

Clinical Management Review

The Clinical Relevance of Pollen Versus Fungal Spores in Allergic Diseases

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Pollen and fungal spores are associated with seasonal and perennial allergies. However, most scientific literature thus far suggests that pollen allergy is more clinically relevant than fungal allergy. Several environmental and biological factors and the difficulty in producing reliable fungal extracts account for this. Biodiversity, taxonomy, and meteorology are responsible for the types and levels of pollen and fungal spores, their fragments, and the presence of free airborne allergens. Therefore, it is difficult to accurately measure both pollen and fungal allergen exposure. In addition, understanding the enzymatic nature of fungal and some pollen allergens, the presence of allergenic and nonallergenic substances that may modulate the allergic immune response, and allergen cross-reactivity are all necessary to appropriately evaluate both sensitivity and exposure. The raw materials and manufacturing processes used to prepare pollen versus fungal extracts differ, further increasing the complexity to properly determine allergic sensitivity and degrees of exposure. The pollen extracts used for diagnosis and treatment are relatively consistent, and some have been standardized. However, obtaining clinically relevant fungal extracts is more difficult. Doing so will allow for the proper selection of such extracts to more appropriately diagnose and treat both pollen- and fungal-induced allergic diseases. © 2021 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2021;■:■-■)

Key words: Pollen allergy; Fungal allergy; Fungal spores; Airborne pollen and fungal allergens; Pollen allergenic extracts; Fungal allergenic extracts; Pollen allergen cross-reactivity; Fungal allergen cross-reactivity

Exposure to outdoor allergens, mainly pollen and fungal spores, is responsible for seasonal allergies worldwide. However, clinical observations suggest that the prevalence and severity of pollen allergy is greater than that of fungal allergy despite the fact that airborne fungal spores are typically present outdoors in larger

amounts and often during more extended periods. Moreover, unlike pollen, fungi grow indoors, particularly in moisture-impacted buildings, and are reported to affect respiratory health.¹⁻³

A total of 10% to 30% of the world's population are affected by allergic rhinitis,⁴ and approximately 7.6% adults and 8.4% children in the USA suffer from asthma,⁵ particularly problematic during the pollen season. Although the medical relevance of fungal exposure is less understood than pollen, the clinical implications of fungal exposure in causing airway diseases and the associated mechanisms have been documented, both IgE- and non-IgE-mediated.⁶⁻⁸ The prevalence of respiratory allergy due to fungal exposure is 20% to 30% in atopic individuals and around 6% in the general population.^{9,10} Likewise, subjects with severe asthma are often sensitized to multiple pollen or fungal species.^{8,11}

Multiple combined factors likely account for the perception that pollen allergy is more relevant than fungal allergy. These include the following: (a) a higher level of natural complexity of fungi compared with pollen, (b) differential exposure to pollen and fungal allergens, and (c) selective value of the pollen and fungal allergenic extracts available for diagnosis and treatment. The objective of this paper is to discuss the differences between pollen and fungi as they relate to allergic diseases. The paper is divided into 2 sections. The first discusses the environmental aspects associated with this topic, whereas the second focuses on biological and technical factors. Recommendations and future directions are provided.

ENVIRONMENTAL ASPECTS

Pollen is composed of the male reproductive cells of many plants, which is aerosolized into air masses and can be transported long distances. Fungi are eukaryotic organisms with a complex genome and ecological adaptability to the environment. Fungal spores are microscopic units of dispersion that germinate when moisture and nutrients are present and grow to produce filaments that form a mass, named mycelium. Because the conditions that allow fungi to grow are present almost everywhere, fungi are ubiquitous outdoors. Fungi absorb their nutrients by secreting digestive enzymes, many of which are allergenic or antigenic, causing several IgE- and IgG-mediated hypersensitivity diseases, including allergic rhinoconjunctivitis, asthma, bronchopulmonary mycoses, and hypersensitivity pneumonitis.¹²

The environmental aspects of pollen and fungi associated with allergic diseases are complex. These include factors responsible for and problems associated with measuring pollen and fungal spores, the impact of weather, and the effect of their aerodynamic characteristics on human exposure.

