

conventional staining methods, this leads to images of higher contrast, thereby enabling optimum description of pollen and spore morphology.

In order of obtaining suitable samplers for testing the staining capacity of Pyrogallol red and its application to fungal spores, a Portable Air Sampler (BPS) for agar plates by Burkard was used in different environments for obtaining a broad spectrum of spore types. For that purpose, dextrose sabouroud agar was the culture medium of choice. After mycelial development and sporulation, both hyphae and spores were mounted in two different ways. On the one hand, the samples were mounted using unstained glycerine jelly as control. On the other hand they were stained with Pyrogallol red previously to being mounted with glycerine jelly.

The results obtained for the different spore types are presented and discussed by means of microscopic slide images of samples taken from the agar plates.

The limitations of aerobiological surveys of fungal spores

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Traditionally, aerobiological surveys are mainly looking at the qualitative and quantitative presence of pollen grains in the atmosphere. The spectrum and diversity of airborne pollen types is limited, the range of (aerodynamic) sizes is rather small, and morphological (microscopical) identification is not extremely difficult. This makes it possible to train people, in one week, to make an almost complete qualitative (pollen types) and quantitative (number/m³) estimation of airborne pollen, as is experienced by the six European Courses in Basic Aerobiology (ECBA).

Contrary to this, the situation with the assessment of airborne fungal spores is far more complicated and difficult. The number of atmospheric spore types is very big, the (aerodynamic) sizes of the spores show a large range, and reliable morphological identification is possible only for a minority of spore types. For a more complete identification, growing colonies from sampled (clumps of) spores is a room and time consuming option, often not compatible with continuous sampling. This means that aerobiological surveys on fungal spores must be restricted to a limited number of spore types and/or to discontinuous sampling procedures. In training courses, people should be made aware of the qualitative and quantitative limitations of aerobiological surveys on fungal spores.

Also, the evaluation of publications of these kinds of surveys must consider these limitations.

Session c3

FORECASTING POLLEN

The reliability of pollen forecast models: their accuracy and problems

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Nowadays Aerobiology is being used as a tool to quantify pollen emission and therefore intensity of flowering in anemophilous plants. This subject has a close application to different disciplines such as Allergy, Agronomy and Ecology. One of the most important goals for an Aerobiologist is to produce forecast models.

Currently two main types of pollen forecasts are provided: long-range forecasts predict the main features of the pollen season, and medium and short range forecasts predict weekly or daily variation once the

season starts. As regards long term forecast, one of the most important features to be predicted is the pollen season start (GARCÍA-MOZO et al., 2002). Chilling Units, Heat Units, Photoperiod, and even rainfall in herbaceous plants during the months prior flowering are usually the most affecting parameters. The importance of these factors will vary depending on the climatic areas. Another important pollen season feature is the severity. In this case, in the Mediterranean Region severity firstly depends on rainfall during the month prior to pollen season start and secondly, on temperature. This behaviour may also vary depending on the climatic and geographical region. To produce these models a long database is needed. They are advantageous and their accuracy is rather high, even they work at local level.

As regards the medium-short term forecast, it is important to consider the influence of the seasonal meteorological parameters. When long series of pollen are available, it is interesting to make a classification of the years and to obtain a model for each different year-class. Most of the researches use regression analysis to produce forecasts but currently other analysis are being used, such as neural network models (SÁNCHEZ-MESA et al., 2002). These models are more complex than the previous ones, and the accuracy is lower. In a similar way, they work at local level. The new statistical tools improve the results, however the assistance of computing engineers is required.

On the other hand, depending on the reproductive phase of the phenological cycle to forecast, either diurnal or night-time temperatures will be the most affecting variables. For example, maximum temperature related with sunlight hours, is usually an important parameter at the beginning of the floral development in spring-flowering trees. It is because the tree is capturing energy through the photosynthesis. However, at the end of the flowering and the start of fruit development, minimum temperatures are usually more important, due probably to the tree captures the energy from the store organs (WIEGOLASKI, 1974).

In recent time, an effort to produce regional forecast is being made. For these new models it is needed to include other variables such as phenological and topographical data, and land use databases. Within the ASTHMA European project, an automatic system for pollen forecast in the Mediterranean Region was developed. The forecast system included one atmospheric physics module, one meteorological module, and three biological models applied to other computational modules, the blossoming, the pollen emission and the dispersion models. Finally, the combined use of Geographical Information Systems (G.I.S.) and Geostatistics using all these type of data allow us to produce regional forecast maps by using lineal interpolation for those places where there are not available data. These last models allow us to produce a forecast at regional level, with a high degree of accuracy due to the variety and number of parameters used on it. However, a disadvantage is that the auxiliary tools (land use maps, meteorological simulation models, etc) are not always available.

