ABSTRACT BOOK

Universidade do Minho

Braga - Portugal

Organised by:

mum
Micoteca da Universidade do Minho

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Workshop Secretariat:

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Our Major Sponsors:

The MUM Acknowledge the Generous Support of

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA E DA TECNOLOGIA
Apoio ao programa Operacional, Tecnologia, Inovação do Quadro Comunitário de Apoio III

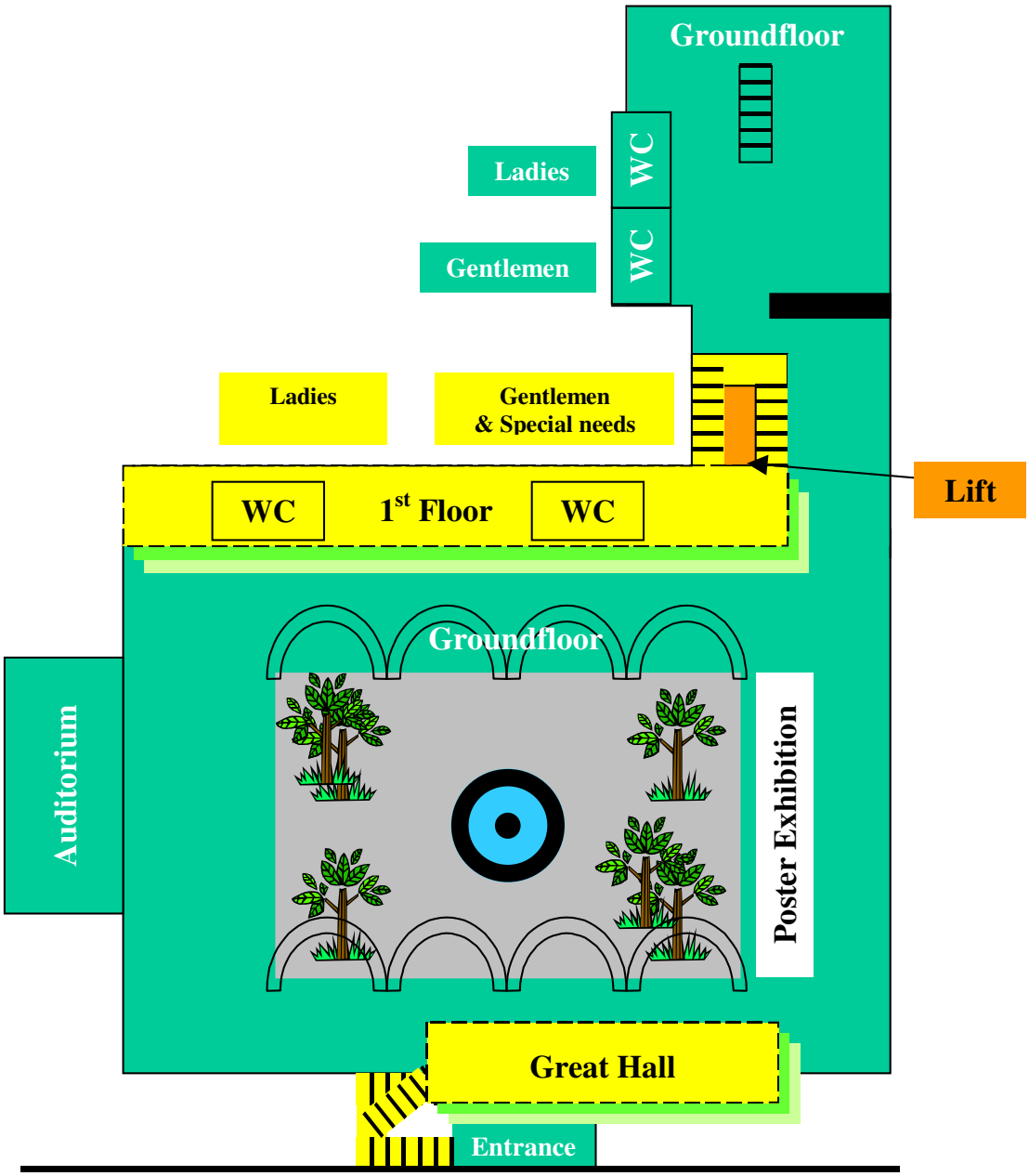
Produtos para Laboratório, Lda

General Information:

CONFERENCE VENUE AND LOCATION

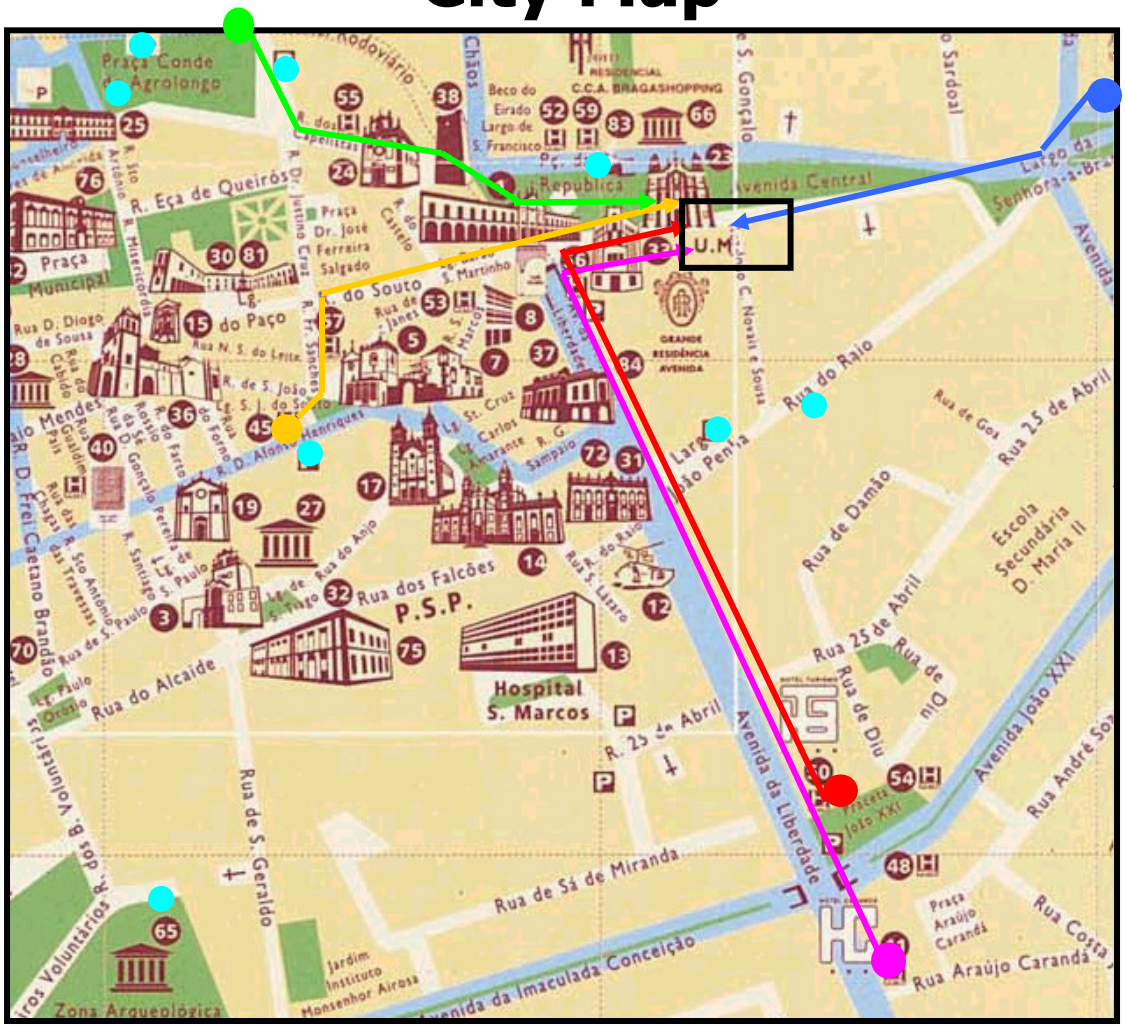
The conference will be held at the ***Convento dos Congregados*** which is located in one of the main avenues of the city centre, "Avenida Central" (door number 100). A floor plan of the meeting venue and a city map may be found on the next pages.
The *Convento dos Congregados* hosts the IEC – UM (Childhood Studies Institute of the University of Minho).

