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An update on pollen and fungal spore aerobiology

Harriet A. Burge, PhD *Boston, Mass*

Changes in climate are altering pollen distribution. Predictive modeling can be used to forecast long- and short-term changes in pollen concentrations. Increasing evidence confirms the presence of pollen allergens on small, respirable particles in the air, explaining the occurrence of pollen-season increases in asthma. Like pollens, aboveground indoor fungal aerosols primarily reflect outdoor concentrations. Basement spore concentrations might be higher and reflective of local sources. Fungal presence in the indoor or outdoor air can be monitored on an area basis or with personal monitors. The samples can be analyzed by means of microscopy, culture, DNA probes, HPLC, or immunodetection. Total fungal biomass can be estimated on the basis of measurements of ergosterol or glucan in environmental samples. Unfortunately, there are no generally accepted standards for interpretation of fungal levels in indoor or outdoor air. At present, the best approach to indoor fungal control is moisture control in the indoor environment. This will essentially prevent fungal growth, except from extraordinary events. (J Allergy Clin Immunol 2002;110:544-52.)

Key words: *Pollen, fungi, aerobiology, spores, allergen, immunodetection*

For airborne disease to occur, one must have a source from which the disease agent is released, some method for aerosolization of the agent, and exposure of susceptible hosts to sufficient agent to cause a response (the disease). The science of aerobiology deals with the agents, the processes of aerosolization, the aerosol itself, and, to some extent, exposure and response. Different disciplines tend to use the term *aerobiology* in different ways. For example, the aerobiologists working for the defense departments around the world consider aerobiology to be only about aerosols of virulent pathogens. Plant pathologists are often also aerobiologists because they deal with transfer through the air of plant-disease agents. Allergists tend to limit the use of the term aerobiology to the characterization of outdoor pollen and spore aerosols. Inextricably bound to these outdoor bioaerosols are those that penetrate and grow in indoor reservoirs. This review will focus primarily on new developments in the literature concerning outdoor and indoor pollen and fungal spores and their sources.

Abbreviations used
CFU: Colony-forming units
MVOC: Microbial volatile organic compound

POLLEN

Historically, outdoor pollen aerosols have been the best known of the allergen sources, and symptoms of hay fever have been reported in ancient literature.

Pollen prevalence

Changes in climate have dramatically changed plant distributions and therefore pollen distributions.[1,2] At present, climate changes have also influenced plant flowering, both in magnitude and time. For example, recent El Niño events have led to early production and release of most tree pollen types.[3] Permanent vegetation changes have occurred in as little as 10 years, perhaps in the aftermath of extensive wildfires.[4] At least in the short run, vegetation in Colorado and Arizona are likely to be extensively affected by this year's western wildfires. Experimentally, Wayne et al[5] observed that increasing concentrations of CO_2 lead to an increase in the mass of ragweed pollen produced. The implications of this finding for global increases in CO_2 are speculative but should be considered.

Predictive modeling

The importance of predictive models for both long- and short-term changes in pollen concentrations lies in the ability to prevent exposure and symptoms, such as with temporary lifestyle changes or medications. Many different groups are working on these models, which almost uniformly use weather data as the main controlling variables. Two types of models are being formulated: those that predict the severity of future pollen seasons and those that predict pollen concentrations in the immediate future.

Compilations of weather data immediately preceding the pollen season have been used to predict the severity of future pollen seasons. Galan et al[6] used early (March) rainfall and temperature up to the flowering period to predict total pollen output for *Olea europaea*. Five-day moving

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Reprint requests: Harriet A. Burge, PhD, Harvard School of Public Health, Landmark Center, Room 404M, West, 401 Park Dr, PO Box 15677, Boston, MA 02215.

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temperature averages best predicted peak timing and values, although rainfall at peak times decreased the maximum value.[6] On the other hand, Emberlin et al[7] used aggregated weather data from 10 days closely preceding the grass pollen season to predict the severity of the season.

