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Aeromycology: studies of fungi in aeroplankton

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ABSTRACT

Air is a natural environment for spores of many genera and species of fungi. Despite its small size and a significant dispersion they have a great impact on human health and different areas of our activities, such as agricultural production. The study on spores of fungi that belong to aeroplankton or bioaerosole is called aeromycology. The most frequent fungi present in the air are *Cladosporium* and *Alternaria* species. Their numbers are abundant regardless of latitude and height above the sea level and above the ground. They mostly originate from agricultural environment. Other frequently listed species of fungi, whose spores are present in the air include of *Aspergillus, Penicillium, Fusarium, Sclerotinia* and *Ganoderma*. The concentration of spores in the air strongly depends on the abundance of their formation during the studied period. This in turn relates to geobotanical region, vegetation, degree of urbanization, climatic conditions, season, current weather, wind force and direction, local microclimate, and many other factors. Changes in humidity affect the concentration of different types of fungal spores. In general they are divided to 'dry' (*Alternaria, Cladosporium, Puccinia, Ustilago, Melampsora, Epicoccum, Drechslera*) and 'wet' (*Didymella, Fusarium, Ganoderma, Gliocladium, Leptosphaeria, Verticillium*). Study of the composition of species and genera are being done using different types of spore samplers, mostly volumetric instruments. Visual identification is based on colony morphology of the fungus and the shape and size of spores. The identification at the species level is possible with molecular tools. Methods based on DNA/RNA amplification are very sensitive and accurate. They allow the identification below the species level, e.g. chemotypes, mating types or isolates with genes or alleles of interest. Aerobiological monitoring is widely used in the epidemiology of human diseases (inhalant allergies) and infections of arable crops (decision support systems for the protection of cultivated plants). Aeromycology is interconnected with such diverse areas as industrial aerobiology, bioterrorism, ecology, climatology or even speleology and cultural heritage.

KEY WORDS: aerobiology, aeromycology, aeroplankton, *Alternaria, Cladosporium*

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Introduction

Air is a natural habitat of numerous fungi, mostly existing there in the form of spores, their clusters, or – on contrast – spore and mycelium fragments. Spores of some fungal genera are very frequent in air samples, often outnumbering pollen grains, on which most of research have focused for many years. Small size and great difficulties to identify the species based on single spores led to great underestimation of their presence in the atmosphere. Recent fast development of aeromycology, supported with molecular tools shows the importance of fungi in aeroplankton. Fungal spores and mycelium fragments passively float in the air and they are often dispersed by air currents (Southworth 1974). The distance of transport greatly depends on the spore size and shape, the height, thickness and location of the air layer, its physical properties such as temperature, as well as air movements. Most of fungal spores are transported in between local habitats; however, there are strong scientific evidences on spores being transported within and between geographic regions or even continents. This phenomenon is called a long-distance dispersal and it is regarded as an important survival strategy for many organisms. Moving of spores with the air currents allows fungi infect new plants of the same host, find new hosts, reach and colonize new regions or move in between summer and winter habitats (Brown & Hovmøller 2002). Modern agriculture, with greatly reduced plant biodiversity and huge monocultures increases the risk of global spread of some plant pathogens. Limited genetic variability of modern plant cultivars and their distribution in different parts of the world make it easy to establish infections by fungal spores blown to new territories. Major pathogens of crop plants such as rice, wheat, soybean, oilseed rape, potato as well as coffee or banana are similar in different parts of the globe, in spite of geographical or political barriers, resulting in no seed or seedling exchange. These obstacles can be easily overcome by spore invasions with the air currents.

Methods used in aerobiology

Fungal spores present in the air are subjected to many studies with the use of spore traps, that may be passive, when spores drop down on certain surfaces or gather in containers, due to their gravity (Koch sedimentation method) or active, when the collection of spores is connected with swirling or sucking, that allows capturing of higher spore loads (Fleischer *et al.* 2006). The most popular and advanced methods of spore trapping use volumetric samplers. They allow to re-calculate the number of observed spores to a given air unit, mostly 1 cubic meter of the air. The first volumetric sampler was used by Pierre Miguel in Parc Montsouris in Paris in 1883. It was an air pump sucking 20 liters of the air via an orifice, with the glass slide covered with glycerin. This equipment allowed the first calculations of fungal spores in the air with high precision; hence this researcher is often regarded as the first professional aerobiologist, and one of the main fathers of aerobiology and aeromycology (Comtois 1997). Since then, the development of volumetric methods has been greatly pushed forward, especially by English researchers Philip Gregory and John Hirst (Hirst 1991). Currently used volumetric spore samplers, such as

Burkard or Lanzoni apparatus, are based on models designed by Gregory and Hirst. The suction power is standardized, what allows the comparison of results obtained by different research teams worldwide.