Floor Plan



IEC/UM – Convento dos Congregados
MEETING VENUE

City Map



Hotel Turismo	
Albergaria Senhora-a-Branca	
Hotel Carandá	
Hotel D. Sofia	
Hotel Ibis	
MEETING VENUE *Convento dos Congregados* IEC – U. MINHO	
P | Parking	

CERTIFICATE OF ATTENDANCE

A Certificate of Attendance may be obtained in the registration desk by request.

LANGUAGE

English will be the official language of the workshop and no simultaneous translation will be provided.

BADGES AND IDENTIFICATION

Upon registration each participant will receive a name badge, which is to be worn during meeting activities please.

POSTERS

The poster session will be held in the cloister and the size of the board on which you will affix your poster is 1.5 m height by 0.8 m wide. The boards will be numerically arranged and push pins will be provided.

COFFEE-BREAKS AND LUNCH

Refreshments during coffee-breaks and lunch are included in the registration fee.

SMOKING POLICY

Smoking is strictly forbidden in all closed areas of meeting venue. The smoking area is the cloister.

E-MAIL ACCESS

E-mail will be available upon request and free of charge. Participants may access their corporate or personal e-mails accounts.

CURRENCY, CREDIT CARDS AND TAXES ON GOODS AND SERVICES

The Portuguese currency is the € (Euro). Common credit cards, including American Express, Dinners Club, Master Card and Visa, are accepted in most hotels, restaurants and shops. In Portugal goods and services are subject to payment of an "IVA" (VAT - value added tax). This is 21%, but some goods pay only 5% or 12%.

MAIN RECOMMENDED HOTEL ADDRESSES

Hotel Turismo Braga
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WELCOME ADDRESS

Dear Participants and Friends,

On the behalf of the Local Organizing Committee (LOC), I am very pleased to welcome you to the **one day meeting in *Trends in Mycology*** and to the University of Minho.

The Micoteca da Universidade do Minho (MUM) is a recent fungal culture collection hosted in the Biological Engineering Centre of the University of Minho. MUM aims to maintain and supply fungal strains for research in biotechnology and teaching, and to become a centre of knowledge, information and training in mycology. To pursue this later aim, MUM has organised recently meetings, workshops and advanced courses. It is a great pleasure to have this year a one day meeting which allow us exchange ideas and discuss trends in mycology.

We are delighted to have 35 participants from 10 different countries and 21 contributions, providing 4 keynote lectures, 6 oral communications and 11 posters. These figures indicate that there will be excellent conditions for a very exciting and enlightening day.

Additionally, the LOC have presented to you the abstracts book of the meeting. We are also gratefully to *Revista Iberoamericana de Micología* for providing us with the possibility of publishing the full papers. On behalf of the LOC I wish to thank all authors who have responded to ours requests in order to achieve this aim. In addition, a report on the meeting will be published in *Mycopathologia*.

I wish also to thank all support that was given by the various institutions and people, namely the: (a) Childhood Studies Institute of the University of Minho, which shares with us this lovely eighteenth century convent and its facilities; (b) sponsors; (c) staff involved in the meeting.

Finally, I wish you a very productive and good meeting and a very pleasant stay in Braga.

Thank you,

Nelson Lima

SCIENTIFIC PROGRAMME

FRIDAY, 23rd SEPTEMBER 2005 MORNING SESSIONS	
08:00 – 09:00	**Registration**
09:00 – 09:15	**Opening Session** N. Lima - Micoteca da Universidade do Minho M. Mota - Vice-Rector of University of Minho
09:15 – 10:00	***Keynote Lecture*** **Pandora's mycological box: Molecular sequences vs. morphology in understanding fungal relationships and biodiversity** D.L. Hawksworth
10:00 – 10:45	***Keynote Lecture*** **Polyphasic taxonomy of fungi in relation to applied mycology** R.A. Samson
10:45– 11:15	**Coffee break**
11:15 – 12:15 **Oral Communications**	**1. Fungi in bottled water: A case study of a production plant** A. Ribeiro, A.P. Machado, Z. Kozakiewicz, B. Luke, M. Ryan, A. Venâncio, N. Lima and J. Kelley
	2. Evaluation of PCR-RFLP of ITS rDNA as a diagnostic tool for a rapid identification of *Penicillium* subgenus *Biverticillium* species J. Dupont, C. Jacquet and M.-F. Roquebert
	3. Geographical similarity analysis of cork colonising *Penicillium glabrum* isolates using DNA fingerprinting M.C. Basílio, R. Gaspar, C. Silva Pereira and M.V. San Romão

