SZENT ISTVÁN UNIVERSITY

AEROBIOLOGICAL STUDIES ON MYCOBIOTA

PhD thesis

Donát Magyar

Gödöllő

2005.

1. INTRODUCTION AND OBJECTIVES

1.1 Introduction

Over land surfaces a quarter of the total airborne particulate may be made up of biological material, a significant proportion of which has fungal origin. Aeromycology as a discipline has been established for the reason to understand the qualitative and quantitative distribution, ecology, biometeorology and deposition patterns of airborne fungal propagules (spores, conidia, and hyphal fragments).

1.2. Actuality of aeromycology in public health

Many fungal genera (e.g. *Alternaria, Botrytis*, *and Fusarium*) are known as an agent responsible for allergic disease and mycoses. Respiration of the conidia of *Alternaria* may cause asthmatic allergy. The number of Hungarian patients with *Alternaria* allergy increased tenfold in the last years (OSVÁTH et al. 1996). *Cladosporium* counts in spore traps increased significantly in the last decade (ERDEI et al. 2001). High concentration of airborne allergens often occurs in the Carpathian Basin, because the lack of land-sea air circulation clearance system (WILKEN-JENSEN and GRAVESEN 1984).

1.3. Actuality of aeromycology in plant protection

Most of fungal species discharge spores into the air, where aerodynamic processes ensure dispersal. Aeromycology should play an important role in forecast, since the presence of pathogenic spores can play a role in disease outbreak. In a study carried out by CRAIGIE (1945) the risk of epidemic were registered by spore traps one or two weeks before any visible symptoms. The costs of chemical plant protection in an Italian tomato field were reduced by 50-80% when a forecast system was used together with aerobiological methods. In this case the risk was revealed by air monitoring two weeks before disease outbreak (BUGIANI et al. 1995). Carpathian Basin is crossed over by a wind trajectory which may carry infective spores (PEYROT 1962), thus air monitoring is necessary in this region. Analysis of air samples provides knowledge for ecology, diversity and distribution of fungi and other micro organisms as well.

1.4. Aeromycobiota

The concentration and composition of the community of airborne fungal spores (airspora or aeromycobiota) varies according to time of day, season, geographical location, weather conditions phisiological factors. (HJELMROOS 1993, LACEY 1981, SALVAGGIO 1986). Studies of HIRST (1953) showed that aeromycobiota should be also divided into dry-air spora and damp-air spora, according to the meteorological conditions.

The dispersal process of many fungi is not well studied: we don't know how frequently a spore type is transported by wind, by rainwater or by insects. Some examples are described in case studies, however a comparative study may reveal quantitative differences. Hirst-type spore trap is an optimal device for air sampling. Honey samples are widely used to determine the composition of pollen grains of nectariferous plants; this method should be applied for fungi as well (PÉREZ-ATANES et al. 2001). The knowledge of the special spore composition of stemflow rainwater comes from Hungarian studies (GÖNCZÖL 1976, GÖNCZÖL and RÉVAY 2004).

1.5. Aim of study

The main goal of my work was to represent an ecological approach of aeromycology. The following objectives were determined:

1. Identification of fungal spores in air samples collected above an vineyard.

2. Reveal the correlation between concentration of observed spore types and weather variables. Provide a detailed biometeorological description of each observed spore type.

