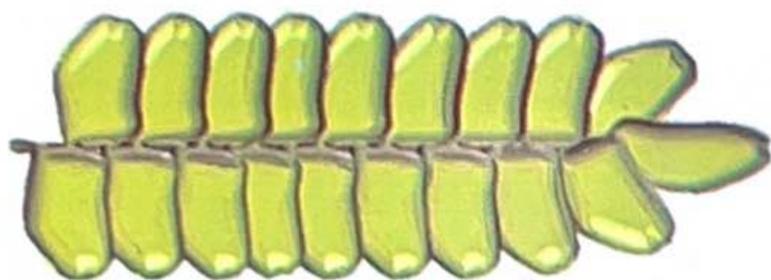


PROGRAM

ICOM6



6th INTERNATIONAL CONFERENCE ON MYCORRHIZA

“BEYOND THE ROOTS”

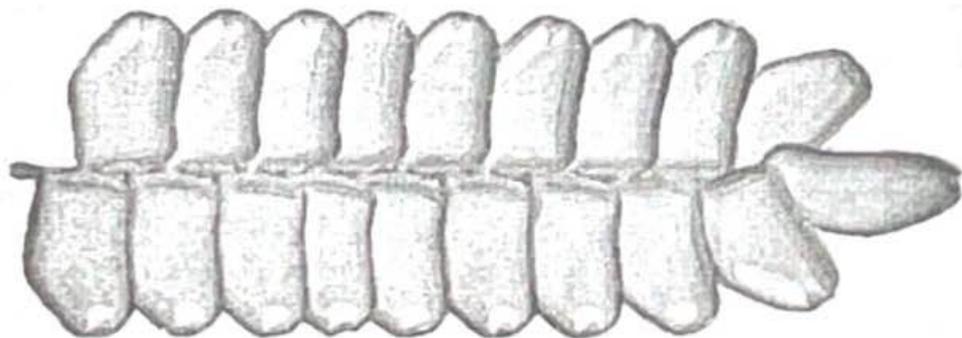
9 to 14 August 2009

Belo Horizonte - Brazil



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"BEYOND THE ROOTS"

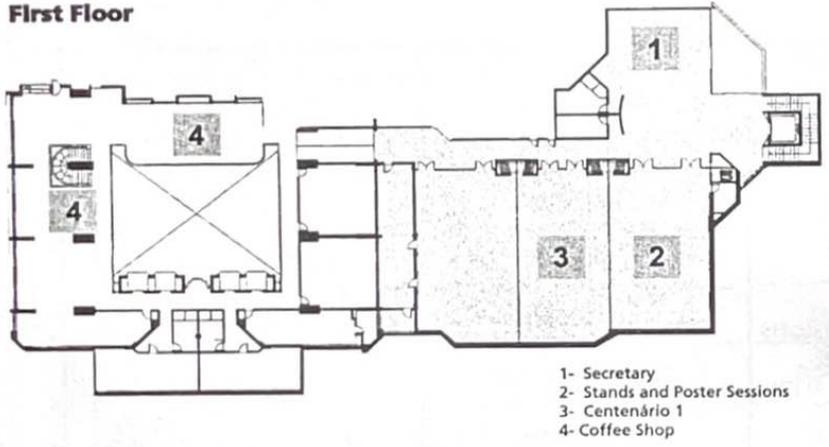
**9 to 14 August 2009
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Summary of the Scientific Program

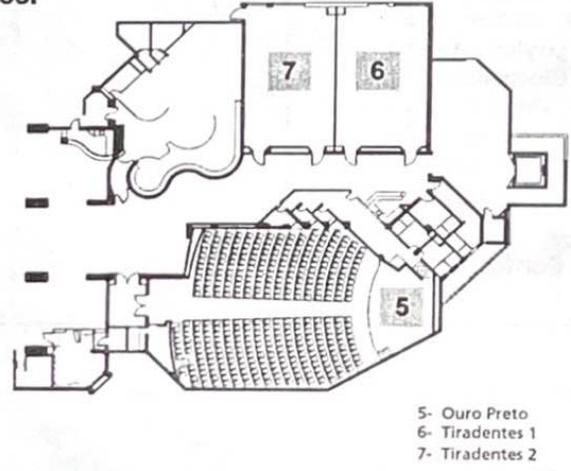
Time	Sunday Aug 09	Mon Aug 10	Tue Aug 11	Wed Aug 12	Thu Aug 13	Fri Aug 14	
09:00 10:00		Keynote lecture	Keynote lecture	Excursions & Field trips	Keynote lecture	Keynote lecture	
10:00 10:30		Coffee break	Coffee break		Coffee break	Coffee break	
10:30 12:30		Symposia	Symposia		Symposia	Plenary session	
12:30 14:00		Lunch break	Lunch break		Lunch break	Lunch break	
14:00 15:30		Poster session	Poster session		Poster session		
15:30 16:00		Coffee break	Coffee break		Coffee break		
16:00 18:00	Registration	Workshops 1. Cell biology and development 2. Fungal communities 3. Mixotrophic and mycoheterotrophic interactions	Workshops 4. Signal perception and metabolism 5. Mycorrhizal fungal population structure in the field 6. Mycorrhizas and plant nutrition 7. Inoculum technology, application, and management of mycorrhizal fungi			Workshops 8. Genome structure, plasticity and evolution 9. Mycorrhizosphere interaction 10. Taxonomy and phylogeny of Glomeromycetes 11. Mechanism of tolerance to biotic and environmental stresses	
19:00 20:00	Opening Ceremony	Inoculum technology meeting	Wines of the World			Conference Dinner	

Map

First Floor



Ground Floor



Opening Ceremony

Sunday Aug 09

Opening conference: "The first thirty years of mycorrhizal research in Brazil". Dr. José Oswaldo Siqueira - Universidade Federal de Lavras/CNPq, Brazil - Ouro Preto

Keynote Lectures

Monday Aug 10

Environmental genomics of ecologically relevant micro-organisms. Dr. George A. Kowalchuk - Netherlands Institute of Ecology (NIOO-KNAW), Vrije Universiteit, The Netherlands - Ouro Preto

Tuesday Aug 11

'Hidden' phosphorus transfer in arbuscular mycorrhizas: what should it mean for you? - Dr. Sally Smith University of Adelaide Australia - Ouro Preto

Thursday Aug 13

Plants and arbuscular mycorrhizal fungi: born to be friends. Dr. Paola Bonfante - Department of Plant Biology of the University of Torino Italy - Ouro Preto

Friday Aug 14

"The *Glomus* genome project: an update". Dr. Peter Young - University of York, UK - Ouro Preto

Workshops

Monday Aug 10

no Centenario 1

Session 1

Cell biology and development of mycorrhizas - Coordinator: Dr. Paola Bonfante - Centenario 1

Dr. Andrea Genre - DBV Università di Torino/IPP-CNR, Torino, Italy - Illuminating plant cellular dynamics during arbuscular mycorrhiza interaction.

Dr. Daniel Wipf - INRA/Université de Bourgogne, Dijon, France - Importance of fungal transporters in successful plant-fungus collaboration.

Dr. Franziska Krajinski - Max Planck Institute of Molecular Plant Physiology, Germany - Correlation of miRNA expression, phosphate homeostasis and arbuscular mycorrhiza development in *Medicago truncatula*.

Session 2

Fungal communities - Coordinator: Dr. Olinto Liparini Pereira - Ouro Preto

✓ Dr. Damase Khosa - Université de Laval, Québec, Canada - Molecular analysis of mycorrhizal communities in forest and agroforest systems.

Dr. Yutaka Tamai - Hokkaido University, Japan - The mycorrhizal status of *Picea glehnii* regenerated on serpentine soil.

no ✓ Dr. Dirk Redecker - University of Basel, Switzerland - From species communities to populations of arbuscular mycorrhizal fungi.

Session 3

no

Mixotrophic and mycoheterotrophic interactions - Coordinator: Larry Peterson - Tiradentes 1

✓ Dr. Marlon Corrêa Pereira - Universidade Federal de Viçosa, Rio Paranaíba, Brazil - Mycorrhizal fungi diversity in Brazilian orchids.

Dr. Juan Pablo Suarez - Universidad Técnica Particular de Loja, Ecuador - Sexual states of Tulasnellales in the tropical mountain forest: correlation between morphology and ITS-5.8S variability.

Dr. Larry Zettler - Illinois College, The Morton Arboretum, USA - An overview of orchid propagation and reintroduction in North America using mycorrhizal fungi as a conservation tool.

Dr. Tupac Otero - Universidad Nacional de Colombia, Colombia - Mycorrhizal interaction between tropical rhizids and *Ceratobasidium* spp.

Tuesday Aug 11

Session 4

Signal perception and metabolism - Coordinator: Vivienne Gianinazzi-Pearson - Tiradentes 1

Dr. Pascale Seddas - UMR INRA/CNRS / Université de Bourgogne, Dijon, France - Deciphering the molecular dialogue between arbuscular mycorrhizal partners during early root interactions.

Dr. Irene da Silva Coelho - Universidade Federal de Viçosa, BRAZIL - Expression of genes during the pre-symbiotic interaction of the ectomycorrhizal association between *Hydnangium* sp. and *Eucalyptus*.

Dr. Valérie Legué - INRA/Univ. H. Poincaré, Nancy University, FRANCE - The ectomycorrhizal fungus *Laccaria bicolor* stimulates lateral root formation in poplar roots through auxin signaling.

Session 5

Mycorrhizal fungal population structure in the field - Coordinator: Francisco Adriano de Souza - Ouro Preto

Dr. Sidney Luiz Stürmer - Universidade Regional de Blumenau and Dr. Francisco Adriano de Souza - Empresa Brasileira de Pesquisa Agropecuária, Sete Lagoas, Brazil - Community structure of arbuscular mycorrhizal fungi in Brazilian ecosystems: patterns, methodologies and perspectives.

Dr. Onguene Awana Nérée - Institute of Agricultural Research for Development (IRAD), Cameroun - Diversity and distribution of mycorrhizal fungal structures in humid forests of Cameroon in relation to ecological significance and land use degradation.

Dr. Nancy Collins Johnson - Northern Arizona University, USA - Biogeography of arbuscular mycorrhizal fungi: distribution and community composition patterns.

Dr. Leho Tedersoo - Institute of Ecology and Earth Sciences, Tartu University, Estonia - Biogeography of the ectomycorrhizal symbiosis.

Session 6

Mycorrhizas and plant nutrition - Coordinator: Dr. Elke Cardoso - Centenário 1

Dr. F. Andrew Smith - University of Adelaide, Australia - Is there a link between phosphate uptake and growth depressions in arbuscular mycorrhizal plants?

Dr. Claude Plassard - INRA, Montpellier France - Ectomycorrhizal fungi and mobilization of phosphorus: novel data and actual role.

Dr. Cristina Cruz - Universidade de Lisboa, Portugal - The importance of AMF on N and P acquisition.

Session 7

Inoculum technology, application and management of mycorrhizal fungi - Coordinator: Dr. Leonor Maia - Tiradentes 2

Dr. Davis Joseph Bagyaraj - Centre for Natural Biological Resources and Community Development (CNBRCD), India - Arbuscular mycorrhizal fungi for sustainable agriculture, horticulture and forestry.

Dr. Chantal Hamel - Agric. and Agri-Food Canada, Canada - AMF in sustainable agriculture: tools for farmers and policy-makers.

Dr. Alok Adholeya - Centre for Mycorrhizal Research, TERI, India - Appropriate host-fungus selection: a key to success of mass production and in depth comparison of cost economics.

Thursday Aug 13

Session 8

Genome structure, plasticity, and evolution - Coordinator: Damase Khasa - Tiradentes 1

Dr. Mohamed Hijri - Université de Montréal, Canada - Non-mendelian inheritance of genomes in arbuscular mycorrhizal fungi.

Dr. Daniel Croll/ Dr. Ian R. Sanders - University of Lausanne, Switzerland - Genetic exchange and recombination in AMF and their consequences on plant growth.

Dr. Greg Douhan - University of California, USA - Population biology and evolutionary genetics of the ubiquitous ectomycorrhizal fungus *Cenococcum geophilum*: species complex or complex species?

Session 9

Mycorrhizosphere interactions - Coordinator: Cristina Cruz - Centenário 1

Dr. Thomas W. Kuyper - Wageningen University, Netherlands - Costs and benefits in the mycorrhizosphere.

Dr. Galdino Andrade - Universidade Estadual de Londrina, Brazil - Correlation among fine root morphology, rhizosphere microbial population and nutrient availability in ecosystems under different successional vegetation status in Southern Brazil.

Dr. José-Miguel Barea - Estación Experimental del Zaidín - Granada, Spain - Co-inoculation of AM fungi and PGPR for helping plant development in either low-nutrient or heavy metal contaminated soils.

Session 10

Taxonomy and phylogeny of Glomeromycetes - Coordinator: Dr. Sidney L. Stürmer - Tiradentes 2

Dr. Arthur Schüssler - LMU Munich, Dept. Biology, Genetics, Germany - Systematics of Glomeromycota - phylogeny based taxa for modern research.

Dr. Fritz Oehl - Agroscope Reckenholz-Tänikon Research Station Zürich, Switzerland - The fairy tale of the difficult morphological identification of arbuscular mycorrhizal fungi - recent advances in the area.

