Andersen sampler and vice versa [88]. On the other hand, this

review shows that *Botrytis* has often been sampled using an Andersen sampler. This difference could be due, for example, to the different sampling times used in the surveyed papers as sampling durations range from 1 minute to 1 day (Tables 1–4).

What is more, Rotem and Aust [86] investigated the viability of spores and spore aggregates. They found the viability of *B. cinerea* was the most vulnerable of the fungal species investigated to the detrimental effects of exposure to darkness under high temperature, UV-radiation and sunlight.

Another possible factor affecting the measured airborne exposure level of *Botrytis* could be the sampling height, which varies from a few centimetres to several metres above ground (Tables 1–4). For example, Rantio-Lethimäki *et al.* [81] compared spore counts sampled at 15 meters height and at ground level. They found that *Botrytis* spores had the highest ground/ roof ratio of the genera studied and that its spore season was 100 days longer at ground level. This could explain the differences in outdoor exposure levels seen for the Netherlands which ranges from 2.7%–17% (Tab. 1).

Tables 1–4 show *Botrytis* can be cultivated and identified on different agar media, though they may not all be optimal media. Indeed, cultivation and identification of fungi is very complicated and often requires spores to be viable and able to germinate, grow and sporulate; therefore, the choice of sampling method, sampling duration, identification method and media affects which fungi can be identified and thus correlated with a following study of health symptoms. With reference to the above reasons, the airborne exposure level of *Botrytis* may well be underestimated.

An alternative route to exposure to airborne *Botrytis* might be oral exposure, thus resulting in higher exposure. As mentioned above, *Botrytis* is frequently isolated from fruits and vegetables [61, 89, 99], also after surface disinfection [89, 99]. Hence, exposure may occur from fruits with no visible *Botrytis* growth.

CONCLUSION

In conclusion, *Botrytis* is found globally with different spore seasons and has low prevalence in ambient air indoors and outdoors. However, the prevalence in indoor complaint homes tends to be higher. The dispersal of spores follows a diurnal pattern where the spore level increases in the early morning hours, decreasing towards the late afternoon. The exposure to *Botrytis* may also increase during rainfall at night. Thus, *Botrytis* allergic patients should ventilate their rooms in the late evening, at night, or in the very early morning hours, except during rainfall.

Furthermore, a substantial amount of patients and workers are allergic to *B. cinerea*, thus it seems to be as important as more prevalent mould genera such as *Cladosporium* and *Alternaria* when investigating allergy towards fungi. Even though the degree of sensibility varies between different geographical groups and test groups, we suggest that *B. cinerea* should be included in standard tests. Furthermore, given the low airborne prevalence of *Botrytis* and other fungi capable of inducing allergy, it is clear that when investigating the correlation between exposure and health effect it is not adequate to only look at the 2–4 most prevalent genera. Although the scientific community agrees that the methods applied to assess fungal exposure to date are a compromise, it would be advisable to agree on what approach should be used to make data comparable.

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