3. Classification of airspora, and description of different type of aeromycobiota.

4. Study the dispersal of *Fusarium* species in the air using a culture plate method.

5. Explain the occurrence of non-anemochor (hydrochor, entomochor) spores in air samples.

2. MATERIALS AND METHODS

2.1. Hirst-type spore trap

A 7-day recording spore trap (Hirst 1952; manufactured by Lanzoni Co. Ltd., Bologna, Italy, Burkard Manufacturing Co Ltd Rickmansworth, England) was used to register the daily concentration of airborne spores. Spore counts were summarized 6- and 24 hourly. In the Italian study, the sampler was located in the middle of a traditional vineyard of Central Italy, near the city of Brufa with Mediterranean climate. Air sampling was conducted during the blooming period of the vine in four years (in 1994 between 27. May and 13. June; in 1995 between 05. June and 03. July; in 1996 between 13. June and 24. June). The spore traps and meteorological instruments were placed on the top of a hill in Brufa (sea level 300m), between the valleys of Assisi and Torgiano in an instrumented tower, at 12 m above ground level. Surrounding country of Brufa is entirely dedicated to vine cultivation, its mayor area, approximately 618 acres kept by Luganotti Co Ltd. The meteorological factors included the daily records of wind speed [m/s], wind gust [m/s], wind direction, cloud cover [%], sun hours [min], relative humidity (RH) [%], atmospheric pressure [Pa], minimum, maximum and average temperatures (Tmin, Tmax, Tavg) [C^o], dew point [C^o], precipitation [mm], duration of precipitation [min], fog and mist [binary data]. Evaporation [mm] was measured simultaneously by two different methods with Wild and Piché evaporimeters.

2.2. Andersen sampler

Andersen sampler (Andersen 1958, Andersen Six Stage Viable Cascade Impactor, Lanzoni Co. Ltd., Bologna, Italy). Airflow provided by a vacuum pump is drawn through a succession of three stages, each perforated with 400 holes. The device was modified for outdoor use. Samples were collected in Piliscsaba (cornfield), Martonvásár (cornfield) and Páty (cornfield) and cocksfoot [*Dactylis glomerata*] lawn. Sampling heights were 10 and 150 cm. Three sieving plate were used. Samples were taken in 10 and 20 minutes, at dry, sunny (28-30 C^o) breezy weather in summer, between 12.00-14.00 hours. Aspiration rate was 20 l/min. Sterile Petri dishes with *Fusarium* selective media (Mycobutanyl-, Pepton-Dichloran, PCNB-Rose Bengal and Malachite green agar) were used. After three days of incubation at room temperature (25 ^oC) in dark, CFU-numbers of 69 samples were determined. *Fusarium*

colonies were transferred on SNA medium by a piece of sterilized filter paper. The plates were incubated in a controlled-environment chamber at 20-25 ^{o}C (night-day) temperature and 12 hours of black-light tubes near ultraviolet on/off cycle to stimulate sporulation. From each strain single-spore culture were made for morphological identification, based on pigmentation of pure PDA culture, sporodochium colour and conidium shape and size on SNA medium. A portable Hirst-type spore trap was used in the same sampling heights (10 and 150 cm) next the Andersen-type device.

2.3. Honey samples

Spores and pollen grains transported by pollinator insects are accumulated in honey, thus entomochor spores also should be studied by means of melissopalinology. Honeydew is produced by piercing and plant-sucking insects (*Rinchota*: *Homoptera*). Some algae and microscopic fungi, especially sooty moulds develop in honeydew. When production is high on forest trees, honeydew drops fall to the ground. When the volume of honeydew on the leaves reaches a certain level, it is collected by honeybees. Honeybees then transport it to hives and process it into honeydew honey. Honeydew honey, often called "forest honey", is commercially valuable. Those from silver-fir, oak-trees, wheat, citrus, etc. are marketed worldwide. I have analysed 83 honey samples. The detailed description of the honeydew honey samples is given in PERSANO ODDO et al. (2000).

2.4. Stemflow samples

To study the relationship between airborne and stemflow mycobiota, 2-10 ml of rainwater samples from trunks of different tree species in persistent rainfall were also collected into centrifuge tubes containing 2 ml of FAA (50 % ethanol 5 % glacial acetic acid, 10 % formaldehyde).

2.5. Statistical analysis

The Spearman's Correlation Analysis was applied to clear the relations between changes of daily airborne fungal propagule concentration and the meteorological factors (SPIEGEL 1988) using the SPSS programme (SPSS Inc 1999 version 10.0.1). Results were sorted by their similarity. Environmental factors with strong correlations were chosen for subsequent Canonical Correspondence Analysis of aeromycobiota. Statistics were computed by SYN-

TAX 2000 programme (PODANI 2001). Comparison of spore components of air-, honey-, and stemflow samples were performed by the „farthest neighbour" method (LANCE and WILLIAMS 1967). Biodiversity of air was computed using Margalet-, Shannon- and „Right Tail Sum" (RTS) biodiversity indices (TÓTHMÉRÉSZ 1997).

