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Identification of Airborne Microfungal Populations from Home Environments within the Dallas-Fort Worth (Texas) Region

Abstract

Airborne mycofloral components of domestic interiors within the Dallas-Fort Worth metropolitan area were examined to elucidate more fully the fungal species most prevalent within home environments of this region. During a 3-year period (1988-1990), a total of 100 indoor home environments were examined for the presence of fungal organisms. Investigations involved the gravity exposure of either 2.0% malt extract or 1.7% corn meal nutrient agar at various locations within each study home. *Cladosporium*, *Alternaria*, *Penicillium*, *Drechslera*, *Epicoccum* and *Aspergillus* are the most abundant groups of fungal genera from these sites. Additionally, data obtained from indoor studies were compared to cumulative outdoor aerometric data to determine the degree of similarity in frequency between indoor and outdoor fungal genera. The routine examination of airborne organisms from inhabited areas, whether home or office, may provide useful information in the initial treatment process for fungal-related hypersensitivity. Clinical evaluation of patients who react to fungal components identified from within their homes could be performed and may provide diagnostic assessment to determine specific individual hypersensitivity.

Key Words

Airborne fungi
 Indoor air quality
 Hypersensitivity
 Respiratory hypersensitivity

Introduction

The relationship of airborne fungal organisms/spores in the cause of allergic hypersensitivity is well documented and has been extensively reviewed by several investigators [1-5]. Adverse reactions to airborne fungi have been reported as early as 1924 by Storm van Leeuwen et al. [6] and today still represent a tremendous source of confusion to many physicians, as only limited fungal species have been investigated for potential antigenicity [4].

Fungal spores represent one of the most common components of air, in that the atmospheric transport of repro-

ductive spore material is the primary mechanism of geographic distribution in almost all fungal groups. Fungal propagules which reach exceedingly high airborne concentrations represent a potentially severe health hazard for many individuals.

To date, considerable literature exists on the identification of outdoor atmospheric fungal organisms from various geographic regions [7-29]. Studies of atmospheric fungal organisms and other biotic propagules (pollen, algae) provide useful information in determining which species predominate in certain geographic regions. Meyer et al. [21] consider airborne fungal populations within any geo-

graphic region as being classified into three principal groups consisting of universal, geographic and local dominants. Aerobiological investigations involving the identification of such fungal dominants can aid in creating, modifying or eliminating antigenic mixtures used in diagnostic screening [30]. However, too often it is hypothesized that prevalent species encountered from outdoor samples will represent the actual incitant in individual reactions.

Recently, microbial contamination of indoor environments has gained increasing attention as a possible cause of indoor-air-related illness at home or work. Energy conservation measures introduced in the US and many countries during the 1970s have resulted in the construction of many homes and commercial buildings which are effectively sealed from the prevailing outdoor environments creating indoor habitats with severely reduced rates of fresh-air exchange or buildings commonly referred to as tight buildings [31, 32]. Indoor environments such as these allow the accumulation and proliferation of microorganisms (bacteria, fungi) and their metabolites (i.e. endotoxins and mycotoxins) as well as other volatile organic compounds to circulate within the indoor air. Tight building syndrome, sick building syndrome and building-related illness are terms which have been much used in the literature over the past 10 years [32–36]. Tight building and sick building syndromes describe a group of nonspecific symptoms and complaints associated with building occupancy. These symptoms/complaints include headaches, lethargy, upper respiratory symptoms, itching eyes, congestion, nervousness, dermatitis and dizziness. Building-related illness typically describes clinically defined illness, such as legionnaire's disease or hypersensitive pneumonitis.

Subsequently, numerous studies have concentrated on the characterization and identification of microbial populations from indoor air of both domestic and commercial interiors [31, 37–61]. In addition, several investigators have recently examined the relationship of indoor humidity/dampness and mold growth relative to respiratory hypersensitivity [62–68]. Since most individuals spend 50–90% of their time indoors, the assessment of microbial populations and other potential pollutants from indoor environments may identify causative agents involved in specific occupant complaints and thus answer the causal factors responsible for an individual's hypersensitivity [60, 69, 70].

