

other biogenic particles. Pyrogallol red sodium molybdate buffer is commonly used for the quantitative determination of total protein in human urine or cerebrospinal fluid, using manual or automated procedures. This test is based on the procedure developed by WATANABE *et al.* (1986) which is a dye-binding colorimetric method utilizing a pyrogallol red-molybdate complex modified to equalize the reactivity of albumin and  $\gamma$ -globulin, thus providing good precision and linearity. The pyrogallol red combined with molybdenum acid forms a red complex (max. absorbance at 467 nm). When this complex is combined with protein in acidic conditions, a blue-purple color develops (max. absorption at 598 nm). VÖLKER *et al.* (1996) reported on the application of a pyrogallol red-vanadium complex for protein staining in animal tissues and cell cultures.

After staining with a modified pyrogallol red-metal complex (0.02 % in 30 % isopropyl alcohol, pH 2.2, light-sensitive), microscopic observation results of different types of native pollen (*Betula*, *Corylus*, *Gramineae*, *Artemisia*, *Pinus*, *Taxus*) collected between 1998 and 2003 as well as material from 2003 pollen monitoring are presented. Furthermore, staining intensities of layers of the pollen exine, intine and cytoplasm are compared. The histochemical reaction of the dye (red-purple to shining blue) in the layers promise improved morphological differentiation due to contrast enhancement and allows visualisation and recognition of protein content and maybe allergenic activity.

For staining pollen, fuchsin or safranin are common dyes applied with glycerine jelly as embedding medium. As pyrogallol red is characteristically staining (glyco)proteins, as a consequence, even protein containing parts of pollen disrupted or damaged by soot and crystal particles (sand) or fungal spores can be visualised. Compared to conventional dyes, the visual differentiation between pollen layers results in images of higher contrast and improved display of pollen morphology, which is particularly valid for the boundary areas of the cytoplasm.

Lactic acid is a widely used mounting fluid in fungal microscopic examination, often used with a dye (cotton blue, aniline blue, trypan blue). For myxomycetes, ascomycetes, rusts and smuts Shear's mounting fluid (potassium acetate) is commonly used. They all stain hyphae and hyaline fungal spores, but with pyrogallol red pigmented hyphal fragments (conidiophores) and spores are stained. Effects of glycerine jelly (Lanzoni), water activity and pH on colour spectra (ranging from purple to blue) and colour intensity are described.

The results of this work confirm the use of pyrogallol red staining solution as a biological indicator in terms of (glyco)protein detection and as an innovative contribution to the identification of both pollen and fungal spores in aerobiological monitoring. In addition to its use in conventional pollen monitoring and detection, the dye allows to display a correlation of staining intensity with the protein content. While protein staining techniques are becoming faster, easier and more sensitive through chemiluminescence and (chemi)fluorescence, traditional reagents such as Coomassie blue and silver stains are still regularly used for certain applications. Among these, pyrogallol red has promising potential to contribute to the validation of the correlation between the allergenic activity and antigen-antibody reactions, especially when image analysis of proteins on SDS gels or nitrocellulose membranes are applied. Furthermore, it may have the potential to value current allergenic activity directly from microscopic observation.

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### Ragweed and mugwort pollen in Szczecin, Poland

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The aim of the study was to analyse the ragweed and mugwort pollination in Szczecin in the years 2000–2003. Measurement were performed by the volumetric and gravimetric method. Pollen seasons were defined as the periods of 90% of the total catch. Ragweed and mugwort pollen are known as potent aeroallergens, often noted to enter into cross reactions. In the four years studied, the lowest concentration of ragweed pollen was observed in 2000 and equalled a few pollen grains in 1 m<sup>3</sup> per 24 h. In 2001 the highest airborne concentration of 30 grains in 1 m<sup>3</sup> per 24 h was noted at the end of August. However, the annual pollen count of ragweed in 2002

