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Lectures

Aerobiology: Pollens and Spores in the Air

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1. Definitions
2. Capturing airborne pollen and spores
3. Pollen grains
4. Fungi spores
5. Applications

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1. Aerobiology. Introduction and definitions

1.2. Index

1.3. Definition of Aerobiology

Aerobiology studies small particles from biological origin that are airborned in the air
They fly passively due to wind transport (airborne).
These particles are mainly pollen grains from plants and spores from fungi.
Aerobiology studies not only their liberation from the sources, transport and deposition, but also their effects on humans, animals, plants and even over food, building, works of art, etc.
Aerobiology is not only concern about outdoors (extramural environment) but also indoors (intramural ones)

1.4. Airborne particle size range

Air we breathe contents inorganic and organic particles airborne (passively) transported.
Inorganic particles come from fires (combustion particles, fumes), dust or mist originated by a mechanical process.
Particles from biological origin include viruses, bacteria, spores and pollen grains.
- Combustion particles: usually start out in the 0.01-0.05 µm size range
- Powder is broken down into smaller particles and released into the air by 0.05 µm
- Biological particles are usually larger than 0.05 µm

1.5. Particles settling in still air

Time that airborne particles spend to settle depends on their size.
Smallest airborne particles can be suspended in the air some hours until they settle (landing). – they are named: submicronic aerosols and paucimicronic spores -
The biggest airborne particles remain in air only a few minutes or seconds.
Nevertheless wind can transport these particles long distances, depending on their speed.
The smaller these particles are the further they are transported

1.6. Particles of biological origin (Microbiology, Botany, Zoology):
Submicronic or paucimicronic. Essentially (basically) studied by Microbiologists
- viruses
- bacteria and actinomycetes
Essentially (basically) studied by Botanists
- fragments of fungi
- **fungi spores**
- lichen fragments (propagules)
- protists (protozoa, algae)
- spores of mosses, ferns
- **pollen grains**
- plant fragments (hairs, cells)
Essentially (basically) studied by Zoologists
- invertebrates (insects, mites, spiders, eggs)
- fragments of invertebrates (butterfly wing scales, hairs, faeces etc.)

1.7. Aerosols

It is a suspension of fine solid particles or liquid droplets in a gas
Examples: smoke, air pollution, smog
Mainly they include inorganic matter floating in air: dusts, mists, haze, and fumes
Nevertheless as there are many small organic particles we can speak of bioaerosol
Bioaerosol: airborne particles that are biological in origin
Bioaerosol can be formed for any process that generate enough energy to separate small particles from organisms: wind, water, or mechanical movement

1.8. Aerobiology pathway

Aerobiology is mainly interested on airborne source, dispersal and impact.
Moreover their interest includes the environmental factors that affect on the take-off and deposition.
Pathway: Source -> Take-off -> Dispersal -> Deposition -> Impact
This pathway gives the different stages in the movement of particles (spores, pollen) from their source to the effect they cause when they land (settle)

1.9. Environmental factors (outdoor air)

Sources (plants and fungi): natural or cultivated landscapes
Weather parameters: temperature, rain, wind speed and direction, relative humidity, etc.
Time: day of the year (season, yearly variations), hour of the day (daily variations)
Geographical barriers: natural (mountains, valleys, island), artificial (building)
These are the main aspects which determine their air concentration (Source strength, Transport, Removal)

1.10. Layers of the atmosphere

Diagrammatic representation of layers of the atmosphere (Gregory 1973)
A profile of the earth's atmosphere, shown on a logarithmic altitude scale
The properties of the atmosphere change most sharply near to the ground
Laminar boundary layer: a still microscopically thing layer of air at the surface of the earth and all objects protruding from it
Turbulent boundary layer (planetary boundary or mixing layer): where most particle dispersion occurs
The content of outdoor air is dynamic, constantly changing with location, weather, season and time of the day

1.11. Environmental factors (indoor air)

It is well known that the environmental conditions or microclimate inside buildings is different and less variable than outdoors
Presumably is more homogeneous with time or season than outdoors
So we have to take into account
-Isolation of room (doors, windows)
-Weather conditions: temperature and humidity
-Ventilation (air conditioned)
-Internal sources: building materials, furniture, food storage
-People movement (particle transport)
-Cleaning practices (frequency, intensity, product used)

1.12. Usefulness of Aerobiology

Many aspects of our lives are affected by biological particles that are carried in the air and are deposited from it
- Dispersal of allergens airborne transported (allergy) and long distance transport
- Indoors air quality: identifying sources of microbial contamination in medical and food processing situation
- Crop disease epidemiology
- Monitoring climate change
- Biodeterioration

1.13. Dispersal of allergens

Air transports allergens mainly as pollen grains and fungi spores
They cause different allergic disorder depending on their size: rhinitis, asthma, alveolotis
Because they reach different part in the respiratory system
-Head airways (nasopharyngeal region) - rhinitis
-Lung airways (tracheobronchial region) - asthma
-Alveolar/pulmonary region - alveolitis

1.14. Long distance transport

Airborne particles can be transported even hundred or even thousands of kilometres away from their sources
For example dust from the Sahara desert can be transported until the American coast
Griffin et al. (2006) studied the trans-Atlantic transport of dust borne microorganisms from Northern Africa to the Caribbean and Americas (May-June 2003)
They reported a statistically significant correlation between daily atmospheric colony-forming-units (CFU) counts at a mid-ocean research site (by 2000 km from coast) and daily desert dust concentrations

1.15. Indoor air quality

Many fungi can appear indoors (inside homes) when circumstances allow their grow (wet and warm)
Apart for the damage they originate, their spores can be the origin of respiratory problems
They may also transport toxins (mycotoxis) that are breath or even deposited over food

1.16. Crop disease epidemiology

Air is the way fungi use to transport their spores (propagules)
Many fungi are crop pathogens and cause important economic losses, hazards or even famines
One of the most important famines was the Highland Potato Famine, causing over 1.7 million people to leave Scotland during the mid-1840s, similar to the Great Irish Famine.
Potato crops were widely spread and they were the main food source. In 1846 potato crops were blighted by and fungi (water mold): *Phythophthora infestans* that spread its propagules by wind
Knowing the airborne spore concentration and environmental conditions it is possible to forecast their spread and regulate the use of fungicides