The diagnosis and effective treatment of an individual's hypersensitivity to airborne fungal agents is typically complex and involves numerous variables [30]. Frequently, physicians perform only narrowly defined diagnostic

procedures by testing individuals only for well-known fungal species. Many physicians may incorrectly assume that the available commercial fungal antigens will represent the prevailing regional mycoflora and, hence, also the local indoor flora. Thus, the examination of indoor air quality, we believe, is of additional importance in the effective diagnosis and treatment of fungal-related inhalant hypersensitivities.

This study seeks to qualitatively determine the micro-fungal populations associated with indoor domestic habitats of mold-sensitive individuals within the Dallas-Fort Worth metropolitan complex. In addition, results of this investigation were compared to regional cumulative outdoor data to determine if findings of indoor mycoflora were consistent with reports of outdoor fungal genera.

Materials and Methods

A total of 100 independent homes were examined for the presence of micro-fungal populations. Isolation of indoor airborne fungi involved exposure of 3–5 polystyrene petri culture plates 100 × 15 mm containing sterilized 2% malt extract agar or 1.7% cornmeal suspension agar (Difco Corporation, Detroit, Mich., USA). Both types of agar media are recommended and routinely utilized for the isolation and cultivation of yeast, phytopathological and saprophytic fungal species [71]. The number of culture plates exposed per individual home was determined by the size of the home and the specific indoor area to be investigated. Most investigations were conducted using a total of 5 culture plates. All culture plates were individually sealed with parafilm and collectively wrapped in aluminum foil to ensure sterility during transport to testing areas. Culture plates were exposed for up to 50-min periods in random areas of the home or in areas known to induce hypersensitive reactions. Petri plates were resealed upon the completion of exposure, as previously described, to avoid contamination upon return to the laboratory. Exposed cultures were incubated at 22–25 °C to promote fungal growth. Cultures were examined for the presence of fungal growth 5 days after initial exposure and sporulating fungi microscopically identified. Further examination was continued over a 4-week period to allow the identification of the slower-growing/sporulating species.

Aerosampling for outdoor atmospheric fungal species was performed using the Aeroallergen Rotorod particulate sampler (Model 85; Sampling Technologies Inc., Los Altos Hills, Calif., USA). The Aeroallergen Rotorod sampler is a nonselective volumetric rotating air impactor capable of obtaining semiquantitative airborne particulate data [72]. Sampling was conducted 24 h a day, 7 days a week and involved direct quantification of airborne fungal propagules retained by the rotorods per cubic meter. The collector head and I-rod assembly were transported to the laboratory, and the I-rods examined microscopically using a compound light microscope at a magnification of × 430. The examination was performed by scanning a partial area along the length of the leading edge of the I-rod. Particles were enumerated within a defined area of the I-rod, identified if possible to the genera level and extrapolated to give the mean number of total fungal spores/hyphae retained per cubic meter over the 24-hour sam-