was very high, three times higher than in 2001. The pollen season started in the second decade of August and lasted till the beginning of September. The highest airborne concentration of 100 grains in 1 m<sup>3</sup> per 24 h was noted at the beginning of September on a sunny day with strong wind. Also in 2003 a high pollen count of *Ambrosia* was recorded with the highest value on Sept. 8<sup>th</sup> equal 84 grains in 1 m<sup>3</sup> per 24 h. The pollination season of mugwort started in the third decade of July and lasted to the end of August in all the years studied. The highest pollen count of this taxon of 187 p/m<sup>3</sup> was noted on July 31<sup>st</sup>, 2002. In the other years the pollen count of *Artemisia* was lower and did not exceed 140 p/m<sup>3</sup>. In sensitive persons the symptoms of pollinosis occur after some threshold pollen count value, which for ragweed is 13 p/m<sup>3</sup>, and for mugwort - 12 p/m<sup>3</sup>. Therefore, the greatest threat by *Artemisia* pollen allergens was noted in the end of July and in August and by *Ambrosia* in the end of August and the beginning of September. Analysis of aeroplankton from different Szczecin city's districts has shown that the highest exposure to ragweed pollen allergens occurs in the Majowe, which is related to the presence of numerous specimens of *Ambrosia* in that district. The pollen count of mugwort was more abundantly present in the district Zelechowa, in the area with villas and gardens or garden allotments than in the district Pomorzany characterised by closely built blocks. Statistically significant correlations have been found between the ragweed pollen count in the air and the maximum wind speed, air temperature and relative air humidity and between the mugwort pollen count in the air and air temperature and relative air humidity. The pollen count of the taxa studied is determined by the weather conditions, especially by the wind speed and relative humidity, diversity of local flora and long distance transportation.

### Session c2

#### BASIC AEROBIOLOGY/ MONITORING/ NEW TECHNIQUES: FUNGAL SPORES

##### The detection airborne plant pathogen spores using immunological assays

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Plant disease epidemics require three conditions to be fulfilled: there must be susceptible host tissue (the crop); the environmental conditions must be appropriate for the pathogen to infect; and there must be pathogen inoculum available. For many plant fungal diseases the inoculum is in the form of airborne spores. Thus, monitoring for the presence of disease causing spores could give useful information on the risk of disease developing in crops. Unfortunately, monitoring airborne disease inoculum using conventional methods for fungal spore detection is difficult. Methods based on microscopy or culturing are often slow, require skilled personnel and can be inaccurate. Recently there has been substantial interest in the development of plant disease diagnostic kits based on the use of serological methods. These methods rely on the production of monoclonal or polyclonal antibodies to detect epitopes specific to the target fungus. The technology used in such immunological assays is usually simpler to use and cheaper than methods based on PCR-assays. Such methods could be potentially exploited for use in detecting airborne plant pathogen inoculum (or other airborne biological particles).

In this paper I will review the potential use of immunological methods for detecting and quantifying airborne plant pathogen inoculum. Although immunological methods have been used to detect airborne allergens there have been few applications to plant pathogens. Immunofluorescence has been used to identify spores of *Botrytis cinerea* deposited on the trapping surface of a Burkard spore sampler. Spores were collected on a modified tape in a Burkard sampler exposed in an infected onion crop and labelled directly on the tape using a *Botrytis*-specific monoclonal antibody. Immunofluorescence has also been used to detect *Mycosphaerella brassicicola* ascospores on Burkard spore trap tapes coated with bovine serum albumin as a support medium and blocking agent. A prototype rotating-arm sampler has been designed for use with ELISA (enzyme linked immunosorbant assay) to detect and enumerate airborne spores. The trap has been tested in wind tunnel experiments using *Alternaria brassicae* conidia and a monoclonal antibody against germinating conidia of *A. brassicae*. A new particle-trapping device, the MicroTiter ImmunoSpore Trap (MTIST), for the rapid detection of

fungal air spora by immunoassay, has been developed by the Burkard Manufacturing Co., Rickmansworth, UK. The device uses a suction system to collect airborne particles direct in microtiter wells. The potential of this device has been demonstrated by using it to sample airborne spores of *M. brassicicola* and *B. cinerea* and detected them using ELISA. The trap has been characterised in wind tunnel tests, and it has been shown that the choice of coating for the microtitre wells may be important, depending on the target spore type.

It is clear that immunological techniques have the potential to play a significant role in the detection and monitoring of airborne plant pathogen inoculum provided that suitable antibodies are available. However, much work is still needed to develop rapid, reliable and easy to use sampling and assay systems suitable for routine use. The paper will, therefore, also consider how such devices could be developed in the future to provide new tools for use in disease risk assessment systems.