Dr. Janusz Blaszowski - University of Technology, Szczecin, Słowackiego Szczecin, Poland - Basic principles of morphological identification of arbuscular mycorrhizal fungi forming glomoid spores.

Dr. Joseph Morton - West Virginia University, USA - Integration of morphological and genetic data to revise hypotheses of evolution in Glomeromycota.

Session 11

Mechanisms of tolerance to biotic and environmental stresses - Coordinator: Paulo Emilio Lovato - Ouro Preto

Dr. Janusz Zwiazek - Department of Renewable Resources, University of Alberta, Canada - Water relations and salt resistance of ectomycorrhizal plants.

Dr. Amadou Bâ - Université Antilles-Guyane, Guadeloupe, France - Alleviation of salt stress by *Scleroderma bermudense* in seagrass (*Coccoloba uvifera* L.) seedlings.

Dr. Jan Colpaert - Hasselt University, Centre for Environmental Sciences, Diepenbeek, Belgium - Heavy metal tolerance mechanisms in the ectomycorrhizal fungus *Suillus luteus*.

Dr. María J. Pozo - Estación Experimental del Zaidín, Spanish National Research Council (CSIC), Spain - Impact of arbuscular mycorrhizal symbiosis on plant resistance to pathogens.

Symposia

Monday Aug 10

Session 2

Fungal communities - Coordinator: Vetúlia Lopes - Ouro Preto

Jean Garbaye - INRA, Tree-Microorganism Interactions, Champenoux, France - Structure and function of ectomycorrhizal communities in response to soil mechanical and chemical disturbances.

Vohnik, M. - Institute of Botany ASCR, Pruhonice, Czech Republic - Symbiotic fungi inhabiting roots of Norway spruce seedlings and neighboring ericaceous dwarf shrubs in a temperate montane meadow.

Przemyslaw Ryszka - Institute of Environmental Sciences, Jagiellonian University, Krakow, Poland - Diversity of arbuscular mycorrhizal fungi from area affected by nuclear plant accident.

Tao Li - Key Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming, China - Biodiversity and ecological functions of AMF and DSE in an ancient Pb/Zn mine tailing plot in Southwest China.

Session 11

Mechanisms of tolerance to stresses - Coordinator: María J. Pozo - Centenario 1

Karin Pritsch - GSF-National Research Center for Environment and Health, Institute of Soil Ecology, Neuherberg, Germany - Functional changes in the soil-mycorrhizosphere-plant system due to ozone and pathogen stress.

Yi Huang - College of Environmental Sciences and Engineering, Peking University, China - Mycorrhizas enhancing the capability of phytoremediation.

Yukari Kuga - Graduate School of Integrated Arts & Sciences, Hiroshima University, Hiroshima, Japan - Micro-XRF imaging using the SPring-8 synchrotron shows cadmium sequestration in intracellular structures of arbuscular mycorrhiza.

Zheng Weishuang - College of Environmental Sciences and Engineering, Peking University, China - Soluble protein and acid phosphatase exuded by ectomycorrhizal fungi and seedlings in response to excessive Cu and Cd.

Session 3

Mixotrophic and mycoheterotrophic interactions - Coordinator: Tupac Otero - Tiradentes 2

R. Larry Peterson - University of Guelph, Guelph, Ontario N1G 2W1, Canada - The myco-heterotroph, *Arachnitis uniflora*, has a unique association with arbuscular mycorrhizal fungi.

Yosuke MATSUDA - Forest Pathology and Mycology, Faculty of Bioresources, Mie University, Japan - Seasonal changes and associating fungi of *Pyrola japonica* mycorrhizas growing under different light environment.

Rafael Borges da Silva Valadares - Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, Brazil - Orchid mycorrhiza diversity in *Coppensia doniana*, a widespread Oncidiinae from Campos do Jordão, Brazil.

Tuesday Aug 11

Session 4

Signal perception and metabolism - Coordinator: Pascale Seddas - Tiradentes 1

✓ Sonia Purin - West Virginia University, Plant & Soil Sciences Div. Morgantown, WV, USA - *In situ* analysis of anastomosis in all genera of glomeromycotan fungi using a unique rhizohyphatron design.

✓ Heike Bücking - Plant Biology Department, Michigan State University, East Lansing, Michigan, USA - Root exudates and their effect on uptake and metabolic processes during presymbiotic growth of arbuscular mycorrhizal fungal spores.

Tatsuhiko Ezawa - Grad. School of Agriculture, Hokkaido University, Sapporo, Japan - Polyphosphate is responsible for the rapid and massive accumulation of phosphorus in arbuscular mycorrhizal fungi.

T. Ishii - Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Japan - Importance of short molecular peptides to axenic culture of arbuscular mycorrhizal fungi.

Session 5

Mycorrhizal fungal structure in the field - Coordinator: Onguene Awana Nérée - Ouro Preto

Irene Sánchez-Gallen - Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico - Changes in AM fungi community structure in fragments of a tropical rain forest at Los Tuxtlas, Veracruz, Mexico.

Juan Carlos Perez - Universidad Nacional de Colombia-Sede Medellín, Colombia - Depth differential colonization and population structure of mycorrhizal fungi in prairie grasses.

Gail W.T. Wilson - Department of Natural Resource Ecology and Management, Oklahoma State University, USA - Local adaptation in mycorrhizal symbioses maximizes nutrient uptake and plant fitness.

Erika Buscardo - University of Coimbra, Portugal - Impact of wildfire frequency on the ectomycorrhizal resistant propagules communities in a Mediterranean open forest.

Session 6

Mycorrhizas and plant nutrition - Coordinator: Andrew Smith - Centenario 1

Ana Corrêa - University of Lisbon, Faculty of Sciences, Environmental Biology Center, Portugal - Is C allocation to the fungus costly to the plant?

Claire P. Humphreys - The University of Sheffield, UK - First evidence of mycorrhizal-mediated phosphorus transfer in a liverwort.

Edith Caroline Hammer - Department of Microbial Ecology, Ecology Building, Lund University, Sweden - Tit for Tat? G. intraradices accumulates P under low plant C availability - C-P trade revealed by PIXE and isotope probing.

Rosemarie Weigt - Institute of Systematic Botany, LMU München, Munich, and Department of Ecology, Ecophysiology of Plants, TU München, Freising, Germany - Nitrogen uptake by mycorrhizal roots: a space-related cost-benefit approach on mature trees.

Session 7

Inoculum technology application and management of mycorrhizal fungi - Coordinator: Chantal Hamel - Tiradentes 2

Alli M. Quoreishi - Symbiotech Research Inc., Canada - Ectomycorrhizal inoculations of seedlings: a biological approach for successful reclamation of the Canadian oil sands disturbed lands.

Ir. Jacqueline Baar - Knowledge Center Triple E, Arnhem, The Netherlands - The Triple-W model for a novel approach enhancing application of mycorrhizal fungi.

Miroslav Vosatka - Institute of Botany, Academy of Sciences of the Czech Republic, Czech, Republic - News, "dēja vus" and challenges of mycorrhiza production, application and marketing.

Dora Trejo - Universidad Veracruzana, Mexico - Parlor palm response to arbuscular mycorrhizal fungus (AMF) inoculation in a commercial nursery scale.

Thursday Aug 13

Session 8

Genome structure, plasticity, and evolution - Coordinator: Janusz Blaszowski - Tiradentes 2

Anders Tunlid - Department of Microbial Ecology, Ecology Building, Lund University, Lund, Sweden - Expansion of signal pathways in *Laccaria bicolor* - evolution of protein kinase and RAS small GTPase gene families.

Caroline Angelard - Lausanne University, Switzerland - Alteration of AMF genotypes and phenotypes caused by segregation and by change in host species.

Dag Åhrén - Microbial Ecology, Department of Ecology, Lund University, Sweden - Diversity and adaptation of metabolism in *Laccaria bicolor*.

Odile Thiéry - Institute of Botany, University of Basel, Basel, Switzerland - Mitochondrial large ribosomal subunit gene evolution in the *Glomus* group A lineage of arbuscular mycorrhizal fungi.

Session 5

Mycorrhizal fungal structure in the field - Coordinator: Fritz Oehl - Ouro Preto

Hassan, S.E. - Institut de Recherche en Biologie Végétale, Université de Montréal, Canada - Impact of long-term manure and inorganic nitrogen fertilization on the community composition of arbuscular mycorrhizal fungi using PCR-DGGE analyses.

Tine Grebenc - Slovenian Forestry Institute, Ljubljana, Slovenia - Molecular diversity in the genus *Tuber* indicates the correlation with the postglacial migration routes of their symbiotic trees in W and E Europe.

Boris Boerstler - Institute of Botany, University of Basel, Basel, Switzerland - Diversity of mitochondrial large subunit rDNA (mtLSU) haplotypes of *Glomus intraradices* in different environments.

Manuela Krüger - Genetics, Dept. Biology I, LMU Munich, Germany - Phylogenetic analyses of the Glomeromycota with species level resolution, based on a 3.3 kb fragment in the rDNA region.

Session 9

Mycorrhizosphere interactions - Coordinator: José-Miguel Barea - Tiradentes 1

André Freire Cruz - Kyoto Prefectural University, Graduate School of Life and Environmental Sciences, Japan - Bacteria living inside and/or on surface of *Gigaspora margarita* spores and their effects on soil-borne plant diseases and P solubilization.

Joanna Dames - Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, South Africa - *Kalaharituber pfeilii* and associated microbial interactions.

Kate Hardy - University of Sheffield, Department of Animal and Plant Sciences, United Kingdom - Biotic weathering: Influences of plants and ectomycorrhizas.

Mayra E. Gavito - Ecosystem Research Center (CIECO), Universidad Nacional Autónoma de México, México - What determines the abundance of AMF and other soil microbes under different land use and management in a tropical dry ecosystem?

Poster session

Monday Aug 10

Session 1

Cell biology and development of mycorrhizas

P1.1 - Efficient gene silencing in the ectomycorrhizal fungus *Laccaria bicolor* by lhpRNA-triggering via *Agrobacterium*-mediated transformation - Kempainen, M.J., Pardo, A.G.

P1.2 - Identification of differentially expressed genes during the pre-symbiotic interaction between *Hydnangium* sp. and *Eucalyptus grandis* - Coelho, I.S., Queiroz, M.V., Costa, M.D., Kasuya, M.C.M., Araújo, E.F.

P1.3 - The ectomycorrhizal morphotype Pinirhiza sclerotia is formed by *Acephala macrosclerotiorum* sp. nov. Münzenberger, B., Bubner, B., Wöllecke, J.

P1.4 - Cellular organization in germ tube tips of *Gigaspora* - Bentivenga, S.P., Kumar, L., Cello, G.J., Roberson, R.W., McLaughlin, D.J.

P1.5 - Role of a transcription factor, GintSTE, from *Glomus intraradices* in the establishment of arbuscular mycorrhiza Gianinazzi-Pearson, V., Tollot, M., Seddas, P., Wong Sak Hoi, J.

P1.6 - Structure-function relationships of GiAGL proteins at plant-fungal interfaces in arbuscular mycorrhiza symbiosis Schultz, C.J., Zulkli, S.M., Harrison, M.J.

P1.7 - Pea genes modulate symbiosis-associated fungal and plant molecular responses during late stages of the mycorrhizal symbiosis with *G. intraradices* - Kuznetsova, E., Seddas-Dozolme, P., Arnould, C.

P1.8 - Genetic exchange in AMF alters plant gene expression - Colard, A., Angelard, C., Sanders, I.R.

P1.9 - Common SYM gene NUP85 affects pollen tube growth in *Lotus japonicus* - Saito, K., Isobe, Y., Kawaguchi, M.

P1.10 - Legume genetics of formation and function of arbuscular mycorrhiza: fundamentals and application. Shtark, O.Y., Ovchinnikova, E.S., Danilova, T.N., Zhukov, V.A., Krashennnikova, A.S., Nemankin, T.A., Borisov, A.Y., Tikhonovich, I.A.

P1.11 - Mycelium growth *in vitro* cultures and morphologic characteristics of three ectomycorrhizae fungi strains from temperate forests in Mexico - Galindo-Flores, G.L., Alvarado-Sanchez, I.Y., Núñez-Rodríguez Emmanuel

P1.12 - Expression profiling of the arbuscular mycorrhiza-specific blue copper proteins in *Medicago truncatula* - Paradi, I., Morandi, D., Robert, F., Ochart, S.

P1.13 - Transformation of ectomycorrhizal fungus *Scleroderma* sp. mediated by *Agrobacterium tumefaciens* - Delazari, F.T., Coelho, I.S., Queiroz, M.V., Araújo, E.F.

P1.14 - The full length of fungal PtSRR1 mRNA encodes a protein that is a potential controller of ectomycorrhiza formation - Vieira, H.E.E., Acioli-Santos, B.A., Calzavara-Silva, C.E., Lima, C.E.P., Malosso, E.

P1.15 - Localization of fungal gene expression at different stages of arbuscular mycorrhizal symbiosis: development of an *in situ* RT-PCR method - Seddas-Dozolme, P.M.A., Tollot, M., Arnould, C., Gianinazzi-Pearson, V.