1.17. Monitoring climate change

The onset of flowering (when flowers blossom) is directly affected by weather parameters
As you know we are in a process of global climate change, apparently with warmer conditions
It seems that the onset of many plants, including crops, is happening earlier than before because winter and spring are warmer
This means that plants seem to flower earlier and pollen is shed in advance
There are many scientific papers that show the pollen season is beginning earlier now, as the onset of olive tree (Galán et al., 2005)
So Aerobiology is a tool to monitor climate change and evaluate its effects over plants

1.18. Biodeterioration over works of art

Historical cultural legacy is often supported over materials that can be damaged by organisms (biodeterioration)
This includes organic materials as books, cloth, wood, etc., but even inorganic substrate can be biodeteriorated too.
Most of the organisms responsible of biodeterioration are fungi, although algae, bacteria and arthropods (insects) can cause important damage.
For example foxing on book is a series of brown dots that appear on many old books, maps, drawings, etc., that have been stored in damp, humid attics or storerooms over a long period of time.
Foxing is a fungal growth on old paper and books if the relative humidity is greater than 65% over a prolonged period of time and can ultimately destroy the cellulose fiber in the paper if left untreated.
Fungi presence can be detected using aerobiology methods and so their effect reduced or avoided.

1.19. Aerobiology and other sciences

Aerobiology is an interdisciplinary science that spans many subjects or disciplines
Botany, Mycology, Zoology, Microbiology as basic descriptive biological disciplines
Disciplines that treat with the environment: Ecology, Meteorology, Ecology
Applied disciplines: Allergology, Agriculture, Genetic, Taxonomy

2.1. Air sampling techniques

2.2. Sampling devices for airborne particles

Pollen grains and fungi spores from air are caught by device named air samplers
They must be suitable for
- viewing particles under a microscope, by now it is necessary to identify and count pollen grains or fungi spores
- provide a way to evaluate concentrations over a period of time and a volume of air known
- allow other forms of analysis, as immunological o molecular techniques, as aeroallergens are present

2.3. Factors affecting particles caught

To take a representative sample we must consider
- time of the day
- day of the year (season)
- weather (wind, temperature, rain, relative humidty)
- location of a sampling site
- duration of sampling (length of time)

2.4. Basic techniques

A. Particles caught over a sticky surface
-Passively (gravimetric):
    Gravitational sedimentation (passive)
    Passively impact (wind)
-Actively (volumetric)
    Sticky surface moving against air (whirling arm traps)
    Air sucked against a sticky surface or media (inertial traps)
B. Particles caught inside a liquid
-Cyclonic (air is led/guided into a container with a liquid)
    Coriolis air sampler

2.5. Passive gravimetric traps

Sedimentation is the simplest way for collecting airborne particles
It is inexpensive and easy
Microscope slide with a sticky surface are used: petroleum jelly, glycerine jelly, silicone grease
If fungi or mold are studied a growing media is used (Petri dishes with agar)

2.6. Principle of sedimentation

Sedimentation (settle) depends directly on
-Particle density
-Particle diameter
-Gravitational acceleration
Sedimentation depends inversely on
-Air viscosity (temperature, relative humidity)
So, considering a homogeneous particle density, terminal velocity depends mainly on particle diameter

2.7. Settling velocity of some airborne biological particles (µm)

These data means that spores or pollen grains take about 30 seconds to three minutes to settle, but always that air is steady, that is an infrequent natural situation.

2.8. Efficiency in passive traps

Passive traps are less efficient for small particles according to Stokes law, so they are underrepresented
Large particles settle more quickly, so, they are overrepresented
In non turbulent air the rate of settling depends on velocity of deposition
In turbulent air depends on particle size
Accurate particle quantification is not possible

2.9. Durham sampler

Sampling surface is exposed in a horizontal position either on the ground or at some elevation
Capture takes place by turbulent impingement as well as by gravitational settling
A glass microscope slide is mounted between two horizontal circular disks
It is usually mounted on a metal rod or pipe support at least several feet above the ground or on a roof top
It was adopted as the standard pollen sampler by the Pollen and Mold Committee of the American Academy of Allergy
Main advantages are that slides are easily loaded and counted, it is inexpensive, does not requires electric power
Main disadvantages are that the volume of air is unknown, efficiency cannot be evaluated, catch is relatively low and it depends on the wind speed, turbulence and orientation of the sampler
Count from different localities is not properly comparable
Data are given as number of particles by a surface unit (one squared centimeter)

2.10. Passive impact traps

Collect particles in the airstreams by impaction
Trap surface (adhesive, filter) is mounted vertically and face to wind direction
Rate of deposition is proportional to
-concentration of particles in air
-wind speed
-shape and width of exposed surface

2.11. Cour

The Cour trap uses 400 cm2 sterile gauze filters impregnated with silicone oil and fitted to a vane.
The apparatus includes a totalizing anemometer indicating the wind flow through the filter during the exposure period.
Sample processing was done according to the methodology proposed by Cour in 1974:
Filters are subjected to a series of chemical treatments in the laboratory to destroy the gauze and release the pollen. The chemical treatment include cold sulphuric acid, cold hydrofluoric acid, hot hydrochloric acid, hot potassium hydroxide, acetolysis (sulphuric acid and acetic anhydride), and coloration with basic fuschin. This method is quite consuming time

2.12. Active impact traps: Whirling arm trap

Consists of a pair of vertical arms which are rotated at a high speed (3500 rpm revolutions per minute)
It uses a small electric motor (12 volt dc)
Particles are impacted on sticky-coated tape mounted on the leading edge of the rotating arms
Air volume (V) sampled by each arm is quantified (≈ 150 l min-1)
V = π D W L S 10-3 l min-1
D: outer diameter (cm)
W: width of collecting surface (cm)
L: length of collecting surface (cm)
S: speed in rpm