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Factors responsible for and problems associated with measuring the level of exposure of particular types of airborne pollen and fungal spores

Several thousand botanical species exist, but only a few hundred studied so far produce pollen associated with allergies. Comparatively, the estimated number of existing fungal species is greater than 1 million. However, only a few are linked to allergies.¹³ Likewise, the natural genetic makeup of fungi and fungal taxonomy likely overestimate fungal biodiversity.¹²

The plants that produce allergenic pollen in different locations are primarily associated with particular climatic zones.¹⁴ In addition, plants pollinate according to specific seasonal patterns. To the contrary, fungi are cosmopolitans and spore production is less clearly defined to a season. However, for example, some basidiomycete species produce spores particularly in the fall.^{15,16} Spore levels arbitrarily considered high can be present year-round in some locations, that is, in the south of the USA.¹⁷

An important environmental aspect about airborne allergen exposure is associated with human activities. Urbanization, increased levels of air pollution, and the introduction of nonnative plants have altered the ecosystem and affect the types and levels of airborne pollen and their allergenicity.¹⁸ Environmental pollution increases the expression of allergens involved in plant defense mechanisms, for example, pathogenesis-related proteins.^{19,20} In addition, increased levels of CO₂ in urban areas increase the Amb a 1 content of short ragweed (*Ambrosia artemisiifolia*) pollen and the number of pollen grains produced compared with rural areas.²¹⁻²⁴

In addition to the effects of CO₂ on pollen, fungi that grow on high levels of CO₂ produce more allergens.²⁵ Urbanization probably has altered airborne fungal spore levels,^{26,27} in particular, land use adjacent to cities previously unused or used for farming and now used for housing and industry.

Aerobiological surveys are performed throughout the world and measure the pollen and spore types and levels present outdoors and are used clinically as a surrogate measure of exposure to their allergens. Although the identification of airborne pollen is possible at the genus level, the classification of airborne fungal spores, even into taxonomic groups, is difficult for many types of small-spored fungi. Likewise, trying to interpret the different pollen and spores as well as their levels as they relate to allergic diseases is difficult.²⁸ First, studies that investigate exposure/response relationships for different species are rare; second, pollen and spore counts do not always correlate with clinical exacerbations; third, individuals differ in their sensitivity to various species; fourth, the counts vary with time and space; and fifth, allergen cross-reactivity considerations are not used to interpret the data.²⁸

Other factors also need to be considered to understand the limitations of using aerobiological data to determine the potential exposure of subjects to outdoor allergens. First, the clinical relevance of many pollen species, and in particular, fungi, is unknown or controversial; second, pollen and fungal allergens are also present in submicronic particles and structures that cannot be measured by microscopy^{29,30}; and third, the allergenic content in pollen grains and fungal spores naturally varies.³¹

Fungal spores are also present indoors, with a taxonomic composition similar to that present outdoors, but at lower levels.³² Spores infiltrate buildings and are transported by building occupants. Building construction and operation, as well

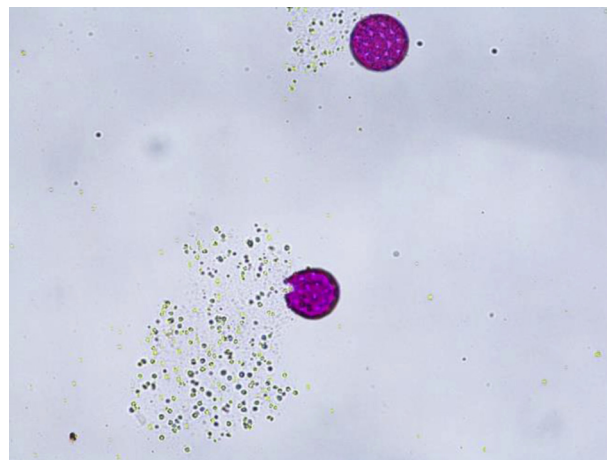


FIGURE 1. *Amaranthus retroflexus* pollen releasing cytoplasmic content as a result of osmotic stress ($\times 400$).

as human activities and habits, are almost exclusively responsible for the presence of indoor fungi; for example, the improper operation of the air conditioning system and the presence of plants and pets contribute to the indoor spore load, which settle from the air and accumulate in house dust.³³ While *Alternaria* spp. and *Cladosporium* spp. are the most clinically relevant fungi outdoors, *Penicillium* spp. and *Aspergillus* spp. contribute to most of the airborne spores present indoors, particularly with high humidity.³⁴ The medical relevance of indoor fungi has been debated for years, but evidence seems to indicate that they can cause respiratory allergies.^{1,35-37}

Because the traditional methods for doing pollen and spore counts are labor intensive and the counts are reported at least 1 day after they are obtained, new technological methods are necessary. More automated pollen and spore counting technology is being developed to provide real-time counts. Although this technology still has many limitations, it is now used in several European countries and Japan.³⁸⁻⁴⁰ It is to be hoped that when better perfected and more affordable, it will be a more efficient and timely method to measure airborne pollen and fungal spores.