4. NEW RESULTS

1. First observation of 65 spore types from air is published. 222 spore types were differentiated, 68 spore types were identified in species level, and 94 spore types were identified at the level of genera. 35 spore types were characterized at higher taxonomical levels (e.g. family) or remained unknown. 57.0 % of the airborne species were mitosporic fungus. Ascospores occupied also a great proportion (25.7 %) of the total airspora. Percentages of the other taxa (Basidiomycetes, Myxomycetes, Oomycetes, Urediniomycetes, Ustilaginomycetes, Zygomycetes) were much lower (1-5 %). Approximately 7000 microphotographs were taken.

2. In this work I published the biometeorological descriptions of 167 spore types, based on the results of the Spearman's rank correlation analysis. **First biometeorological characterization of 91 spore types is described.** 41.7 % of the spore types were found in dry, warm days, however some spore types (28.3 %) were recorded in wet weather. The latter group contained mostly Ascomycetes (69.0 %) and gloiospores of Deuteromycetes (15.1 %). Some spore types (39.2 %) showed positive correlation with the wind alone, these fungal particles were considered to be immigrant spores from farther sources.

3. Morphological variants (broken, aggregated, desiccated forms) of 25 spore types were studied. **Biometeorological analysis of morphological variants** is a new method to reveal the environmental circumstances which are responsible for the development of different variants.

4. Biodiversity of the air was expressed by three different indices for fungal spores (Margalet's-, Shannon- and Right Tail Sum-biodiversity) for the first time. My results show, that biodiversity positively depended on the daily maximum temperature. Wind velocity and rain decreased biodiversity.

5. Airborne dispersal of the *Fusarium* species was studied using a 3-stage Andersen-sampler for the first time in Hungary. Concentration of airborne *Fusarium* spores should be high in dry conditions as well. Aerodinamical diameter and number of spores were considerably larger near ground level, than at 1.5 m height.

6. **Honey and stem-flow mycobiota:** to study the fungal communities dispersed by rainwater and insects, I examined 24 stemflow samples (94 spore types identified) and 83 honey samples (198 spore type identified). Honeydew honeys were sudied first time by mycological aspect and the presence of stauroconidia in the samples was recorded. The similarity and connection of these mycobiota with the airborne dispersal was illustrated with a dendrogram generated by the „farthest neighbour" method. The common and frequent spore types both in honeys and stemflow were *Excipularia*, *Tripospermum* and *Oncopodiella* species, which were rarely found in the air, only in case of wind gusts and rain splash. Honey samples can be divided into groups on the basis of their spore content Honeys with floral origin, and honeys with honeydew origin separated markedly. Honeydew honeys from firs formed two small distinct groups (*Pinus burtia* and *Abies*). *Abies* honeydew honeys from Greece and Italy were similar. These honeydew honeys contained many of the members of the stemflow mycobiota. The explanation of this amazing observation could be, that Mediterranean honeydew honeys covers the plant surface and flows on the stems and trunks of the host trees. Bees collect the arboreal mycobiota immersed into the honeydew to produce honeydew honey. Because of the climate of the Carpathian Basin for mass production of the honeydew is unfavourable, honeydew can not flow on the stems. This theory explains the similarity measured between air samples and Hungarian honeydew honeys.

8. The most important meteorological factors on the basis of the arrows of the **canonical correspondence analysis** were found to be relative humidity, temperature, and wind. The first axis (X) is defined by RH and Tmax (species-environment correlations 0.716 and-0.702), the second axis by wind. A high proportion (56.14 %) of the variance in the species-environment relations was interpreted on the canonical axis X and Y. The unexplained remainder of the variance should be in connection with other environmental factors (e.g.: maturation of spores, phenology of the host plants, long-range transport).