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SÁNCHEZ-MESA, J.A., C. GALÁN, J.A. MARTÍNEZ-HERAS, & C. HERVÁS-MARTÍNEZ. 2002. The use of an neural network to forecast daily grass pollen concentration in a Mediterranean region: the southern part of the Iberian Peninsula. *Clinical and Experimental Allergy*, 32:1-7.

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WIEGOLASKI, F.E. 1974. Phenology in Agriculture. In LIETH & SCHWARTZ, *Phenology in seasonal climates* Lvol. 8, pp. 369-381.

Forecasting the aerial transport of soybean rust spores (*Phakopsora pachyrhizi*) to North America

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Aerial movements of biota occur at a variety of spatial and temporal scales. Consequently, operational aerobiology transport models should be constructed in a flexible manner to provide accurate forecasts over a corresponding range of scales. The Integrated Aerobiology Modeling System (IAMS) that combines two previously applied model types within a well-tested design framework is presented and applied to forecast the aerial transport of soybean rust spores to North America. The IAMS uses a "parcel-box model" approach, combining transport (parcel) models and local, in situ (box) models. In the example system, the aerial dispersal of

soybean rust is described in a set of iterative steps from surface to air to surface. The pathogen is first affected by the environment at the earth's surface prior to its escape from a canopy, secondly, while airborne, by atmospheric conditions during transport, and then, after deposition, once again by the surface environment. The pathogen can be envisioned as developing in a terrestrial habitat or "box" having well-defined environmental conditions, and traveling in another volume or "parcel" having well-defined atmospheric conditions. In this modeling system, an organism can be subjected to varying surface and atmospheric conditions as it moves generation-by-generation in and out of boxes and parcels, at a wide range of spatial or temporal scales.

D-media, a new parametre to be used in forecasting models

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Many aerobiological works in which short or long-term forecasting models are developed have been published. Commonly, different meteorological parameters are used as predictive variables. Some models also include the one-day-before pollen concentration or indexes obtained after recombining several parameters.

Due to most meteorological centres only facilitate predictive information about temperatures and rainfall, in many occasions variables that hardly will be obtained in advance are included in the equations, such as wind speed, sunshine hours or relative humidity. So, we could distinguish between useful and no useful data.

Many authors have pointed out the importance of floral phenology in the behaviour of pollen curves, but it is difficult to introduce this parameter in an equation as predictive variable. A useful data that we can obtain in advance and use in forecasting models is that we have so-called "D-media" or "date-media". This is the mean value obtained for the concentrations of a pollen type during several years for the same date of the calendar, which is a way to introduce, with figures, the pollination phenology of the different taxa observed during years of study. For softening this value we can even use running media.

In this work we have developed forecasting models for the airborne pollen of *Olea europaea* and Poaceae in Malaga (southern Spain), the more important taxa from an aerobiological point of view. For this, together with a correlation study, stepwise multiple regressions have been made in which, besides meteorological parameters, one-day-before concentrations and D-media have been used as predictive variables. The data correspond to the years 1992-1999, both inclusive, 2000 being reserved in order to test the fitting between observed and predicted data.

The highest correlation coefficient values were obtained between pollen concentrations and one-day-before pollen concentrations, followed by D-media. They were significant for $p \leq 0.001$. Regarding stepwise multiple regressions, the predictive variables were taken in the following order: one-day-before pollen concentration and D-media, followed by relative humidity and sunshine hours in the case of Poaceae and by 4th quadrant wind frequency and maximum temperature in the case of *Olea europaea*.

Airborne-pollen map for *Olea europaea* L. in eastern Andalusia (Spain) using GIS. Prediction models

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Geographic Information Systems are computer applications that have enabled advancement in the study and analysis of geographic phenomena by incorporating solid analysis tools and facilitating the statistical treatment of large spatial databases. The application of these new techniques in Aerobiology enable optimal management of information provided by aerobiological networks through the different communication media.

The seasonal evolution diagrams that are currently generated in each sampling station offer highly precise information but lack a spatial dimension in which to record the variations in airborne pollen on a regional

scale. To offer regional information through the aerobiological information networks, we have drawn maps of seasonal evolution for pollen from the olive tree (*Olea europaea* L.) while simultaneously introducing the variable "space" and "time". Furthermore, the application of classical predictive models will enable simulations of pollen dispersion in future periods.