P1.16 - Cellular and subcellular studies of the arbuscular mycorrhizal symbiosis in *Medicago truncatula*: a proteomic survey - Zeina Daher, Ghislaine Recorbet, Franck Robert, Benoit Valot, Thierry Balliau, Daniel Wipf, Eliane Dumas-Gaudot, Benoit Schoefs

Session 2

Fungal communities

P2.1 - P Doses on the prevalence of native arbuscular mycorrhizal fungi - Moreira, M., Baretta, D., Tsai, S.M., Cardoso, E.J.B.N.

P2.2 - Responses of arbuscular mycorrhizal fungal community to the zonal distribution of coastal sand dune vegetation - Kawahara, A., Osaki, M., Ezawa, T.

- P2.3 - The effect of weed and cover crop management on mycorrhizae of grapevine (*Vitis vinifera*) roots - Baumgartner, K., Phillip Fujiyoshi, Fujiyoshi, P.T., Smith, R.F.
- P2.4 - Community diversity and structure of arbuscular mycorrhizal fungi across distinct land uses in Western Brazilian Amazon - Sidney Luiz Stürmer, José Oswaldo Siqueira
- P2.5 - Ectomycorrhizal communities of *Eucalyptus urograndis* monocultures and ectomycorrhizal colonization of *Psidium guajava* - Pickford-Gilbert, J., Kasuya, M.C.M., Khasa, D. P.
- P2.6 - Diversity of arbuscular mycorrhizal fungi and glomalin - Kil, Y.J., Lee, J.E., Shin, H.C.
- P2.7 - Diversity of Glomeromycota in El Palmar National Park (Entre Ríos, Argentina) - Velázquez, M.S., Cabello, M.N., Barrera, M.
- P2.8 - Linking coexistence with genetic distance in arbuscular mycorrhizal fungi - Roger, A., Sanders, I.R.
- P2.9 - Forest management and ectomycorrhizal communities of cork oak stands - Seddaiu, S., Lancellotti, E., Schiaffino, A., Corda, P., Franceschini, A.
- P2.10 - The effects of long-term fertilization and rotation cropping regimes on the AMF community in the Loess Plateau of northwestern China - Feng, H., Liu, Y.J., Dong, M.X., Wu, F.S.
- P2.11 - ITS TRFLP profiling limitations: problematic fungal genera - Alvarado, P., Manjón, J.L.
- P2.12 - The ectomycorrhizal community and cork oaks' state of health - Lancellotti, E., Seddaiu, S., Schiaffino, A., Franceschini, A., Corda, P.
- P2.13 - Arbuscular mycorrhizal fungi, a tool to access the side effects of pesticides used in grapevine production - van Tuinen, D.; Rivera-Becerril, F.; Martin-Laurent, F.; Chatagnier, O.
- P2.14 - Arbuscular mycorrhizal fungi in the Sete Cidades National Park (Piauí, Brazil) - Silva, G.A., Mello, C.M.A., Goto, B.T., Santos, S.S., Maia, L.C.
- P2.15 - New data on *Tuber pseudohimalayense* Chinese truffle - Manjón, José Luis, Alvarado, Pablo; García-Montero, Luis G., Moreno, Gabriel, Di Massimo, Gabriella
- P2.16 - Ectomycorrhizal fungal community of *Pinus thunbergii* in a coastal pine forest and a plantation in east coast of Korea - Obase, K., Cha, J.Y., Lee, J.K., Lee, S.Y., Lee, J.H., Chun, K.W.
- P2.17 - Arbuscular mycorrhizal fungi community structure at the Colombian Amazon region - Peña-Venegas, C.P., Cardona, G.J., Mazorra, A.
- P2.18 - Ectomycorrhizae fungi richness from Conifers forests of Central Mexico - Galindo-Flores, G.L., Alvarado-Sánchez, I.Y.
- P2.19 - Diversity of ectomycorrhizal fungi (Basidiomycotina) in Brazil. A brief study at the Parque das Dunas de Natal, RN - Neves, M.A., Baseia, I.G.
- P2.20 - Symbiotic fungi inhabiting roots of Norway spruce seedlings and neighboring ericaceous dwarf shrubs in a temperate montane meadow - Vohnik, M., Kohout, P., Malinova, T., Jersakova, J.
- P2.21 - Arbuscular mycorrhizal fungi in Tropical Atlantic Forests of Brazil with different regeneration periods - Silva, D.K.A., Freitas, N.O., Oliveira, K.C.
- P2.22 - Arbuscular mycorrhizal fungi diversity from timber plantations of *Gmelina arborea* and *Pachira quinata* in the Colombian Caribbean region - Serralde, D.P., Ramírez-Gómez, M., Baquero, C., Martínez, J., Roveda-Hoyos, G.
- P2.23 - Glomeromycota fungi diversity in agricultural systems of Central Venezuela - Rosas, J.V., Alban, R.E., Toro, M.
- P2.24 - Management of soil microbial quality using chickpea cultivars and endophytic inoculation to improve the productivity of subsequent wheat crops - Ellouze, W., Hamel, C.; Perez, J.C.; Bouzid, S.; St-Arnaud, M.
- P2.25 - Diversity of arbuscular mycorrhizal fungi in semiarid areas with Caatinga vegetation in Brazil - Albuquerque, P.P., Mala, L.C., Carrenho, R.

- P2.26 - Impacts of 90-year-incorporation of chemical fertilizer on the community structure of arbuscular mycorrhizal fungi - Cheng, Y., Kuriyama, Y., Ishimoto, K., Osaki, M., Ezawa, T.

Session 3

Mechanisms of tolerance to biotic and environmental stresses

- P3.1 - Proteome analysis of a Cd-tolerant and a Cd-sensitive *Suillus luteus* after cadmium exposure - Verstraelen, K., Vangronsveld, J., Colpaert, J.
- P3.2 - Proteomic analysis of *Glomus mosseae* colonized tomato plants during early infection of the plant-parasitic nematode *Meloidogyne incognita* - Vos, C., Panis, B., Carpentier, S., De Waele, D., Robert, F., Guillier, C., Dumas-Gaudot, E., Eisen, A.
- P3.3 - Biocontrol mode of action of AM fungi against *Pythium* root diseases - Ravnskov, S., Larsen, J.
- P3.4 - *In vitro* study of the combined effects of elevated CO₂ and temperature on arbuscular mycorrhizal associations - Dupré de Boulois, H., Voets, L., de la Providencia, I.E., Zavalloni, C., Nijs, I., Ceulemans, R., Declerck, S.
- P3.5 - Plant accumulation of organic contaminants and their dissipation in soil as affected by arbuscular mycorrhizal (AM) fungus *Glomus etunicatum* - Zhang, S.Z., Huang, H.L., Wu, N.Y.
- P3.6 - Arbuscular mycorrhiza alters coffee seedlings response to increasing Zn concentrations in soil - Andrade, S.A.L., Silveira, A.P.D., Schiavinato, M.A.
- P3.7 - Inoculation of arbuscular mycorrhizal fungi and arsenic uptake by plants: implication for As bioremediation - Zhang, J.L., Wang, Z.H., Christie, P.
- P3.8 - Sex-specific responses to mycorrhiza in a dioecious species - Varga, S., Kytöviita, M.M.
- P3.9 - Field inoculation studies of arbuscular mycorrhizal fungi - Kandru, A.
- P3.10 - Corn (*Zea mays* L.) growth as affected by soil compaction and arbuscular mycorrhiza - Miransari, M.
- P3.11 - Extraradical mycelium of arbuscular mycorrhizal fungi decreases cadmium toxicity in the rhizosphere of mycorrhizal plants - Janouskova, M., Pavlikova, D., Szakova, J.
- P3.12 - Arbuscular mycorrhizal fungi contribute to the phytoremediation of a highly alkaline anthropogenic sediment through pH amelioration - Oliveira, R.S., Dodd, J.C., Vosátka, M., Castro, P.M.L.
- P3.13 - Impact of DDT stress on laccase - Huang, Y., Li, X., Zhao, X.
- P3.14 - Mycorrhizal colonization in plants growing in lead contaminated soil - Ribeiro, P.R.A., Souza, A.P., Thompson Júnior, U., Marques, L.F., Silva, M.C.S.
- P3.15 - Studies on arbuscular mycorrhizal profiles in tsunami affected coastal soils: a case study from India - Sambandan, K.
- P3.16 - Growth response of ornamental fountain grass (*Pennisetum setaceum*) to mycorrhizae in compost amended media contaminated with NaCl, Na₂CO₃ or CaCl₂ - Nowak, J., Kunka, M.
- P3.17 - Protective effect of *Glomus* darum of the root-knot nematode *Meloidogyne incognita* in micropropagated banana plant - Meira-Hadad, L.S.A., Moreira, B.C., Guimarães, U., Freitas, L.G., Ottoni, W.C.
- P3.18 - Systemic resistance to the nematode *Xiphinema index* induced by a mycorrhizal fungus in grapevine - Gianinazzi, S., Hao, Z.P., Fayolle, L., van Tuinen, D.
- P3.19 - Vacuolar zinc storage in zinc sensitive and adaptive tolerant *Suillus luteus* (L.-Fr.) isolates - Ruytinx, J., Vangronsveld, J., Colpaert, J.V.
- P3.20 - Mycorrhiza and copper toxicity: proline accumulation and changes in amino acid composition in leaves of *Coffea arabica* seedlings - Andrade, S.A.L., Silveira, A.P.D., Mazzafera, P.

P3.21 - Environmental plasticity of AM fungi with herbaceous plant species - Charest, C., Audet, P., Benothmane, F., Tbaileh, T.F., Boutin, C.

P3.22 - Arbuscular mycorrhiza on plant growth and protection against heavy metals toxicity in contaminated soil - Soares, C.R.F.S., Siqueira, J.O., Klauber-Filho, O.

P3.23 - Beneficial effect of arbuscular mycorrhizal fungi and indigenous filamentous fungi on chromium accumulation by plants grown on tannery sludge - Sharma, S., Uppal, H.S., Adholeya, A.

P3.24 - Effect of soil salinity on growth and P uptake by *T. alexandrinum* L. - Gharineh, M.H.

P3.25 - Proteomics of Vitis root cuttings: a step forward to understanding molecular mechanisms involved in mycorrhizal symbiosis of a woody plant species - Guillier, C., Rebollo, M., Cangahuala-Inocente, G.

Session 4

Mixotrophic and mycoheterotrophic interactions

P4.1 - Mycorrhizal association on seed germination and seedling acclimatization of *Cyrtopodium cardiochilum* (Orchidaceae) - Pereira, M.C., Guimarães, F.A.R., Oliveira, S.F., Torres, D.P., Felício, C.S., Bocayuva, M., Liparini, O.P., Kasuya, M.C.M.

P4.2 - *Epidendrum* (Orchidaceae) and *Epulorhiza* (mycorrhizal fungus) compatibility - Torres, D.P., Pereira, M.C., Guimarães, F.A.R., Oliveira, S.F.O., Silva, C.F., Bocayuva, M., Pereira, O.L., Kasuya, M.C.M. (Universidade Federal de Viçosa, Brasil).

P4.3 - Characterization of mycorrhizal fungi of *Epidendrum secundum* (Orchidaceae) - Pereira, O.L., Pereira, M.C., Coelho, I.S., Torres, D.P., Guimarães, F.A.R., Oliveira, S.F.; Araujo, E.F., Kasuya, M.C.M.

P4.4 - Uninucleate *Ceratobasidium* sp. is a mycorrhizal fungi of native Brazilian orchid *Oncidium pirarense* - Oliveira, S.F., Pereira, M.C., Torres, D.P., Guimarães, F.A.R.; Coelho, I.S.; Bocayuva, M., Pereira, O.L., Kasuya, M.C.M.

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Session 5

Signal perception and metabolism

P5.1 - Root exudates of Anatto in the pre-infection stage of *Glomus clarum* - Leite, T.S., Oliveira-Neto, R.R., Silva, M.C.S., Almeida, A.M., Otoni, W.C., Kasuya, M.C.M.

P5.2 - Characterization of GlnT, a gene encoding a glutamate synthase in *Glomus intraradices* - Tienda, J.P., Azcón-Aguilar, C., Ferrol, N.

P5.3 - Metabolomic analysis of arbuscular mycorrhizal symbiosis - Jérôme Laparrie, Fabien Létisse, Virginie Puech-Pagès, Jean-Charles Portais and Guillaume Bécard

P5.4 - Evolutionary origin of strigolactones - Pierre-Marc Delaux, Bécard Guillaume and Nathalie Séjalon-Delmas

P5.5 - Dynamics of inorganic and organic cations during polyphosphate-hyperaccumulation in an arbuscular mycorrhizal fungus - Hijikata, N., Ohtomo, R., Osaki, M., Ezawa, T.

P5.6 - Optimizing extractant concentration for glomalin removal from soil - Balke, J., Janos, D.P.

P5.7 - Correlates of glomalin abundance and extractability - Janos, D.P., Garamszegi, S., Fierer, N.

P5.8 - A *Medicago truncatula* mutant hyper-responsive to mycorrhiza and defective for nodulation - Morandi, D., Le Signor, C., Gianianzi-Pearson, V.