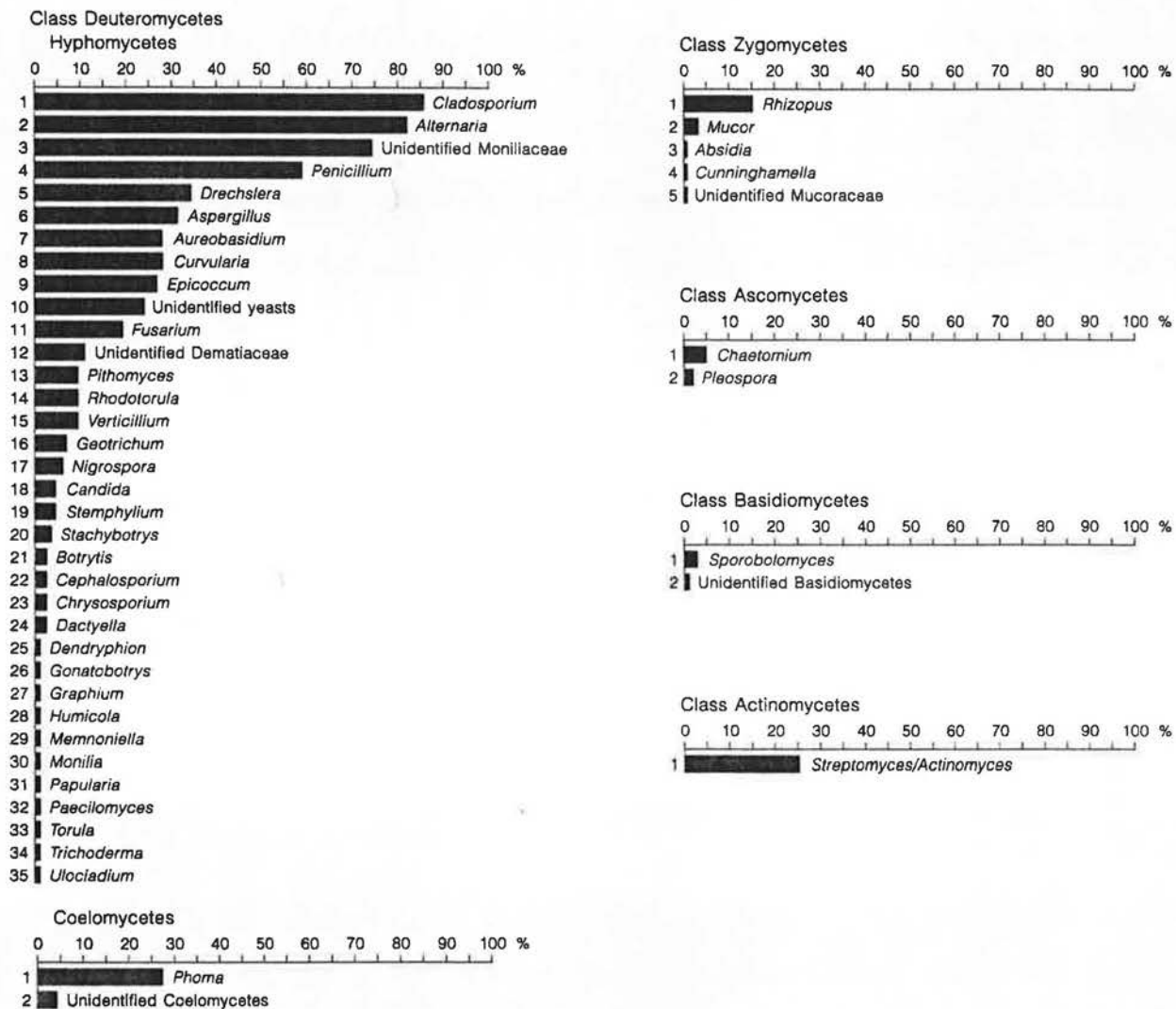


Fig. 1. Percentage frequency of occurrence of fungal taxa identified from gravity-exposed culture plates from indoor home environments.

pling period [30]. Sampling was conducted at a single site within the northern city limits of Dallas. Though not definitive for mycoflora citywide, the sampling is considered an impartial representation of the prevalent fungal components for this North Texas region [30].

Results

Composition of fungal organisms identified from exposed gravity cultures comprised the total fungal genera and encompassed four separate classes of fungi: Deuteromycetes, Zygomycetes, Ascomycetes and Basidiomycetes. The relative abundance in terms of percentage frequency for fungal organisms isolated during this investigation is

summarized in figure 1, denoting percentage occurrence values for individual fungal taxa. Additionally, aerometric data listing fungal genera retained and identified from rotorod impactor samples during outdoor atmospheric investigations are summarized in figure 2, indicating percentage occurrence values for fungal taxa collected from 1988 to 1990.