The basic methodology used for the mapping the airborne-pollen was geostatistical estimation which, from a dataset such as the stations of aerobiological monitoring located in geographical space, enabled us to estimate the values corresponding to places lacking a sampling station but which were included in a given spatial domain. In this context, Alba et al. (2003) proposed the formulation of three-dimensional coordinate systems that, together with classical geostatistical analyses, enabled the construction of spatio-temporal models of pollen dispersion. Finally, the use of thematic digital cartography provided airborne-pollen maps with a high degree of reliability.

One of the results derived from the aerobiological information provided by each of the monitoring stations was the mapping of pollen isolines for the olive tree in eastern Andalusia. The pollen maps drawn bear reliable information concerning the onset of olive pollination according to geographic distribution, the estimation of the critical period of maximum pollen release, area of influence of pollen clouds, and the determination of the end of the pollen season in altitudinal clisere.

In the second phase, we show the results after applying classical predictive models based on *multiple stepwise regression* at the sampling points considered. The previous values from the series of pollen and meteorological parameters were taken as independent variables. The percentage of the seasonal dynamics of the olive tree which can be forecast exceeded 40% in all cases. Finally, estimation by interpolation enabled simulations of dispersion of this pollen in future periods, with an acceptable error.

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The reliability of geostatistic interpolation in olive field floral phenology

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Most of natural phenomena shows, as a inherent feature, a high degree of spatial continuity (MORAL GARCÍA, 2003). Interpolation by using lineal geostatistic analysis allow us to estimate phenological data of wide areas in base of a limited number of samples. Given the economic and timing cost of taking field data from faraway sites it is needed to optimise the number and location of sampling points and the number of the observed plants chosen in each place.

A total of 128 olive trees (*Olea europaea* L.) grouped in 19 sampling sites were observed during all the pre-flowering and flowering period of 2003 (March to July). All the sites are distributed trough extended olive crop area, around a transect from the city of Córdoba (37° 55'N, 4° 45' W) up to the Subbetica mountains (37°26'N, 4°11'W) in the south-west of the Province. The information about the olive crop distribution has been obtained by mean of Geographic Information Systems (GIS). The program Geostatistic for the Environmental Sciences® (GS+) was the geostatistic software used. The demarcated studied area was interpolated to a rectangular grid of 1675 km² (67x25).

Space models were constructed using three co-ordinates, x, y and z. were x and y are the UTM coordinates and z represents the phenological data. Numerical floral phenology data, altitude, cumulated chilling hours below 7.2°C, cumulated Growing Degree Days^o, cumulated sunshine hours from the previous week, and cumulated rainfall were the parameters used for geostatistic interpolation.

The shape of the average pollen olive curve from the city of Cordoba shows three main peaks that clearly reflect the phenology of the different areas located at different altitude (GALÁN et al., 2003). In the present work authors analyse the reliability of applying lineal geostatistic for the interpolation of olive floral phenological data. The main prior hypothesis is that 7 sampling points (with 10 olive trees in each site) uniformly distributed at different altitudes through the main olive crop areas would be enough to interpolate the floral values in order to create computerized geostatistic phenological maps.

Previously to create the interpolation maps the isotropic variograms were analysed by comparing the semi-variance confronted with the distance among samplings to detect the degree of autocorrelation. Obviously

the degree of autocorrelation was higher between the olive trees situated at the same sampling point, therefore an average phenological value from each site was included in the kriging interpolation analysis in order to obtain a direct estimation (KITANIDIS, 1996). Preliminary analyses give us reliable results of the interpolation maps based to the 19 samplings points, although a deeper analysis must be done to determine the distribution of the minimum number of olive sampling points through the Subbética mountains.

The combined use of GIS and geostatistics has been demonstrated as essential partners for spatial analysis (BURROUGH, 2001). The application of those combined tools on floral phenology will allow us to optimise human and economic resources on field phenology campaigns applied to aerobiology forecasting. Moreover, an appropriate use of geostatistic software to create phenological maps will be an essential complement in pollen aerobiological studies, given the increase interest on obtaining automatic forecasting maps.

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Variability of meteorological requirements in winter-spring pollination of different arboreal species

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One of the most important aims in Aerobiological studies is to find forecast models that enable us to know the start date the pollen season. The woody plants of temperate regions have evolved mechanisms during the periods of adverse meteorological conditions in order to preserve their cells from risk of frost. The strategy involves a requirement for winter chilling followed by another of warm temperatures, to break a physiologic state of inactivity denominated dormancy. The quantification of chilling and heat requirements to overcome this period of dormancy enables us to determine the onset of pollination, which is of great importance to allergic individuals. During the last few years studies to determine chilling and heat requirements using aerobiological data have been conducted (FRENGUELLI & BRICCHI 1998; JATO et al. 2000; RODRÍGUEZ-RAJO et al. 2003). In these studies, the beginning of pollination in arboreal plants with winter flowering is more closely related to the thermal time rather than calendar time. The aim of this work is to determine temperature requirements to trigger pollination of six different species and to know as other factors, such as photoperiod, could to interfere with this process.