There are also other types of apparatus, e.g. the MicroBio or Air Ideal samplers as well as the Andersen impactor – the facilities that combine volumetric methods with the culture on media. Both of them allow growing fungal spores into colonies on microbiological substrates. Moreover, the Andersen impactor allows sieving the spores into groups of different sizes. The recognition of fungi present in the air are then based on traditional visual assessments (Kasprzyk & Worek 2006, Grinn-Gofroń & Strzelczak 2011, Stępalska *et al.* 2012, Pusz *et al.* 2013), as well as on molecular tools (Kaczmarek *et al.* 2009, Karolewski *et al.* 2012, Piliponyte-Dzikiene *et al*. 2014).

Identification of fungal spores in air with molecular tools

The recognition of fungal spores based on spore shape and size is possible for such frequent 'air-flyers' as *Botrytis, Chaetomium, Coprinus, Didymella, Entomophthora, Epicoccum, Erysiphe, Ganoderma, Nigrospora, Pithomyces, Polythrincium, Stemphylium, Torula, Ustilago* and some others (Kasprzyk 2008). However, numerous spores of different species or even different fungal genera can be mixed up in studies based on microscope analysis only. Possibilities created by impactors allowing to subculture these fungi partly solve this problem, as they allow identifying fungal genera, species and variants below the species level, but they demand much time and laboratory space. Moreover, great parts of fungal spores present in the air are produced by the biotrophs, which are unculturable on conventional microbiological media. The broadcasting of spores by wind is crucial for all these species as their survival relies on quick finding the relevant host. Their detection and proper identification is possible due to molecular methods.

PCR-based methods allow precise spore identification s of many fungal species, including the most dangerous plant pathogens (West *et al.* 2008a). Moreover, recent introduction of Real-time PCR is designed to quantify the numbers of spores or gene copies (Kaczmarek *et al*. 2009, Karolewski *et al*. 2012). Molecular tools allow proper identification of fungal species with similar or identical spores and study the genetic changes in fungal populations, such as fungicide resistance (Fraaije *et al.* 2005) or AVR gene alleles (Kaczmarek *et al.* 2014b). Molecular methods allow simultaneous studies of several fungal species, allergens or genetic changes. The greatest hope is currently connected with isothermal methods, such as LAMP, allowing a simplified DNA isolation and greatly reducing the possibilities of reaction inhibition (Jędryczka *et al.* 2013). Molecular methods are sometimes criticized for no distinguishing the difference between viable or dead spores, as they both contain DNA. This may be solved by RNA-based studies, as this nucleic acid is produced by living cells only and it is rapidly broken down in dead tissues, unlike DNA. Due to difficulties with RNA stability and extraction, current RNA-based studies are confined to RNA viruses and human pathogens, rather than to plant pathogens (Schmale *et al*. 2005).

Most frequent fungal spores in air

Although a lot of scientific literature in aeromycology is devoted to fungal plant pathogens, the majority of spores belong to species that do not infect plants directly, but grow saprophytically on their surfaces. Skjøth *et al.* (2012) proved that at the time of crop harvest local load of airborne spores greatly increases and it is an agricultural area, which substantially contributes to the sudden raise of fungal spores captured by samplers located in cities. Major part of air spora is composed of *Alternaria* spp. and *Cladosporium* spp., and in general – these two genera constitute the greatest part of each spore lot in the air, at almost all geographical latitudes (Kasprzyk 2008) and heights (Pusz *et al*. 2013). Other frequently encountered spores belong to *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. Due to great similarities in their spore size and shape it is not possible to identify them based on morphological characteristics. To properly identify these spores DNA- or RNA-based detection methods are required, alternatively, the use of suction trap which impacts the spores on microbiological media.

Air contains spores of different modes of appearance. Telio- and urediniospores of rusts (e.g. *Puccinia*, *Ustilago* or *Melampsora* spp.), as well as *Alternaria*, *Cladosporium*, *Epicoccum* or *Drechslera* are present in the air of low humidity, and therefore they are called 'dry spores', whereas *Didymella*, *Fusarium*, *Ganoderma*, *Gliocladium*, *Leptosphaeria* or *Verticillium* fungi release spores after rainfall events, that puts them to the group of fungi producing 'wet spores'. In general, numerous ascospores of plant infecting fungi belong to 'wet spores', that directly relates to their biology. High humidity is a prerequisite for the successful infection of a host-plant. This is why natural mechanisms evolved fruiting bodies with layers reacting to the presence of atmospheric water that directly causes osmotic changes and allows ascospore release to air currents.