Differentiation of the aeromycobiota above Brufa was based on my results of Canonical correspondence analysis and Spearman's rank correlation analysis. The dry *(AD)* and wet *(AW*) weather airspora separated clearly. Four new subdivision of airspora were determined (1.: Drying weather spores (AD*e*), 2.: Dry shake-off (or windstorm) spores (AD*d*), 3.: Warming wet weather spores (AD*b*), 4.: Wet shake-off (or splash) spores (AWs).

a) Dry weather spores (AD): In this case, concentrations of the fungal components of the air (*Alternaria, Arthrinium, Cladosporium, Epicoccum, Stachybotrys, Stemphylium, Torula*, hyphal fragments) depend on high temperature. Many of these saprotrophic fungi are capable

to trigger allergic symptoms. Relative abundance of *Cladosporium* species was high (80-95 %). Most of these spores correlated positively with Tmax.

b) Drying weather (or evaporation) spores (AD*e*): Some spore type took place between high and low relative humidity regarding to their projected position along the RH vector. Based on the statistical results, some species were considered to belong to this group (*Chaetomium*, *Trichothecium roseum*, *Venturia*, *Badhamia* sporeball). Real members of the AD*e* could have an active spore liberation mechanism (e.g. hygroscopic movements). Some spore components should be called as secondary members, because of their concentration can grow when environmental humidity reduces and passive liberation methods take place. In the AD*e* several plant pathogenic fungal spore can be found. Their concentration reaches its maximum in the mornings. Effects of two types of evaporation (elicited by wind or by rising temperature) on the airspora can be differentiate with Wild- and Piché-evaporimeters.

c) Dry shake-off (or windstorm) spores (AD*d*): Higher airflows (>4 m/s) or wind gusts can suspend previously deposited mycoparticles or strongly attached spores into the air. I found in this group some spores of soilborne, coprophylic, corticolous fungi, sooty moulds, staurosporae, „giant spores" (*Bipolaris*, *Podosphaera*, *Phragmidium, Tetraploa*), fruit bodies, sclerotia and fungal particles aggregated with pollens and plant debris. Real members of this aerobiota should be any spore type which needs a relatively „extra high" kinetic energy to became airborne. Daily maxima should be observed in certain regions, where diurnal high winds occur regularly (e.g.: in afternoons or in springtime, GREGORY 1961, OGDEN et al. 1969). This group deserves attention from the point of view of public health: out of season outbreak of allergy can be provoked by resurfaced allergens in dust- and sandstorms.

d) **Warming wet weather spores (AD*b*):** A well defined group of basidiospore types were displayed by Canonical Correspondence Analysis in the area between rains of the previous days and maximum temperature. I conclude that this effect was owing to the growth of mushroom fruit bodies. Rapid increase of the temperature in 48 hours had a positive effect on the spore counts of *Agrocybe, Boletus* and *Coprinus* species as well. Diurnal maximum of spore number was measured at night and early dawn. My results should take into account by allergic patients in the period of „agaric climax".

e) Wet weather spores (AW): Most of the points representing the Ascomycetes and Coelomycetes species in canonical correspondence analysis flocked around the RH-vector. Substantial rainfalls elevated the spore counts of hialodidymae and *Leptosphaeria* species. Less humidity were sufficient to increase the spore concentration of some other spore types

(e.g. *Chaetosphaerella, Dyatripaceae, Lophiostoma vicinum* and *Nectria*-type). Because of this, the effect of the precipitation is not uniform between AW fungi.

f) Wet shake-off (or splash) spores (AWs): Some fungal spore types (*Colletotrichum*- and *Tilletiopsis*-type, *Excipularia*, *Caloplaca*, *Leptosphaeria rubicunda* and 3-septate fuziform spores) appeared during longer rains. I annexed *Ophiobolus* and *Rebentischia unicaudata* to the AWs as well, on the basis of projecting points of this species on the RH vector.