12:15 – 14:30	**Lunch and Poster Exhibition with Presence of Authors**
Posters	**P1. Efficacy of plant extracts against stored product fungi** A. Magro, M. Carolino, M. Bastos and A. Mexia
	P2. Marine fungi on *Spartina maritima* baits exposed in the Mira salt marsh M. Barata
	P3. The ambrosia fungi in the insect-fungi symbiosis and their influence to forest decline J. Henriques, M.L. Inácio and E. Sousa
	P4. New and simple test plating for screening relative transfructosylation activity of fungi A. Dominguez, I.M. Santos, J.A. Teixeira and N. Lima
	P5. Airborne fungi in Portuguese schools S. Rodrigues, S. Carvalho, R. Moreira, J. Madureira, O. Mayan and E. Pinto
	P6. Notes on a plant parasite fungus in Portugal: *Gymnosporangium cornutum* M.C. Lopes and V.C. Martins
	P7. Filamentous basidiomycetes for testing biological toxicity of pollutants M. Vandrovcová, M. Šušla, K. Malachová and Č. Novotný
	P8. New synthetic compounds as inhibitors of mycotoxin synthesis L. Abrunhosa, M. Zaki, F. Areias, F. Proença and A. Venâncio
	P9. Ochratoxin A and filamentous fungi in red wine grapes from Santa Catarina, Brazil E.O. Nunes, J.J.M. Xavier, R. Serra, A. Furigo, V.M. Scussel and A. Venâncio
	P10. Fungal growth and mycotoxins in corn silages V.M. dos Santos, A. Venâncio and J.E. Matos
	P11. FISH and Calcofluor staining techniques to detect *in situ* filamentous fungal biofilms in water A.B. Gonçalves, I.M. Santos, R.R.M. Paterson and N. Lima

SCIENTIFIC PROGRAMME (cont.)

FRIDAY, 23rd SEPTEMBER 2005 AFERTNOON SESSIONS	
14:30 – 15:15	***Keynote Lecture*** **The renascence of classifications in mycology: extrolites, ecophysiological features and morphology allow clear-cut classifications that are complementary to cladifications** J.C. Frisvad
15:15 – 16:00	***Keynote Lecture*** **Multilocus sequence analysis of *Penicillium* and *Eupenicillium* species** S.W. Peterson
16:00 – 16:30	**Coffee break**
16:30 – 17:30 **Oral Communications**	**4. A novel approach for mycotoxigenic fungi identifications based on uncomplicated microscopy and mycotoxin characters** R.R.M. Paterson, A. Venâncio and N. Lima
	5. Pigment chemistry, taxonomy and phylogeny of the *Hypoxyloideae* (*Xylariaceae*) M. Stadler
	6. Portuguese regional differences in the wine grapes mycoflora R. Serra, A. Lourenço, O. Belo and A. Venâncio
17:30	**Closing session and concluding remarks** R.R.M. Paterson - Micoteca da Universidade do Minho

KEYNOTE LECTURES

PANDORA'S MYCOLOGICAL BOX: MOLECULAR SEQUENCES *VS.* MORPHOLOGY IN UNDERSTANDING FUNGAL RELATIONSHIPS AND BIODIVERSITY

D.L. Hawksworth

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Delving into molecular phylogenetics with fungi is like opening the mythical box of Pandora, the first woman in Greek mythology. The box contained either all manner of miseries and evils, or according to later versions of the myth, blessings. The contents were unknown until released, but then the miseries or blessings flew rapidly all over the Earth, and there was no escape from their impact. And there is no escape for mycologists from the impact of molecular insights. Fundamental reappraisals of traditional ideas have become necessary, from the level of determining what organisms belong in the *Fungi,* through reorganizing systems of orders and families, to the remodelling of genera, and the realization that many fungal "species" are several and not one. These different situations are discussed in relation to: the positions of microsporidia, slime moulds and oomycetes; the basal position of lichen fungi in the evolution of ascomycetes forming fruit bodies; remodelling of orders and families in the larger basidiomycetes (*e.g.* gasteromycetes, mushrooms, and polypores); changed generic concepts (*Coprinaceae,* parmelioid lichens); the issue of whether permitting a dual nomenclature for the different states of pleomorphic fungi should be continued; and the recognition of additional species within a "species" (*Cantharellus, Fusarium, Ganoderma, Ophiostoma, Parmelia s. str., Trichoderma*). The molecular data has stimulated a fundamental reassessment of the systematic importance of fruit body types, but at the same time ultrastructure, micromorphological, cultural, physiological, and ecological features are now assuming of major importance in species recognition. The techniques also open exciting horizons in placing precisely totally sterile genera of hitherto completely unknown position (*Coscinocladium, Lepraria, Racodium*), and undreamed of abilities to identify non-sporing fungi in ecological samples and plant material enabling fresh insights into pathogenicity, biological relationships, breeding systems, and ecosystem processes – as well as revealing unexpected levels of diversity in habitats as disparate as rock surfaces, insect guts and discovering hybrid fungi (*Melampsora, Phytophthora*). We can also anticipate major advances in understanding how fungi operate through total genomic approaches as more fungi are completely sequenced. The issue is not so much one of molecules *versus* morphology, but the need for a wholesome marriage in which the partners fully respect and input their complementary skills in a synergism taking the subject to new heights. The Pandora's box of molecular surprises is to be seen as one of blessings and not one of miseries and evils, though reaping those blessings can involve some short-term pain.

POLYPHASIC TAXONOMY OF FUNGI IN RELATION TO APPLIED MYCOLOGY

R.A. Samson

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The role of filamentous fungi in applied mycological research is increasing. Many species are involved in biotechnology and the production of numerous useful products. In addition, fungi play an essential role as spoilage and biodeteriorating agents. In the presentation some examples are given of the fungal spoilage of food and mycotoxin production. Also, mould problems in indoor environments are discussed. One of the major problems is that characterization of the species remains difficult, especially when dealing of representatives of the genera *Penicillium* and *Aspergillus*.

In *Penicillium*, classification and identification of the species have been problematic for many years. This is particularly the case in subgenus *Penicillium*, where many species are spoilage agents of food. Classification schemes were based on only phenotypical characters which proved to be extremely variable depending on the isolate and methods of cultivation. Consequently this phenotypic plasticity has rendered the taxonomy of species within terverticillate penicilli very unstable and features other than micromorphology (and the few other characters employed) are needed to stabilize the taxonomy and recognize/discover species. The introduction of DNA sequencing data have improved the situation, but they are more suited to phylogenetic studies and are less satisfactory for classification and identification than phenotypic data. Samson and Frisvad [1] proposed a stable taxonomy of these species based on a polyphasic study of a large number of isolates, using phenotypical characters, extrolite profiles and β-tubulin sequence data. The phenotypic characters used include (a) micro- and macro-morphology, (b) physiology (including growth at 5, 15, 25, 30, 37 ºC), (c) growth at 5 % NaCl and 15 % sucrose, (d) growth inhibition in presence of 1 % propionic acid, and (e) nutritional characters (including growth on urea, nitrite and creatine). All species were analyzed for extrolites and the profiles are highly species specific, and often of high consistency. In general features based on fungal differentiation (morphology and extrolites) are the most diagnostic and consistent, but the classification proposed is also supported by the physiological and nutritional characters. Some examples will be discussed of taxa with relevance to applied research.

