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New trends in aerobiology

G. FRENGUELLI

Dipartimento Biologia Applicata, University of Perugia

Summary

From the beginning of this century most of the published papers in aerobiology have concerned above all the following fields: the presence of pollinic allergens in the atmosphere and not only pollen, long-distance transport models, modelling of the presence of pollen and spores in the air, the influence of air pollution and climate change on bioparticles, the phenology of allergenic plants, pollen released by alien species, phytopathology of crops, the productivity of forest plants, indoor monitoring for human health and cultural heritage protection. In many cases the problems linked to aerobiological monitoring have been handled and new monitoring techniques for counting and identifying particles and following their development have been put forward. What could be the challenges in the future for aerobiology? Perhaps three major directions of development can be foreseen: renovating theoretical aerobiology, expanding monitoring, and aerobiological information systems. In any case, aerobiology should be more proactive, in other words it should be able to facilitate and stimulate further research, therefore not only producing data used by other branches of learning. These goals could be obtained by means of more active systems, capable of providing information on the ecological dynamics of the species, and of integrating with phenological networks to improve the measurement rather than making estimates of the bioparticles dispersal. This idea could facilitate research in order to take advantage of most modern research techniques so as improve knowledge on the dynamics of bioparticles in the atmosphere, their dispersion and the effects of deposition.

Introduction

Eighty years have passed since F.C. Meier of the U.S. Department of Agriculture first used the word "Aerobiology" ¹ to describe a research project on microbial life in the atmosphere. In the mid-1930s he published a series of preliminary work that reported the results of monitoring of micro-organisms that flying at about 1,000 m in various regions of the United States, the Arctic and Caribbean, mounting slides on a plane piloted by Lindbergh. The new discipline was finally launched by a Symposium precisely devoted to indoor and outdoor aerobiology and the proceedings were published by the American Association for the Advancement of Science in 1942. Aerobiology is therefore a relatively young science and over the decades has had various interests mainly related to the consequences of the fallout of particles of biological origin on living organisms, able to cause various diseases.

Gregory in his book "Microbiology of the atmosphere" ² devotes the last page to highlighting possible future studies of the atmosphere, showing that which until then several studies were made

essentially to study allergy and plant disease, but the atmosphere was not explored systematically and simultaneously in different parts of the world by methods comparable, making possible to use the data obtained. Thus Gregory argued the need of new studies in aerobiology, conducted over long periods of time and using equipment tested and calibrated to analyze the presence of particulate in the atmosphere, its concentration in the air, the extent of his relapse and the deposition on the surfaces. When these methods were developed, it would be necessary for international cooperation to organize a chain of sampling stations in a variety of climates and vegetation, coordinated with particular interest to cities, agriculture and medicine.

Similarly, Edmonds also published a monograph, "Aerobiology: the ecological systems approach" ³, dedicating the last chapter, written with Benninghoff, to future challenges of aerobiology. These authors advanced the need to develop a global monitoring network to establish the knowledge base for the construction of simulation models to provide information to assess bio-environmental changes and their causes and advocated new tools for sampling and storage of data. The monitoring aerobiological

system would have consisted of three levels: urban, regional and global levels in order to provide knowledge and predictions about human diseases, animals and plants, detection of the major changes in the productivity of natural vegetation, but also of the microflora and microfauna, build predictive models to allow adequate protective measures and contribute to a better management of natural resources. Actually, these issues have been for over 20 years the major successes and achievements of aerobiology, such as the introduction of national monitoring networks and, in Europe, continental networks, the improvement in the use of sampling equipment, computerization of data collected, their distribution via the Internet and the development of predictive models, all research largely focused on the protection of human health and plants.