Impact of weather on types and levels of pollen and fungal spores

Although the pollination seasons associated with particular plant species can be anticipated to some extent, the environmental parameters that affect pollen release, such as ambient humidity and temperature, fluctuate and are not predictable. Another consideration is that rainfall washes pollen from the air or decreases its concentration; however, it also can release pollen cytoplasmic materials containing allergens due to osmotic stress on the pollen itself⁴¹ (Figure 1). Therefore, airborne pollen allergens and their associated submicronic particles, not measured, are also present outdoors. This phenomenon, which occurs particularly during thunderstorms,^{27,42} is associated with asthma outbreaks during the pollen season.⁴³

Local weather also influences the types and levels of both airborne pollen and fungal spores. For example, rainfall and elevated ambient moisture cause the release of large amounts of ascospores and basidiospores (Figure 2), both linked to the severity of asthma and allergic rhinitis and to asthma

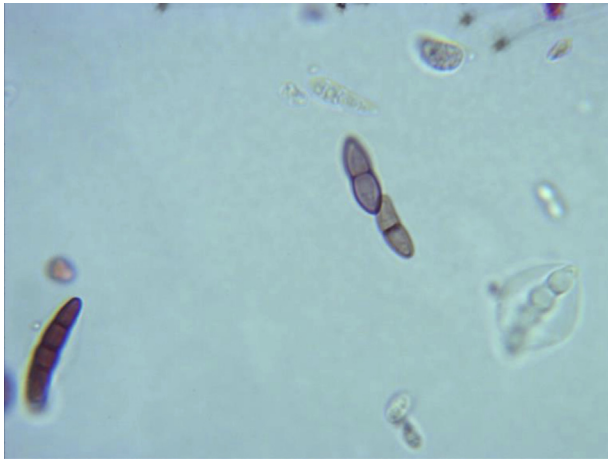


FIGURE 2. Microphotograph illustrating the presence of airborne ascospores and basidiospores during a rainy day ($\times 1000$).

exacerbations.^{44,45} Also, airborne spores of *Alternaria* spp. and *Cladosporium* spp. predominate during dry, windy days (Figure 3) and are associated both with emergency room admissions for asthma and severe asthma.^{44,46,47}

Various combined meteorological parameters, such as changes in barometric pressure, wind speed and direction, temperature, and rainfall, are also responsible for spore plumes, that is, large increases in the levels of airborne spores over few hours. For example, *Alternaria* spp. and *Cladosporium* spp. are associated with this phenomenon.⁴⁸⁻⁵⁰

Immunochemical methods to identify a few pollen and fungal allergens present in nonintact pollen and fungal spores are available,^{51,52} but not widely available. For example, a study that measured the major allergen of olive pollen, Ole e 1, identified a large variation between the levels of this airborne allergen versus olive pollen counts, indicating that counts are not necessarily an accurate assessment of exposure to Ole e 1.⁵³

Effect of the aerodynamic characteristics of pollen and fungal spores on exposure

The size, shape, and density of airborne particles are responsible for their aerodynamic characteristics, the time they remain airborne, and their physical integrity. The most clinically relevant pollen types are typically globoid with an average tridimensional size of 20 to 50 μm . However, the shape and size of fungal spores are much more variable (Figure 4). Typically, large and elongated spores, that is, *Alternaria* spp., are released in small amounts, do not remain airborne for extended periods, and their structures are easily disrupted. This means that fungal fragments can be airborne in the absence of, or in addition to, intact spores.⁵⁴ However, small and round or near-round spores, that is, *Cladosporium* spp, *Aspergillus* spp., and *Penicillium* spp., often are released in clumps or chains. They remain airborne for extended periods and are not easily disrupted. These illustrate 2 different scenarios with potentially different outcomes.