P5.9 - fHANT-AC gene expression and regulation in the ectomycorrhizal fungus *Laccaria bicolor* - Kempainen, M.J., Alvarez Crespo, M.C., Pardo, A.G.

Session 6

Mycorrhizal fungal population structure in the field

P6.1 - Arbuscular mycorrhizal fungi (AMF) in native pitahaya (*Hylocereus undatus* Britt & Rose) plants under three environmental conditions of the dry tropic - Michel-Rosales, A., Peña-Beltrán, E., Farias-Larios, J., Bazán-Tene, M., Radillo-Juárez, F., Michel-López, C.Y., Michel-López, S.E.

P6.2 - Arbuscular mycorrhiza fungi and glomalin as soil quality indicators for the rehabilitation of a riparian forest of Velhas river in Brazil - Muzzi, M.R.S., Marques, M.M., Vilela, I.B., Silva, C., Moreira I., Lima, G.A.

P6.3 - Survey of arbuscular mycorrhizas in preserved and impacted riparian environments - Pagano, M.C., Persiano, A.I.C., Cabello, M.N., Scotti, M.R.

P6.4 - Occurrence of arbuscular mycorrhizal fungi in avocado orchards in Mexico - Bárcenas-Ortega, A.E., Chávez-Bárcenas, A.T., Stürmer, S.L., Varela-Fregoso L.

P6.5 - Arbuscular mycorrhizal fungi associated with *Aniba perutilis* - Ocampo, M.L., Mesa, N.

P6.6 - Effects of botanical and synthetic pesticides on the structure and function of arbuscular mycorrhizal fungi - Ipsilantis, I., Samourelis, C., Papadopoulou, K., Karpouzas D.G.

P6.7 - Arbuscular mycorrhizal fungi in agricultural soil under disposal of tannery sludge - Nakatani, André S.; Mescolotti, Denise L.C.; Martines, Alexandre M.; Nogueira, Marco A.; Stürmer, Sidney L.; Cardoso, Elke J.B.N.

P6.8 - Mycorrhization of teak (*Tectona grandis* L. F) in the Caribbean region of Colombia - Diaz, L. A., Masmela, X.

P6.9 - Arbuscular mycorrhizal fungi in a desertification nucleus - (Vabrobó, PE, Brazil) - Mello, C.M.A., Goto, B.T., Silva, I.R., Silva, G.A., Maia, L.C.

P6.10 - A COST 870 workshop on molecular ecology of AM fungi - Field Katile, Gollotte Armelle, Gianinazzi Silvio, Orfanoudakis M., van Tuinen Diederik, Redecker D., Blaszkowski Janusz

P6.11 - Diversity and density of spores of arbuscular mycorrhizal fungi under different land use systems - Bini, D.; Nogueira, M.A.; Miyauchi, M.Y.H.; Santos, C.A.; Mescolotti, D.L.C.; Andrade, G.; Zangaro, W.; Cardoso, E.J.B.N.; Stürmer, S.L.

P6.12 - Ectomycorrhizal fungi: the genus occurring associated to *Eucalyptus* spp. in Mato Grosso State, Brazil - Campos, D.T.S., Morais, L.P.V.X.C., Nonato, J.J.

P6.13 - Effect of agricultural practices on growth and diversity of arbuscular mycorrhizal fungus in lily (*Lilium longiflorum* Thunb) cropping. - Trejo, D., Guadarrama, C., Lara, L., Jiménez, T., Barois, I.

P6.14 - Abundance and diversity of ectomycorrhizas in native and nursery cultivated *Nothofagus nervosa* specimens - Fernández, N.V., Fontenla, S.B., Gallo, L.

P6.15 - Diverse tomato cropping systems affect arbuscular mycorrhizal fungal community diversity and structure - Johnson, E.G., Chellemi, D.O., Wu, T., Graham, J.H.

P6.16 - Mycorrhizal community of a relict Balkan shrub species from the upper forest border - Kraigher, H., Bajc, M., Hrenko, M., Grebenc, T., Ballian, D.

P6.17 - Diversity of Arbuscular Mycorrhizal Fungi in natural and disturbed *Polytepis australis* forests in Central Argentina - Becerra, A.G., Cabello, M., Renison, D.

P6.18 - Spatial patterns of mycorrhizal fungal genets in the field: evidence of vertical niche partitioning by two closely related species of Rhizopogon - Beiler, K.J., Durall, D.M., Simard, S.W.

P6.19 - Diversity and function of the arbuscular mycorrhiza in a fifty year old long term experiment - Tauschke, M., Dalpe, Y., Fox, C.

P6.20 - Effects of irrigation on the functional and structural diversity of ectomycorrhizal fungi - Hutter, S.C., Egli, S., Peter, M.

P6.21 - Morphological and molecular characterization of ectomycorrhizas occurring in commercial plantations of *Eucalyptus grandis*, in Minas Gerais, Brazil - Zubieta, M.P., Coelho, I.S., Queiroz, M.V., Kasuya, M.C.M., Araújo, E.F.

- P6.22 - Arbuscular Mycorrhizal Fungi in natural and disturbed *Polylepis australis* forests in Central Argentina - Becerra, A.G., Cabello, M., Renison, D.
- P6.23 - Comparative functional diversity of ectomycorrhizal fungal communities in contrasted physico-chemical environments - Franc A, Ali A, Courty P-E, Damon C, Duchemin, Egli S, Ernst J, Hutter, Legname, Marmeisse, Peter, Plassard.
- P6.24 - Arbuscular mycorrhizal diversity and root colonization potential of agricultural soils from Doukkala regions, Morocco - Boudarga, K., Kandil, M., Dalpé, Y.
- P6.25 - Diversity of AMF isolated from *Araucaria angustifolia* forest soils using *Mimosa scabrella* and *M. bimucronata* as trap plants - Cardoso, E.J., Lammel, D.R., Mescolotti, D.L., Stürmer
- P6.26 - Density and diversity of arbuscular mycorrhizal fungus an oxisol of cerrado (Savannah) under different systems of management of the soil - Carneiro, M.A.C., Fernandes, R.A., Ferreira, D.A.
- P6.27 - Arbuscular mycorrhizal fungi in different ecosystems on Tibetan Plateau - Li, X., Gal, J.P., Zhang, J.L., Cai, X.B.
- P6.28 - Long-term phosphorus fertilization impacts on AM fungi and other soil microbial communities in alfalfa rhizosphere - St-Arnaud, M., Beaugard, M.S., Hamel, C., Nayyar, A.
- P6.29 - Arbuscular mycorrhizal fungi diversity in conventional and organic vineyards of Central Chile - Von Bennewitz Álvarez, E.
- P6.30 - Plant invasion-induced biogeographical shift in the arbuscular mycorrhizal communities: a morpho-molecular approach - Shah, M.A., Khasa, D.P., Reshi, Z.A.
- P6.31 - Are AMF more resilient under agricultural practices of severe disturbance and intensive nutrient inputs than previously thought? - Rasmann, C., Graham, J.H., Chellemi, D.O., Datnoff, L.E.
- P6.32 - Mycorrhizal fungi for sustainable production of Christmas trees in the French Morvan region - Armelle, G., Helbecque, C., Houis, V., Wipf, D., Lemoine M.C.
- P6.33 - Nickel tolerance and population structure of *Pisolithus albus* in New Caledonia - Ducouso, M., Jourand, P., Majorel, C., Riss, J., Prin, Y.
- P6.34 - Response of arbuscular mycorrhizal fungi and soil enzyme activities to *Eupatorium adenophorum* invasion - Sun, X., Guo, L.D., Liu, X.Z.
- P6.35 - Illuminating the hyphal world: mapping fungi to soil phosphatase activity - Brooks, D.D., Jones, M.D., Grayston, S.J.

Session 7

Mycorrhizas and plant nutrition

- P7.1 - Influences of improved fallow systems and phosphorus application on arbuscular mycorrhizal fungi symbiosis in maize in western Kenya - Muriithi, M.N., Bashir, J., Othieno, C., Okalebo, R., Odee, D., Machua, J., Jansa, J.
- P7.2 - Responses of fungal root colonization to long-term exposure of elevated atmospheric CO₂ and warming in a sub-arctic birch forest understory - Olsrud, M., Michelsen, A., Svensson, B., Carlsson, B., Melillo, J.M.
- P7.3 - Fe nutrition in mycorrhizal *Pinus sylvestris* - Wevers, J.H., Colpaert, J.V., Vangronsveld, J.
- P7.4 - Effect of *Glomus intradices* and nitrogen source on growth and fruit production of strawberry plants. - Cárdenas-Navarro, R., Ruiz-Corro, R., Villegas, J., Salgado-Barreiro, C.S.
- P7.5 - Short-chain polyphosphate in arbuscular mycorrhizal roots colonized by *Glomus* spp.: a possible phosphate pool for host plants - Takanishi, I., Ohtomo, R., Hayatsu, M., Saito, M.
- P7.6 - AM symbiosis enhances the salinity tolerance of *Pistacia vera*. - Taheri, A.E., Riginjad, N., Farzmand, R.
- P7.7 - The effect of symbiotic association between arbuscular mycorrhizal fungi and *Physalis peruviana* in crop nutrition - Roveda-Hoyos, G., Ramirez-Gómez, M., Peñaranda, A.
- P7.8 - Evaluation of arbuscular mycorrhizal fungi isolates associated with two forest species (*Gmelina arborea* and *Pachira quinata*) in the Colombian - Ramirez-Gómez, M., Roveda-Hoyos, G., Serralde-Ordoñez, D.P.

- P7.9 - Overcoming growth depressions in arbuscular mycorrhizal symbiosis: Can we make the mycorrhizal and direct P uptake pathways additive? - Grace, E.J., Tester, M., Smith, S.E., Smith, F.A.
- P7.10 - The tripartite symbiosis between diazotrophic bacteria, arbuscular mycorrhizal fungi, and *Araucaria angustifolia* seedlings - de Lima, J.E., Vasconcellos, R.L.F., Cardoso, E.J.B.N.
- P7.11 - P Inflow and mycorrhizal growth response of two sorghum cultivars as affected by drought stress - Nadian Ghomsheh, H., Jafari, S.
- P7.12 - Influence of exogenous treatment by plant growth regulators on development of *Medicago lupulina* with mycorrhizal fungus *Glomus intradices* - Pavlovich, A., Yurkov A.P., Jacobi, L.M., Stepanova, G.V., Kojemiakov, A.P.
- P7.13 - Survival and growth of micropropagated physic nut (*Jatropha curcas* L.) plantlets associated to *Glomus clarum* - Folli, M.S., Rodrigues, A.L., Meira, L.S., Lani, E.R.G., Otoni, W.C., Silva, L.C.; Kasyua, M.C.M.
- P7.14 - Do arbuscular mycorrhizal fungi influence caesium uptake by *Medicago truncatula*? - Lea Wiesel, Martin R. Broadley, Philip J. White
- P7.15 - Fe nutrition in mycorrhizal *Pinus sylvestris* - Wevers, J.H., Colpaert, J.V., Vangronsveld, J.
- P7.16 - Estimate of symbiotic efficacy for *Acacia mangium* inoculated with different mycorrhizal fungi species - Angelini, G.A.R., Saggin Júnior, O.J., Silva, E.M.R.
- P7.17 - Enhancement of plant growth by arbuscular mycorrhizal fungi under N-limited conditions - Hayatsu, M., Takanishi, I., Saito, M.
- P7.18 - Arbuscular mycorrhiza and cattle manure in the establishment of *Tocoyena selloana* Schum. in dune areas Souza, R.G., Goto, B.T., Silva, D.A.
- P7.19 - Absorption of stable forms of phosphorus by arbuscular mycorrhizal maize - Tavares, R.C., Cardoso, I.M., Mendonça, E.S.
- P7.20 - Mycorrhizal colonization in sugarcane after herbicide application - Silva, M.C.S., Tironi, S.P., Reis, M.R., Belo, A.F., Costa, M.D.
- P7.21 - Rhizosphere and root mycorrhizal diversity of maize genotypes contrasting for phosphorus efficiency - Gomes, E.A., Oliveira, C.A., Oliveira, F.A.S., Lana, U.G.P., Guimarães, C.T., Parentoni, S.N.
- P7.22 - Contribution of arbuscular mycorrhizal fungi inoculation to the reduction of N fertilization on forage grasses - González, P.J., Arzola, J., Rivera, R., Plana, R., Fernández, F.
- P7.23 - Effect of *G. intradices* on the growth and yield of garlic (*Allium sativum*) and the usage of its oil extract as antimicrobial agent - Aboulnasr, A., Omar, S., Gouda, M.
- P7.24 - Arbuscular mycorrhizal fungi in two mangroves of south China - Wang, Y.T., Xin, G.R., Qiu, Q.
- P7.25 - Limiting factors for the growth of four woody legumes in an Oxisol - de Carvalho, T.S., Moreira, F.M.S., Siqueira, J.O.
- P7.26 - Phosphorus efficiencies and responses of maize (*Zea mays* L.) to arbuscular mycorrhizal fungus *Glomus caledonium* in an experimental field as affected by long-term fertilizer management - Hu, J.L., Lin, X.G., Wang, J.H., Chen, R.R., Dai, J., Chu, H.Y., Yin, R., Zhang, J.B.
- P7.27 - Response of mycorrhizal wheat genotypes to saline soil under field conditions - Ardakani, M.R., Rejali, F., Daei, G., Teimuri, S., Miransari
- P7.28 - Response of the neem (*Azadirachta indica* A. Just.) to mycorrhizal inoculation - Cavalcante, U.M.T., Lima, E.L., Miamoto, R.Y.
- P7.29 - Synergic interaction between arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* and its effect on plant phosphorus uptake - Ordoñez, Y.M., Marulanda, J.J., Uribe, D., Rodríguez, A.
- P7.30 - The opportunistic behaviour of *Glomus intradices* in relation to phosphorus acquisition - Van Aarle, I.M., de la Providencia, I.E., Rouhier, H., Declerck, S.
- P7.31 - Physiological parameters influenced by artificial inoculation of arbuscular mycorrhizal fungi *Glomus clarum* to the achievement of the rubber rootstock - Patrícia Fabian de Araújo Diniz, Luiz Edson Mota de Oliveira, Marcelo Pedrosa

- P7.32 - Influence of Phosphorus homeopathic medicine on growth and root colonization by AMF native in sorghum de Marins, J.F., Carrenho, R., Bonato, C.M., Novelino, J.O.
- P7.33 - Do ectomycorrhizal fungi have a role in the formation of Ca stores as oxalate crystals inside eucalypt roots? Costa, M.D., Gonzalez, J.A.Z., Pereira, G.M.D., Pylro, V.S.
- P7.34 - Field validation and in vitro culture and mass production of AMF with particular reference to low-input wheat-rice and wheat-pulse cropping systems - Uppal, H.S., Shukla, K.K., Singh, R., Adholeya, A.