2.13. Rotorod trap

Tape is stuck onto the leading edge of the arms and trimmed to size
The tape strips are made sticky with and adhesive
After use the tape strips are mounted on microscope slide

2.14. Inertial impactors samplers

Air is actively sucked by an electric pump
Air flow rate is known
Air impact against (impinges on) a sticky surface or over a growing media (a narrow slit to direct the airstream onto a glass slide coated with a sticky surface)
Efficiency depend on
-speed of the airflow through the inlet
-width of the inlet
-separation between inlet and collecting surface
-adhesive properties (temperature)

2.15. Hirst spore traps

Standard device used for outdoor pollen and spore monitoring
Air is drawn through an intake slit (2 x 14 mm) by a built-in pump
Air intake is protected from rain by shield
Face into the wind by the action of an attached wind vane
Flow rate of 10 litres / minute

2.16. Hirst spore traps (figures)

2.17. Burkard trap

Comprise a chamber into which air is drawn through an intake slit by a built-in pump
Device face into the wind by action of an attached wind vane. Air intake is protected from rain by shield
Air-stream impact on a slowly rotating drum (clockwork mechanism) revolving once every seven days (2 mm per hour)
Drum is covered by a transparent plastic tape (Melinex) coated with an adhesive where particles are trapped
Tape is replace every week (or for less time length) and then cut into day-length sections and mounted for microscopy

2.18. Preparing the drum for a new sample

First of all the drum is cleaned thoroughly with a dry tissue
The trapping tape (Melinex tape) is secured to the drum with a piece of double-sided sticky tape, sticking the edge of the Melinex tape only on one half of the sticky/adhesive tape
The Melinex tape is wound tightly around the drum until it can be stuck onto the other half of the adhesive tape, then it is cut using a razor blade, leaving no gap or overlap
The tape is coated with the adhesive (petrolatum jelly) using a brush, rotating the drum, then heating the drum to permit the adhesive extend smoothly on a fine coat
Drums can be prepared in advance and stored in containers

2.19. Changing the drum

The revolving head of the trap is locked by placing the anti-swivel pin horizontally in the block of the base plate (for safety as well as making the trap easier to work with)
The tape is marked using a dissecting needle through the orifice and marking a line (furrow)
The locking bar is moved and the lid assembly is removed and put on a firm surface
Holding the knurled ring on the side of the aluminum drum unscrew on the clock spindle, the drum is removed, taking care not to touch the trapping surface

The air flow rate must be checked regularly, there is a screw on the bottom inside of the container (sampling chamber)

Mounting the tape from the drum
The double-sided sticky tape is cut with a razor, and the Melinex tape is transferred from the drum to the cutting template
The start of the tape must be placed in the right position
The tape is cut in segment of 48 mm (24 houres) with a razor; you can be helpful using forceps (tweezers)
Each segment of tape is put over a slide covered previously with gelatin or water, the sides of the tape must be parallel to the microscope slide
Slides must be labelled to avoid any confusion
A cover slide with heated glycerin jelly is put over each slide; the glycerin jelly contains basic fuchsine to stain the pollen grains
Slides are firstly gently pressed and the using clothespins

2.20. Cyclonic sampler

The Coriolis air sampler is a cyclonic type of instrument based on the principle described by Decker et al. (1969)
Air is pulled into a conical collection sampler tangentially with a swirling buffer
The air is drawn into a conical vial in a whirling type motion using suction
Airborne particles are collected in this liquid buffer
They are pulled against the wall by centrifugal force.
Airborne particles are separated from the air and collected in a liquid medium.
The buffer can be assayed after sampling for the presence of micro-organisms using whichever technique is thought to be propper.
This sampler allows rapid analysis by several techniques including PCR assay and serological assay in order to measure the antigenicity/allergenicity of pollen grains and fungal spores.
Also, traditional counting of pollen grains or taxa identification by optical microscopy can be done.

2.21. Suitable place of spore traps (Placing the trap)

Location should be chosen according to the type of particles under investigation and the scale of interest
High location (roofs and tall building) will sample air that has been mixed thoroughly by the turbulent boundary layer, data obtained represent that of relatively large surrounding are
Conversely, traps located at ground level are likely to reduce emphasis on sampling the background air-stream in favor of particle produced primary from local sources
Building that could obstruct air circulation of location close to pollen or spore source should be avoided
For work in plant pathology, traps must be located between plants or around the crop taking into account wind direction

Cascade impactor
Four stage suction trap
Air impinges on a sticky microscope slide
Each successive slit is narrower and the air travels faster causing progressively smaller particles to be impacted

Andersen sampler
It uses a stack of perforated metal sections or stages
Air impact over open Petri dishes
The diameters of perforations in each stages reduce from the top to the bottom of the stack
A electric pump is used at set of flow rate

It mimics the deposition of particles in the human respiratory tract, with larger particles penetrating only into the nose and trachea or first state of the Andersen sampler and smaller particles penetrating into the lungs or to the lowest stage of the Andersen sampler

3.1 Pollen grains from plants

3.2. Index

3.3. Pollen grains and reproduction

Higher plants accomplish their reproduction by both sexual and asexual means.
Sexual reproduction produces offspring by the fusion of gametes.
Gametes are produced by meiosis (haploids) and their fusion restores the diploid status in the zygote.
In higher plants male gametes travel through the air inside pollen grains until they reach a place close to the female gametes
Pollen grains are produced in seed plants in male-cones (conifers) or in flowers (flowering plants),

3.4. Life cycle in seed plants

3.5. Male cones and flowers

Gymnosperms include conifers, brushes or trees as
Pines (Pinus), firs (Abies), cedars (Cedrus), spruces (Picea), larches (Larix) from the Pinaceae family
Cypresses (Cupressus) and junipers (Juniperus) from the Cupressaceae family
Yews (Taxus) from the Taxaceae family
They are woody seed plants that produce their pollen grains and ovules in cones
Pollen is always wind-pollinated, and male cones produce a high amount of pollen grains

3.6. Conifers pollinations

Pollen grains are dispersed from male cones by wind and have to reach the ovules in female cones
After pollination by wind if pollen grains settle next to ovules and they can fertilize them, after that ovules become seeds
Two main pollen types are identify from conifers: Pinaceae and Cupressaceae pollen types

3.7. Pinaceae pollen

Pollen grains are quite big and bear two bladders than allow better airborne buoyancy.
Pollination takes in last spring in the north and middle spring in south Europe.
Pollen are produced in high amounts but they do not provoke (set up) allergy.