In a model to predict how high pollen levels will be from one day to the next, windy conditions, low humidity, little rain, and temperatures of greater than 6°C predicted high levels.[8] Chaos theory and fractals have also been applied to daily pollen concentration forecasting.[9]

Indoor-outdoor relationships

Although the majority of pollen exposure probably occurs outdoors, the amount of time spent indoors today makes even low-level indoor exposures important. In the majority of cases, indoor pollen aerosols are controlled by those outdoors. A recent study from Houston reported that 62% of indoor pollen variability was explained by outdoor levels.[10] Although keeping windows closed and entry air filtered will reduce this problem, grass and other pollen allergens do accumulate in house dust and remain beyond the grass pollen season. Frequent vacuuming appears to reduce this potential source of exposure.[11]

Pollen allergens

Pollen grains themselves are large, and few penetrate the lower airways to lead to asthma. The existence of pollen allergen associated with particles of less than 5 μm in size continues to be of significant interest. Grass pollen allergens have been most extensively examined, with increasing evidence available that supports rain release of small-particle grass allergen. Some of these small particles on which allergen resides are starch grains that are released from the pollen grain during light rainfall.[12,13] Similar findings have been reported for birch allergen (Bet v 1), with smaller particulate allergen present on days with light rain compared with on days with heavy rain.[14] Some grass allergen might bind to diesel exhaust carbon particles, possibly favoring deposition in the airways.[15] Holmquist et al[16] recovered birch and grass pollen allergens from inside ground-level shops and hypothesized that they had entered on diesel exhaust particles.[16]

Pollen exposure and asthma

Historically, pollen exposure has been considered to lead primarily to hay fever. However, data are increasingly supporting a role for pollen allergen exposure and asthma. Of particular interest is pollen-associated asthma related to rain and thunderstorms. Some studies supporting this relationship are retrospective clinical studies with no exposure assessment.[17] Others report increased pollen levels either during or before the thunderstorm event and grass-specific IgE levels in patients experiencing exacerbations during the event.[18-21] One group of researchers included use of inhaled steroids in their epidemiologic study, and their data suggest that the use of steroids could ameliorate associated asthma attacks related to small-particle allergens, pointing out the importance of predicting these events.[22] Other studies, however, have not supported this as a strong relationship. Anderson et al[23] found that thunderstorms did result in an increase in asthma attacks but found no relationships between pollen or fungal spore counts and these attacks.

FUNGI

Fungal exposure occurs primarily outdoors, but as for pollen, fungal spores do penetrate indoor environments, and some fungi might also colonize indoor materials, resulting in unique exposure situations. Some occupational settings are also rich in fungi.

Fungal sources and prevalence: Outdoor sources

Sources of outdoor fungal aerosols are usually considered to remain constant from year to year. However, because the primary source for airborne fungi is plant materials, it should be obvious that major changes in plant ecology will also change the fungal air spora. For example, although we have no documenting data, it is probably true that our changing agricultural practices have changed the outdoor air spora over time. Fungal aerosols above forests and grasslands are likely to be considerably different than those above fields of corn, wheat, soybeans, and so forth.[24]

As for pollen sources, changes in the earth's atmosphere also might change both types and concentrations of fungi on substrates. Global warming and increasing levels of atmospheric CO_2 are examples of such changes. In one experimental study concentration of airborne fungal propagules, mostly spores, increased 5-fold in leaf litter under twice-ambient CO_2 concentrations.[25]

Another case of human-induced changes in fungal sources is the use of fungi as biocontrol agents, both indoors and outdoors. For example, *Epicoccum nigrum* conidia sprayed onto sunflowers shows promise for the control of sunflower head rot and, if used successfully, might increase the relative proportion of this important allergenic fungus in the outdoor aerosol.[26] *Beauveria bassiana* is an entomophilic fungus that is being studied for use in control of the Mexican fruit fly.[27] Some fungi parasitize other fungi and are used for control of some of the fungal plant pathogens.[28] Fungi can also control harmful nematodes.[29]

Indoor-outdoor relationships

In the majority of cases, indoor fungal aerosols are controlled by outdoor concentrations.[30] Two recent studies from Houston reinforce the fact that most indoor fungal aerosols are derived from outdoor air. In one study outdoor concentrations explained a large proportion of indoor fungal spore variability.[10] In another, indoor and outdoor levels and types of fungi were consistently and highly correlated over an 18-month period in a large building, in spite of the fact that the building became colonized during the investigation.[31] Levy et al[31] collected over 100 samples in one intensive day of sampling in a bakery. Concentrations and types of spores in the air