In the genus *Aspergillus*, classification of taxa producing important mycotoxins have attracted much interest. In two studies [2,3] the taxonomy of the sections *Circumdati* and *Nigri* were elucidated with the descriptions of several new taxa. Refining the taxonomy of these important groups and the recognition of taxa using a polyphasic approach also contributes to our knowledge of potential mycotoxins problems and the rich biodiversity of these fungi.

References:

[1] Samson, RA, Frisvad JC (2004). *Studies in Mycology* 49: 1-257.
[2] Frisvad JC, Houbraken JAMP, Kuijpers AFA, Frank, MJ and Samson RA, (2004). *Studies in Mycology (Utrecht)* 50: 23-43.
[3] Samson RA, Houbraken JAMP, Kuijpers AFA, Frank, MJ and Frisvad JC (2004). *Studies in Mycology (Utrecht)* 50: 45-61.

THE RENASCENCE OF CLASSIFICATIONS IN MYCOLOGY: EXTROLITES, ECOPHYSIOLOGICAL FEATURES AND MORPHOLOGY ALLOW CLEAR-CUT CLASSIFICATIONS THAT ARE COMPLEMENTARY TO CLADIFICATIONS

J.C. Frisvad

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A very clear trend during the last 20 years in mycology, bacteriology, zoology and botany has been to use DNA sequence data rather than phenotypic features for classifications, cladifications, identification (bar-coding) and fingerprinting. It is suggested here to use the word phylotype for the phylogenetically relevant part of the genotype, selected parts of DNA, RNA and protein sequence data, and use such data for cladifications and eventually hypotheses concerning phylogeny. Similarly the ecotype is the part of the phenotype that is ecologically relevant and data based on the ecotype can be used for classifications. Classification can then be the basis for species recognition, nomenclature, identification and annotation of ORFs. Classification is therefore regarded as the core discipline in taxonomy and should ideally be based on the full phenotypic reaction norm. To deliver a full phenotypic description of any one isolate of fungus would be very difficult and time consuming, so in contrast to earlier taxometric principles it would be necessary to weight different features unequally, for example based on ecological relevance. Groups of ecotypic features of high relevance could be extrolites, morphology, nutrition, ecophysiology and resistance. Any of these features can be described in broad terms or in fine detail, depending of how advanced is the image analysis, spectrometric and chromatographic equipment available. Identifications, however, need to be based on standardized methods and equipment that is available. It is important to note that classification and identifications can only be made in a particular space of time, as evolution and co-evolution will of course take place. Thus classification, description, identification and cladification should all be used in full biosystematic studies, *i.e.* monographies. Examples will be given for *Penicillium* subgenus *Penicillium*. In this subgenus species are clear-cut, with no intergrading strains, and classification and cladification point to the same species and series. Individual extrolites such as ochratoxin A and citrinin are produced in a species specific way, but often by species in different clades, so it is important to stress that it is the profile of extrolites that is species specific *sensu stricto*.

MULTILOCUS SEQUENCE ANALYSIS OF *PENICILLIUM* AND *EUPENICILLIUM* SPECIES

S.W. Peterson

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Taxonomy of Hyphomycetes has always been a challenging problem, with experts viewing species in different ways and modifying the taxonomy of groups to reflect their best evaluation of species limits and concepts. The advent of phylogenetic analysis, relatively easy DNA sequencing techniques and PCR have provided an opportunity for mycology to move from a strictly morphological analysis of species to phylogenetic analysis of DNA sequences. Phylogenetic theory dictates that data from different loci will produce congruent or at least non-contradictory evolutionary histories of a clonal lineage. Tests of tree congruence such as the index of association can show whether lineages are clonal, and has revealed that some species long thought to be clonal are cryptically recombining. For those species, a biological species concept can be applied. For other species a phylogenetic species concept that groups isolates by descent from a common ancestor rather than simple similarity can be applied.
The impact has been immense in *Penicillium*. Subgenus *Biverticillium* (along with *Talaromyces* species) was proved to represent a distinct lineage, with *Aspergillus* and the remaining species of *Penicillium* and *Eupenicillium* on the sister branch. Subgenera *Aspergilloides* and *Furcatum* were proved to be paraphyletic groupings. Of the subgenera associated with *Eupenicillium* teleomorphs only subgenus *Penicillium* is a monophyletic lineage. Use of these theoretical and technical advances promises to stabilize the taxonomy of *Penicillium* while it improves our understanding of the evolution of critically important aspects of phytopathology and mycotoxicology.

ORAL COMMUNICATIONS

FUNGI IN BOTTLED WATER: A CASE STUDY OF A PRODUCTION PLANT

A. Ribeiro[1,2], A.P. Machado[1,2], Z. Kozakiewicz[3], B. Luke[3], M. Ryan[3], A. Venâncio[2], N. Lima[1,2] and J. Kelley[3]

[1]Micoteca da Universidade do Minho and [2]Centro de Engenharia Biológica da Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal. [3]CABI Bioscience, Bakeham Lane, Egham, Surrey TW20 9TY, United Kingdom.
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A one year fungal survey of a water bottling plant was conducted in order to evaluate the incidence and fluctuations of the biota. The dominant fungal genera in order of highest numbers were *Penicillium, Cladosporium* and *Trichoderma* followed by *Aspergillus, Paecilomyces*, and others. As expected, highest number of isolates collected were during the summer months, particularly May and June. Indeed during these two months there were more fungi present in the water after it had passed through the filtration system (0.4μm filter), indicating that during those times of the year when fungal contamination is high, filters should be changed on a more regular basis. In order to assess whether contamination was single or multi-loci, molecular methods based on PCR were used. Overall fungal contamination arose from multiple sources. Some fungal strains were very "alike" and were detected during different sampling times, indicating that some strains were endemic to the plant. There was little evidence to suggest that fungi detected in the source water passed through to other parts of the plant. However, there was evidence that fungal strains isolated from the water filter were detected elsewhere in the factory, confirming the need to change filters more regularly during periods of high fungal contamination. In order to improve quality control a HACCP programme was implemented and Best Practice Guidelines introduced.

Acknowledgments:
This work was supported by European project COMBOW - Control of Mycological Contaminations in Bottled Water (CRAFT/QLK1-2002-70843 contract).