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Session 8 Inoculum technology, application and management of mycorrhizal fungi

- P8.1 - Arbuscular mycorrhiza (AM) for reforestation - Krüger, C., Krüger, M., Stockinger, H.
- P8.2 - Misleading mycorrhiza? - Ragonezi, C., Castro, M.R.; Santos-Silva, C.; Lima, M.; Klimaszewska, K.; Vaz, M.; Zavattieri, M.A.
- P8.3 - Growth of the ectomycorrhizal fungus *Pisolithus microcarpus* on different nutritional conditions - Rossi, M.J., Oliveira, V.L., Angioletto, E.
- P8.4 - Ectomycorrhizal inoculum viability for *Nothofagus* species from the Patagonian forest of Argentina - Bassani, V.N., Barroetaveña, C., Rajchenberg, M.
- P8.5 - Bioreactor for the production of ectomycorrhizal inoculants - Rossi, M.J., Duarte Filho, P.F., Fúrgio Júnior, A., Oliveira, V.L.
- P8.6 - Mycorrhizae inoculated vegetable seedling production and use for field experiments - Ortas, I.
- P8.7 - *Ostryopsis davidiana* seedlings inoculated with ectomycorrhizal fungi facilitate formation of mycorrhizae on *Pinus tabulaeformis* seedlings - Bai, S.L., Li, G.L., Liu, Y.
- P8.8 - Development and test of a counting equipment for in-vitro produced AM-spores - Spindler, A.; Rathmann, T., Morawietz, M.
- P8.9 - *Eucalyptus grandis* Hill ex Maiden essential oil biostimulation in ectomycorrhizal fungi isolates in vitro. Sulzbacher, M.A., Steffen, R.B., Steffen, G.K., Pazzini, D., Antonioli, Z.I.
- P8.10 - IBG: a tool for promoting ecosystem services in plant production systems - Mercy, L., Gianinazzi-Pearson, Wipf, D.
- P8.11 - Responses of grain legumes to rhizobia and arbuscular mycorrhizal fungi: a meta-analysis in the perspective of photosynthate limitation of symbioses. - Kaschuk, G., Leffelaar, P.A., Giller, K.E., Alberton, O., Hungria, M.
- P8.12 - Mycorrhiza as a component of organic approach in fruits and vegetables cultivation - Vosátka, K., Albrechtová, J., Vohník, M., Latr, A., Posta, K.
- P8.13 - Growth response of sago palm plantlets, *Metroxylon sagu* (Rottboll) to vesicular arbuscular mycorrhiza Chan, M.K.Y.
- P8.14 - Survival of *Castanea sativa* Mill. plants mycorrhizal with *Pisolithus tinctorius* after transplantation to soils infected with *Phytophthora cinnamomi* - Martins, A., Pais, M.S.
- P8.15 - Yield of Welsh onion (*Allium fistulosum* L.) inoculated with arbuscular mycorrhizal fungi at different soil phosphorus levels in the field - Tawarayama, K., Hirose, R., Wagatsuma, T.
- P8.16 - Effect of dual inoculation (*Glomus mosseae*-*Pseudomonas fluorescens*) on the fruit quality of tomato (*Lycopersicon esculentum* Mill.). - Zulueta, R., Chiquito, R., Zárate, J.
- P8.17 - Glomerospores density (*Glomus etunicatum*) for neem seedlings production. - Cavalcante, U.M.T., Lima, E.L., Miamoto, R.Y., Souza, R.G.
- P8.18 - Arbuscular mycorrhizal colonization and soil enzyme activities early in the organic transition: effect of soil solarization and organic matter applied - Karasawa, T., Nagaoka, K., Takenaka, M., Takebe, M., Sato, S.

- P8.19 - Effect of arbuscular mycorrhizal fungi and green waste compost on growth and nutrient uptake of selected container grown ornamental nursery crops - Bozena, M., Falkowski, G., Koniarski, M.
- P8.20 - Native Glomeromycota biofertilization to improve maize development under agroecological management Toro, M., Hernandez, R.M., Lozano, Z., Rivero, C.
- P8.21 - Mass propagation of VAM inoculum - Palipane, J.B.
- P8.22 - Production of in vitro mycorrhized plants: learning of the nature. - Fernández, K., Fernández, F., Declerck, S.
- P8.23 - Desinfection efficiency of *Glomus* spores for in vitro culture - Moreno, P.A., Ocampo, M.L.

Session 9 Genome structure, plasticity, and evolution

- P9.1 - Organization of the respiratory chain of *Glomus intraradices* deduced from genome analysis - Formey, D., Roux, C.
- P9.2 - Glomeromycota (AM fungi) host a pan-global, monophyletic lineage of endobacteria - Naumann, M., Schübler, A., Bonfante, P.
- P9.3 - Identification and expression analysis of isocitrate lyase and malate synthase genes in *Pisolithus microcarpus* Campos, A.N.R., Costa, M.D., Martin, F.
- P9.4 - Analysis of the microtranscriptome of *Medicago truncatula* during symbiotic interactions - Formey, D., Sallet, E., Gouzy, J., Niebel, A., Roux, C.
- P9.5 - ITS rDNA of six ectomycorrhizal fungi isolates - Antonioli, Z.I., Lupatini, M., Oliveira, V.L.

Session 10 Mycorrhizosphere interaction

- P10.1 - Heat shock protein 60 (Hsp60 protein) from *Glomus intraradices*, sequence alignment and phylogenetic analysis - Seobi, T., Dames, J.F., Blatch, G.L.
- P10.2 - Interaction between arbuscular mycorrhizal fungi (AMF) and phosphate solubilizing fungi. Effect on AMF hyphal growth and alkaline phosphatase activity - Scervino, J.M., Peña, R., Godeas, A.M., Della Mónica, I.F.
- P10.3 - Occurrence of ecto- and arbuscular mycorrhizas and nitrogen fixing bacteria in *Dimorphandra wilsonii*, a threatened species from the Brazilian Cerrado - Scotti, M.R., Cruz, C., Bacelar, M., Gomes, M., Castro, M., Miranda, P., Pereira, R.A., Sprent, J.; Silva, I.S.; Silva, M.C.S. and Kasuya, M.C.M.
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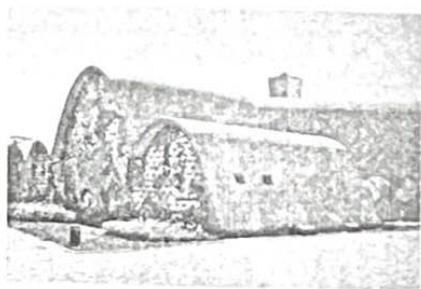
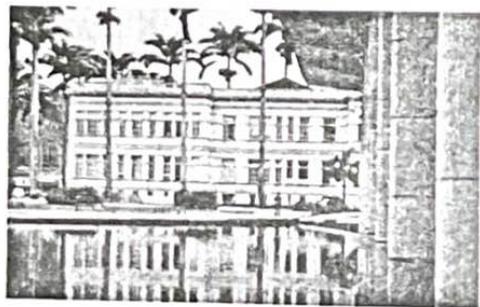
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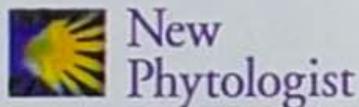
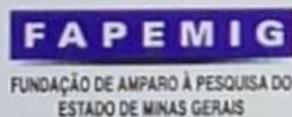
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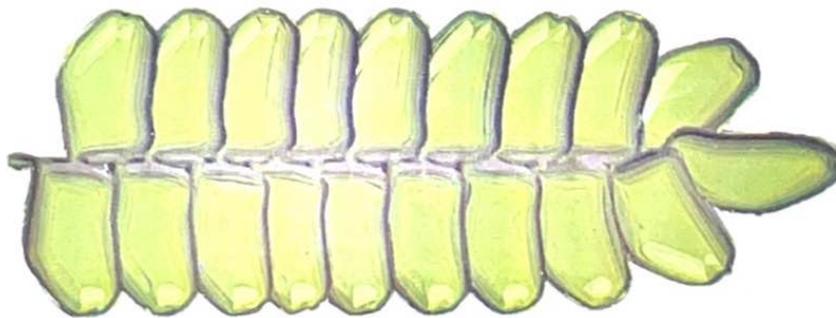


PRODUCTION



ABSTRACTS

ICOM6



6TH INTERNATIONAL CONFERENCE ON MYCORRHIZA

“BEYOND THE ROOTS”



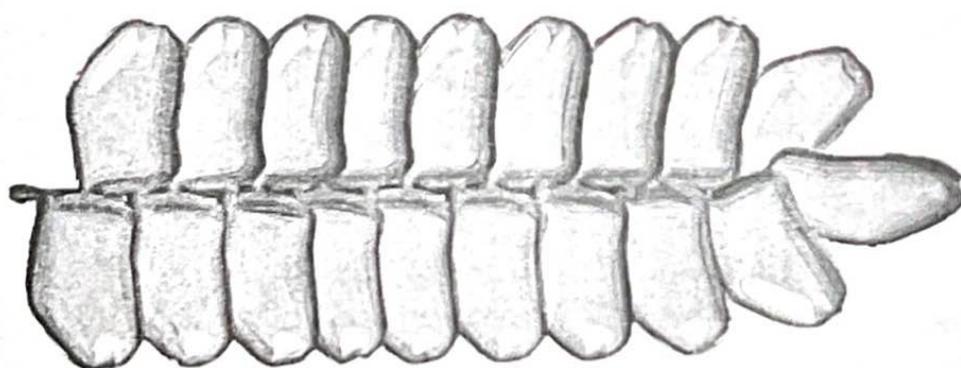
9 to 14 August 2009

Belo Horizonte - Brazil



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Edited by Maria Catarina Megumi Kasuya and Mauricio Dutra Costa

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Preface

The Organizing Committee is very pleased to host the 6th INTERNATIONAL CONFERENCE ON MYCORRHIZA (ICOM6), in Belo Horizonte, Minas Gerais, Brazil. The theme chosen for ICOM6 is "Beyond the roots". The Conference is jointly held by the Federal University of Viçosa and the International Mycorrhiza Society.

Several Brazilian research institutions are actively working to improve the country's agricultural and forest production, generating technology that has been exported both to other regions of Brazil as well as to other countries. Mycorrhizal research in our country is completing 30 years of activities in almost every aspect of the mycorrhizal associations and it will be a pleasure to celebrate that with you. The Organizing Committee hopes that the Conference will be a place for promoting mycorrhizas as important ecological components, broadening the views on mycorrhizas from their local surroundings to far beyond the roots.

The symbol of our Conference was designed by the ICOM6 secretary Dr. Maurício Dutra Costa and represents a leaflet of the leguminous plant *Caesalpinia echinata* Lam., known as pau-brasil. The name "Brazil" was derived from the common name of this species, whose red wood resembles live embers (brasas, in Portuguese, and thence, Brasil). Pau-brasil wood was widely exported to Europe during the colonial times and used to dye cloth. Today, the species is under the threat of extinction and is highly protected. Due to its historical and economic contribution to Brazil, pau-brasil has been chosen as the symbol of ICOM6 as an example of how the importance of mycorrhizal plants can go far beyond the roots.

The Conference bags were made with corn straw by a group of small farmers from Brás Pires, Minas Gerais, under the support and guidance of technicians from EMATER-MG and Instituto Xopotó. Each bag contains a wooden tag made with pruning remains of *Samanea tubulosa* (Benth.) Barneby & Grimes, commonly known as sete-cascas (seven-barks) because of its thick, corky bark. The Conference badges were also made with sete-cascas wood.