TABLE I. Comparisons of fungal taxa in indoor and outdoor air, as reported by the listed authors from specified locations

	Outdoors	Indoors	
		Summer	Winter
Ren et al, 1999,[33] New England	*Cladosporium* species	*Cladosporium* species	*Penicillium* and *Aspergillus* species
Burge et al, 2000,[31] Texas	*Cladosporium* species	*Cladosporium* species	*Cladosporium* species
Su et al, 2001,[34] Taiwan		*Cladosporium*, *Aspergillus*, and *Penicillium* species	
Khan et al, 1999,[35] Kuwait	*Aspergillus fumigatus* and *Alternaria fusarium*	*Cladosporium*, *Penicillium*, and *Bipolaris* species	
McGrath et al, 1999,[36] Texas	*Cladosporium* and *Penicillium* species	*Penicillium* species	
Shelton et al, 2002,[30] United States (many locations)	*Cladosporium* and *Penicillium* species and nonsporulating *Aspergillus* species	*Cladosporium* and *Penicillium* species and nonsporulating *Aspergillus* species	*Cladosporium* and *Penicillium* species and nonsporulating *Aspergillus* species

Dominant organisms are listed for each site.

were related to activities in the bakery, but effects were different on different days, and outdoor air concentrations were variable and strongly affected indoor concentrations.[32] Table I[30,31,33-36] presents a summary of studies comparing taxa across indoor and outdoor air.

Most evidence supports a consistent fungal spora throughout the aboveground living space in houses and, as mentioned above, supports the notion that this spora varies with outdoor populations. On the other hand, basement spore concentrations might be consistently greater than those in the rest of the house, and the types present are more constant and not related to those outdoors.[33,37]

The well-known effects of disturbance of dust on fungal levels has been affirmed in several studies. Mahieu et al[38] documented that *Aspergillus* species spore concentrations increased during remediation and that use of an air cleaner significantly reduced concentrations. Interestingly, remediation activities did not increase prevalence of *Aspergillus* species in the nasal cavities of patients. On the other hand, remediation of obvious fungal contamination does routinely result in increased airborne concentrations.[39]

Fungal sources and prevalence: Indoor sources

Water sources. Water is the primary controlling factor for fungal growth indoors, and different fungi colonize susceptible materials under different moisture conditions.[40] Water within materials is the important factor for fungal growth. Relative humidity, which is usually the parameter measured in field studies, controls fungal growth only to the extent that it leads to excess water in materials. Unless cold surfaces that result in condensation are present, relative humidity must be consistently very high (>85%) to lead to fungal growth (unpublished data).

One place that condensation can be particularly problematic is inside the structure of walls with improperly installed vapor barriers. In cold climates warm moist indoor air diffuses through walls and contacts cold vapor barriers, leading to condensation and growth of fungi and bacteria.[41] Release of spores and other particles from these sources continues to be investigated.

Water conditions in large studies are usually assessed by means of questionnaire. However, evidence is mixed with respect to the relationship between answers to dampness questions and quantitative measures of fungal concentration.[37,42,43]

Building materials. The trend over the past 50 years toward more and more paper products used as building materials in houses has increased the availability of easily digested fungal substrates, all of which contain spores as they come off the manufacturing line. In a study of organic and inorganic ceiling tiles, Karunasena et al[44] determined that the inorganic type did not support the growth of *Penicillium*, *Cladosporium*, and *Stachybotrys* species. However, it should be emphasized that any substrate can become colonized by fungi if it remains wet for a long period.

Biocontrol. Fungi are beginning to be used for control of indoor insect infestations. Both *Metarrhizum anisopliae* and *Beauveria bassiana* are used for cockroach control.[45,46] Both of these fungi sporulate on the dead insect bodies, and the small spores could become abundant indoors. The fungus has produced asthmatic responses in sensitized mice and could add to the allergen burden in cockroach-infested homes.[47]

Predictive models

Increasing efforts are underway to develop models for predicting outdoor spore concentrations from seasonal and meteorologic data. Such models will have to be specific for individual spore types because of the many different modes of spore release that occur in the fungi. Among the most important factors overall appear to be temperature and dew point.[48] Several efforts have also been made to predict indoor fungal levels. Table II[37,42,49-52] documents the factors that have been consistently significant in the models.