EVALUATION OF PCR-RFLP OF ITS rDNA AS A DIAGNOSTIC TOOL FOR A RAPID IDENTIFICATION OF *PENICILLIUM* SUBGENUS *BIVERTICILLIUM* SPECIES

J. Dupont, C. Jacquet and M.-F. Roquebert

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PCR-RFLP of ITS rDNA is proposed as a useful tool for molecular identification of the most common species of biverticillate penicillia. 60 isolates were analysed representing 13 species and 21 sequences were produced. The combination of five restriction enzymes was successful to discriminate between the 12 species investigated. The variety *Penicillium purpurogenum* var. *rubrisclerotium* remained however indistinguishable from *P. funiculosum*. *P. funiculosum* appeared as the species most confusedand was misidentified with (a) *P. miniolutum* and *P. pinophilum* which were originally part of the species and (b) *P. purpurogenum* probably because of the red pigment produced. *P. variabile* was difficult to investigate as introns were found for half of the isolates. *P. piceum, P. rugulosum, P. loliense, P. erythromellis* and *P. purpurogenum* were homogeneous molecularly and morphologically, and corresponded to well circumscribed taxa. Further, intraspecific variability was observed within *P. pinophilum* and *P. funiculosum* where, particularly, the ex-type isolate produced a unique pattern far removed from the others isolates. This method is sensitive, rapid and inexpensive and can be used for the identification of biverticillate species. It is recommended particularly when large numbers of samples have to be authentified prior analysis.

Acknowledgments:
We wish to thank the curators of the culture collections who provided some isolates. This work was supported by the « Ministère de la jeunesse, de l'Education et de la Recherche » for the programme « Création du Centre Français de Ressources Fongiques d'intérêt agro-industriel », 2002-2005.

GEOGRAPHICAL SIMILARITY ANALYSIS OF CORK COLONISING *PENICILLIUM GLABRUM* ISOLATES USING DNA FINGERPRINTING

M.C. Basílio[1], R. Gaspar[1], C. Silva Pereira[1] and M.V. San Romão[1,2]

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The cork manufacturing process includes an operation known as stabilisation, where humid cork slabs are extensively colonised by fungi. The repercussion of fungal growth over cork is yet to be completely understood and is especially important with respect to "cork taint". To address this it becomes essential to identify environmental constraints which define the appearance of the colonising fungal species, tracing their origin either back to the forest, and/or as residents in the manufacturing space. The present data correlates two rounds of systematic sampling carried inside two manufacturing units, in distant zones of the country: north and south. Fungi from various genera were detected in different units or in the same unit in different years. Following *Chrysonilia sitophila* dominance, the appearance of a high diversity of *Penicillium* species was apparent. The inter-species variability of the *Penicillium taxon* was solved through morphological analysis. *P. glabrum*, was found consistently in all samples as the most frequent species. Moreover, *P. glabrum* intra-species variability was studied by DNA fingerprint tests that identify high discriminative polymorphic markers in the genome. Cluster analysis of *P. glabrum* data was used to discuss their geographical similarity.

Acknowledgments:
This work was partially supported by Amorim & Irmãos company (Sta. Maria de Lamas, Portugal).

A NOVEL APPROACH FOR MYCOTOXIGENIC FUNGI IDENTIFICATIONS BASED ON UNCOMPLICATED MICROSCOPY AND MYCOTOXIN CHARACTERS

R.R.M. Paterson[1,2], A. Venâncio[2] and N. Lima[1,2]

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Species concepts within fungi are (a) subject to frequent revision and (b) unusually protean. However, this allows novel schemes some scope to be considered. The taxonomy of the penicillia is unstable particularly in the important antibiotic and mycotoxin-producing subgenus *Penicillium*. Non-specialists in particular encounter difficulty with assigning names to taxa. Name changes of important fungi have occurred frequently and recently. There are difficulties relating identifications to mycotoxin production. This is necessary for controlling mycotoxin contamination in food, drink, homes, etc. Patulin is an important mycotoxin produced by various fungi and has strict limits in the European Union and elsewhere. Also, it is a useful model mycotoxin *per se*. The mycotoxin and/or the isoepoxydon dehydrogenase (IDH) gene of the metabolic pathway have been assessed in 318 strains predominately of subgenus *Penicillium*. These data were used to classify the isolates, although the applied aspects of the scheme are emphasised over the purely taxonomic. Of course, the issue of whether to apply a dual nomenclature to different states of pleomorphic fungi is relevant. Subgenus *Penicillium* contained most of the IDH and patulin positives. Many of species contained positive and negative members, which demonstrates that identifications based on morphology do not indicate patulin production. The species and varieties in subgenus *Penicillium* which have been associated with patulin production can be reduced to one name, *viz. Penicillium* Pen p^+, where p is the abbreviation of patulin. This basic concept has been extended to other mycotoxin producing fungi within the subgenus using published data to indicate the utility of the scheme. The classification will lead naturally to the number of taxa being reduced. In addition, more meaningful results are obtained in terms of assessing the potential for patulin production. The possibilities of direct analysis of environmental samples are also discussed. The scheme could be used with advantage for other fungi.

Acknowledgements:
R.R.M. Paterson is grateful for grant SFRH/BPD/14923/2004 from Fundação para a Ciência e a Tecnologia (FCT), Portugal.

PIGMENT CHEMISTRY, TAXONOMY AND PHYLOGENY OF THE *HYPOXYLOIDEAE* (*XYLARIACEAE*)

M. Stadler

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The biological and chemical diversity of *Xylariaceae* with emphasis on genera with *Nodulisporium*-like anamorphs (*e.g.*, *Daldinia*, *Hypoxylon*) was evaluated by a HPLC-based chemotaxonomic survey of >2000 specimens and cultures, accompanied by extensive morphological studies and SEM [1,2]. Conspecificity of recent records with ancient type specimens by comparison of HPLC profiles was established in many cases, since the characteristic metabolites may remain stable for over 200 years [3-5]. Most of them constitute novel natural products that were identified from stromata of *Xylariaceae* in the course of concurrent "mycochemical" studies [6-8]. These results were largely in agreement with those of concurrent molecular studies, involving 5.8S/ITS nrDNA, ß-tubulin and actin sequences [9,10]. While anamorphic morphology and secondary metabolism of cultures agreed well at the generic level and above, a combination of chemical and morphological traits complied with molecular data and are better suited for species discrimination than PCR-based approaches because relatively few taxa in these genera have been sequenced [4,10,11]. The utility of chemotaxonomy to predict and support phylogenetic relationships as inferred by molecular methods was demonstrated by: i) validation of the recent segregation of the new genus *Annulohypoxylon* (=*Hypoxylon* ss. *Annulata*) from *Hypoxylon* [10], which was largely reflected by the diverging HPLC profiles of these fungi [3]; ii) revealing the discoid to peltate species in *Hypoxylon* ss. Ju & Rogers 1996 [12] (*H. placentiforme* group) to be ancestral to *Daldinia*, suggesting they should be placed in the latter genus as a separate section; iii) establishing the affinities of *Pyrenomyxa* and *Pulveria* to one another and their close relations to *Hypoxylon* by a combination of all the above methods [13]; and iv) recognition of *Creospheria* as a member of a separate evolutionary lineage that may have diverged from the mainstream of *Xylariaceae* and *Diatrypaceae* a long time ago in evolutionary terms [9,14]. A polythetic approach appears most useful to achieve a stable phylogeny of these and other fungi, and the utility of chemotaxonomy to assess biological diversity should not be underestimated.