Once achieved these objectives, from the early years of this century aerobiology have devoted more energy to other researchs, broadening their interests, so the majority of the work that the aerobiology of the century has produced have concentrated primarily on the following areas: presence in the atmosphere of pollen allergens as well as pollen grains, models of long-range transport, modeling the presence of pollens or spores in the atmosphere, influence of air pollution and climate change on bioparticulate, plant phenology as a source of allergenic pollen, appearance in the atmosphere of pollens released by alien species, diseases of agricultural environments, productivity of forest species, indoor monitoring for the protection of human health and for the protection of cultural heritage. In all cases it is often repeated, moreover, the sampling problem of aerobiology: how to capture the bioparticulate, how to recognize and quantify it, which is the evolution over time and space.

Standardization of sampling methods

One of the major problems that has always characterized the aerobiological studies is related to the difficulty of sampling the particulates and its recognition and quantification. Over the years, the means of capture have evolved considerably and the first rudimentary "traps" as the Durhan or Tauber, moved to Rotorod and finally to the current volumetric samplers in low-and high-volume ⁴. Being a biological sample, it is difficult to achieve the standards, and recognition and quantification is often linked to the operator's ability to recognize and count the particles. So, over the years, the activity of aerobiology was partly devoted to the training of operators, with an increasing demand for the use of image analysis to automate the "reading" of the slide under a microscope. Some attempts have been made and still are being increasingly sophisticated analysis results that are reaching gradually more significant as

the analysis of DNA pollen by the polymerase chain reaction (PCR), suitably adapted for the identification and quantification of mixtures pollen ⁵.

Currently, a serious problem is the standardization of the method of reading the aerobiological sample and the percentage of reading of the slide. The examination of the slide has two main purposes: 1) actuopalinology research purposes, such as studying the daily rhythm of the presence of certain pollens in the atmosphere, control the degree of biodiversity pollen in a certain area, to study the phenological rhythms of certain taxa, etc., 2) for control purposes of the tendency of the phenological stage of a plant with wind pollination or the onset and persistence in the atmosphere of a certain fungal spore.

In the first case the methods of analysis of the sample may follow various methods and it may be necessary to analyze the entire slide or read only certain times, etc. In this case it is not important the factor "time" as the end user is the researcher, the world of scientific research which must be an exact figure that would give useful information to improve our knowledge. In the second case, the individual captors are often placed in the network for purposes related to the study of allergenic pollen or phytopathogenic spores. In this case the analysis of the slide must take into account the factor "time" to be able to launch a "warning". Indeed, it is important to know as soon as possible the trend of the presence of some pollens in the atmosphere, if it starts its presence, whether it is increasing or decreasing, if this is disappearing and to allow the user, allergist or plant pathologist to intervene in time and limit the damage. In this second case the monitoring performed routinely must require short time and thus the counting and recognition of pollen is carried out only on a fraction of the slide. What is the minimum fraction required to have a good relationship between the time required to read and data quality?

A level Italian has come to the application of a rule, UNI11108/2004 which provides some basic operations that follow standardized Mandrioli ⁶ has proposed in the wake of the first experiences at the international level were maturing. The Italian method is not followed by other researchers conducting aerobiological studies in various parts of the world and this can make it difficult and not always significant comparative research conducted by various laboratories.

This is a subject studied and debated, of great importance and requires a joint effort by the whole community of aerobiologists to get to have guidelines acceptable to all and which renders the data obtained from various research centers comparable ⁷⁻⁹. A Working Group constituted within the European Aerobiology Society (EAS) has laid down some minimum recommendations to achieve a standardization of monitoring aerobiological in Europe ¹⁰.

Monitoring allergens

The pollen counts performed with volumetric samplers may not reflect the total exposure to allergens in the atmosphere since the allergen pollen can also be transported in microaerosol suspension. It follows that, as important information and complementary to pollen counts, it would be useful to measure the variations of atmospheric organic aerosols and their allergenic activity to establish correlations with clinical signs and estimate the difference in risk for patients with asthma and rhinitis sensitive to pollen allergens ⁴.