A 7-day volumetric pollen trap Lanzoni VPPS 2000 was used, placed 25 m above ground level on the terrace of the town hall of the city of Vigo (N.W. Spain). Data of airborne pollen concentrations from 1995 to 2004 were used to determine the start of the pollination period of the arboreal species selected: *Alnus*, *Corylus*, *Populus*, *Platanus*, *Betula* and *Olea*. Weather data were supplied by the National Institute of Meteorology, from a station located at 100 m of the sampling point.

To predict the date of the beginning of pollination, the quantification of chilling and heat requirements to overcome the dormancy period were considered. In the present study, to determine chilling requirement we chose a thermal time model (ARON 1982). Heat requirement (H.R.) was estimated as a function of the sum of the daily maximum temperatures from the end of the chilling period to the beginning of the pollen season, taking into account different threshold temperatures: $H.R. = \sum (T_{max} - threshold)$ (RODRÍGUEZ-RAJO et al. 2003). Different threshold temperatures (5.5-6-6.5-7-7.5-8-9-10-11°C) were tested for chilling and heat requirements, and the lowest standard deviation and variation coefficient were used to identify the most adequate threshold temperature, in both the chilling and forcing-temperature period.

The chilling period is close similar for all the studied species and it undertakes a short period of time of around 50 days of duration as average. A slightly higher quantity of 680 Chilling Hours are needed to satisfy the requirements of cold.

By the contrary, heat requirements present a high variability depending on the taxa considered, so much in what refers to their length as the quantity of growth degree days needed. Depending on the threshold temperature considered, in the case of taxa with early winter flowering as *Alnus* and *Corylus*, the requirements were satisfied in a short period of time with around 50 GDD°C. Trees that flowers at the end of the winter such as *Populus*, heat requirements takes place during a longer period of time and they are necessary around 200 GDD°C. Finally when the pollen season start at the beginning of the spring, these requirements rise to a quantity of 400-500 GDD°C (*Platanus*, *Betula* and *Olea*). In this case, besides temperature effect, other meteorological factors also influence the start pollen season as sun hours.

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Forecasting: A useful tool for Public Health

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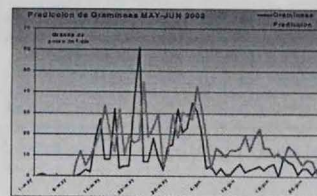
Palinocam network is a multidisciplinary organization, which has been working since 1993. The principal aim of this network is watching for the aerobiological content of air in Madrid. Ten Hirst-type volumetric samplers, Burkard spore-trap, have been working during ten years in ten different sites: three in Madrid Capital, two along Henares River, one in the north, one in the northwest, two into the south, and another one in the meridian point of Madrid (Aránguez & Ordoñez, 2001).

Daily and weekly bulletins with data and charts have been elaborated and distributed to Population, Sanitary professionals, Hospitals, Healthy centres, Scientific societies and several press media such a radio, television and newspapers. This information has been sending by email, fax, internet and telephonic information service, carrying out the Public Health Service of the Palinocam Network (Cervigón & al., 2002a).

All the aerobiological information is made with real data, so our principal effort has been to give some predictive data every day in a short term way. Pollen levels are directly influenced by meteorological parameters, so forecasting has been elaborated with the diary meteorological predictive data, so the information is changed every day.

The first forecasting model has been elaborated with Autoregressive and Mobil Average Models (ARIMA), using Poaceae pollen data and meteorological parameters temporal series of eight years (Cervigón & al. 2002b). Poaceae pollen is a very allergenic pollen type, responsible of a very high percentage of rhinoconjunctivitis (64%) and asthma (58%) in spring in Madrid (Subiza, 2001; Tobías & al. 2003). Forecasting of this pollen type has been made successfully since 2002, giving an individual bulletin every day.

Predictive Models are very accurate tools for giving more complete information to health care professionals and population, so we keep on working on them for improving the public health service that we have been offering until now (Cervigón & al., 2003).