g) When the Melinex-tapes of the Hirst-type spore traps was examined at low magnification of the microscope, a novel phenomenon was observed. In tapes representing rainy days, some circlets of 100-3500 μm were present, traced by the mass of fungal spores. These dried droplets coincided with longer rainfalls. At 12 and 23 meters, where spore traps was located the so-called „horizontal" or „driving rains" may occur, delivering spores from the wetted canopy. „Drift-droplets" should have an important role in the field of plant protection (spore viability, attachment, germination, and infection rate, should be altered). Spore carrying droplets may facilitate the adhesion and germination of some phytopathogenic fungal spores. **By these „drift-droplets" long-range dispersal may became possible for the gloiospores**.

h) On the basis of the results of the statistical methods mentioned above, I composed a **theoretical, comprehensive model for the terrestrial systems of fungal spore groups formed during dissemination.** In the model (like in Canonical Correspondence Analysis) axes correspond to the stronger meteorological factors. Circadian rhythm is also indicated. Connection between anemo- entomo- and hydrochor mycobiota are also indicated.

5. CONCLUSIONS AND SUGGESTIONS

1. The monitoring of airborne spores help to predict the risk of plant disease. Forecasting models based unilaterally on agrometeorological data should be completed with aeromycological monitoring to gain a more reliable insight into the development of epidemics.
2. The presented results suggest a high diversity of airborne spores, which merit the attention of the allergologist. The „mono-allergen" approach of the allergenic background should be replaced by the „poly-allergen (aerobiota)" concept. The effect of allergens should be estimated cumulatively.
3. Some gaps in the knowledge of fungal ecology should be fulfilled using aerobiological methods. The dispersal of many fungal species is not well studied yet. Spore resurfacing and long distance dispersal by drift droplets from splashing rain could be important topics.

6. PUBLICATIONS

Publications related with the PhD thesis

Papers-in English

Magyar D, Gönczöl J, Révay Á, Grillenzoni F, Seijo-Coello MdC (közlésre elfogadva) Stauro- and scolecoconidia in floral and honeydew honeys. *Fungal Diversity*

Szécsi Á, Bartók T, Varga M, **Magyar D**, Mesterházy Á. (2005) Determination of trichothecene chemotypes of *Fusarium graminearum* strains isolated in Hungary. *Journal of Phytopathology* 153: 445-448

Magyar D (2002) The Aerobiology of the Ascospores. *Acta Microbiologica et Immunologica Hungarica* 49(2-3): 227-234

Magyar D and Tóth S (2003) Data to the Knowledge of the Microscopic Fungi in the Forests around Budakeszi (Budai Hills, Hungary). *Acta Phytopathologica et Entomologica Hungarica* 38(1-2): 61-72

Papers-in Hungarian

Magyar D és Szécsi Á (2002) A levegőmikológia növénykórtani alkalmazása. Növényvédelem 38(8): 397-407

Magyar D (2004) A természetes spóracsapdák levegőmikológiai szerepe. Allergológia és Klinikai Immunológia 7:206-212

Szécsi Á, Bartók T, Varga M, **Magyar D**, Mesterházy Á (2004) A 8-ketotrichotechén típusú mikotoxinok kemotípusainak azonosítása a hazai *Fusarium graminearum*-populációban. Növényvédelem 41(2):45-51

Proceedings- in English

Magyar D and Manningerné K (2004) Effects of meteorological conditions on uredo- and teliospores of rusts. Cereal Rusts and Powdery Mildews Bulletin [www.crpmborg/2004/1029magyar]

Proceedings- in Hungarian

Magyar D, Frenguelli G, Bricchi E, Tedeschini E (1999) A szőlő aeromikoflórája. A Környezeti Ártalmak és a Légzőrendszer 9:248-258

Magyar D, Szécsi Á, Barasits T (2003) Andersen és Hirst típusú levegőmintavevők összehasonlítása növény-és közegészségügyi vizsgálatokban. Környezeti Ártalmak és a Légzőrendszer 13: 239-250

Magyar D, Frenguelli G (2004) Az időjárás hatása az *Alternaria*–eredetű légköri gombaelemek morfológiai változékonyságára. Környezeti Ártalmak és a Légzőrendszer 4:229-240
előadás