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PORTUGUESE REGIONAL DIFFERENCES IN THE WINE GRAPES MYCOFLORA

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The recent discovery of mycotoxins in wine, in particular ochratoxin A, caused concern and motivated an extensive survey to the mycoflora of Portuguese grapes. It is known that the mycoflora of agricultural commodities can vary according to the geographical origin, and therefore, regional differences in the mycoflora of Portuguese were investigated. Four regions were selected for a 3-year study: Alentejo, Douro, Ribatejo and Vinhos Verdes. The mycoflora of grapes was evaluated by plating methods. A total of 32 grape samples were taken, of 50 berries each. The differences in the mycoflora of grapes between regions were analyzed using the non-parametric test Kruskal-Wallis H. Ostensibly, the classification of the grapes into their geographical origin based on its mycoflora was attempted using a decision tree algorithm (C4.5) based on the Shannon Information Theory. Of the 27 fungal genera identified, 3 varied its incidence significantly according to the region of origin of the samples: *Aspergillus*, *Botrytis* and *Ulocladium*. The only species that varied significantly its frequency between regions was *A. niger* aggregate. Six *Penicillium* species differed significantly between regions: *P. brevicompactum*, *P. citrinum*, *P. glabrum/spinulosum*, *P. expansum*, *P. implicatum* and *P. thomii*. Using decision trees it was possible to classify successfully 91% of the samples according to 3 sample classes: Vinhos Verdes, Douro and South samples (Alentejo and Ribatejo). The classification was based on the incidence of *A. niger* and *P. thomii* in the grape samples. The estimated predictive ability of the model in the 3 classes was 82%.
The data presented here indicate that grapes are consistently exposed to a particular mycoflora that varies according its geographical origin, which may be of importance to establishing risk areas for mycotoxin contamination of grapes and wine.

Acknowledgements:
The authors gratefully acknowledge the support of the EC, Quality of Life Program (QoL), Key Action 1 (KA1) on Food, Nutrition and Health; contract number QLK1-CT-2001-01761 - Wine-Ochra Risk.

POSTERS

EFFICACY OF PLANT EXTRACTS AGAINST STORED PRODUCT FUNGI

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There is an increasing public concern about the level of pesticide residues in food, which has encouraged researchers to find alternative solutions. Plant extracts are generally assumed to be more acceptable and less hazardous than synthetic compounds. This means that those extracts could be considered as alternative antifungal treatments. In this work, we have evaluated the fungistatic activity of six aqueous extracts namely: chamomile (*Anthemis nobilis* L.), cinnamon (*Cinnamomum verum* J. Presl.), garlic (*Allium sativum* L.), malva (*Malva sylvestris* L.), peppermint (*Mentha piperita* L.) and rosemary (*Lavandula stoechas* L.). They were tested against *Aspergillus* sp., *A. niger*, *Penicillium* sp. and *Fusarium culmorum*. Results are presented and discussed.

MARINE FUNGI ON *SPARTINA MARITIMA* BAITS EXPOSED IN THE MIRA SALT MARSH

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The occurrence of higher marine fungi (Ascomycota, Basidiomycota and mitosporic fungi) associated with baits, made of pieces of stems of *Spartina maritima*, on the Mira salt marsh was investigated for 12 months. The method involved the exposure of baits to different conditions: permanent and temporary submersion at the upper and lower limits of the intertidal zone of the marsh.
The substrate was observed for fruit bodies and spores directly after collection and monthly following an incubation period (up to 8 months) in moist chambers.
In the course of this work, 26 marine species were identified (17 Ascomycota, 2 Basidiomycota and 7 mitosporic fungi). 24 out of all taxa reported in this survey are new records for Portugal. *Nia globospora* has been published as a new species.
Based on the collected data, species were characterized with regard to their frequency of occurrence, colonization capability and succession in the substrate. The following parameters were also determined the: diversity of fungi on baits exposed to three different conditions based on the Shannon diversity index; similarity of the mycota associated with baits exposed to three conditions based on the Sorenson similarity index.

THE AMBROSIA FUNGI IN THE INSECT-FUNGI SYMBIOSIS AND THEIR INFLUENCE TO FOREST DECLINE

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Ambrosia fungi live associated with beetles (Scolytidae and Platypodidae) and their galleries in host trees. The symbiotic relations between insect and fungi seem to have a special importance in the colonizing strategies of host trees by beetles, and offer several advantages for insect and fungi. Typically, ambrosia fungi are dimorphic: they grow as ambrosial or yeast-like form and as mycelium. The fungi are highly specialized, adapted to their specific beetle as well as to the biotope where they both live. In addition to fungi directly involved in insect nourishment, other fungi have been found associated with them, such as tree pathogenic fungi that may play a role in insects' host colonization success. Several saprobes are also frequently present in insects' galleries. The permanent presence of these fungi associated with insects indicates their importance in the symbiosis, for example, for the decomposition of cellulose and lignin, and/or antagonism of other fungi. With this study, we summarize the importance of ambrosia fungi and the interaction with insects and hosts to forest health condition. The hypothesis of the transport of pathogenic fungi by *Platypus cylindrus* to cork oak thus contributing for its decline will be discussed in particular.

NOVEL AND SIMPLE TEST PLATING FOR SCREENING RELATIVE TRANSFRUCTOSYLATION ACTIVITY OF FUNGI

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Fructooligosaccharides (FOS) have received particular attention recently because of their excellent biological and functional properties, namely, as prebiotic compounds that promote the growth of intestinal microflora. They are also low calorie non-carcinogenic sweeteners with numerous suggested health benefits. These include immune system activation, resistance to infections, synthesis of B-complex vitamins, calcium absorption. They can be used as a treatment for breast cancer, diarrhoea, and constipation.

Although FOS are present in trace amounts in fruits, vegetables and honey as natural products, its mass production is limited by seasonal restrictions and the inherent inefficiencies of these systems. Hence, microbial FOS production by fungi in bioreactors is more realistic.

Several microorganisms are reported to have transfructosylation activity due to fructosyltransferase (EC 2.4.1.9) and/or fructofuranosidase (EC 3.2.1.26) activities. However, the search for other fungi with higher transfructosylation activity is still a challenge.