3.8. Cupressaceae pollen

Pollen is medium size, spherical and with rough surface covered with small granules.
Pollination takes in spring (North) and winter-early spring (South).
They have a medium-high allergenic importance.

3.9. Flowers and pollen

In flowering plants, with true fruits that contain seeds, pollen is produced/developed inside stamens.
The ovules are produced/developed inside carpels, that they become the fruits after fertilization.
Both, stamens and carpels, are usually surrounded by petals and sepals, this are flowers.

3.10. Pollen release, germination and fertilization

In flowering plants pollen grains are released from stamens.
Pollen grains have to reach one part of the carpel named stigma.
Pollen grains over the stigma germinate and develop a pollen tube transporting the male gamete to the ovule.
Only pollen of the same species can germinate on the stigma.
Male gametes are transported through the pollen tube to contact with the female gamete and fertilize it.

3.11. Pollination in flowering plants

Insect pollinated plants (biotic)
- about 80%, entomophily process, entomophilous plants
- well developed flowers, with perfume
- low pollen production
- pollen grains with sticky surface
- rewards: nectar, pollen

Wind pollinated plants (abiotic)
- about 20%, anemophily process, anemophilous plants
- mainly in grasses and deciduous trees
- small flowers, simple, small or without perianth
- high pollen production
- pollen grains dry

3.12. Insect pollinated plants

Daisy flowers

3.13. Wind pollinated plants

Canarygrass

3.14. Pollen grains

Pollen grains are developed inside pollen sacs from the stamens.
In the beginning a diploid cells suffers meiosis and then four haploids cells are developed.
Each of the haploids cells become a pollen grains but they are at first together in a tetrad.
Position in the tetrad allow to define a imaginary polar axis and an equatorial plane.
When pollen grains germinate the pollen tube is developed through specific areas named apertures.
Apertures basically can be circular named porus (pori), or elongated named colpus (colpi).
Often apertures appear around the equatorial plane and then we can define a polar view or an equatorial view.
Less often apertures are around all the surface and then is not possible observe poles in the pollen grains.
Shape, number and disposition of apertures allow us describing different pollen types; this is object of study of Palynology

3.15. Wind pollinated plants

Flowering plants that interests to Aerobiology are wind pollinated ones.
They can be separated in two groups, woody and herbaceous plants
Woody plants
-Birch (Betula), Alder (Alnus), Hazel (Corylus), (Betulaceae family)
-Oak (Quercus, Fagaceae family)
-Ash (Fraxinus, Oleaceae family)
-Olive trees (Olea, Oleaceae family)
-Planes trees (Platanus)
-Elm (Ulmus, Ulmaceae family)
Herbaceous plants
-Grasses (Poaceae family)
-Nettle (Urticaceae family)
-Plantains (Plantago, Plantagicaceae family)
-Goosefoots and pigweeds (Amaranthaceae-Chenopodiaceae)
-Docs and sorrels (Rumex, Polygonaceae family

3.16. Birch (Betula)

Triangular pollen with tree vestibulate pori in equatorial zone
Pollination spring (North) and winter (South)
With a medium to high allergenic importance

3.17. Alder (Alnus)

Polygonal, mainly pentagonal, in shape with five pori in equatorial zone
Pollination early spring (North) and winter (South)
Low allergenic importance

3.18. Hazel (Corylus)

Triangular pollen with tree pori in equatorial zone but not vestibulate as in Betula
Pollination early spring (North) and winter (South)
Low allergenic importance

3.19. Oak (Quercus)

Elliptic in shape with three colpi in equatorial zone
Pollination late spring or early summer (North) and spring (South)
None or very low allergenic importance

3.20. Olive trees (Olea)

Elliptic to spherical in shape, tricolporate, well developed reticulate surface
High allergenic importance
Spring pollination
Mediterranean crops and woods

3.21. Ash tree (Fraxinus)

Spherical in shape, trizoporate, finely reticulate surface
Low-medium allergenic importance
Early spring pollination
Eurosiberian woods and Mediterranean rivers

3.22. Elms (Ulmus)

Spherical to polygonal in shape, 5-6 pores in equatorial zone, Rugulate surface with light microscope
None o low allergenic importance
Eurosiberian woods and Mediterranean rivers

3.23. Planes trees (Platanus)

Elliptic to spherical in shape, tricolporate, finely reticulate surface
Medium-high allergenic importance
Early spring pollination
Ornamental trees

3.24. Grasses (Poaceae)

Spherical to elliptical in shape, smooth surface (light microscope), monoporate
High allergenic importance
Spring to summer pollination

3.25. Nettles (Urticaceae)

Spherical in shape, pantoporate, smooth surface
None (Urtica) or high (Parietaria) allergenic importance
Winter and early spring pollination

3.26. Goosefoots and pigeweeds (Amaranthaceae and Chenopodiaceae)

Spherical in shape, pantoporate, surface with small granules
Medium allergenic importance
Summer pollination

3.27. Plantains (Plantago)

Spherical in shape, pantoporate (many pori around all grain)
medium-high allergenic activity
Late spring – early summer pollination

3.28. Docs and sorrels (Rumex)

Elliptic to spheroidal in shape, tricolporate, finely reticulate surface
Low-medium allergenic importance
Spring pollination

3.29. Pollen calendar of Scotland

Start in February and end in September
The most abundant pollen type is Grasses with nearly 40 grains/m3, from June to September
The second is Nettle with the same time of pollination
The third is Birch in April and May, with more than 10 grains/m3

3.30. Pollen calendar of Badajoz (SW Spain)

There are pollen all the year
The first pollen type is Grasses, with nearly 300 grains/m3 (weekly average), from March to August but mainly in May
Second is Olive tree pollen, also mainly in May
The third is Cypress pollen, in winter