Fungal spores of different taxa are subjected to specific diurnal and seasonal cycles, which greatly depend on climate, weather conditions, circadian clock (changes of day and night) as well as the availability of fresh substrates, necessary for fungus development (Calderon *et al*. 1995). In moderate climate most of spores occur in summer or early autumn (Kasprzyk & Worek 2006), whereas in tropical areas they are more abundant in cold seasons of the year (Hasnain 1993). Microclimate greatly influences the release and appearance of spores, what relates not only to local rainfalls or the effect of ponds, lakes and water reservoirs, but it may also relate to temperature. The delayed presence of airborne spores in the season caused by lower temperatures and snow cover were found in the mountainous regions (Pusz *et al*. 2013). On contrast, in 'urban heat islands' plants usually produce more pollen and their seasonal occurrence starts earlier and lasts longer (Calderon *et al.* 1997), what partially applies also to the abundance of fungal spores. Microclimate and local turbulences make it difficult to accurately predict the occurrence of spores. The studies are easier when airborne fungal spores are transported by big air currents, which can be tracked by back-trajectories (Sadyś *et al*. 2014).

Disease forecasting systems

Forecasting of spore release, based on biology of a particular fungal taxon and the weather finds direct use in plant and human protection against infectious diseases. The systems check if a regional or local weather favored the production and release of pathogen inoculum and the infection of the host (West *et al*. 2008b). Disease forecasting systems used in modern agriculture are usually based either on mathematical models, which were built up following the experiments on pathogen biology, or they refer to stages of development of plant pathogens, controlled at time points that are crucial for plant epidemics. The effective plant control is currently a compulsory policy of the European Union (Kaczmarek *et al.* 2014a). The challenge of plant disease control is a key component of increasing food production and food security (West 2014). It was proven that the use of decision support systems brings economic rewards to farmers and better protects the environment.

Poland runs the System for Forecasting Disease Epidemics SPEC (Jędryczka *et al.* 2012) – the third world's biggest system based on aerobiological methods. The system has been constantly operating since 2004 (Jędryczka *et al*. 2006). Similar attempts have been recently undertaken in the Czech Republic (Jędryczka *et al*. 2010). The studies concentrate on *Leptosphaeria maculans* and *L. biglobosa* – the pathogens infective to oilseed rape (*Brassica napus*), which belongs to one of top cash crops. Air samples captured with volumetric spore traps are quantified with light microscope and molecular techniques (Kaczmarek *et al*. 2009, 2014b). Airborne spores that form inoculum responsible for disease epidemics allow predicting the risk of yield losses as well as its quality. Timely applications of fungicides are beneficial to agriculture. High precision of plant protection treatments, based on aerobiological data, allows combating the pathogen with the highest efficiency.

Outdoor and indoor air spora allergenic to humans

High concentration of airborne spores may lead not only to plant diseases, but often cause skin, eye or nasal irritation and diseases of human respiratory systems, resulting in shortness of breath, alveolitis and asthma. Rapiejko *et al*. (2004) elaborated threshold values for the spores of *Alternaria* and *Cladosporium* spp. necessary to evoke allergic symptoms. In case of the other fungi such values have not been published. It is also clear that these numerical values are only indicative figures, and the allergic reaction greatly depends on patients' age, health condition and numerous individual characteristics. Modern traps enable the collection of bioaerosols with allergenic proteins that can be detected with ELISA tests or other molecular diagnostic methods. Initially the detection concerned *Penicillium roqueforti*, the fungus responsible for food spoilage and serious diseases of cheese workers (Campbell *et al.* 1983). The symptoms ranged from cough, dyspnea and malaise to reduced lung volumes and hypoxemia. The studies revealed that serum and lavage fluids of the patient contained antibodies to *P. roqueforti*. This is one of numerous cases allowing understanding a serious influence of airborne fungi on human health, which started a series of studies on indoor air spora in numerous countries, including Poland (Lipiec 1997, Górny & Dutkiewicz 2002, Lipiec &

Samoliński 2002, Karwowska 2003, Filipiak *et al.* 2004, Stryjakowska-Sekulska *et al.* 2007, Dumała & Dudzińska 2013). Since then, the whole selection of different apparatus and methods has been elaborated to monitor fungal spores and allergens in the air, ranging from static samplers to minute, portable instruments. Most studies were done in public buildings such as universities, schools and hospitals. The monitoring revealed that the concentration of microorganisms only rarely exceeded its recommended limits. However, some of fungal species present in the indoor air of studied rooms could negatively affect human health.