Fungal disease agents

Spores and allergens. The size of airborne fungal particles controls both how efficiently various samplers collect them and how they penetrate the human respiratory tract.[53,54] Most fungal spores are in the 2- to 10-μm

TABLE II. Predictors used in models of fungal growth or fungal concentrations in homes

Authors	Factors	Effect on fungal levels
Ren et al, 2001[37]	Temperature	+
	Relative humidity	+
	Season	Summer, fall
	Cats	+
Dharmage et al, 1999[49]	Ceiling fan	–
	No visible mold	–
	Frequent vacuuming	–
	Solid fuel	–
	No pets	–
	More than one cat	++
	Old fitted carpets	+
Hirsch et al, 2000[50]	Insulated windows	+
	Central heating	+
	Ceiling fan	–
	No visible mold	–
	Frequent vacuuming	–
Takatori et al, 2001[42]	Wooden board construction	+
	Reinforced concrete construction	–
Dales et al, 1997[51]	Moldy odors	+
	Water damage	+
	Dampness	+
Gehring et al, 2001[52]	Carpets in the living room	+ (glucan)
	Keeping a dog inside	+
	Use of the home by ≥4 persons	+
	Use of the living room for >180 h/wk	+
	Lower frequency of vacuum cleaning and dust cleaning	+
	Presence of mold spots during the past 12 months	+

TABLE III. Mycotoxins recovered from a group of common indoor fungi

Fungus	Toxins	Strains
Penicillium chrysogenum	Meleagrin (antibiotic)	1 of 4
Penicillium polonicum	3-Methoxy-viridicatin, verrucosidin, and verrucofortine	2 of 2
Penicillium brevicompactum	Mycophenolic acid	2 of 5
Chaetomium species	Chaetoglobosins A and C	6 of 6
Aspergillus ustus	No toxins	
Aspergillus niger	Naphtho-γ-pyrones and tetracyclic compounds	
Ulocladium species	No toxins	
Alternaria species	Alternariol and alternariol monomethyl ether	5 of 6
Paecilomyces species	No toxins	
Aspergillus versicolor	Sterigmatocystin, 5-methoxysterigmatocystin	

Modified from Nielsen et al.[57]

range, are efficiently collected by most commonly used particle samplers, and penetrate into the lower airway.

Specific studies on *Cladosporium* species aerosols have indicated that between 1 and 1.3 spores were associated with each dispersion unit and that grouping did not seem to affect penetration into the respiratory tract. Average size of airborne *Cladosporium* species spores on the basis of size-selective sampling was 2.2 μm. This might be surprising to some because large clumps are so easily recognized on spore traps and appear to dominate the aerosol. Smaller spores, which are present in abundance, are often overlooked.[55]

Calvatia species spores have also been studied for penetration into the respiratory tract. More than 60% of these small spores penetrated into the alveoli. Although not necessarily representative of the most common basidiospores in air, these studies confirm that this class of important allergen-bearing particles do reach the lower airways.[56]

Mycotoxins. Many different fungi produce mycotoxins both in culture and when growing on building materials. Table III represents a summary of data presented by Nielsen and Madsen.[57]

In most cases investigated for suspected mycotoxin-related disease, *Penicillium* and *Aspergillus* species comprise the majority of airborne spores (23,000 colony-forming units [CFU]/m^3 total culturable fungi vs 43 CFU/m^3 culturable *Stachybotrys* species).[58] *Stachybotrys* species spore measurements in a building to be remediated were initially between 0.1 and 9.3 spores/m^3 air, and the toxicity of air particulates was correspondingly low. During remediation, airborne spore counts and toxicity increased.[59] At least when wet, *Stachybotrys* species growth releases few spores or other particles and insufficient toxic or irritant material to acutely affect mice.[60]

Most studies of mycotoxins in buildings use analysis of bulk samples.[61] Although mycotoxins in reservoirs do confirm the presences of sources, interpretation of this kind of data as evidence of sufficient exposure to cause disease is risky. None of the published studies reviewed provided evidence of high exposures to the usual suspect (*Stachybotrys* species), often because only bulk sampling was used. High-level exposures to *Aspergillus* and *Penicillium* species are often documented in cases in which *Stachybotrys* species are found in reservoirs. However, these small, dry spores are rarely blamed for disease outbreaks.