4.1. Fungi spores

4.2. Fungi characteristics

Fungi include very diverse organisms, than although traditionally studied by Botany or Mycology include organism that are not truly fungi. Fungi share only a few characteristics:
Eukaryotes. True nucleus
Heterotrophic. Feeding by excretion of enzymes into the substrate and by subsequent take-up of digested compounds
Cell wall. Glucan, chitin (exceptionally cellulose or chitosan)
Reproduction. Spores (meiospores: sexual, mitospores: asexual)

4.3. Fungi biodiversity

Over 100 000 species have been described
They are the largest group of plant pathogens
About 100 species are regularly involved in human and animal mycoses (illness from fungi)
By a few hundreds occur as opportunists: fungi that normally would not cause infections in otherwise healthy people but are able to cause infection under certain circumstances such as immunodefficiency, cancer, organ transplant, neutropenic patients, diabetes, debilitated patients and patients on long term antibiotics

4.4. Mould spores provoking allergy

One of the most common problems to humans is allergy to fungi spores, mainly mould spores
By the contrary of pollen grains mould spores are frequent in indoor environment
Nearly twenty genuses have species that are allergenic to humans

4.5. Most common allergenic moulds

The importance of those fungi is not the same.
About one third of allergy from moulds are provoked by Cladosporium, a worldwide mainly saprophytic fungi.
The second in importance is Alternaria.

4.6. Fungi spores by origin

There are basically two great types of spores in fungi depending on their origin
Mitospores (asexual), originated only by simple divisions
Sporangiospores (Zygomycetes), spores inside a sporangium
Conidia (Deuteromycetes, Ascomycetes), spores developed by cells over a mycellium
Teliospores (Basidiomycetes), asexual spores of rusts and smuts
Meiospores (sexual), there is meiosis (meiotic division) in their formation, and permit differentiate three great groups of truly fungi
Ascospores (Ascomycetes)
Basidiospores (Basidiomycetes)
Zygospores (Zygomycetes)

4.7. Fungi life cycle

Fungi can reproduce only by asexual spores, only by sexual spores, or both, asexual and sexual spores
They are many fungi that have both types of reproduction but they have been described and named separately and now have two scientific names
The anamorph name (asexual cycle)
The teleomorph name (sexual cycle)
The correct name in this cases is the teleomorph name, that include both cycles (holomorph: teleomorph + anamorph)
Excepcionally some fungi have more than one teleomorph name

4.8. Anamorphic fungi

Many fungi are know only by its asexual cycle, they are anamorphic fungi and most of them are included inside an artificial group named Deuteromycetes or imperfect fungi
Some of these fungi were discovered that over special conditions they show their sexual cycle and most of then are Ascomycetes. Some of them are yeasts
Deuteromycetes, imperfect fungi

- Hyphomycetes: mycelium of septate hyphae, conidia, fruit bodies absent, stroma can be present
  - Moniliales (spores on conidiophores)
  - Stilbellales (spores on synnemata)
  - Tuberculariales (spores in sporodochia)
- Coelomycetes: mycelium of septate hyphae, conidia in fruit bodies (pycnidia, acervuli)
  - Melanconiales (spores in acervuli)
  - Sphaeropsidales (spores in pycnidia)

Yeasts: unicellular no hyphae, reproduction by budding

4.9. Truly fungi

Zygomycota (white moulds)
Ascomycota (morels, truffles, green and black moulds, yeasts, apple scab)
Basidiomycota (mushrooms, puffballs, boletes, smuts, rusts)
Chitridiomycota (chytrids)

4.10. No truly fungi treated by Mycology

Oomycota (Chromista) – pseudofungi (potato blight and other blight), water moulds
Mycetozoa, Mesomycetozoa (protista)
Microsporidium (protozoan)
Actinomycetes: bacteria (prokaryotes)

4.11. Fungal diseases in humans

Systemic mycosis (primary or opportunistic pathogens)
    aspergilosis, candidiasis, cryptococcosis, zygomycosis, histoplasmosis, blastomycosis
No systemic mycosis: superficial, cutaneous and subcutaneous infections
    Tinea, dermatophytes (richophyton, Microsporum, Epidermophyton)
Allergy to fungi (spores, propagules),
Mycetisms: mushroom poisoning
Mycotoxicosis: poisoning associated with exposures to mycotoxins

4.12. Common fungal diseases in plants

Powdery mildews (Ascomycetes, Erysiphales, Oidium)
Rust (Basidiomycetes, Puccinia)
Smut (Basidiomycetes, Ustilago)
Late blight (Oomycetes, Phytophthora)
Fusarium wilt disease (Ascomycetes)
Apple scab (Ascomycetes, Venturia)

4.13. Mycotoxins (characteristics)

Toxic secondary metabolite produced by fungi
Their productions depend on the environmental conditions
Their function is not yet clearly known, some have antibiotic effects, some act as allergenic
Can appear in the food chain as a result of fungal infections of crops, either by being eaten directly by humans, or being used as livestock feed
Mycotoxins greatly resist decomposition and could remain in food chain
Even temperature treatments (cooking, freezing) do not destroy mycotoxins
There are regulatory limits for mycotoxins (legislation)

4.14. Mycotoxinis (types)

Aflatoxins (Aspergillus sp., some are potent carcinogen), in nuts
Ochratoxin (Penicillium and Aspergillus sp.), in coffee, wine, raisins
Patulin (Penicillium expansum), in apple juice
Sterygmatocystin
Satratoxins (Stachybotrys)
Fusarium toxins (Fusarium sp.): T-2 toxin, vomitoxin (DON Deoxynivalenol), zearalenone, diacetoxyscripenol, fuminosins
Trichothecenes (Trichoderma), in cereals
Ergot alkaloids (Claviceps)

4.15. Mycotoxins effects

Immune supression
Carcinogenic
Nephrotoxic
Idiopatic pulmonary hemosiderosis
Dermal toxicosis
Gastro-intestinal disturbances
Parasynpathetic nerve system
Haemorrhage of liver
Hepatic necrosis
Oestrogenic
Haemorrhage of the lung and brain
Tremorgenic activity