The compositions of fungal air spora in man-made places greatly differed, even at sites located in seemingly similar conditions. Speleomycological research done in unfinished Nazi military complex "Riese" located in underground complex Osówka in the Sowie Mountains showed that *Cladosporium* spp. were most frequently isolated both from internal and external atmosphere (Pusz *et al*. 2014b). However, in shafts of copper mine located in Lubiń mining site (property of KGHM Polska Miedź SA) the most numerous fungi belonged to *Penicillium* spp. and *Aspergillus* spp., with *P. notatum* and *P. urticae* found as best adapted to grow in these specific conditions (Pusz *et al.* 2014a). Significant differences between the composition and size of fungal species between the shafts and sample collection sites supported the hypothesis on substantial influence of microclimate on fungal air spora. Fortunately, in this particular case the concentration of fungal spores also did not present a health risk to the mine workers.

Concluding remarks

The potential of fungal spores in the air is in contrast to their 'invisibility' caused by vast dispersion and small size of particular spores. However the impact on humans and their economy may be enormous, with great influence on such crucial branches as agriculture or human safety. Based on a review on primary biological aerosol particles in the atmosphere, written by Després et al. (2012), it is clear that aeromycology is connected to numerous basic and applied sciences, such as allergology, bioclimatology, biological pollution, biological warfare and terrorism, mycology, biodiversity studies, ecology, plant pathology, microbiology, indoor air quality, industrial aerobiology, speleology, cultural heritage and many other disciplines.

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Streszczenie

Powietrze jest naturalnym środowiskiem dla zarodników licznych rodzajów i gatunków grzybów. Pomimo niewielkich rozmiarów i znacznego rozproszenia mają one wielki wpływ na zdrowie ludzi i różne kierunki ich działalności, w tym w szczególności na produkcję rolniczą. Badania nad zarodnikami grzybów stanowiącymi część aeroplanktonu są przedmiotem aeromykologii. Niezależnie od szerokości geograficznej i wysokości nad poziomem morza w powietrzu szczególnie często występują grzyby z rodzajów *Cladosporium* i *Alternaria*, a ich źródłem jest najczęściej środowisko rolnicze. Innymi często notowanymi rodzajami grzybów, których zarodniki występują w powietrzu są m.in. *Aspergillus*, *Penicillium*, *Fusarium*, *Sclerotinia* i *Ganoderma*. Stężenie zarodników w powietrzu jest ściśle uzależnione od obfitości ich tworzenia w danym okresie, co jest pochodną regionu geobotanicznego, szaty roślinnej, stopnia zurbanizowania danej lokalizacji, warunków klimatycznych, pory roku, aktualnej pogody, siły i kierunku wiatru, lokalnego mikroklimatu i wielu innych czynników. Zmiany wilgotności powietrza wpływają na stężenie zarodników różnych rodzajów grzybów, określanych na tej podstawie jako „suche” (*Alternaria*, *Cladosporium, Puccinia*, *Ustilago, Melampsora*, *Epicoccum*, *Drechslera*) lub „mokre” (*Didymella*, *Fusarium*, *Ganoderma*, *Gliocladium*, *Leptosphaeria*, *Verticillium*). Badania składu rodzajowego i gatunkowego prowadzone są przy zastosowaniu różnego rodzaju chwytaczy zarodników, a identyfikacja wizualna na podstawie morfologii kolonii grzyba oraz kształtu i wymiarów zarodników uzupełniana jest obecnie przez wyjątkowo czułe metody detekcji molekularnej, specyficzne względem rodzajów, gatunków, chemotypów, a nawet składu genów i kompozycji poszczególnych alleli. Monitoring aerobiologiczny znajduje bezpośrednie wykorzystanie w epidemiologii chorób ludzi (alergologia) i roślin uprawnych (systemy wspierania decyzji w ochronie roślin uprawnych). Badania z zakresu aeromykologii znajdują zastosowanie w tak

różnych kierunkach jak aerobiologia przemysłowa, bioterroryzm, ekologia, dziedzictwo kulturowe, klimatologia lub speleologia.