The technical excursions were chosen to provide a set of sites with distinct climates, vegetation, and soils, representative of the geographical diversity of Minas Gerais State. Some of these sites, with restricted access, represent areas of permanent conservation where threatened plant and animal species are protected.

This book contains the abstracts of the works presented during the Conference and has been organized as: 1 - keynotes; 2 - workshops; 3 - symposia with oral presentations; 4 - poster presentations.

We hope you enjoy the Conference and wish you an excellent stay in Brazil!

Opening Ceremony

The First Thirty Years Of Mycorrhizal Research in Brazil

SIQUEIRA, J.O.

Professor at Universidade Federal de Lavras on license to be Director of CNPq in Brasília

Although reports on mycorrhiza in Brazil dated back to the beginning of XX century, this subject was highly neglected here until mid 1970's, when Higher Education and Science and Technology were structured and also it was offered an intensive training course on "Endomycorrhiza" at Instituto Agronômico de Campinas, event that became the milestone of mycorrhiza research in Brazil. In the 80's, research groups were set up in several institutions, which became national leaders on research and training and support the expansion of mycorrhizal research that reach its highest point with the First Brazilian Meeting on Mycorrhiza in 1985. Since this event, mycorrhiza continued to be an attractive subject, experiencing a steady growth up the following decades. Along with these thirty years, we built a good competence in terms of human resources, research facilities and capacity to generate new knowledge. We are currently, awarding 40 master and doctoral degrees majoring on mycorrhiza and according to the Web of Knowledge (Thomson Reuters) we stand the 11th world position in the publication of mycorrhiza papers in indexed journals. Brazilians have given significant contributions to the understanding and application of this symbiosis in tropical environments and such contributions were gathered and critically reviewed in a book that is at a final stage of editing. Our major findings will be highlighted in this lecture, giving special emphasis on the biology and ecology of AMF in Brazilian ecosystems and field studies dealing with the benefits of inoculation and management of indigenous communities for crop production and sustainability in low fertility soils. We provide evidence that mycorrhiza play a significant functional role in our agro-ecosystems and that these benefits goes beyond the economic viewpoint.

Keynote Lectures

1 - Environmental genomics of ecologically relevant micro-organisms

George A. Kowalchuk^{1,2} (¹Netherlands Institute of Ecology (NIOO-KNAW), Centre for Terrestrial Ecology, Department of Terrestrial Microbial Ecology, Boterhoeksestraat 48, 6666 GA Heteren, The Netherlands . ²Institute of Ecological Science, Vrije Universiteit, De Boelelaan 1085, 1081 HV, Amsterdam, The Netherlands)

Plant-soil systems harbor a tremendous storehouse of genomic information that steers the functioning of terrestrial ecosystems. However, the majority of such activities are carried out by organisms that have remained difficult to study, principally because of our difficulties in isolating them in pure culture. Furthermore, the complexity of plant-soil communities lessens the relevance of reductionist approaches that seek to examine a single feature of the system by itself. The recent application of molecular and more comprehensive genomics approaches in microbial ecology is, however, now providing access to the untapped reservoir of soil-borne microbial diversity, providing the opportunity to better understand how soil-borne communities function, and how microbial activities and plant-microbe interactions might better be exploited. In this presentation, I will provide a number of examples of how we are attempting to gain insight into the organization of soil-borne microbial communities and the forces that drive microbial community structure, diversity and function in plant-soil systems. Special emphasis will be paid to the effects of environmental perturbations and climate change, specifically the effects of global warming, rising atmospheric CO₂ concentrations and changing land usage. With this focus, I seek to highlight how polyphasic approaches, including several methods out of the expanding "-omics" toolbox, can help to provide a more mechanistic and integrated understanding of terrestrial soil-borne communities functions.

2 - 'Hidden' phosphorus transfer in arbuscular mycorrhizas: what should it mean for you?

Smith, S.E.(School of Earth and Environmental Sciences, The University of Adelaide, Australia), Christophersen, H.M.(School of Earth and Environmental Sciences, The University of Adelaide, Australia), Facelli, E.(School of Earth and Environmental Sciences, The University of Adelaide, Australia)

Recent physiological and molecular research shows that the arbuscular mycorrhizal (AM) pathway of phosphorus uptake (AMPU) functions in both growth-responsive and non-responsive plants, often delivering a very high proportion of plant P. In non-responsive plants in particular, this contribution remains 'hidden' unless it is quantified with radioactive P. 'Hidden' AMPU has wide-ranging consequences for interpretation of many aspects of AM symbioses, from cell biology to ecology. A large AMPU contribution in non-responsive plants (and possibly responsive ones) means that direct P uptake (DPU) via root hairs and epidermis is reduced, contrary to long-held belief. The mechanisms need attention, because they will impact on research to improve P uptake efficiency of field-grown plants, including crops, and because reduction in DPU may underlie growth depressions in plants that are not highly colonised. Lower DPU activity may also provide increased arsenic tolerance in AM plants, where P uptake is maintained by AMPU; the latter is likely to have very high P/As selectivity. 'Hidden' AMPU may give

competitive advantages to non-responsive AM plants competing with non-mycorrhizal ones, that are not apparent when plants are grown singly. 'Hidden' uptake of nutrients other than P may play a significant role in plant performance. Overall, new information on the integration AM and direct P uptake offers an explanation for evolutionary persistence of AM in non-responsive plants.

3 - Plants and arbuscular mycorrhizal fungi: born to be friends

Bonfante, P. (Department of Plant Biology of the University of Torino Italy)

Arbuscular mycorrhizas (AMs) are symbiotic associations, which are commonly described as the result of co-evolution events between fungi and plants where both partners benefit from the reciprocal nutrient exchange. The presentation will focus on the process of colonization by AM fungi from an evolutionary perspective. Data from fossil records, recent characterizations of AM fungi in basal plant taxa, and live cell imaging of angiosperm colonization processes will be presented to discuss evolutionary-developmental events. The uniformity of plant cell responses to AM colonization in haploid gametophytes and diploid sporophytes, in non-root organs and throughout many seed plant clades highlights the ancient origin of the interaction. This is corroborated by evidences of common cellular mechanisms (i.e. the disappearance of starch accumulation in liverwort thalli and in angiosperm roots; the constant presence of a perifungal membrane). These features are may be at the basis of the plant cell reprogramming and suggest that pre-existing mechanisms, like those involved in plant cell growth, were recruited by plants to accommodate AM fungi. Molecular determinants, which are involved in signalling or are considered functional markers of AMs, have been identified in legumes and in other model plants, but so far not in naturally collected plants or in lower taxa. Answers to these questions may help to solve ongoing debate on plant-fungal co-evolution and on the functional role of AMF in natural systems.

4 - The *Glomus* Genome Project: an update

Peter Young (Department of Biology, University of York, UK)

A complete genome sequence is a vital tool for many investigations aimed at understanding biological systems, so it is important to pursue this information for arbuscular mycorrhizal fungi. As with many aspects of their biology, however, the quest for a genome sequence has proved more arduous than for many other organisms. In the past year there has been some dramatic progress, though, with the help of next-generation sequencing. We now have about 350 Mb of genomic sequence from *Glomus intraradices*. We can see from this that the effective "genome space" is probably at least ten times the original estimate of about 16 Mb that was based on nuclear DNA content. Within a single isolate, there are multiple "allelic" variants of most genes, as well as large numbers of repetitive elements and much noncoding DNA. As a result, assembly is still very fragmentary, although the complete mitochondrial genome has been assembled. Genome sequencing is continuing with the high-throughput sequencing of fosmid and attempts to isolate the DNA from single nuclei. We also have more than 300 000 EST sequences, which are providing an important overview of the *Glomus* transcriptome and will be used in microarray studies to explore gene expression throughout the fungal life cycle.

Workshops

Cell Biology and Development of Mycorrhizas

1.1 - Illuminating Plant Cellular Dynamics During Arbuscular Mycorrhizal Interaction

A. Genre¹, M. Chabaud³, S. Ivanov², A. Faccio¹, E. Fedorova², D. Barker³, T. Bisseling², P. Bonfante¹ (¹DBVUniversità di Torino/IPP-CNR, Torino, Italy; ²MOLBI, Wageningen, Netherlands; ³LIPM CNRS/INRA Toulouse, France)

During the development of arbuscular mycorrhizas (AM), the root cells of the host plant anticipate fungal colonization by organising extensive prepenetration responses. Epidermal and cortical cells that are about to be colonised by the symbiotic fungus develop the so-called prepenetration apparatus (PPA), a columnar cytoplasmic aggregation that predicts the route of intracellular hyphae.

The PPA is considered to be directly involved in the construction of the symbiotic interface compartment where the fungus is hosted. The interface is composed of plant cell wall materials and is surrounded by an invagination of the plant plasma membrane. Such membrane proliferation and cell wall material deposition require the set up of an intense, focussed secretory activity.

We here presents our recent advancements in the identification of the plant cell components and mechanisms involved in the PPA organisation and interface compartment formation. By combining *in vivo* confocal imaging of specific GFP tags with transmission electron microscopy, our results highlight important changes not only in in the nucleus, cytoskeleton, endoplasmic reticulum, but also in Golgi stacks, secretory vesicles, endosomes and plasma membrane. They suggest that the perifungal membrane is the result of an intense secretory process based on

proliferating Golgi stacks, endosome recycling and membrane dynamics. PPA response can involve basic cell mechanisms such as those responsible for cell division and polar growth.

1.2 - Importance of fungal transporters in successful plant-fungus collaboration

J. Doidy¹, A. Schüssler², D. van Tuinen¹ and D. Wipf¹ (¹UMR INRA/CNRS /Université Bourgogne Plante-Microbe-Environnement, BP 86510, 21065 Dijon Cedex, F - ²LMU Munich, Genetics, Department Biology Grosshaderner Str. 4, 82152 Planegg-Martinsried, D)

Understanding mechanisms that underlie high nutrient (phosphate, nitrogen) use efficiency by mycorrhizal plants, and carbon allocation in a context of multitrophic interactions between biotrophic organisms (symbiotic, pathogenic, neutral), is critical for sound management of both crop lands and forests. Indeed, availability, uptake and exchange of nutrients in biotrophic interactions will govern plant growth and modulate biomass allocation, both of which are central to plant yield, a major outcome in the context of high biomass production. Uptake and exchanges of nutrients and/or metabolites at biotrophic interfaces are controlled by membrane transporters, of which the regulation patterns are essential in determining the outcome (beneficial, antagonistic) of plant-fungal interactions and in adapting to changes in soil nutrient quantity and/or quality. The identification and functional analysis of fungal transporters in mycorrhizal systems will provide key information about those central to uptake by soil-growing hyphae, those responsible for efficient nutrient and/or metabolite export and import at the plant-fungus interface in the symbiosis, and those involved in successful nutrient competition with other soil micro-organisms. The presentation will focus on the current state of the art and highlight powerful tools for the identification and characterization of new fungal transporters driving biotrophy in arbuscular mycorrhiza.

1.3 - Correlation of phosphate homeostasis, arbuscular mycorrhiza development and microRNA expression

Emanuel Devers, Anja Branscheid, Daniela Sieh and Franziska Krajinski (Max Planck Institute of Molecular Plant Physiology, Science Park Golm, 14476 Potsdam (Golm), Germany, krajinski@mpimp-golm-mpg.de)

Pi-replete plants are much less colonized by arbuscular mycorrhizal (AM) fungi than Pi-deplete plants. This indicates a link between plant Pi-signaling and AM development. MicroRNAs (miRs) of the 399-family are systemic Pi-starvation signals important for maintenance of Pi-homeostasis in *Arabidopsis thaliana*, and might also qualify as signals regulating AM development in response to Pi availability. We investigated the miR399 family in *Medicago truncatula* and experimentally confirmed ten new miR399 family genes. Expression of the miR399 genes was studied in shoots and roots of *M. truncatula* plants in dependence of Pi-status and AM symbiosis with *Glomus intraradices*. Pi-deplete plants showed increased expression of mature miR399 and miR399 primary transcripts (pri-miR399s), and unexpectedly, some pri-miR399 species were higher in leaves of mycorrhizal plants than in leaves of non-mycorrhizal plants. Compared to non-mycorrhizal Pi-deplete roots, mycorrhizal roots of Pi-deplete *M. truncatula* and tobacco plants had increased Pi-contents due to symbiotic Pi-uptake, but displayed higher mature miR399 levels. Expression levels of *MtPho2* remained low and PHO2-dependent Pi-stress marker transcript levels remained high in these mycorrhizal roots. Hence, an AM symbiosis-related signal appears to increase miR399 expression and decrease PHO2 activity. In order to identify other miRs, which are involved in AM symbiosis, we sequenced small RNAs of *M. truncatula* mycorrhizal and non-mycorrhizal roots. This revealed the prediction of more than 200 novel *M. truncatula* pri-miRs and the identification of novel and conserved miRs regulated during AM symbiosis.

Fungal Communities

2.1 - Molecular analysis of mycorrhizal communities in forest and agroforest systems.