For the sampling of these aero-diffused allergens suction samplers should be used at high volume and able to evaluate the concentration of allergens in different fractions using immunochemical detection methods such as RAST-inhibition of IgG subclasses, IgE-RAST, the IgG-ELISA, immunoblotting and chemiluminescence. This method also allows to establish correlations between concentrations of aeroallergens and clinical symptoms of pollen morphologically similar and not distinguishable with the common pollen monitoring, but with different potential allergens such as common nettle and pellitory of the wall ¹¹.

Air pollution and airborne particulate

One of the most common explanations of the increase in pollen allergies is the negative impact of conventional pollutants on airborne pollen. The interaction with these products may occur along several lines. First, pollutants can influence the production of pollen and/or its allergens. Second, they can influence the dispersion of pollen and its transformation into the atmosphere. Third, they can determine the reactions of the organism due to exposure allergenic. This area is highly uncertain with, at the time, limited research. For example, there are data that pollen originated in urban areas or at least stressed, allergologically may be more powerful than those issued in rural and non-polluted sites. It is comparatively well documented that high concentrations of gaseous pollutants such as NO_x and O₃ can damage the grains of pollen and cause the release of allergens ¹²⁻¹⁴.

The plants, in addition to producing reactive oxygen species (ROS) during normal aerobic metabolic processes, increase their production when they are exposed to environmental stresses, both biotic to abiotic. ROS are also produced by pollutants at the level of the mucous membranes and when the production exceeds the capacity of the tissue of their neutralization by means of antioxidants, an oxidative stress occurs ¹⁵. The ragweed pollen, which is one of the most allergenic pollens, when it is hydrated, releases particles of 0.5 to 4.5 microns which contain Amb a 1 and other allergenic proteins and have NADPH oxidase activity. These particles significantly

increased ROS levels in the tissues and induce allergic inflammation in the airways ¹⁶.

A pollen "stressed" may contain more protein than normal pollens, changing its allergenicity. Under stress conditions, the pollen would produce a certain amount of defense proteins and many allergenic molecules, such as Bet v 1 and Cor a 1, are precisely proteins that are part of the defense system of plants by pathogens or by abiotic stress. Ragweed pollen stressed with O₃ ¹⁷ shows a significant increase in NADPH oxidase generating ROS, but the expression of the allergen Amb a 1 is not affected instead.

The quantification of these processes and identification of others essentially requires strong efforts by various sectors of aerobiological research ¹⁸.

Climate changes

For almost twenty years, perhaps due to the release into the atmosphere of large amounts of CO₂ and other greenhouse gases, there was in the world, especially in the northern hemisphere, a clear gradual increase in the temperature of the air caused changes in the development of the vegetation and, as a consequence, changes in abundance and temporal distribution of pollen of different species in the atmosphere ¹⁹. The increase in CO₂ and temperature may also lead to an increase of the production of pollen and, indirectly, of fungal spores which together may alter the immediate future exposure to allergen bioparticulate ²⁰.

The impact of the climate in these processes, however, is not unique and is due to a large number of factors. Some of them are of geological and geographical origin (such as soil type, constant local insolation, etc.) and thus do not change, while others do (first of all the temperature, cloud cover and precipitation). As a result, in many cases even the direction of changes in vegetation due to climate forcing may be uncertain, taxon-dependent and region-specific ¹⁸.

The data collected in various regions suggest an increase in the last decades of the effects of aeroallergens in patients with pollenosis, which may also imply a more unexpected development of respiratory problems in sensitized individuals and exacerbations in symptomatic patients, although it is difficult to make certain predictions for immediate future ^{21 22}.

Forecasting models

For over two decades have been published numerous works dedicated to the creation of models of varying complexity that would allow, through the study of historical aerobiological data and the performance of the various climatic factors, to predict what will be

the beginning of the pollination of a particular taxon, or when the maximum peak of the presence of its pollen in the atmosphere is reached, when the season will end or other parameters.