Prevalent fungal taxa observed from indoor environments throughout the sampling period comprise common, ubiquitous species, particularly members of the Deuteromycetes. A total of 34 genera, representing 80% of the total number of fungal organisms isolated and identified during the investigation, were Deuteromycetes. In

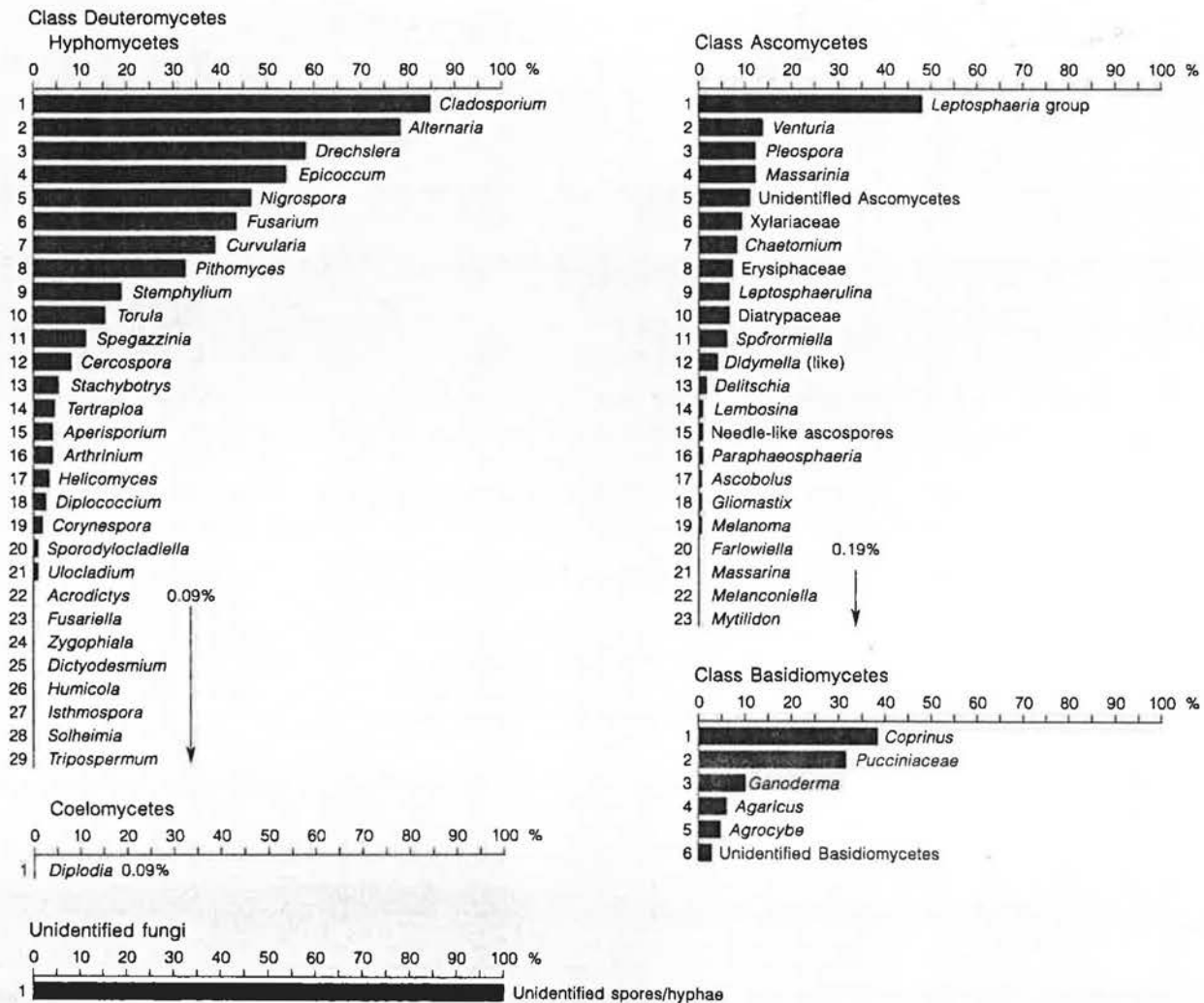


Fig. 2. Percentage frequency of occurrence of fungal taxa identified from rotorod particulate samplers from 1988 to 1990.