Microbial volatile organic compounds. Fungi growing on building substrates release a variety of volatile organic compounds. Although some compounds are released by most fungi, others appear to be species specific and might eventually be useful for identifying fungal growth.[62] Specific identification might depend on the substrate, which is not always controllable in natural substrates. Growth on substrates favoring terpene synthesis produced the most characteristic volatile compounds in one study.[63] On insulation material, interestingly, one study demonstrated that volatile organic compound concentrations were lowest when fungal growth was present.[64]

One study of microbial volatile organic compounds (MVOCs) determined that levels of 2 common compounds necessary to cause sensory irritation in mice were

considerably higher than levels measured in contaminated environments. Mixtures of 5 MVOCs resulted in effects at a lower total concentration than the individual compounds, but the authors conclude that MVOCs are less likely than previously thought to play a large role in building-related symptoms.[65]

Sampling strategies

Questionnaires as monitoring tools. Many studies designed to assess the health effects of indoor exposure to fungi use questionnaires to measure exposure. Questions to indicate fungal exposure include dampness, water damage, and moldy odors. Many of the studies using these questions have revealed relationships between dampness and respiratory disease.[66-69] However, the connection between dampness and fungal concentrations is not well documented, and the actual cause of the relationship has not been elucidated.[70,71] Bias has been reported in these kinds of studies, and recommendations have been made that appropriate objective measures of fungal exposures be developed and used to test questionnaire responses.[51]

Sample analysis choices. Many investigators have noted that some fungi (particularly *Stachybotrys chartarum*) can be abundant on surfaces but rare to absent in air. The conclusions made from these observations have considered this to be a false-negative finding.[72] It is a false-negative finding if the point of the sampling was to determine whether the organism was present in any reservoir. If the point of air sampling was to determine whether the fungus was present in air, then the result might still be false negative if nonculturable spores were present. However, the results could also mean that no spores were present. A method not relying on culturability would have to be used to be sure.[59]

Update on sampling equipment

Personal sampling. Until recently, virtually all studies of pollen and fungal prevalence that were used as exposure measures were based on ambient sampling. Several studies have indicated that outdoor fungal monitoring with spore traps is generally representative of local established fungal populations rather than long-distance transport.[73] Personal exposure measures are especially useful in some situations. Recently, a nasal sampler has been developed that allows the number of pollen grains actually entering the nose of a subject to be quantified.[74] This study documented that exposure can vary significantly from one individual to another and is related to both location and activity. Personal exposure measurements are important to document exposures during specific activities. Lawn cutters, for example, are exposed to higher levels of both pollen and fungal spores than the general population.[75] These results are in accord with reports of high-level fungal exposure during farming activities.[76,77]

Several other personal samplers are now available for bioaerosol sampling. Kenny et al[78,79] have developed a size-fractionating personal sampler and tested it for protection of sensitive microorganisms. The button sampler improves distribution of particles on filter cassette samples, allowing epifluorescence analysis for fungal spores.[80] Fiorina et al[81] used a personal sampler to assess bioaerosol exposures on high-altitude expeditions.

Sampler comparisons. Aizenberg et al[82,83] have done laboratory evaluations of the Air-O-Cell impaction cassette, the Burkard personal volumetric air sampler, and the button aerosol sampler. All were approximately equivalent for particles larger than 2 μm, but only the button sampler (a filter collector) was 100% efficient for smaller particles. The comparative ease of analysis was not studied.

Andersen two-stage, Burkard portable, RCS Plus, and SAS Super 90 samplers were compared by Mehta et al.[84] The devices were more or less equivalent, except for the RCS Plus, which had lower collection efficiency than the other three. Bellin and Schillinger[53] compared the Andersen N6 and the SAS samplers. They performed equivalently for large spores, but the SAS sampler collected only about one third as many *Aspergillus* species, *Penicillium* species, and other small spores as the Andersen sampler.[53]

Update on analytic methods

New data on old analytic methods. The Burkard spore trap is becoming the most commonly used ambient pollen and spore sampler. Recent data confirm a strong correlation between longitudinal traverses of a slide and confirm that analysis of a single longitudinal traverse is an acceptable approach except when counts are low.[74]

A number of studies have compared culture media for use in cultural sampling. Most recent studies have compared malt extract agar and DG-18. No study clearly shows an important difference between these media, although some small, consistent differences in types and numbers of colonies have been noted.[85] Either is acceptable for use in environmental investigations.