The aerodynamic characteristics of pollen and fungal spores also are partially responsible for the likelihood of reaching the upper or lower airways or being deposited onto the conjunctiva. In addition, the physical and chemical properties of the mucosal



FIGURE 3. Microphotograph illustrating the presence of airborne *Alternaria* spp. and *Cladosporium* spp. spores during a dry, windy day ($\times 400$).



FIGURE 4. Microphotograph of short ragweed (*Ambrosia artemisiifolia*) and *Alternaria* spp. ($\times 400$).

tissues are partially responsible for allergen exposure and presentation to the immune system, variables not often appreciated.

In summary, biodiversity, taxonomic considerations, and meteorological parameters are primarily responsible for the types and levels of pollen and fungal spores and their fragments present outdoors. However, fungi can also grow indoors and potentially impact human health. The technology used to perform aerobiological surveys has not evolved for decades. Automated equipment and newer techniques are needed to more accurately determine exposure and its relevance for allergic diseases. Aerodynamic factors are necessary to consider to properly interpret the data reported in aerobiological surveys. Understanding such data also would help to understand the clinical relevance of the types and levels of particular pollen and fungal spores.

BIOLOGICAL AND TECHNICAL ASPECTS

Some of the biological characteristics of pollen and fungal allergens are unique and important to diagnose and treat the

allergic patient. Another aspect relevant for the clinician is the appropriate selection of pollen and fungal extracts for diagnosis and treatment.

Nature of pollen and fungal allergens

Many fungal and some pollen allergens are enzymatically active and can damage the epithelial barrier and increase allergen uptake, for example, Alt 6 from *Alternaria alternata* and pollen proteases from different species. These include olive tree (*Olea europaea*), grass Group 22, and various allergens from short ragweed (*A. artemisiifolia*). Such enzymatic activity probably plays a role in sensitization.^{55,56}

Both pollen and spores also contain substances other than allergens that may be relevant and/or play a synergistic role in inducing an immune response. For example, allergen-deprived low-molecular-weight fractions from short ragweed (*A. artemisiifolia*) pollen promote the production of specific IgE.⁵⁷ Pollen grains also release lipids, adenosine, and nicotinamide adenine dinucleotide phosphatase hydrogenase, products that may act as immunologic adjuvants.^{58,59}

Like pollen, products other than allergens are produced by fungi, such as B-glucans, ergosterol, extracellular polysaccharides, and mycotoxins, and could similarly influence the immune response.⁶⁰ The potential allergenic or immunologic effects associated with exposure to these products remain controversial, but chitin and B-glucans from *Aspergillus* spp can induce eosinophilic inflammation in mouse lungs.^{61,62} Such exposure could modify the immune response to allergens, their presentation to the immune system, induce Th2 polarization, and recruit neutrophils that augment allergic inflammation.⁶³

A high level of cross-reactivity exists among pollen species, even among phylogenetically distant plants. This cross-reactivity is not limited to pollen but extends to fruits and vegetables due to the presence of pan-allergens like lipid transfer proteins and profilins, which are contained in both.^{64,65} However, cross-reactivity among different fungal groups is less defined, even though many allergic subjects are sensitized to multiple fungal species.⁶⁶ However, highly conserved sets of allergen orthologues, including enolase, heat shock proteins, cyclophilins, proteases, redoxins, and disulfide isomerases, are suspected to be possible fungal pan-allergens.⁶⁷

Understanding the different types of cross-reactivity associated with various pollen and fungi is necessary to appropriately evaluate a patient's sensitivity. Ideally, similar considerations should apply to manufacture efficient allergenic extracts for diagnosis and treatment.

Pollen and fungal allergenic extracts and vaccines available for diagnosis and treatment

The prevalence and degree of sensitization to fungi is less than that for pollen, as determined by *in vivo* and *in vitro* tests, leading to the concept that fungal allergenic extracts and vaccines have less diagnostic and treatment value than those from pollen. Because this hypothesis is probably correct, it is important to understand how pollen and fungal extracts are manufactured.^{68,69}

Allergenic extracts are made from both pollen and fungal raw materials and should contain the allergens relevant to diagnose and treat sensitized individuals. Natural raw materials are a mixture of both allergenic and nonallergenic constituents. A different strategy is to use single or combined purified or

recombinant allergens for diagnosis or treatment purposes. One of the main advantages of molecular-based diagnosis is that it can differentiate true sensitization from cross-reactivity.^{70,71} However, the use of such allergens is still under investigation and is not universally accepted.