So, a presumptive and indirect colorimetric plate assay for the evaluation of transfructosylation activity in fungi was developed by the simultaneous determination in the same plate of glucose and fructose released from sucrose. The method entailed the coupling of two dye systems, namely the glucose oxidase-peroxidase coupled reaction using phenol and 4-aminoantipyrine for determination of glucose; and the fructose dehydrogenase oxidation in the presence of a tetrazolium salt for determination of fructose. In order to have a standard assay, the fungi were grown on Czapek Dox (CD) agar. 1 disc of mycelium (8 mm diameter) was cut from the edge of each colony and then put in contact with CD agar plates. After incubation at 25 ºC for 72 h each assay plate was overlaid with soft agar containing the reagents. The presence of enzymes with transfructosylation activity was identified by the formation of pink (presence of glucose) and blue (presence of fructose) halos around the discs. In conclusion, the results showed that the method is suitable for screening a large number of fungi due to its simplicity, reproducibility and rapidity.

Acknowledgements:
This work was supported by Agência de Inovação (AdI), Portugal, project BIOLIFE ref. PRIME 03/347.

AIRBORNE FUNGI IN PORTUGUESE SCHOOLS

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Fungi are ubiquitous with species found in soil, vegetation and air. Their spores are common even in indoor environments and may be harmful to health. The main diseases caused by fungi are conceivably infection, allergy, and mycotoxicoses. Because children breathe a greater volume of air relative to their body weight when compared to adults, they may be more sensitive. Additionally, people in school buildings may be close physically, with schools having a four times greater occupation density in comparison to office buildings (Heath and Mendell, 2002). In Portugal data were not available concerning indoor air quality in schools. The aim of this study is to understand in what environment our children are learning and to which fungal health risks they are exposed.
Airborne viable fungi were collected on plates of standard sterilized malt extract agar (MEA) by impaction using an environmental Mair T sampler according to the method 0800 described in NIOSH (1998). Media plates were removed from the sampler and incubated at 25 ºC for 7 days. Different volumes of aerosol were taken at a height of 0.9 m. Samples were collected in 9 schools of different cities and urban areas. Table 1 indicates the bioload obtained.

Table 1 - Average concentrations (and range) of fungi in different urban areas, CFU/m^3.

	Matosinhos city Industrial area	Oporto city Coastal area	Vila Real city Inland area	Guidelines
Fungi	353 (240-451)	431 (176-741)	1704 (202-7000)	500

The main species already identified in the classrooms are *Penicillium* (*P. crustosum, P. thomii, P. oxalicum, P. olsonii, P. rugulosum, P. variabile), Aspergillus (A. versicolor, A. niger, A. fumigatus, A. candidus), Alternaria* sp. and *Cladosporium* sp. These results demonstrate that there was a large variation in the concentration of airborne fungi. The average levels of fungi recorded in the classrooms, was higher than the guidelines for Vila Real city. Our study showed that the most prevalent indoor molds are *Penicillium*, *Aspergillus* and *Cladosporium,* which are in accordance with Husman (1996).

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NOTES ON A PLANT PARASITE FUNGUS IN PORTUGAL: *GYMNOSPORANGIUM CORNUTUM*

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In August 2004, a rust fungus identified as *Gymnosporangium cornutum* was found on *Sorbus aucuparia* in Serra da Estrela (Manteigas – Torre road), and the disease caused by this pathogen was severe at that location. Despite the abundance and worldwide occurrence of the genus *Gymnosporangium*, floristic studies in Portugal are still lacking.

Acknowledgments:
This study was supported in part by PIDDAC 105/02.

FILAMENTOUS BASIDIOMYCETES FOR TESTING BIOLOGICAL TOXICITY OF POLLUTANTS

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A simple test using the inhibition of growth of filamentous basidiomycetes on agar medium for the estimation of toxicity of pollutants was developed. Out of nine species of wood-rot fungi, *Pycnoporus cinnabarinus* and *Pleurotus ostreatus* were selected as suitable for toxicity testing because of regular and rapid growth. The rate of concentric growth of the fungal colony measured in the presence of a toxicant and related to the growth without the toxicant was demonstrated to be a effective parameter for toxicity testing. Exposure times of 6 and 7 days were used for *P. cinnabarinus* and *P. ostreatus*, respectively. The function relating growth inhibition (per cent inhibition expressed in probits) to the toxicant concentration (log c, mg l^{-1}) was represented by a straight line ($R^2 \geq 0.95$). Potassium dichromate and 3,5-dichlorophenol were used as the reference toxicants: The respective EC50 values for *P. cinnabarinus* were 409.8±50.8 and 9.2±0.1 mg l^{-1} and for *P. ostreatus* 150.7±9.0 and 11.4±0.4 mg l^{-1}. The fungal test could be used for the evaluation of toxicity of heavy metals (Cd, Zn, Pb) and organics such as pentachlorophenol (PCP) and synthetic dyes. The toxicity of azo dyes (Congo Red, Reactive Orange 16) and anthraquinone dyes (Remazol Brilliant Blue R, Disperse Blue 3) was much lower compared to PCP.

Acknowledgments:
The work was supported by the following projects: No. IAA6020411 (Grant Agency of the Czech. Acad. Sci.), No. 011-2004-05 (Ministry of Education, Youth and Sports of the Czech. Rep), No. AVOZ5020903 (Institutional Research Concept).

NEW SYNTHETIC COMPOUNDS AS INHIBITORS OF MYCOTOXIN SYNTHESIS

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Ochratoxin A (OTA) is a mycotoxin produced by some *Aspergillus* and *Penicillium* species which is often detected in beer, cereals, coffee, feeds, figs, sultanas and wine. Some fungicides have been found to be effective in preventing fungal growth, but, in other cases, an increase in the synthesis of mycotoxins was reported. Also, the pressure to use less harmful compounds to the environment stimulates the exploration of new and more benign compounds.
Synthetic compounds were tested on the growth and OTA production of one strain of *A. alliaceus*, *A. ochraceus*, *A. carbonarius* and *A. niger*. These new synthetic compounds have a linear structure incorporating urea and/or a phenolic unit. Fungi were grown in yeast extract sucrose (YES) medium supplemented with 50 µM to 200 µM of each one of 20 test compounds in triplicate, for 6 days. Growth was recorded by measuring the diameter of colonies every 24 hours, and OTA was quantified after 5 days of growth using HPLC and fluorescence detection.
Growth of the *A. ochraceus* and the *A. carbonarius* strains were not inhibited by most of these compounds. However, some led to a decrease in OTA detection. Compounds without the phenolic unit were found to be less effective, while those compounds with urea and phenolic units were the most effective. Growth of the *A. alliaceus* and of the *A. niger* strains were inhibited by compound-X by 22 and 27% respectively.
This approach will lead to the selection of functional groups able to inhibit the synthesis of OTA which could be incorporated into more powerful antifungal compounds.