4.16. Sources of mycotoxins

- Food
    - Primary (contaminated food by fungi)
        - Fruits and vegetable not well preserved
        - Cereals and nuts in bad storage conditions
        - Spices, etc. from tropical countries
        - Wine, juices, beverages, etc elaborated with contaminated sources
        - Out-of-date packaged food
    - Secondary (accumulated in meat)
        - Sheep, cattle, pigs, chicken fed with contaminated food
- Indoor environment (buildings)
    - Dampened and flooded homes
    - Food warehouses or grocers

4.17. Fungus growing requirement

For growing fungi require this factors:
Moisture, relative humidity over 60% is necessary for fungus grow
Temperature includes a wider variation: 4-38ºC, from psycrophilic fungi to termophilic ones
Organic matter is necessary, although sometimes in few amounts
And obviously there must be a source of spores or propagules

4.18. Moisture requirements

Depending on water requirement there are Xerophilic, Mesophilic, Hydrophilic fungi

4.19. Methods of study for airborne fungi

Airborne sampling propagules
- spores, hyphae, sporangia

No viable methods
- propagules captured on a sticky surface

Viable methods (Petri dishes)
- propagules grow developing colonies
- different culture (growing) media
  - SDA Sabouraud Dextrose Agar, MEA Malt Extract Agar, PDA Potato Dextrose Agar
- different growing temperature (25, 27º)

4.20 Common airborne fungi spores types

Deuteromycetes (conidia)
- Cladosporium, Penicillium, Aspergillus, Alternaria, Botrytis, Fusarium, Curvularia, Stemphyllium, Drechslera

Ascomycetes (ascospores)
- Venturia, Leptosphaeria, Pleospora, yeasts

Basidiomycetes (basidiospores, teliospores, uredospores)
- Coprinus, Agaricus, Ustilago, Puccinia

Oomycetes (sporangia)
- Peronospora

4.21. Cladosporium

Often extremely abundant in outdoor air.
Commonly found on living and dead plant material. Some species are plant pathogens.
Rarely pathogenic to humans (but have been reported to cause infections of the skin and toenails, as well as sinusitis and pulmonary infections. If left untreated, these infections could turn into respiratory infections like pneumonia).
The airborne spores of Cladosporium species are significant allergens, and in large amounts they can severely affect asthmatics and people with respiratory diseases.
Cladosporium species produce no major mycotoxins of concern, but do produce volatile organic compounds (VOCs) associated with odours.

4.22. Aspergillus

Cosmopolitan and ubiquitous fungus found in nature.
It is commonly isolated from soil, plant debris, and indoor air environment.
Around 20 species have so far been reported as causative agents of opportunistic infections in man.

4.23. Penicillium

Widespread and are found in soil, decaying vegetation, and the air.
They are commonly considered as contaminants but may cause infections, particularly in immunocompromised hosts.
Additionally to their infectious potential, Penicillium species are known to produce mycotoxins.
There are occasional causes of infection in humans and the resulting disease is known generically as penicilliosis.

4.24. Alternaria

Cosmopolitan, commonly isolated from plants, soil, food, and indoor air environment.
Alternaria alternata is the most common isolated from human infections.
Some species have emerged as opportunistic pathogens (particularly in patients with immunosuppression., it colonizes the paranasal sinuses, leading to chronic hypertrophic sinusiti
It is one of the causative agents of phaeohyphomycosis, onychomycosis, sinusitis, ulcerated cutaneous infections, and keratitis, as well as visceral infections and osteomyelitis
It is among the causative agents of otitis media in agricultural field workers)

4.25. Drechslera

Mainly from soil and plants
Many of the species in this genus are plant pathogens
The spore type include Helminthosporium, Bipolaris, Exserohilum, that has been reported to cause human diseases

4.26. Fusarium

It is widely distributed in soil and in association with plants.
Most species are harmless saprobes and are relatively abundant members of the soil microbial community.
Some species produce mycotoxins in cereal crops that can affect human and animal health if they enter the food chain.
The main toxins produced by these Fusarium species are fumonisins and trichothecenes.
Some species may cause a range of opportunistic infections in humans.
(In humans with normal immune systems, fusarial infections may occur in the nails (onychomycosis) and in the cornea (keratomycosis or mycotic keratitis).
In humans whose immune systems are weakened in a particular way (neutropenia, i.e., very low count of the white blood cell type called neutrophils), aggressive fusarial infections penetrating the entire body and bloodstream (disseminated infections) may be caused by members of the Fusarium solani complex, Fusarium oxysporum, Fusarium verticillioides, Fusarium proliferatum and rarely other fusarial species)

4. 27. Botrytis

Isolated from decaying plants.
No infections due to *Botrytis* have been reported in humans or animals.
Facultative pathogen in plants.
Botrytis cinerea is a necrotrophic fungus, known as grey mould or gray mold. It is economically important on soft fruits such as strawberries, grapes and bulb crops. Unlike wine grapes, the affected strawberries are not edible and are discarded.

4.28. Leptosphaeria

It is often the most abundant ascospore during rainy weather.
They are known to produce positive skin-prick test reactions.
It is an occasional cause of human infections.
Leptosphaeria spp. are the among the causative agents of human mycetoma and phaeohyphomycosis

4.29. Venturia

Species in the genus are plant pathogens.
V. inaequalis cause the apple scab, anamorph of *Fusicladium dendriticum*

4.30. Pleospora

Teleomorph of Stemphyllium and Phoma
Plant pathogen, mainly on fruits as apples

4.40. Yeasts

They are non filamentous fungi except in specific conditions.
Some of then are very useful as baking by fermentation
Some species are opportunistic pathogens and they can cause infections (Cryptococcosis, Candidiasis)

4.41. Coprinus

Genus of mushrooms with lamellae autodigested to release their spores.
Elliptic basidiospores in black colours and with a porus

4.42. Agaricus

Include many mushrooms with similar basidiospores
light brown with a porus

4.43. Puccinia

Different kind of spores are found: uredospores, teliospores, basidiospores
Many of the species in this genus are plant pathogens (parasites): rust
Great econocomic importance becuase cause damage to cereal crops

4.44. Ustilago

Mass of black teliospores cover mainly grasses inflorescences.
Ustilago is a genus of smut fungi parasitic on grasses.
Pathogenic role of Ustilago in humans is unclear.