### The contribution of leaf-surface fungi to the air spora

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High concentrations of airborne fungal spores frequently occur from spring through fall in temperate areas of the world. Although it is generally assumed that fungi on leaf surfaces are contributors to the air spora, little data are available comparing the types of fungi found on leaf surfaces with those in the atmosphere. The present study was undertaken to address this comparison.

Air sampling was carried out with a Burkard Spore Trap located on the roof of a building on the University of Tulsa campus. The sampler was set for 7-day sampling onto Melenex tape that was coated with a thin film of Lubriseal. Each week the drum was changed and the tape cut into one-day segments. These were stained with glycerin-jelly mounting medium and examined microscopically at 1000X. Results were expressed as spores/m<sup>3</sup>. Leaf samples were aseptically collected from *Ulmus americana* and *Quercus palustris* trees on campus, placed in sterile plastic bags, and brought to the lab. Each week three leaves were collected from the lower canopy of each tree. For each leaf, 4 cm<sup>2</sup> areas of both upper and lower leaf surfaces were separately wiped with a sterile cotton swab which was then rinsed in 1 ml of sterile distilled water. The resulting spore suspension was diluted plated on malt extract agar with streptomycin. Cultures were incubated at room temperature for 5 to 7 days and then examined microscopically. Results were expressed as colony forming units (CFU)/cm<sup>2</sup>.

Twenty-three fungal taxa were identified from the air samples. The most abundant taxa were *Cladosporium*, Ascospores, basidiospores, and *Alternaria*; together these four spore types comprised over 90% of the yearly total. Yeasts were the most abundant fungi isolated from both leaf types. Among the mycelial fungi were *Phoma*-type species, followed by *Cladosporium* spp and *Alternaria* spp. These four taxa occurred on 100% of the samples. Overall 21 genera of mycelial were identified. Mean concentration of total fungi on *Ulmus* leaves was 67 CFU/cm<sup>2</sup> and 54 CFU/cm<sup>2</sup> on *Quercus* leaves. Although yeasts and *Phoma* are not readily airborne, eleven taxa of leaf-surface fungi were common in the air samples. In addition, *Phoma* is the anamorphic stage of some *Leptosphaeria* species, and *Leptosphaeria* ascospores were quite common in the air samples. It is possible that the some of the leaf surface *Phoma* developed the sexual stage and produced some of the airborne *Leptosphaeria* spores. Crude estimates of the leaf surface area of each tree suggest that the total fungal load was 1.0 x 10<sup>9</sup> CFU for *Ulmus* and 5.4 x 10<sup>8</sup> CFU for *Quercus*. These data support the assumption that leaf-surface fungi are major contributors to the air spora.

### The potential for the use of Polymerase Chain Reaction (PCR) assays in detection airborne fungal spores

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Conventionally, airborne fungal spores are detected and identified using microscopic or culturing techniques. Both approaches can be time consuming and usually require skilled personnel. In addition, the

accuracy of identification, that is often needed in some applications, such as in the detection of plant pathogens, can be difficult to achieve. For example, appropriate selective cultures may not be available for the target organism, or the fungus may be slow growing and cultures easily swamped by non target fungi. Microscopic identification can also be difficult especially when the target spore has few easily identifiable morphological characteristics. Recently there has been interest from a number of disciplines (medicine, plant protection, biohazard detection) in the development of rapid accurate methods for detecting airborne bioparticles.

The Polymerase Chain Reaction (PCR), a widely used tool in molecular biology, can be used to detect very small amounts of DNA from a specific organism. Essentially, PCR amplifies a region of DNA specific to the target organism and the amplified product is then detected. PCR-based assays are therefore potentially both very specific and very sensitive. In some circumstances it may be possible to detect the DNA from a single fungal spore. PCR, thus has the potential to be used in conjunction with air sampling to detect the presence of specific organisms in the air.

Until recently little had been done to apply PCR methods to the detection of airborne biological particles. Most of the work using DNA-based methods has been to detect medically relevant bacteria, mycoplasmas and fungi, but they are now also being used to monitor plant pathogenic fungi. This paper will review recent progress on the application of PCR based assays to the detection of airborne micro-organisms.