The relationship between atmospheric concentrations of some pollens and meteorological factors are well documented in the literature²³⁻²⁵, but recently models referred as Artificial Neural Networks (ANN) are applied in aerobiology which have the advantage, compared to programs for algorithm processing of information, to “learn”, that is to use new knowledge not present in the processes of education. ANN have become a very useful tool in the analysis of situations “predictable” analytically, and lend themselves very effectively to the modeling of a variety of problems, from the financial to the biological one, as is happening now for some years in phenology.

The phenological stage of flowering and subsequent dispersal of pollen in the atmosphere depends on climatic factors modified by geographical location. These specific relations are well analyzed and modeled by neural networks. The use of these new techniques of analysis of the data is able to provide daily concentrations of certain pollens or airborne spores²⁶⁻³⁰ with the aim of predicting the pollen in the atmosphere and provide accurate information to patients sensitive in order to help them to optimize their treatment processes.

Other modeling to predict the performance of the pollen season with the intention of giving a meaningful result as early as possible has led to the development of other analytical mathematical models, increasingly complex as multivariate regression models for olive³¹ or integrated models based on analysis polynomial regression and multiple processed by Sabariego et al. for the cypress³².

Long-distance transport of pollen

In models of expected development on the pollen season in a particular area we must not overlook the influence that may have pollen transported from other regions with different climatic conditions and vegetation. These episodes are particularly evident in areas with few mountains that might otherwise constitute natural barriers to the transport of pollen in the atmosphere. The long-distance transport of pollen, spores and allergens has two obvious consequences: first, short-term changes in the concentrations of pollen over large regions receptor, second, rapid redistribution of large-scale genetic material along paths into the atmosphere¹⁸. Without doubt one of the most important cases studied in Europe concerns the birch. The birch pollen, in fact, is frequently transported across the continent contributing significantly to determine even very high concentrations of its pollen from thousands of miles away from the source. These episodes were recorded more often during the season and tend to anticipate or lengthen the

presence of a certain pollen in the atmosphere, the origin of which is the number of plants in the area. This can increase the severity of the pollen season as a result of the sum of native pollen and allochthonous pollen with a negative impact on allergic subjects. Aerobiological and phenological observations carried out in parallel on certain areas, as recently reported for birch in Denmark³³, have in fact found as concentrations of hundreds of pollen grains per cubic meter of air can be present in the atmosphere in regions where the birch still has not started flourish and certainly from southern regions, such as Germany and Poland, where the plants begin to release pollen in advance. It is evident that in such cases a forecast on the concentration of pollen in the atmosphere must take into account not only the local conditions but also those of neighboring regions that have significantly anticipated pollination³⁴.

The long-distance transport of pollen also relates to a topic as sensitive as the studies on genetically modified organisms (GMOs). The research that are being carried out in various parts of the world and which relate to some food plants, such as maize, soya, potatoes, ryegrass and others, face the problem of dispersion of pollen released from these plants with the risk that it comes in contact with other plants with obvious consequences, not only biological³⁵. Some of these plants have a strictly anemophilous pollination, but the problem also affects those with entomophilous pollination with the involvement of insects that could represent significant vectors of transport of pollen from genetically modified plants.

The aerobiology is concerned with the long-range transport of other bioparticles over pollens and several publications addressed transport of small seeds, powder or various microorganisms such as bacteria, viruses, fungi, that when passively transported by air currents are able to survive for long time suspended in the atmosphere and to across entire continents before falling to the ground, still retaining their pathogenicity³⁶.

Phenology

The phenology of allergenic plants is of great support for a correct interpretation of the aerobiological data on certain taxa. The estimation of occurrence of phenological stages of indicator species to better predict and interpret the aerobiological data is only one aspect, but also other information on the dynamics of source plants and their correct identification to the species level support aerobiology. It is known that the morphology of the pollen is species-specific, but unfortunately with the means of routine investigation is not always feasible to recognize the species source of airborne pollen and a careful examination of the vegetation present in a given region and of the subsequent phenologic stages can provide very useful indications.