4.45. Rhyzopus

Found in soil, decaying fruit and vegetables, animal feces, and old bread.
They are common contaminants
They are also occasional causes of serious (and often fatal) infections in humans (zygomycosis).

4.46. Peronospora

Genus of plant pathogens, belonging to the class of water moulds.
Water moulds are not true fungi
The Phytophthora group is a genus that causes diseases such as dieback, late blight in potatoes (the cause of the Great Hunger or Potato Famine of the 1840s in Ireland and other parts of Europe)

4.47. Outdoor airborne spore concentration

These data are for Badajoz
Cladosporium is the most abundant spore type in air everywhere, monthly average maxima is more than 4000 conidia/m3, in October
Basidiospores are the second, also in October, about 800 spores/m3.
Some fungi spores are more frequent in summer: Alternaria, Botrytis, Ustilago, Drechlera, Torula, and also fungi Hyphae

4.48. Indoor airborne spore concentration

Data are from the main regional hospital. Indoor and outdoor analysis was done.
There is a close relationship between outdoors and indoors in Cladosporium and Alternaria, but not for Aspergillus-Penicillium
Indoor spore concentration of Aspergillus-Penicillium conidia can rise 200 spores/m3, but the colonies formed are fewer than 20 per cubic meter of air.

5.1. Applications

5.2. Index

5.3. Airborne pollen and allergy

There is a strong relationship between airborne pollen concentration and allergy.
A Japanese study (done in 1999 with Durham sampler) shows a close significant correlation between airborne pollen concentration of Cupressaceae and mean symptoms scored.
Patients kept a record of their nasal and eye symptoms they experienced.
This is a single example that suggests the importance to avoid exposure to pollen.

5.4. Variation of allergy (geographical variation)

Many studies have reported the prevalence of allergy sensitization using skin prick tests.
However, comparisons between studies and between regions are difficult because the number and the type of allergens tested vary widely.
In the study shown the geographical variation in 15 developed countries of sensitization to environmental allergen was measured by skin tests o nine common aeroallergens: Dermatophagoides pteronyssinus, timothy grass, cat, Cladosporium herbarium, Alternaria alternata, birch, Olea europea, common ragweed and Parietaria judaica.
There was substantial geographical variation in the prevalence of sensitization to each of the nine allergens tested and in the prevalence of sensitization to any allergen.
Sensitization to grass pollen were usually the most prevalent pollen sensitization.
This geographical variation is due, in part, to the geographical variation of pollens sources.

5.5. Temporal variation of pollen concentration (yearly variations)

There is also a temporal variation of pollen concentration.
Variation between years (yearly variation)
Variation between days of one year (daily variation or seasonal variation).
Variation between hours of one day (hourly variation).
Most of this temporal variation is a consequence of weather (meteorological) variation.

5.6. Pollen monitoring networks and media application

Asthma and hay fever (seasonal rhinitis) is the cause of an important loss of working hours and impact on the medical services.
Awareness of pollen concentration can permit suffers to modify their habits and subsequently reduce their effects or even get a better administration of its medicines.
As there is a geographical and temporal variation in pollen concentration national pollen monitoring networks has been developed in many European countries.
Each national network receives and collects pollen data by the pollen counters throughout the season European Aeroallergy Network (EAN) in Vienna (Austria) act as centre collecting this data
The most important application is the provision of allergenic and spore data to the media: press, radio and television networks.
Research into data analysis can help in predicting the onset of some pollen types release.
The application of pollen curves from different regions in the temperate zones, is being employed to produce predictive computer models

5.7. Indoor Air Quality

We spend most of our time indoors (80-90%), and indoor air is no absent of aerobiologic particle.
The indoor airborne spore concentration is about ten times the concentration of pollen grains.
It seems that there is a relationship between the features of houses and moulds growing indoors
The study examined 242 houses in Izmir (west coast of Turkey with a mild climate).
The moulds most commonly isolated were Aspergillus, Penicillium, and Mucor spp.
- Aspergillus growth in houses older than 20 years was more common than other species when the features of houses and isolated fungi were compared.
- Penicillium grew more frequently in houses where visible mould was present and birds were bred.
- Mucor grew significantly more in houses where the air was humid, the temperature was cooler and there were pot plants.

Mould growth is much affected by many conditions and the environment in a house is one of many factors that may facilitate growth.

5.8. Fungi presence at home

Because fungi in the indoor environment strongly affect not only damage to and the deterioration of building materials, but also affect human health, it is important to know the distribution of fungi within an indoor environment.
This study examined fungi in houses over a period of 1 year and attempted to produce an indoor fungal contamination map for Japanese houses.
Fungi were collected in 81 ordinary houses around the Kanto District (Japan) between 1999 and 2000.
The indoor environment in Japanese homes was classified into three areas:
- relatively wet areas (bathroom, lavatory and kitchen) with hygrophilic fungi and yeasts
- relatively dry areas (living room), where xerophilic fungi are often detected.
- areas where wet and dry parts coexist (bedrooms and closets containing futons and clothes with moisture) where both hydrophilic and xerophilic fungi, as well as yeasts, were detected.

5.9. Size of fungi spores and effects on health

There is a strong relationship between fungi spore diameter and the illness they can provoke in respiratory system.
The biggest spores (Alternaria, Fusarium, Curvularia) affects manly the upper parts.
Medium size spores (Cladosporium, Memnoniella, Stachybotrys) affects the middle.
The smallest spores (Aspergillus, Panicillium and Cladosporium) are the most dangerous because can reach alveoli.