Damase Khasa¹, V. Chiffot¹, A. Gagné¹, M.A Shah², M.E. Beaulieu¹, S. Sokolski¹, Y. Dalpé³, and Y. Piché¹ (¹Canadian Research Chair in Forest and Environmental Genomics and Centre for Forest Research, Pavillon Marchand, Université Laval, Québec, Canada G1K 7P4, e-mail: dkhasa@rsvs.ulaval.ca ²Department of Botany, University of Kashmir, Srinagar-190 006, J&K, India ³Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, K.W. Neatby Building, 960 Carling Avenue, Ottawa, ON K1A 0C6)

Forest and agroforest ecosystems provide many socio-economic goods and ecological services. Besides supporting major industries, healthy agroforest and forest ecosystems provide efficient water filtration services, diverse wild life habitats, sinks for sequestration and storage of carbon and facilitate effective pollination. Knowledge of interrelationships between ecosystem components is essential for effective and long-lasting management strategies. This knowledge can be gained through combined use of traditional and more recent molecular ecological approaches. Mycorrhizal symbioses are widespread in terrestrial ecosystems and underpin their health and productivity. In this paper, we present the progress made in our laboratory for the development of robust molecular markers for mycorrhizal fungi. These markers include rDNA PCR-RFLP and protein-coding genes (clone libraries or not) coupled with sequencing or denaturing gradient gel electrophoresis, terminal restriction fragment length polymorphism or single-strand conformation polymorphism, and microsatellites, for describing soil mycorrhizal

community composition, structure and diversity within and among habitats and for establishing phylogenetic relationships between unknown and known mycorrhizal fungi. Case studies using these molecular approaches are illustrated to: i) assess the effects of soybean intercropping on the arbuscular mycorrhizal diversity of poplar by fungal SSU rDNA sequence analysis; ii) molecularly discriminate species in the genus *Glomus* (Glomeromycota) by protein-encoding genes; iii) molecular analysis of plant invasion-induced biogeographical shift in the arbuscular mycorrhizal communities; and iv) assess ectomycorrhizal fungal communities and persistence of nursery inoculated seedlings outplanted on clearcut and reclaimed sites in northern Alberta, Canada. Mycorrhizal metagenomics studies, like all DNA-based microbial community analyses, face significant biases. They produce a snapshot of the mycorrhizal community genome at a specific point in time and space. The challenge for future is to resolve the genetic structuring of mycorrhizal fungi so that we may address ecological questions in a holistic framework and ultimately establish the link between above- and below-ground ecosystem diversity; analyses of the proteomics or specific assays for mycorrhizal fungal activities will be needed.

2.2 - The mycorrhizal status of *Picea glehnii* regenerated on serpentine soil

Tamai, Y., Miyakawa, M., Kitagawa, M., Miyamoto, T., Yajima, T. (Laboratory of Forest Resource Biology, Graduate School of Agriculture, Hokkaido University, Japan), Miyakawa, M. (Laboratory of Forest Resource Biology, Graduate School of Agriculture, Hokkaido University, Japan)

Serpentine soils are distributed in Hokkaido northern part of Japan along a central mountain range, give rise to unusual and sparse associations of edaphic plants that are tolerant of extreme soil conditions such as a low Ca-Mg ratio, lack of essential nutrients such as N, P, K, and high concentrations of the heavy metals. Sakhalin spruce (*Picea glehnii*) has tolerance, and forms dominant vegetation on the serpentine soil. Naturally regenerated spruce seedlings with several age classes were collected and compared their mycorrhizal status. We assessed relative abundance of mycorrhizal morphotypes in each age class. Colonized ectomycorrhizal fungi in each morphotype were identified by DNA sequences of the internal transcribed spacer region. Collected seedlings of 75% were ectomycorrhizal colonized, and non-ectomycorrhizal were observed in 0-1 year seedlings. The non-ectomycorrhizal seedlings were partially colonized by arbuscular mycorrhizal fungi (16-46%). Ectomycorrhizal colonization rate was not different among age classes, but the colonized fungal diversity and abundance increased with age. We found 20 morphotypes of ectomycorrhizas from roots of all age classes. The dominant mycorrhiza in each age class was species of *Tuber*. *Amphinema byssoides* and *Cenococcum geophilum* were ubiquitous in most of the classes.

2.3 - From species communities to populations of arbuscular mycorrhizal fungi

Redecker, D. (Botanical Institute, University of Basel, Switzerland)

Molecular markers are an indispensable tool to analyze phylogeny and evolutionary ecology of arbuscular mycorrhizal fungi (AMF). Molecular tools based on nuclear-encoded ribosomal RNA genes have been used extensively in field studies for the identification of AMF symbionts within colonized roots, revolutionizing our views of AMF communities in natural and human-influenced ecosystems. To better understand diversity and dynamics of AMF communities, the influence of environmental factors, host specificity and fungal colonization strategies was analyzed. In these field studies, an AMF lineage commonly assigned to *Glomus intraradices* was found to dominate AMF communities across a surprisingly broad range of habitats and environmental conditions. This fungal lineage has also been selected for genome sequencing, therefore there is considerable interest to unravel the mystery of its ubiquity. We therefore developed molecular tools to assess intraspecific diversity within this group, which will be useful to analyze the population structure of this fungus. These markers will now allow to address a new level of questions in AMF ecology.

Mixotrophic and Mycoheterotrophic Interactions

3.1 - Mycorrhizal fungi diversity in Brazilian orchids

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The association with mycorrhizal fungi is essential to the life cycle of orchids in nature. Lacking a store of nutrients, orchid seeds take in carbon and minerals from their symbionts during germination and embryo development, in a kind of metabolism named myco-heterotrophism. As adults, achlorophyllous orchids remain myco-heterotrophic, while chlorophyllous orchids need the symbiont mainly to absorb minerals required to their development and may transfer to fungi up to five times more carbon than they receive. A great diversity of *Rhizoctonia*-like fungi establishes association with orchids, and these fungi play different ecological roles. Fungi belonging to genera *Ceratohiza*, *Epulorhiza*, *Rhizoctonia*, and *Opadorhiza* have been isolated from Brazilian orchids, and the diversity among them, based on morphological and molecular characteristics, has been studied to comprehend mycorrhiza specificity in orchids. Specificity, indeed, is a critical factor in orchid distribution, and its

comprehension is fundamental to develop conservation programs of orchids, their fungal symbionts and their natural habitats (Supporting agency: CNPq and FAPEMIG).

3.2 - Sexual states of Tulasnellales in the tropical mountain forest: correlation between morphology and ITS-5.8S variability

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The genus *Tulasnella* has been morphologically most studied in Europe, but this genus is wide-spread around the world. Recently investigation showed Tulasnellales as frequently associated to epiphytic orchids in the Reserva Biologica San Francisco (RBSF), South Ecuador. The taxonomy of Tulasnellales species is not consistent. Morphological information on 77 records of fruiting bodies is available but taxonomic clarification is needed. Molecular phylogenetic studies on ITS-5.8S sequences of Tulasnellales was used to correlate with teleomorph data of fruiting bodies. Resupinate fruiting bodies near to epiphytic orchids were collected from pieces of decayed wood in the tropical mountain rain forest of RBSF. Fruiting bodies were morphologically characterized and illustrated, using light microscopy. PCR amplification and sequencing of ITS-5.8S were performed using primers ITS1/ITS4Tul and molecular cloning was used for this PCR products. The sequences were aligned separately within subclades to phylogenetic calculations and these were compared with morphological results. We propose two different morpho-species defined by hyphae diameter, basidia and spore size and shape. Molecular analyzes of ITS-5.8S showed sequences of Tulasnellales in several clades. The clade A and B appear near *T. calospora* sequences, and the clade C is clustering between *T. asymmetrica* and *T. violea* sequences. All clades found are close to sequences previously reported from mycorrhizal fungi of pleurothallid orchids in this region.

3.3 - An overview of orchid propagation and reintroduction in North America using mycorrhizal fungi as a conservation tool

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All members of the Orchidaceae have diminutive, dust-like seeds that generally lack an internal source of nutrition (endosperm). Upon landing on a substrate, orchid embryos must physically encounter and then consume free-living, mycorrhizal fungi as a carbon source (mycotrophy) prompting seedling development to a leaf-bearing stage. Following the initiation of photosynthesis, orchids – especially terrestrials – continue to utilize fungi into adulthood (mixotrophy), whereas non-photosynthetic orchids rely entirely on mycotrophy. Thus, for an orchid to complete its life cycle *in situ*, there must be suitable mycorrhizal fungi available in the substrate. Given that orchids are mycoheterotrophs, especially seedlings, the orchid-fungal association has been described as a “predator-prey relationship”, with the orchid serving as the “aggressor” and the fungus the “prey”. Many techniques exist that allow growers to cultivate orchids in the laboratory without using fungi (e.g., asymbiotic germination), but doing so has given conservationists the illusion that rare species grown in this manner are safe from extinction. In reality, the establishment of rare orchids *in situ* from artificially-grown stock will be successful only if mycorrhizal fungi (“prey”) are available to spawn the next generation of seedlings. Clearly, maintaining this source of “prey” in a given habitat is crucial to sustainable orchid conservation. In light of climate change now underway, habitat preservation alone can no longer be viewed as a reliable means to safeguard orchids and their associated biotic agents (e.g., pollinators, mycorrhizal fungi). Instead, the conservation of this remarkable plant family will probably require considerable human intervention on multiple levels, including assisted migration via symbiotic seed germination, and the recovery, preservation, use and release of orchid mycorrhizal fungi. The aim of this talk is to provide an overview of orchid propagation and reintroduction in North America using mycorrhizal fungi as a conservation tool. Case studies will focus primarily on taxa endemic to Hawaii (e.g., *Platanthera holochila*), prairie ecosystems (e.g., *P. leucophaea*), and the Florida Panther National Wildlife Refuge (e.g., *Epidendrum nocturnum*).

3.4 - Mycorrhizal Interaction Between Tropical Orchids and *Ceratobasidium* spp.

Joel Tupac Otero, PhD (Universidad Nacional de Colombia, Sede Palmira)

All orchids require mycorrhizal associations for successful germination, growth and establishment and an important number of them depend on the fungi *Ceratobasidium* spp., including most Oncidinae and Pterostylidinae. Mycorrhizal interactions vary from generalist; when orchids interact with several fungi, to specialized, with interactions limited to one fungal group. Theoretically, the dependence of orchids on mycorrhizal fungi could lead to coevolution. I observed considerable variation in the specificity of mycorrhizal associations amongst nine tropical orchid species, even among related species. Germination studies showed that specialized orchids were more

effective at exploiting fungal symbionts than generalist ones. I assessed levels of coevolutionary response within the Australasian terrestrial orchids, the Pterostylidinae. Results of cross-inoculation germination experiments and fungal DNA phylogenies revealed strong correlations between certain fungal clades and particular orchid genera. However, the congruence between the orchid and fungal phylogenies do not suggest strict co-cladogenesis between these orchids and their mycorrhizal fungi. The available evidence suggests that *Ceratobasidium* diverged before the orchids, and orchids have subsequently traced the mycorrhizal fungi phylogenetic pattern.

Signal Perception and Metabolism

4.1 - Deciphering the molecular dialogue between arbuscular mycorrhizal partners during early root interactions

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The arbuscular mycorrhiza symbiosis results from a successful interaction between the genomes of the two symbiotic partners. In order to gain better insight into the molecular dialogue involved, we investigated whether symbiosis-related *Medicago truncatula* genes affect *Glomus intraradices* gene expression at the appressorium stage of root interactions. Transcripts of a subset of fungal genes, identified in expressed sequence tag libraries of mycorrhizal *M. truncatula* roots by *in silico* expression analyses and with predicted functions in transcription, protein synthesis, primary or secondary metabolism, or of unknown function, were monitored during interactions with roots of wild-type or mycorrhiza-defective (Myc-) mutants of *M. truncatula*. All the fungal genes were expressed when *G. intraradices* spores germinated in wild-type *M. truncatula* root exudates or when appressoria or arbuscules were formed in association with wild-type *M. truncatula* roots. Most of the fungal genes were up-regulated or induced at the stage of appressorium development. Inactivation of the *M. truncatula* genes DMI1, DMI2/MtSYM2, or DMI3/MtSYM13 was not only associated with modulation of plant gene expression but also with plant cell wall responses, altered fungal gene expression (non activation or inhibition) and modified appressorium structure, providing first evidence that cell processes modified by symbiosis-related plant genes impact on root interactions by directly modulating AM fungal activity.

4.2 - Gene expression during pre-symbiotic interaction between *Hydnangium* sp. and *Eucalyptus grandis*

Irene da Silva Coelho (Universidade Federal de Viçosa)

Ectomycorrhizas are mutualistic symbiotic associations between plant roots and compatible fungi. During development and maintenance of ectomycorrhiza it is observed extensive changes in the genes expression that control signaling and metabolic pathways in both partners. The formation of the ectomycorrhizal root-tip takes place in four stages: pre-symbiotic, colonization, differentiation, and functioning. A complex series of molecular mechanisms is activated at the earliest or pre-symbiotic stage that precedes contact between plant and fungus. A suppressive subtractive cDNA library of the fungus *Hydnangium* sp. during the pre-symbiotic stage was constructed. Among the genes differentially expressed in the pre-symbiotic stage, genes that encode proteins related to carbohydrate and energy metabolism, transcription and synthesis of protein, cell communication, and response to stress were identified. The identification of genes through suppressive subtractive hybridization of *Hydnangium* sp. will allow the study of different genes related to mycorrhization, facilitating the understanding of the molecular mechanisms involved in the ectomycorrhizal association. Now, the next objective of our project has been constructing the suppressive subtractive cDNA library of the *Eucalyptus grandis* during the pre-symbiotic stage to identify what genes are activated during this stage in the plant.