addition, a large proportion of unidentifiable sterile fungal colonies and unicellular yeasts were observed from the samples. All were taxonomically categorized within the Deuteromycetes due to the lack of teleomorphic expression in culture. Sterile fungal colonies were further subdivided into either unidentifiable Moniliaceae or Dematiaceae based on the specific mycelial pigmentation in culture. *Cladosporium* and *Alternaria* comprised the most frequently encountered genera, occurring 86 and 82%, respectively (fig. 1). Other species observed include *Penicillium*, *Drechslera*, *Aspergillus*, *Aureobasidium*, *Curvularia*, *Epicoccum* and *Fusarium* ranging from 59 to 19%, respectively (fig. 1). Twenty-three additional genera with frequencies less than 9% were also encountered.

Zygomycete fungi followed representing 10.8% of the total fungi identified. Species of *Rhizopus* and *Mucor* were the most abundant, with minor representation of *Absidia*, *Cunninghamella* and a single unidentifiable isolate with coenocytic hyphae. Ascomycetes and Basidiomycetes constitute the least-observed fungal organisms, both representing 4.6% of the total. *Chaetomium* and *Pleospora* were the only two species of Ascomycetes observed. *Sporobolomyces* represented the most frequent species of Basidiomycetes with only the single occurrence of an additional unidentifiable isolate producing hyphal clamp connections.

Fungi retained and identified from outdoor aerometric spore impactors comprise the three principal groups Deu-

teromycetes, Ascomycetes and Basidiomycetes (fig. 2). The Deuteromycetes were the predominant mycoflora observed during the 1988–1990 sampling period, comprising 49% (29 species) of the total fungi identified from rotorods. Species of *Cladosporium*, *Alternaria*, *Drechslera*, *Epicoccum*, *Nigrospora*, *Fusarium*, *Curvularia* and *Pithomyces* were most abundant with percentage frequencies ranging from 85 to 37%. Other minor species encountered include an additional 21 genera with frequencies ranging from 19 to 0.09%.

Ascomycetes constitute 39% (23 species) of the total fungi identified outdoors. The *Leptosphaeria* 'group' of species were the most prevalent Ascomycetes encountered in the rotorod samples. Fungi comprising this group include species of *Leptosphaeria*, *Phaeosphaeria* and *Keissleriella*, *Lophiostoma*, *Arenariomyces* and *Massariosphaeria*. Other less frequently encountered genera include species of *Venturia*, *Pleospora*, *Massarina*, *Chaetomium*, *Leptosphaerulina*, *Sporormiella* and members of the Xylariaceae, Erysiphaceae and the Diatrypaceae. Minor genera observed during sampling periods include an additional 10 genera with frequencies < 1%. Basidiomycetes genera accounted for 10% of the total fungi identified from air samples, with *Coprinus* and rust fungi (Pucciniaceae) the most frequently encountered.

A portion of fungal material retained and observed from rotorods remained unidentifiable due to the lack of morphological characteristics for definitive identification. Questionable spore material observed from rotorods during the sampling period was divided into four categories: ascospores, basidiospores, spherical phaeosporae and unidentifiable 'other'. Unidentifiable Ascomycete spores detected from rotorods included primarily hyaline didymosporous and slightly pigmented needle-like ascospores resembling those of immature *Venturia*, *Didymella* and *Ophiobolus* (*Leptosphaeria*). In addition, spores were occasionally observed which clearly resembled ascospores, many with the production of a mucilaginous sheath about the spore. Unidentifiable Basidiomycete species were grouped based on the existence of a distinct spore apiculus. The unidentifiable group designated as spherical phaeosporae comprises frequently encountered spore assemblage that encompasses fungal organisms which produce characteristic spherically dark-pigmented spores. Airborne spores grouped into this category ranged from 1 to 7 μm in spore size and may possibly contain several fungal groups, including: Zygomycetes (*Mucor*, *Rhizopus*), species of *Aspergillus*/*Penicillium*, smuts, Myxomycetes species or various other Deuteromycetes species. The exact identity of these spores, however, can-

not be determined, as spores retained on rotorods are morphologically similar and no sporulating reproductive structure is available for accurate generic identification. The unidentifiable group designated 'other' constitutes both unknown spore types and hyphal fragment material. Hyphal material was frequently encountered from rotorod samples and was clearly unidentifiable. Actual fungal spores placed into this category were either physically damaged due to spore impaction or desiccation, obscured by debris or lacked specific morphological information.