Various types of samplers have been used to collect spores prior to PCR analysis including cyclone separators, rotating-arm traps and Hirst-type spore traps. Experiments, particularly with airborne plant pathogens suggest that the PCR-assays combined with air sampling can be very sensitive. For example wind tunnel tests using *Penicillium roqueforti* spore suggested a detection limit of less than 10 spores in the assay. PCR-assays can also be highly selective. Experiments with *P. roqueforti* demonstrated that they could readily be detected in a background of spores of six other unrelated species. Similarly *Sclerotinia sclerotiorum* spores could be distinguished from the closely related species *Botrytis cinerea* even when there was more than 20 times more *B. cinerea* spores in the sample.

Conventional PCR assays are essentially non-quantitative, thus when combined with air samples they can only reliably detect the presence of the target organism. The development of "real-time" PCR allows the original amount of target DNA in the sample to be estimated. In real-time PCR the PCR product is monitored during the reaction and this can be related to the amount in the original sample. Real time PCR has been used successfully to analyse medical air samples and it has been used at Rothamsted Research to study airborne *Blumeria graminis* spores, wheat powdery mildew.

It is now clear that PCR-based methods can detect and quantify biological material in air samples. The paper will consider how this technology can be further developed to become a reliable tool for use in plant, human and animal disease management.

### Fungal spore control in the atmosphere of the Cave of Nerja

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Although there are many aerobiological works carried out indoors, references about this kind of studies made inside caves can hardly be found. The Cave of Nerja is situated in eastern end of the province of Malaga (southern Spain) and takes up a space of 250,000 m<sup>3</sup>. It is characterised by its huge halls in which a constant temperature and high relative humidity can be appreciated throughout the year. These temperature and humidity conditions, together with the high affluence of visitors (550,000 people per year, as average) favours mould proliferation and spreading of their spores in the air.

The aim of this work is to analyse the fungal spore content in the air of the Cave of Nerja, as well as to determine the seasonal behaviour of the different spore types detected, in order to prevent health diseases. For that, a volumetric spore-trap, Hirst type (Lanzoni VPPS 2000) was situated on the ground level of the Cave, at the first hall, next to the entry, called "Sala del Belén". The study was carried out, from January 2002 to December 2003, period in which the spore-trap was uninterruptedly kept operational.

During the period studied, 95 airborne spore types were detected, 66 out of them were identified. The highest concentrations of fungal spore occurred in late July-early August due to the presence of high levels of Aspergillaceae spores, the most abundant spore type detected, followed by *Cladosporium*, *Agaricus*, *Ustilago* and *Coprinus*.

### A comparison of recently introduced samplers for indoor aeromycology studies

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Over the past five to 10 years a number of samplers have become available for estimating airborne spore concentrations by direct microscopic examination (DME). In the United States the most popular DME-type sampler for indoor air investigations is the Air-O-Cell (Zephon, St. Petersburg, Florida) with many fewer investigators using the Burkard Portable Spore Sampler (Rickmansworth, England), the Allergenco MK-3 (San Antonio, Texas), or the EMS Cyclex samplers (Charleston, South Carolina). Fewer still use filtration samplers such as the Aircuity Optima monitor (Newton, Massachusetts), or the Bi-Air sampler (Placentia, California).

Collection efficiency, ease of use, portability, price, and ease of analysis, all are factors to be considered when selecting a sampler for indoor investigations. These factors, along with data from side-by-side field collections will be presented and compared.

Comparisons by other laboratories<sup>1</sup> and manufacturers' claims have stated that the impaction samplers have cut-points ( $d_{50}$ ) between 2  $\mu\text{m}$  (Allergenco MK3 and Cyclex-d samplers) and 2.5  $\mu\text{m}$  (Burkard Portable and Air-O-Cell samplers). The Bi-Air samplers use a filter with a 1.2  $\mu\text{m}$  rated pore size; theoretically it should be the most efficient collector of small fungal spores among the above samplers. While these characteristics are important to help characterize the samplers, inlet efficiency, sample preparation procedures, and analytical methods also impact the reported recoveries from samplers.

Samples are collected indoors, under low air-velocity conditions, over a range of spore concentrations. Different spore size categories are analyzed to determine sampler collection/analytical efficiency over a range of particle sizes. Within, and between, sampler variability will be evaluated.

Sample processing and spore counting procedures are performed according to currently accepted practices used by analytical laboratories across the United States.

These findings will contribute to the information currently available on portable spore samplers, enabling investigators to make better-informed decisions when selecting and using air samplers for indoor aeromycology investigations.