This is true for example for grasses, which have pollens morphologically similar among species; only a joint analysis aerobiological / phenological may be able to interpret at best the source of allergenic pollen identifying the succession of blooms of different species that combine to form the seasonal aerospora ³⁷.

Phytopathogenic fungi

Fungi or mycetes reproduce and spread primarily by means of spores. They will produce different types that may rise for sexual reproduction or asexual reproduction (conidia). Of all the various types of biological particulates suspended in the atmosphere of every part of the world, fungal spores constitute the largest part, both in indoor and outdoor environments: in "natural" condition, the concentrations of over 100,000 spores per cubic meter 'air are not exceptional. The number of types of spores, as their concentration in the external environment, at any time depends on many variables interreacting as seasonal climatic factors, including temperature, precipitation, wind, humidity, alternation of hours of light and darkness and concentration of CO₂ ³⁸.

The presence of fungal spores in indoor environments regards above all their incidence of some respiratory diseases, fungal contamination of air (in particular from *Alternaria* and *Cladosporium*) and mycotoxins (*Aspergillus*/*Penicillium*, *Fusarium*, *Stachybotris*), but also of environments for the conservation of food stocks ³⁹. Particular attention was paid to assess the damage caused by the proliferation of fungal spores on materials of cultural significance, such as paintings, printed materials, sculptures, of particular historical and cultural value ⁴⁰.

Considerable attention has also received the assessment of air quality, both indoor and outdoor, thanks to comparative tests between indoor and outdoor environments, checking the periodicity allowance for the presence of different biological particles such as windblown, as well as spores, bacteria, viruses and mites in special conditions and places such as air conditioners, central heating, piping, gardens, factories or other workplaces ^{41 43}.

However, as from the origin of aerobiology, the main research on airborne fungal spores relate to diseases of cultivated plants, the dynamics of spread, appearance and recurrence of phytopathogenic with serious damage to agricultural areas. Their collection,

isolation and rapid identification and knowledge about environmental factors that favor the development allows to make predictions about their effects on the soil and then promptly intervene with programs aimed at optimizing plant protection and limit the use of anticrotopogamics ⁴⁴⁻⁴⁶.

Future challenges for aerobiology

Comtois ⁴⁷ following the same criteria as Gregory and Edmonds, taking note of the research and the results obtained in the last two decades, has suggested three main areas on which future aerobiological research will be directed: theoretical aerobiology, new monitoring systems (based on biochemical or genetics and image analysis), aerobiological information systems. The theoretical aerobiology requires a new vision, bi-or tri-dimensional, and requires studies from a mathematical and physical point of view and to know the laws, on different scales, regulating the movement of pollens or other particles in the atmosphere.

New monitoring systems should enable us to study not only the presence of particulate matter in the soil, but also an interest of the highest layers of the atmosphere, i.e. to get to have three-dimensional data of the vertical "clouds" of pollen or spores as, on the other hand, it is necessary to arrive to identify the thresholds of particulate which indicate the levels of risk to human health or plant health ⁴⁸.

Finally, aerobiology should become more proactive, i.e. able to act to facilitate and stimulate further research, then produce not only data used by other branches of research ⁴⁷. These objectives could be achieved with a system more active, able to give information on the ecological dynamics, for example of invasive species, integrating at best with the phenological networks to improve the measure, rather than estimate, the dispersion of bioparticulate. Aerobiology no longer or not only as a multidisciplinary discipline, but discipline in itself, integrated, such as ecology, able to involve aerobiological researchers and not, as hitherto, botanists who are interested in "even" aerobiology or allergists who study "also" airborne pollens. This concept could help to conduct research that can use the most modern techniques of investigation in order to acquire deeper understanding and fundamental dynamics of the bioparticulate.

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- Correspondence: Giuseppe Frenguelli, Dipartimento Biologia Applicata, Università di Perugia, Borgo XX Giugno 74, 06121 Perugia - E-mail: freng@unipg.it