Acknowledgements:
L. Abrunhosa, F. Areias and M. Zaki are grateful for grants SFRH/BD/11228/2002, SFRH/BD/3185/2000 and SFRH/BPD/12044/2003 respectively from Fundação para a Ciência e Tecnologia – FCT, Portugal.

OCHRATOXIN A AND FILAMENTOUS FUNGI IN RED WINE GRAPES FROM SANTA CATARINA, BRAZIL

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The quality of wines has been evaluated traditionally according to sensorial properties. Recently, safety issues have been raised, such as pesticide residues and mycotoxins, with the introduction of new agricultural practices and the development of analytical methods with higher sensitivity. Ochratoxin A (OTA) is such a mycotoxin, produced by some *Aspergillus* and *Penicillium* species and is one of the most recent safety issues for wine.
The mycobiota of, and the occurrence of OTA in Southern Brazilian grapes are not known. The presence of these contaminants was assessed by collecting 30 samples of grapes, from 16 vineyards, from the two most important wine sub-regions in the State of Santa Catarina, Brazil. The mycobiota was evaluated by plating 10 grapes from each sample in Dichloran Rose Bengal Chloramphenicol Agar and Sabouraud Dextrose Agar, supplemented with chloramphenicol. Production of OTA by black *Aspergillus* strains was estimated after growing in Czapeck Yeast Agar. OTA was analysed in 9 grape samples by chromatography with immunoaffinity clean-up, as stipulated by the European regulation.
Three hundred and eighty seven strains were isolated. The dominant genera were *Cladosporium* (found in 86.7% of plated berries), *Alternaria* (80.0%), *Botrytis* (70.0%), *Aspergillus* (66.7%), and *Penicillium* (63.3%). Sixteen *A. niger* aggregate strains (26 % of total *Aspergillus* strains) were isolated, and OTA was not detected from any of these strains. No *A. carbonarius* was isolated. OTA was found in 6 grape samples, with a range of values from 0.16 µg/Kg to 0.77 µg/Kg.
In conclusion, no OTA producing black *Aspergillus* strains were found in grapes, although some grape samples contain the mycotoxin. The fungal source of OTA requires further investigation.

FUNGAL GROWTH AND MYCOTOXINS IN CORN SILAGES

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Ensiled forages and grains are very important for feeding dairy cows in the Portuguese Azores islands. Fungal spoilage of animal feed silage occurs frequently. Moulds have no significant beneficial purpose to the ensiling process, and their ability to proliferate results from silage environments that are aerobically unstable, leading to unstable silage, loss of nutritive substances and mycotoxins contamination.

A total of eighty maize silos produced in Azores were collected. Samples (from middle, surface, and critical points - with visible moulds contamination) were examined for the total fungi and particularly for the presence of *Aspergillus fumigatus*, the mycotoxicological evaluation of fumonisin B_1 and deoxynivalenol, were done to twenty five samples from the silo middle, that were considered good silos concerning the dry matter and the silo pH.

All samples present fungi contamination. High levels (over 10^4 CFU/g) of yeasts were found in 70 samples (89% of total samples). Thirteen samples (54%) of the samples from the silo middle, 21 samples (72%) from the surface and 19 samples (86%) from the critical points were infested by *A. fumigatus*. *A. fumigatus* is the dominant spoilage mould in maize silage in Azores. Other fungi that were identified belong to the genera *Absidia*, *Aspergillus*, *Cladosporium*, *Monascus*, *Mucor*, *Penicillium*, *Phoma*, *Rhizopus*, *Sepedonium*, *Trichoderma*, *Verticillium*.

The mycotoxicological evaluation indicated contamination of 14 samples (56%) with fumonisin B_1 and 10 samples (40%) with deoxynivalenol.

FISH AND CALCOFLUOR STAINING TECHNIQUES TO DETECT *IN SITU* FILAMENTOUS FUNGAL BIOFILMS IN WATER

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Filamentous fungi (ff) are a ubiquitous and diverse group of eukaryotic organisms. However, fungal involvement in biofilms has not been demonstrated unambiguously. They may be responsible for the production of tastes, odours and mycotoxins in water [1] and so rapid detection is important. Detection of ff by conventional methods is complex, indirect and time consuming: results can be obtained only after 3-5 days because of slow growth. In order to study the presence of ff in water biofilms two methods for direct detection were used: (a) the FISH technique which employed the EUK516 probe, 5'-ACCAGACTTGCCCTCC-3' labelled with the red Cy3 dye at the 5' terminal; (b) Calcofluor White M2R fluorescent dye which stained the fungal cells walls blue. *Penicillium brevicompactum* was used in pure culture to establish the methods and then real water biofilm samples in PVC-C and cast iron coupons were studied.
The FISH method demonstrated the filamentous structures we assume were fungi after approximately 5 hours. In contrast, the Calcofluor method revealed ff in less than one hour, and so it is possible for a rapid assessment of the presence of fungi in biofilms. When the two methods were combined additional information was possible to extract such as combining the images of empty filaments (blue) with intact protoplasm (red).
In conclusion, FISH and Calcofluor staining provide rapid, direct and unambiguous information on the involvement of ff in biofilms which form in water.

Acknowledgements:
This work was undertaken as part of the research project supported by the European Union within the Fifth Framework Programme, "Energy, environment and sustainable development programme", n° EVK1-2002-00108. There hereby follows a disclaimer stating that the authors are solely responsible for the work. It does not represent the opinion of the Community and the Community is not responsible for any use that might be made of data appearing herein. R.R.M. Paterson was supported by the grant FRH/BPD/14923/2004 from Fundação para a Ciência e Tecnologia (FCT), Portugal.

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FORTHCOMING MEETINGS

3rd International Medicinal Mushroom Conference
12-17 October 2005.
Port Townsend, Washington, USA.
www.fungi.com/conference

Ochratoxin A in Grapes and Wine: Prevention and Control
20-21 October 2005.
Sala S. Pietro, Marsala (TP), Sicily, Italy.
http://www.ochra-wine.com

XXV European Culture Collections' Organisation (ECCO) Meeting
7-9 June 2006.
Budapest, Hungary.
www.eccosite.org

V Latin American Congress of Mycotoxicology - MicotoxLatinAm5
18-21 June 2006.
Florianópolis, Santa Catarina, Brazil.
http://www.labmico.ufsc.br/micotoxlatinam5

2nd FEMS Congress of European Microbiologists
4-6 July 2006.
IFEMA Centro de Convenciones, Feira de Madrid, Madrid, Spain.
www.fems-microbiology.org/congress

8th International Mycological Congress (IMC8)
21-26 August 2006.
Cairns Convention Centre, Cairns, QD, Australia.
www.sapmea.asn.au/imc8

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