New analytic approaches. Many new techniques have been developed to analyze samples for fungal content (Table IV).[52,57,86-105] Although many of these show great promise, it is important to remember that although they are extremely useful for testing specific hypotheses, none are survey instruments that can evaluate the range of bioaerosols present in an environment.

Molecular methods

The development of DNA probes has promise for enabling the identification of specific fungal taxa in samples. Many probes have been developed for the identification of specific fungi and for groups of fungi. A fungal-specific PCR assay using only one primer set has been developed for detecting any indoor fungus.[86] The authors stress the extreme sensitivity of the assay, but it should be noted that there will always be more than this limit (2 spores) in any environmental sample. Williams et al[87] developed a model method for detecting *Penicillium roquefortii* using PCR directly on samples collected in Eppendorff tubes.

An especially important use of PCR methods is to compare fungal isolates from different sources. The use of PCR

TABLE IV. New methods for fungal detection and the type of data that result from their use

Authors	Approach	Data recovered
Day et al, 2002[95]	Flow cytometry	*Phytophthora infestans*
Williams et al, 2001[87]	PCR	*Penicillium roquefortii*
Leenders et al, 1999[88]	PCR	*Aspergillus fumigatus*
Roe et al, 2001[96]	PCR	*Stachybotrys* conidia
Olsson et al, 1998[89]	PCR	*Pneumocystis carinii*
Zhou et al, 2000[86]	PCR	Fungal spores
Cruz-Perez et al, 2001[97]	PCR	*Aspergillus fumigatus*
Razmovski et al, 2000[103]	Immunodetection-microscopy	*Lolium perenne* allergen
Wijnands et al, 2000[98]	Immunodetection	*Alternaria alternata*
Thrane et al, 2001[94]	HPLC, image analyis	*Trichoderma* strains
Dales et al, 1999[104]	Ergosterol	Fungal biomass
Nielsen and Madsen, 2000[57]	Ergosterol	Fungal biomass
Szponar and Larsson, 2000[91]	Ergosterol	Fungal biomass
Charcosset and Chauvet, 2001[99]	Ergosterol	Aquatic hyphomycetes
Nielsen and Madsen, 2000[57]	Ergosterol	Fungal biomass
Wouters et al, 2000[105]	Glucan, EPS (Pen/Asp)	Fungal biomass, *Penicillium* and *Aspergillus* species
Wan and Li, 1999[93]	Glucan	Fungal biomass
Rylander, 1999[92]	Glucan	Fungal biomass
Thorn et al, 2001[100]	Glucan	Fungal biomass
Gehring et al, 2001[52]	Glucan	Fungal biomass
Douwes et al, 1999[90]	EPS (*Penicillium*/*Aspergillus* species)	*Penicillium*/*Aspergillus* species biomass
Elke et al, 1999[101]	MVOC analysis	Active fungal growth
Keshri et al, 2002[102]	MVOC analysis (electronic nose)	Active fungal growth

EPS, Extracellular polysaccharide.

to compare airborne and clinical isolates of *Aspergillus* species has been reported.[88] Another increasingly common clinical fungus, *Pneumocystis carinii*, does not grow in culture, and documentation of its airborne nature has only been achieved recently with PCR techniques.[89]

Immunodetection

Immunodetection is an important approach for detecting specific allergens. For the fungi, the method is only partially successful because of the enormous number of potential allergens that might be in a sample and the variability of allergen production depending on strain, age, substrate, and other factors. Antibodies are commercially available for a number of fungal allergens, but the method has yet to come into widespread use. Immunostaining can be used to visualize allergen-bearing particles on Burkard spore-trap slides. Razmovski et al[103] used *Lolium perenne* antibodies to visualize allergen-containing particles on Burkard spore-trap slides. This method enables one to localize the types of particles on which specific allergens are borne.