The raw materials used by various companies to manufacture pollen and fungal extracts may differ both in origin and botanical or fungal characteristics. The pollen used is a natural product obtained from different plant species, and both genetic and environmental factors, previously described, influence their qualitative and quantitative allergenic composition.⁶⁸ Such factors are much easier to address for pollen versus fungal raw materials.

The materials and procedures used to prepare pollen allergenic extracts by manufacturers, in general, are analogous and reproducible.⁶⁸ To the contrary, multiple factors are responsible for the characteristics and allergenic composition of the fungal raw materials, obtained from cultures. Therefore, the particular fungal strains used, the composition of the culture medium, and growth conditions differ among various companies. This leads to a much more heterogenic qualitative and quantitative composition of the commercially available fungal extracts.⁶⁹ Such differences should be minimized. This is one of the reasons why standardized fungal extracts are not yet available and the extracts provided by different manufacturers cannot be considered equivalent to both diagnose and treat allergic subjects.⁷² More than 200 different fungal allergenic extracts are commercially available in the USA, but around 30% of them have been identified as lacking medical evidence to support their use.⁷³

This concept of differences in quality is illustrated by the relatively "high" protein and allergen concentrations in pollen versus fungal extracts. To date, carbohydrates, which are not usually allergenic, comprise most of the components present in fungal extracts.⁷² These factors are responsible for the general perception that fungal extracts have less diagnostic and treatment value than pollen extracts. This concept is enhanced by the fact that there are so many different commercial extracts available, the importance of many of which is unclear.

In summary, the chemical structure and function of pollen and fungal allergens, together with other naturally occurring substances, should be considered to better understand the immune response to these allergens. Cross-reactivity should also be considered to understand the clinical relevance of individual pollen and fungal allergens. The raw materials and procedures used to prepare pollen extracts are much more reproducible than those used to manufacture fungal extracts. This is reflected by the large number of different fungal allergenic extracts available with selective clinical relevance from different manufacturers.

CONCLUSIONS, RECOMMENDATIONS, AND FUTURE DIRECTIONS

Complex subjects, such as those associated with environmental exposures and their relation to disease, are difficult to understand. There is no clear-cut evidence of how a subject is exposed, to what extent, and under what circumstances. The source of exposure and the mechanisms by which such exposure occurs need to be clarified. Appropriate tools to accurately quantitate such exposure and associate it with the onset of symptoms are necessary.

TABLE I. Comparison of the main characteristics of pollen and fungal spores and the associated allergenic extracts

Characteristic	Pollen	Fungal spores
No. of species	Few thousand	>1 million
Presence	Seasonal	Seasonal and perennial
Size	20-50 μm	1.5-200 μm
Shape	Globoid	Highly variable
Fragmentation	No	Yes
Response to osmotic stress	Rapid	Resistant
Presence indoors	Less common	Common
Allergens	Well defined	Less defined
Cross-reactivity	Well defined	Less defined
Standardized allergenic extracts	A few	None
No. of commercial extracts	Many	Excessive
Consistency of commercial extracts	Yes	No

Pollen allergy is considered more clinically relevant than fungal allergy. Several combined factors account for this, including environmental, biological, and technical aspects. Among the most relevant environmental aspects, biodiversity, taxonomy, meteorology, and aerodynamic factors are primarily responsible for the types and levels of pollen and fungal spores, their fragments, and products present outdoors (Table I). In addition, the technology and analytical methods used to perform aerobiological surveys have not evolved for years. Proper understanding of these parameters, together with technological updates, would help to understand the clinical relevance of the types and levels of particular pollen and fungal spores. Because the presence of fungi indoors also can affect human health, the physician also should evaluate this in clinical practice.

From a practical perspective, the large number of fungal extracts on the market, versus those from pollen, much more characterized and some standardized, help the medical community understand the relevance of pollen versus fungal allergy (Table I). Characterized fungal extracts and their relevance are necessary. Although there are no ideal solutions, a first approximation should focus on obtaining clinically relevant fungal extracts from appropriate species and withdrawing nonrelevant products from the market. This activity likely would allow for the proper classification and selection of individuals and populations to participate in further studies to ascertain the clinical relevance of pollen versus fungi and for the preparation of reagents that could be used to characterize and perhaps standardize fungal extracts. More funding and research is necessary to better understand the complexity of pollen and fungal exposure in various environmental conditions.

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