AIZENBERG V, REPONEN T, GRINSHUPUN SA, WILLEKE K. Performance of Air-O-Cell, Burkard, and Button Samplers for total enumeration of airborne spores. American Industrial Hygiene Association Journal 2000; 61:855-64.

### Modelling airborne fungal spore data with the gamma distribution

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We present a discussion on the performance of the gamma distribution as a model for airborne fungal spore daily concentrations, using data extracted from the Aerobiological Network of Catalonia (Xarxa Aerobiològica de Catalunya, XAC). It is the sequel of a previous paper, dealing with similar models for pollen concentrations.

In the first part of this communication, we discuss the advantages of the gamma distribution with respect to other models, and how successful has been the application of the gamma model to pollen data. The gamma distribution has two parameters, a shape parameter and a scale parameter. The interpretation of these parameters and the relationship between them and the usual statistics are also discussed.

The second part contains the results of the data analysis, which includes the eight spore types that are more abundant in the area covered by the network (*Alternaria*, *Arthrinium*, *Aspergillus/Penicillium*, *Cladosporium*, *Coprinaceae*, *Leptosphaeria*, *Ustilago* and Total spores). For each spore type, location (6 in the XAC) and year (period 1994-2003), we fit a gamma model, checking the stability of the parameters across time, for each site. The fit is assessed by means of a chi square statistic. The rationale of this yearly approach is that we

expected the scale parameter to change from year to year, depending on the meteorological conditions, but there was a reasonable hope that the shape is fairly stable.

In general, the shape parameter estimates obtained for spore data are higher than those obtained for pollen, but, except for Total spores, they are still lower than 1, so that the probability density function is still decreasing and asymptotic in zero. For Total spores, the visual aspect of the probability density is not far from the lognormal curve.

Although the shape parameter seems to be less stable than in pollen distributions, we also group the spore types according to the shape estimates, as we have done with pollen types in our previous research. These groups can also be based on the coefficient of variation, which is a function of the shape parameter.

### An estimation of fungal spore concentrations obtained using two counting methods

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The aim of the study was to estimate the spore counts of *Alternaria* and *Cladosporium* using the single longitudinal traverse and twelve transverse traverse methods. Outdoor air samples were collected by the Burkard trap. The study was carried out during peak period (June, July, August, September) of the season in Cracow (Poland) in 1997-1999. Both the single and twelve traverse methods generally showed similar average daily concentration fluctuations of the two studied spore types on the same days although the single traverse method usually presented higher spore concentrations. However, analysing the distribution of concentrations obtained using both methods there were days when single or twelve traverse method showed a rise or fall in concentration which was not reflected by the other method. In case of *Cladosporium* higher daily concentrations measured using the twelve traverse method occurred more frequently in months of the highest spore concentrations. On the other hand, the higher concentrations of *Alternaria* spores measured using this method occurred more frequently in months of the lowest concentrations. Analysis of correlation (Pearson's correlation coefficient  $r$ ) between those variables (number of days with higher concentration and monthly concentration) showed that correlation was non significant statistically at a significance level of  $\alpha = 0.05$  (t test).

To determine statistically significant differences between the two method values the nonparametric Wilcoxon Paired-Sample Test was applied. Significant differences were obtained at significance level of  $\alpha = 0.025$  (one-sided test). The results of the Wilcoxon's test indicated that for both *Cladosporium* and *Alternaria* the average daily concentrations obtained using the single traverse method were significantly higher than using the twelve traverse method (*Cladosporium*  $n = 366$ ,  $z = 12.3995$ ,  $p < 0.0000$ ; *Alternaria*  $n = 366$ ,  $z = 13.1792$ ,  $p < 0.0000$ ).

### The use of pyrogallol red for staining fungal spores

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Most of the aerobiological studies made indoors are carried out with the aid of volumetric spore traps designed for collecting viable particles in agar plates, such as the Andersen model or the Portable Air Sampler for agar plates by Burkard. After that, fungal spores are cultivated and the mycelia and spores observed and identified. Generally, the use of fuchsin is not frequent because fungal spores cannot easily be coloured by this stain.

Pyrogallol red is a staining solution for determining proteins and glycoproteins. Staining is based on interaction between the staining solution and certain proteins. The intensity of staining depends on the current protein charge status, which is advantageous in determination of allergenic potential. In comparison with

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/m<sup>3</sup>) 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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#### 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