Extracellular polysaccharides

Douwes et al[90] have developed an assay for extracellular polysaccharides that is specific for *Aspergillus* and *Penicillium* species as a group. This is an especially promising approach because these fungi are the most common early invaders of wet building materials. However, many other fungi can be important in both indoor and outdoor air, and it would be a mistake to rely only on this assay for building investigations. For epidemiologic studies, the hypothesis (ie, that the group of fungi comprising the genera *Penicillium* and *Aspergillus* is the environmental variable of interest) must be clearly stated.

Fungal biomass methods

Ergosterol in environmental samples is a clear sign of fungal content, and assays for this fungal component have been used for many years to detect fungal growth in food products. However, it should be noted that ergosterol content of cultured fungi is dependent on the type of nutrient medium on which they are growing.[91] This might limit comparison between biomass measures on different types of building materials. Also, one study did not find any relationship between respiratory symptoms and ergosterol measures in spite of the fact that fungal growth reported by questionnaire was predictive of symptoms.[104]

Glucan (1-3 b-d-glucan) forms a part of the cell wall of most fungi. Glucan measurement is used as an indicator of the presence of fungal biomass, as a surrogate for allergen or spore exposure, and as an indicator of exposure to the glucan itself. Data on the relationship between symptoms and glucan are contradictory.[92,93,105]

Chemical methods

HPLC is used to separate complex mixtures of molecules, and the resulting banding patterns are evaluated to identify compounds. Thrane et al[94] used HPLC to analyze culture filtrates from the growth of *Trichoderma* species strains. Using image analysis techniques, they were able to correctly identify all strains for which they included a standard.

Standards-guidelines

The push for some concentration standard for fungal levels in houses continues. Unfortunately, differences in sampling and analytic approaches and the relative incon-

TABLE V. Some measured fungal concentrations in indoor environments

Authors	CFU/m³ of air	CFU/g of dust
McGrath et al, 1999,[36] one building	150-567 (*Penicillium* species)	
Dharmage et al, 1999,[106] Australia, 485 houses	55% >500	
Dales et al, 1997,[51] Canada, 403 houses		255,000 when moldy odors were reported and 155,000 otherwise
Takahashi, 1997,[107] Japan, 288 houses	13-3750	
Li and Kendrick, 1995,[108] Taiwan, 92 houses	411-602	
Verhoeff et al, 1994,[43] The Netherlands, 60 houses		3530-12,880
Martinez Ordaz et al, 2002[109]	930-10,230	
Ren et al, 2001,[37] Northeast United States, 1000 homes	1033.5 (mean)	
Su et al, 2001[34]	4380.9-9672.1 (mean)	

sistency of sampling plans continue to make this an elusive goal. Table V[34,36,37,43,51,106-109] presents ranges of fungal levels in air and dust and exhibits the wide variability in methods and in concentrations across studies with similar methods.

No official standards exist for interpretation of pollen or fungal data. The American Academy of Asthma, Allergy and Immunology publishes guidelines for interpretation of pollen data that are based on national averages for groups of pollen types. Outdoor fungal aerosol concentrations are also listed. Whether these guidelines relate to disease in any way remains unknown.

For indoor air, many groups have proposed guideline levels that range from as low as 50 to 2000 total CFU/m³. Unfortunately, average levels in non–air-conditioned homes during the growing season often exceed 2000 CFU/m³, depending on the outdoor aerosol. None of these proposals consider that different types of fungi have different health effects and that the effects depend on many factors that cannot presently be predicted. Until we have accurate dose-response data for the many different types of fungi that can grow in indoor environments, standards are unlikely to be supportable. Unfortunately, several states have established or are in the process of establishing fungal standards for indoor environments. The federal government is also considering such a standard. Unless such standards and laws are based on common sense, they will do more harm than good. Already, insurance companies are pulling out of some home insurance markets, and where coverage is available, mold damage is often excluded.

Because no dose-response data exist for most fungal and pollen disease agents, interpretation of data must rely on comparison with control environments or with qualitative decisions regarding whether an unusual exposure situation exists.[110] A common-sense approach to moisture control would solve the indoor fungal problem. Moisture standards for houses could be formulated that would essentially prevent fungal growth, except from extraordinary events. Prompt action in response to unusual events would prevent mold-related damage. Finally, rational responses to fungal exposure would allay fears and prevent hysteria.

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