Discussion

The predominant representation of Deuteromycetes fungal species from air samples during this investigation corresponds with previously conducted indoor air studies [38–40, 42, 48, 49, 51, 53, 56, 58–60] and outdoor atmospheric investigations [7–10, 14, 16, 19, 24, 26, 28, 29]. The predominant genera observed throughout this investigation represent well-known soil-inhabiting species, which are active participants in the decomposition of plant and animal debris [73]. With adequate moisture and nutrient conditions, these species can proliferate rapidly by producing a large number of spores which disseminate by atmospheric or substrate disturbance.

The principal genera observed from indoor samples comprise species of *Cladosporium*, *Alternaria*, *Penicillium*, *Drechslera* (i.e. *Drechslera*, *Bipolaris*, *Exerohilium* sp.) and *Aspergillus*. These genera represent well-known airborne fungal organisms which are most often the predominantly encountered mycoflora in aerobiological samples. *Cladosporium* and *Alternaria* comprise two of the most thoroughly investigated species which have received considerable attention with respect to fungal-related hypersensitivity. The frequent occurrence of both *Aspergillus* and *Penicillium* species from gravity exposures during this investigation is not consistent with Solomon [74] and Burge and Solomon [75] who indicate that 'smaller' conidial-producing species are less likely to be isolated and/or totally missed by gravity collection devices versus semi-quantitative volumetric samplers. The frequent isolation of *Aspergillus* (33%) and *Penicillium* (58%) from simple gravity collection during this investigation indicates that these smaller conidial fungal organisms may occur in airborne concentrations great enough to be successfully recovered within the homes investigated.

Compiled outdoor atmospheric mycoflora data demonstrate many similarities to indoor fungal data (fig. 2). Genera observed during indoor and outdoor investiga-

tions comprise many of the same predominant species, with *Cladosporium* and *Alternaria* species almost equally represented. However, the identification of less common fungal genera from indoor habitats suggests the presence of fungal agents not commonly observed by rotorod sampling during outdoor atmospheric monitoring. The sporadic occurrence of such fungal organisms from indoor air samples increases the scope of potential causal factors regarding individual hypersensitivities expressed among patients within a home environment. These infrequently encountered fungal species may have remained undetected if indoor air sampling had not been performed and thus not incorporated into possible diagnostic screening/testing.

Ascomycetes and Basidiomycetes fungi were more frequently identified from outdoor samples than indoor samples, as one would expect. Fungi encompassing these groups are most often involved with the decomposition of woody cellulosic debris in the soil. In addition, many are found in mycorrhizal associations with higher vascular plants (i.e. Basidiomycetes) or as plant pathogens. Subsequently, both fungal groups have varying nutrient requirements for somatic growth and sporulation, which could limit the ability of many species to proliferate within the indoor environment and thus their identification in culture. The majority of Ascomycetes genera recorded from outdoor air samples represent the Loculoascomycetes, with *Leptosphaeria*-like genera being the predominant organisms observed during the sampling period. Many of the taxa reported exhibit varying existences and habitats, ranging from lichenized species to holobiotrophic, hemibiotrophic or saprophytic species [76]. In addition, many are restricted to particular host plants or specific groups of plants [76]. The presence of many Ascomycetes was observed during and immediately after rainfall. During periods of rainfall, Ascomycetes genera, including *Leptosphaeria*, *Venturia* and members of the Diatrypaceae, predominated over the more common Deuteromycetes flora. The occurrence of such species within air samples remains a subject of continual interest, as the increased abundance of these organisms during and just after significant precipitation may answer clinical questions concerning individuals who experience asthmatic respiratory symptoms during rain/thunderstorms [77].