5.10. Sampling airborne fungi in hospitals

There is a great concern about presence of fungi in hospitals.
To identify a potential source of nosocomial aspergillosis is extremely important, so, sampling is a routine but methodology is no absent of doubts.
Levels of fungal spores vary by several orders of magnitude during the course of a day due to: activity levels, fluctuations in temperature, humidity, air flow, even changes in light level. Even using (HEPA High Efficiency Particulate Air filters)
A single air sample will often underestimate the fungal contamination in the air, son multiple air sampling has to be performed.
No strict numerical guidelines are available which are appropriate for assessing whether the contamination in a particular location is acceptable or not

5.11. Environmental control

Is often accepted that building or ground removal increase airborne spore concentration.
In this study the demolition of a building next to a hospital provided the opportunity to study the load of filamentous fungi in the air.
Outdoor and indoor air was sampled at least daily during the week before the demolition, and post-demolition.
A significant increase in the colony count of filamentous fungi occurred after the demolition.
Mechanical demolition on day 4^{th} also produced a significant difference between outdoor and indoor air and the counts returned to baseline levels on day 11th.
Demolition work was associated with a significant increase in the fungal colony counts of hospital external and non-protected internal air.
Effective protective measures may be taken to avoid the emergence of clinical infections.
These findings underline the importance of environmental surveillance and strict application of preventive measures under circumstances of fungal propagule overload.

5.12. Aerobiology and climate change

The epidemiological implications with respect to climate change and public health are beginning to be acknowledged.
Less recognized however, are the potential links between climate, plant biology and public health.
This study undergoes the relationship between airborne pollen of ragweed (Ambrosia) depending on the level of urbanization: a gradient between rural and urban areas of Baltimore and the Maryland countryside (USA).
Because cities constitute a “heat-island”, and produce many of the gases (e.g., carbon dioxide, ozone) that are responsible for environmental change at regional and planetary levels, it has been suggested that cities may provide analogy for studying ecological responses to global change.
Overall, ragweed monocultures demonstrated significantly earlier flowering and greater pollen production in urban areas, relative to rural or semi-rural areas.
These data do suggest that the higher carbon dioxide concentrations and increased air temperatures associated with urbanization may be a harbinger of what could be expected with respect to pollen production and allergic rhinitis / asthma with global climate change.

5.13. Allergy trends

In recent decades, a large number of epidemiological studies investigating the change of prevalence of hay fever showed an increase in the occurrence of this disease.
However, other studies carried out in the 1990s yielded contradictory results.
Many environmental factors have been hypothesized to contribute to the increasing hay fever rate
Epidemiological data for hay fever in Switzerland are available from 1926 until 2000
This allows an investigation as to whether these data are correlated provided the same time spans are compared.
It is shown in this paper that the pollen exposure has been decreasing in Basel since the beginning of the 1990s whereas the rate of the hay fever prevalence in Switzerland remained approximately unchanged in this period but with a slight tendency to decrease.

5.14. Crop pollination in olive trees

Reliable crop volume estimations are an increasing necessity to allow optimised and effective agronomic management.
It is possible to evaluate future olive crop yield several months in advance.
Olive tree phenology, airborne pollen concentrations, meteorological data and fruit production data were analysed in the province of Córdoba (Andalusia, Spain) over a period of 20 years (1982–2002).
Data were integrated to obtain models for predicting fruit production.
In this study, annual Olea pollen emission is shown to be a reliable bio-indicator to forecast olive fruit production up to 8 months in advance.
Hirst volumetric pollen traps were found to be an accurate tool in olive crop yield forecasting.

5.15. Crop pollination in cereals

It is possible to establish a relationship between grass pollen production and cereals yield, although pollens from cereals plants are not likely airborne
This relationship can therefore only be understood at the level of the physiological relationship between the status of the wild grasses and that of the cited crop species, with the expectation that factors favouring the pollination of the former will favour the fruit-set of the latter.

5.16. Crop pollination and GM organisms

One aspect that most concern in food is Genetic Modify organisms, (GM) as some corn crops.
Corn is a dioicus plant, with big pollen grains, and many farmers are worried about the possible contamination of their crops from close GM crops.
It is estimated that about 5% of the corn pollen released by a source could be dispersed at least 60 meters by wind.
It is likely that this percentage would be higher under convective atmospheric conditions and that a significant portion of the pollen remaining airborne could be dispersed by wind much farther.
So to avoid possible contamination with pollen from GM plants crops must be separated at leas 100 meters.

5.17. Crop disease epidemiology

Alternaria represents an important part of the pathogenic fungus in potato crops.
The objective of this work is to determinate the aerial concentration of Alternaria conidia over a potato crop in A Limia (NW Spain), the main crop on this county.
Alternaria represents an average of 1.9–3.1% of the spores collected, after the Cladosporium type (which represented 80% and 64%).
A Hirst volumetric sampler was used in order to determine the airborne concentration of Alternaria conidia over a potato crop and finally to try to establish the most suitable prediction models for Alternaria attacks and how they might be controlled.
In the first year of study the efficiency of this method was lower as alternariosis did not proliferate to the same degree due to the adverse meteorological conditions registered for the development of the fungus.

5.18. Biodeterioration

Biodeterioration can be defined as the irreversible loss of value and/or information of an object of art following the attack by living organisms
Aerobiology can provide very useful information for conservation of works of art
It is possible to identify sources, access flow and areas of major accumulation of airborne microorganisms, including their time variations (daily, seasonal)
Mural paintings, stone, wood, metals, other raw material to develop manufacture products, monuments and buildings, can be damaged by microorganisms airborne transported.

5. 19. Organisms that promote biodeterioration

The problem of deterioration of works of art is particularly relevant in Countries like Italy that are rich in Cultural Heritage.
Many kind of organisms can biodeteriorate these materials: bacteria, fungi, algae, lichens, a mosses.
Fungi are among the most harmful organisms associated to biodeterioration of organic and inorganic materials, this mean a wide range of materials

5.20. Fungi biodeterioration in libraries

Fungi in libraries can attack over: paper and cardboard, leather, parchment, inks, adhesive substances of animal or vegetal origin, synthetic materials, tissues, wax seals, photographies, and magnetic tapes.
A total of 23 genus are listed in the table, all of then easily found in airborne samples

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