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Continental Slovenia and Croatia as a common information area for pollen forecasting

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Pollen has been definitely proven as one of the most potent allergens. Allergenic plants are characteristic for particular areas, depending on the geographic-climatic zone and vegetation. Pollen concentrations of such plants depend on particular species phenological stage and weather conditions in the area. Providing preventive information on the occurrence of pollen allergens in the residential and industrial areas is crucial for allergic individuals to improve the quality of their lives. More over, modern human life style and population migration allows pollen allergens to aggressively spread, and this is actually a macro-regional problem.

Due to these reasons a lot of European countries conduct pollen monitoring on their and neighbouring territory. That was the reason why we started a bilateral project "Continental Slovenia and Croatia as a common information area for aeropalinological researches". The project started at the beginning of 2003 and we now have first results.

We are compiling aerobiological researches of the similar climatic-phytogeographic area of the continental parts of Slovenia and Croatia. Specific attention was devoted to the most invasive aeroallergen *Ambrosia artemisiifolia*. From the beginning of the project we collected pollen at four stations in Slovenia (Ljubljana, Hrasce, Zalec and Maribor) and four in Croatia (Samobor, Zagreb, Ivanić Grad and Osijek). Reports about pollen appearance were made monthly and after the pollen season we prepared yearly reports and diagrams for the researched area, which will be presented here. So far we can conclude that the most important allergenic plants in continental area of Slovenia and Croatia are *Betula*, *Corylus*, *Urticaceae*, *Cupressaceae*, *Poaceae*, *Alnus*, *Ambrosia*, *Fraxinus*, *Quercus*, *Carpinus* and *Castanea*.

The project is ongoing and we expect it will help in prevention of the symptoms of allergic reactions in individuals with regard to pollen hypersensitivity and in the future in forecasting the specific pollen season in this region.

From Pollen Counts to Allergy Risks or Meaningful Aerobiology

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Even if there are now standard methods and techniques to collect and measure the atmospheric pollen content, the way this content is then reported as so called "pollen counts" is at best confusing and at worst misleading. Indeed, most pollen reports will differ when they are presented by different groups, even if they are coming from the same region. Pollen counts are "transformed" into risk by rules of thumbs that have no scientific basis what so ever. Moreover, there are a lot of factors that can explain these differences different regions are considered: phytogeography, phenology, allergenicity. In this regard, we would like to propose a multi-faceted methodology that would try to make sense of all these factors. First, instead of having risk reports linked to raw counts, these should be modulated by the different species. Then, the site representativeness should be taken into account. Finally, a statistical estimate of the risk, as compared to the average expected for one region, should be taken into account. This can be based on the Main Pollen Season Average Minimum (MPSAM), Mean Pollen Season Mean (MPSM), Minimum Yearly Maximum (MYM), Maximum Yearly Average (MYA). Examples from Canadian and Swiss sites will be exposed. From these standards, it should be possible to obtain comparable risks from different settings.

Session c4

POLLEN AND FUNGAL SPORE DISPERSAL / LONG DISTANCE TRANSPORT

Long distance transport of allergenic pollen – a common phenomenon?

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Long-distance transport (LDT) of biological particles is well known everywhere; for instance, phenomena called 'yellow snow' or 'yellow rain' containing foreign pollen from distances of several thousands of kilometres have been reported repeatedly. Pollen from far origin have lost their protein content, the grains are empty and therefore not allergologically important. However, pollen from 1000 km distance has been shown to retain the potency and might thus also cause allergic symptoms. Strange results in many published papers concerning the start of pollen period could find explanations in LDT pollen. Early separate peaks in several pollen calendars certainly reason in LDT and not only in abnormal meteorological conditions as often suggested. The LDT phenomenon is not easy to study if no local phenological observations exist. Extreme geographical areas like Alaska or Lapland in the North, or respectively, Southern Spain or Italy are natural LDT study areas. When non-local pollen (i.e. marijuana pollen in Spain or pollen of southern herbs in Alaska) occur, LDT is the obvious explanation. To find out the origin of foreign pollen, trajectories of air currents backwards are necessary.

In allergological point of view LDT pollen is especially important at northern latitudes, where high frequencies of foreign pollen often occur 2-3 weeks before the beginning of local flowering. Global warming has been reported to induce generally an earlier start in flowering of allergenic plants in Europe, but results concerning the northernmost areas of the continent have been contradictory. Instead, LDT pollen in general has increased and changes in air currents due to climate change is one of the suggested explanations. The separation of LDT pollen from local production was studied at Kevo, Finnish Lapland. Two approaches were used to mark the beginning of pollen season: cumulative degree days (d.d.), and the date of anthesis based on phenological observations. Using