Books (editor)- in Hungarian

Magyar D (ed) (1998) Az ÁNTSZ Aerobiológiai Hálózatának tájékoztatója, 1998. Fodor József Országos Közegészségügyi Központ, Országos Környezetegészségügyi Intézete, Budapest

Magyar D (ed) (2000) Az ÁNTSZ Aerobiológiai Hálózatának tájékoztatója, 1999. Fodor József Országos Közegészségügyi Központ, Országos Környezetegészségügyi Intézete, Budapest

Magyar D (ed) (2001) Az ÁNTSZ Aerobiológiai Hálózatának tájékoztatója, 2000. Fodor József Országos Közegészségügyi Központ, Országos Környezetegészségügyi Intézete, Budapest

Magyar D (ed) (2001) Az ÁNTSZ Aerobiológiai Hálózatának tájékoztatója, 2001. Fodor József Országos Közegészségügyi Központ, Országos Környezetegészségügyi Intézete, Budapest

Magyar D (ed) (2003) Légköri allergének tér- és időbeni eloszlása Magyarországon. NEKAP, Budapest

Publications not related with the PhD thesis

Papers-in English

Török K, Botta-Dukát Z, Dancza I, Németh I, Kiss J, Mihály B, **Magyar D** (2003) Invasion gateways and corridors in the Carpathian Basin: biological invasions in Hungary. Biological Invasions 5:349-356

Papers-in Hungarian

Farkas I, Fehér Z, Erdei E, **Magyar D** (1998) Allergia prevenció, parlagfű mentesítési program. Egészségtudomány 42:116-128

Fehér Z, Farkas I, Erdei E, Gallovich E, Csoltkó G, Wimmer J-né, Klatsmányi J, Laczik M-né, Szintainé Dobrádi J, Borsányi A, Oravecz A, Farkas L, **Magyar D** (1998) Parlagfű szezonok vizsgálata az ÁNTSZ aerobiológiai hálózat adatai alapján (1992-1997). Egészségtudomány 42:61-69

Farkas I, Erdei E, **Magyar D** (1999) A környezet és az ember ökológiai integritás zavara: az allergia és a parlagfű. Ökotáj 22:64-68

Farkas I, Fehér Z, Erdei E**, Magyar D** (1999) A parlagfű térhódítása. Allergológia és Klinikai Immunológia 2:104-109

Varró H, Záhonyi L, Andrásofszky Zs, Virágh Z, **Magyar D**, Farkas I, Bánházi A, Kertész M, Rudnai P (1999) Az ózon és különböző pollenek légköri koncentrációja, valamint allergiás légúti kórképekkel kezelt gyermekek légzőrendszeri paraméterének és tüneteinek összefüggései Mosdóson, 1996 nyarán. Egészségtudomány 43:351-362

Farkas I, Erdei E, **Magyar D** (2000) Pollenallergiát okozó növényeink- magyarországi pollennaptár. Családorvosi Fórum 4:40-44

Magyar D, Farkas I, Nagyné Horváth E, Bíró E (2001) A parlagfű mentesítési propaganda hatása az általános és középiskolás gyerekekre. Egészségnevelés 42:28-31

Proceedings- in Hungarian

Farkas I, **Magyar D**, Erdei E, Oravecz A, Gallovich E, Kulja A (1999) Parlagfű allergia rizikója Magyarországon, a parlagfű pollenszám alakulása az 1999 évi pollenszezonban az ÁNTSZ pollencsapdáinak adatai alapján. A Környezeti Ártalmak és a Légzőrendszer 9:38-41

Book and homepage- in Hungarian

Farkas I, **Magyar D**, Fehér Z (1997) Parlagfű mentesítési program- Önkormányzati tájékoztató. Pharma Press, Budapest

Farkas I, **Magyar D**, Orbánné Erdei E (1998) Tanároknak röviden az allergiáról. Pharma Press, Budapest

Magyar D és Magyar M (2003) Légköri allergének tér- és időbeni eloszlása Magyarországon. [http://wwwantszgovhu/oki/V14/index.html]. NEKAP, Budapest