Basidiomycetes mycoflora from outdoor samples represent the Uredinales and Agricales. *Coprinus* was the predominant genus observed during sampling periods. Basidiospores of rust fungi (Uredinales) were primarily represented by uredospores of yellow and brown rust. Uredospore morphology resembled that of the genus *Puccinia*. Several other investigators have reported the occur-

rence of significant basidiospore concentrations in atmospheric samples [14, 26, 78]. Subsequently, many researchers have recently concentrated on the antigenicity of airborne Basidiomycetes propagules [79-81].

It is of basic clinical interest to understand whether mold-sensitive individuals react to one or several fungal species and, more importantly, whether such species are common to both outdoor and indoor environments of the patient being treated [30]. Answers to such key questions concerning outdoor and indoor mycoflora are important for proper diagnosis and treatment [30]. Analysis of indoor air may complement outdoor aerobiological investigations and thus define more clearly the possible incitants of hypersensitive reactions. However, culture plate analysis for atmospheric fungi cannot be considered truly definitive. Gravity samples are only a spot check for indoor flora and do not provide semiquantitative data. In addition, any nutrient culture media used to determine airborne fungal populations will clearly discriminate against species which do not readily germinate and grow under those laboratory conditions. Some fungal organisms may germinate and grow in culture, but fail to sporulate and thus remain unidentifiable (sterile), giving little diagnostic information concerning the identity of the fungal contaminants. The high percentage of unidentifiable (sterile) fungal colonies reported in this investigation may reflect such nutrient-requiring species, possibly Ascomycetes or Basidiomycetes contaminants. Such fungal taxa continue to represent a tremendous source of confusion in aeromycological investigations. Previous researchers have investigated various culture media for the collection and examination of airborne fungal organisms and suggest the use of media which restrict overall vegetative growth while still supporting the formation of reproductive structures [82-85]. However, any media used for the isolation of airborne fungi will provide a competitive nutrient advantage for certain fungal species.

Volumetric semiquantitative studies of indoor fungal organisms using the Andersen sampler, Burkard spore trap and other such samplers are advantageous in the determination of atmospheric mycoflora from indoor habitats. Such equipment is routinely used and has been extensively studied by numerous investigators in determining the quality of indoor air. However, such instrumentation is expensive and laborious for the general routine screening of patients' homes, as personnel are also required for transporting and operating the equipment. In addition, like gravity plate exposure, nutrient culture plate analysis of atmospheric fungi using the Andersen sampler clearly discriminates against those fungal genera

which do not readily germinate and grow under laboratory culture conditions. The Burkard sampler, often used in conjunction with the Andersen sampler, provides a further understanding of the airborne fungal component within a given environment, as airborne propagules are collected without discrimination, including nonculturable and nonviable fungal species. However, both such samplers are only spot checks for atmospheric fungal populations and give no information concerning long-term diurnal spore content.

The environment (both macrohabitat and microhabitat) will no doubt ultimately influence the fungal populations within a given area. All biotic and abiotic components of the particular environment, whether indoor or outdoor, will potentially influence fungal organisms in a positive or negative way, by causing proliferation, sporulation and subsequent distribution of fungal propagules or by limiting fungal growth [30]. The condition of the indoor environment in terms of temperature, humidity,

utilizable substrates and adequate ventilation will ultimately determine the presence of fungal populations within the environment and the severity of proliferation. Patients complaining of mold-related problems within their homes may well have fungal spore/propagule concentrations high enough to be successfully recovered and identified by gravity plate exposure alone and thus yield useful information. Gravity culture plate exposure, although limited, is a convenient and economical procedure for identifying such fungal contaminants. Such simple investigations may lead to a greater understanding of the fungal populations to which patients are exposed and thus further facilitate diagnosis and treatment. Thus, we believe that the examination of indoor dwellings for contaminating fungal organisms complements outdoor aerobiological investigations; it therefore should not be overlooked in the routine clinical evaluation and maintenance of patients within the region [30].

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