4.3 - The ectomycorrhizal fungus *Laccaria bicolor* stimulates lateral root formation in poplar roots through auxin signalling

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Establishment of the ectomycorrhizal symbiosis between tree roots and ectomycorrhizal fungi is accompanied by a number of developmental changes in root architecture. The use of an *in vitro* sandwich culture system allows us to demonstrate that a stimulation of lateral root development in poplar roots is induced in the early phase of poplar root interaction with *Laccaria bicolor*. Analysis of the root transcriptome using Nimble oligo-arrays permitted identification of genes with altered expression in this early phase and revealed the differential expression of 2945 genes. We focus our studies on differentially expressed genes involved in auxin transport and signaling pathways including several components of polar auxin transport (*PtaPIN* and *PtaAUX* genes), auxin conjugation (*PtaGH3*) and

auxin signaling (*PtaIAA*). Time-course expression analysis showed that transcripts of *PtaPIN9*, the homolog of Arabidopsis *AtPIN2* protein, and several *PtaIAAs* accumulated specifically during the early interaction phase. Interestingly, inhibition of auxin polar transport by NPA during the early poplar/*L. bicolor* interaction significantly inhibited lateral root induction and altered the expression level of genes normally induced by *L. bicolor*.

Mycorrhizal Fungal Population Structure in the Field

5.1 - Community structure of arbuscular mycorrhizal fungi in Brazilian ecosystems: patterns, methodologies and perspectives.

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Brazil is characterized by distinct types of biomes including tropical and subtropical Forests, tropical Savannas (Cerrado), arid and semi-arid Caatinga, and grasslands (Pampas) and one of the most important agricultural producing countries in the world using intensive to very conservative agricultural systems. The high plant diversity occurring in Brazil are predominantly arbuscular mycorrhizal and studies on occurrence, diversity and community structure of arbuscular mycorrhizal fungi (AMF) are one of the main research lines in the country. We will approach the community structure of AMF in Brazilian ecosystem through a series of case studies. Patterns of community structure indicate that most of the fungal communities are dominated by few species of fungi when sporulation is considered and they tend to follow a log normal distribution. Diversity indices of AMF communities were calculated for some selected studies and the most frequent species in Brazilian ecosystems were detected. Latitudinal patterns of diversity will also be demonstrated. Most of the research was carried out based on spore counting and morphological identification, only few studies used the tools of molecular biology to study changes on community structure in the field and the use of both approaches will be discussed.

5.2 - Diversity and distribution of mycorrhizal fungal structures in humid forests of Cameroon in relation to ecological signification and land use degradation

Onguene Awana Nérée (Institute of Agricultural Research for Development (IRAD), Cameroun)

In humid forests of South Cameroon, arbuscular mycorrhizal structures occur as an underground mycelia continuum not overlapping "island" of ectomycorrhizal systems of various sizes that can extend around a single isolated host tree species such as *Berlinia* or *Azelia* or over large oligo or monodominant patches of several kilometer squares. However, the functional attributes of both mycorrhizal fungal structures within the same ecosystem forest remain unknown, at both the ecological and evolutionary scale. In the humid forests of South Cameroon, from the Nigerian border down to the Dja world heritage, through the coastal forest of Kribi, both types of mycorrhizal fungal structures co-exist as distinct patches of different sizes and composition. Ectomycorrhizal (ECM) fungal forests are rich in ectomycorrhizal fungi and ectomycorrhizae but poor in plant speciation. As much as two dozen ECM morphotypes and 125 ECM fungal carpophores had been identified from the Bipindi-Lolodorf-Akom II forests while a large number of ECM fungi had also been identified from Korup National Park. Many unknown species are yet to be identified from *Gilbertiodendron* clumps in the Dja forest or in small *Uapaca* clumps scattered close to numerous streams resorting the area. The examination of numerous root samples of major food crops such as cassava, maize, groundnut, beans... and of important timber species also revealed that most of them were colonized by vesicular arbuscular mycorrhizae with abundant intra- and intercellular hyphae. Extraction of soil samples also reveals a high diversity of spores of arbuscular mycorrhizal fungi from all land use types (farms, vegetative fallow, cocoa and coffee farms, secondary and primary forests, wood lots, skidding trails...). This suggests that agricultural productivity in such agroecosystems is strongly related to the promiscuity of indigenous arbuscular mycorrhizal fungi. Occurrence of ectomycorrhizal fungi as distinct patches implies niche partitioning between both mycorrhizal types and may explain the persistence of relictual ECM clumps. Hence, sustainable management of humid forests of Cameroon as well as of the Congo basin requests urgent knowledge on the functioning of such dual forests.

5.3 - Biogeography of Arbuscular Mycorrhizal Fungi: Distribution and Community Composition Patterns

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Despite the abundance and ubiquity of arbuscular mycorrhizal fungi (AMF) in most ecosystems, surprisingly little is known about the factors that control their distribution and community composition. We compiled spore

community data sets from sites in Costa Rica, Mexico, Arizona, Utah, Colorado, and Minnesota USA to study the factors that influence the diversity (richness and evenness) and species composition of AMF communities. Our fourteen sites included tropical rain forests, tropical dry forests, temperate forests, grasslands, arid shrublands, croplands and old fields. Our analysis indicates that species richness and evenness of AMF is not correlated with plant species richness; however, evenness is higher at tropical latitudes than temperate latitudes. Disturbance either decreases or increases the richness and evenness of AMF communities depending upon the type of disturbance. Across all of our sites, the species composition of AMF communities is associated most strongly with latitude, temperature and precipitation. Archaeospora and Diversispora spores are more dominant at high latitudes and Acaulospora, Ambispora and Scutellospora spores are more dominant at low latitudes. This study suggests that AMF spore communities exhibit macro-scale distribution patterns that are related to both biotic and abiotic factors.

5.4 - Biogeography of ectomycorrhizal symbiosis

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Ectomycorrhizal (EcM) symbiosis involves ca 6,000 plant and 20,000 fungal species and has independently evolved in at least 24 plant and 53 fungal lineages, respectively. The orders Pezizales, Agaricales, Helotiales, Boletales and Cantharellales include the largest number of EcM fungal lineages. Analysis of herbarium records from 11 major biogeographic regions revealed three main patterns in distribution of EcM lineages: 1) Austral; 2) Panglobal; 3) Holarctic (with or without some reports from the Austral or tropical realms). The Northern temperate regions host the largest number of EcM fungal lineages; no lineages are restricted to a tropical distribution with Dipterocarpaceae and Caesalpiniaceae hosts. EcM plant lineages are most numerous in mountainous regions of the north temperate zone, where subalpine and temperate vegetation occur in close proximity. However, semiarid Australia and rain forests of SE Asia support the largest number of EcM host species. Distribution of the oldest extant EcM plant lineage, Pinaceae, coincides with the distribution of many fungal lineages suggesting the origin of EcM symbiosis in temperate-subtropical mountains. Paradoxically, the few published phylogenies of globally distributed *Russula*, *Inocybe* and *Hysterangium* lineages argue against their north temperate origin. The highest below ground diversity of EcM fungi occurs in mixed forests of the north and south temperate regions, depending on many factors and sampling artifacts.

Mycorrhizas and Plant Nutrition

6.1 - Is there a link between phosphate uptake and growth depressions in arbuscular mycorrhizal plants?

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Conventional ideas about physiological mechanisms that underlie mycorrhizal growth responses (MGRs) are being overturned by new experimental evidence. This is reviewed by SE Smith et al. (this ICOM) mainly in relation to plants with high positive MGR and little or no MGR. Here we consider plants which show negative MGR, i.e. where there are growth depressions. These are conventionally thought to occur where the fungus 'cheats' (parasitizes) the plant, in that net growth benefit from P uptake (if any) via the fungus is less than the C cost due to fungal growth. Recent results show that in fact there can be considerable P uptake via AM fungi (supported by expression of AM-dependent genes for P transport) even where there are growth depressions. Further, large growth depressions can occur when colonization (and hence fungal C cost) is very low. The simplest explanation is that 1) during the onset of colonization P uptake via the plant epidermis is suppressed (as it is when plants show no positive MGR: SE Smith et al., this ICOM), but 2) restricted colonization results in little P uptake via the fungus; hence P stress and growth depression. Our conclusions are supported by a finding that shading did not effect growth of AM wheat that had negative MGR at high light, contrary to expectation if growth was C-limited. All these results suggest that AM fungus/plant interactions are much more subtle than usually believed in cellular, whole-plant, ecological and evolutionary contexts.

6.2 - Ectomycorrhizal fungi and mobilisation of phosphorus: novel data and actual role

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Ectomycorrhizal (ECM) symbiosis improves markedly the P nutrition of the host plant, but the mechanisms sustaining this positive effect are not all understood. In our group, we focused on (1) the identification of fungal P transporters and (2) the role of fungal phosphatase (Pase) released into the soil solution on organic P (Po) mobilisation. To address the first question, we worked on the model fungal ECM species *Hebeloma cylindrosporum*. We identified two phosphate (Pi) transporters (HcPT1 and HcPT2) able to mediate Pi:H⁺ symport in yeast with different affinities for Pi. Expression patterns of the two genes in ECM plants grown in a low-P or high-P soil indicate

that HcPT1 and HcPT2 might be involved in the uptake of Pi from the soil solution in the two conditions of soil P availability used. To address the second question, we worked on the quantification of Pase released by ECM tips collected in various forest ecosystems through Europe differing by their levels of water and nutrient availability. We used also *H. cylindrosporium* as this species is able to release huge amounts of Pase activity when grown in vitro in P-starved conditions. The results obtained so far showed that the regulation of Pase activity evaluated in situ is complex. *H. cylindrosporium* released four acid Pase fractions displaying different efficiency to release Pi from Po extracted from podzol. A novel scheme of the possible role of ECM fungal Pase in Po mobilisation from forest soil will be proposed.

6.3 - The importance of AMF on N and P acquisition

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In most of the natural and semi-natural ecosystems productivity is limited by P and/or N. In agro-systems N and P availability is higher due to the use of fertilizers, but crops have high growth potential and therefore represent big sinks for these nutrients. It is widely accepted that the establishment of AM symbiosis is strongly inhibited under high levels of P and N (Smith & Read 2008). However some works highlight the importance of the P source and others stress that the symbiosis formation depends on the P status of the plant and not on the external P concentration. On the other hand, works on assessing AMF diversity and spores density in agricultural soil confirm the existence of inoculums in considerable amounts. Most cultural practices completely based on the use of fertilizer are target at a single objective- increased production. AMF give a plant the opportunity to become better attuned to the surrounding circumstances, resulting in an increased ability to absorb nutrients such as P and N and water, or simply in better-regulated growth.

The work focus on the direct and indirect nutritional effects of AMF to plant. It is shown that AMF soil community responds to N and P availability (form and concentration) and that AMF form symbiotic associations with plant roots even in presence of high soil P concentrations. The scientific relevance of the work relies on the design of a strategy to increase nutrient use efficiency while producing "climate proof plants".

Inoculum Technology Application and Management of Mycorrhizal Fungi

7.1 - Arbuscular Mycorrhizal Fungi for Sustainable Agriculture, Horticulture and Forestry

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The role of arbuscular mycorrhizal fungi (AMF) in improving plant growth is well documented. The beneficial effects being improvement in the uptake of diffusion-limited nutrients, synergistic interaction with beneficial soil microorganisms, production of plant growth promoting substances, greater ability to withstand water stress and root pathogens. Though these endophytes are not host-specific there is enough evidence of their host preference, thus enabling the possibility of selecting an efficient AMF for a particular crop. AMF being obligate symbionts mass production is not as easy as other bio-inoculants. Hence, it will be more practical to concentrate on crops normally grown in nursery beds, root-trainers or polybags and then transplanted to the field. Our investigations with plants important in agriculture, horticulture and forestry, including medicinal and aromatic plants, have shown great potential in using AMF inoculation for improving growth and productivity. The micro-propagated plantlets as well as perennial crops already established in the field respond positively to inoculation with efficient AMF. In horticultural plants it was found that AMF inoculation made the root stocks ready for budding/ grafting much earlier than uninoculated plants. Inoculation made flowering ornaments to flower early and to have a longer vase-life. In plantation crops like cashew AMF helped in overcoming the transplant shock when planted in the field. Studies on delivery systems brought out the optimum method and level of inoculum placement. Co-inoculation of AM fungi with other beneficial soil microorganisms is more useful in improving plant growth thus suggesting the need for development of suitable 'microbial consortia' for inoculating different crop plants. Recently, we brought out that 'microbial consortia' developed using efficient AMF + PGPRs helped in the successful re-vegetation of degraded forests.

7.2 - AMF in sustainable agriculture: Tools for farmers and policy makers

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Variation in the level of root colonization of durum wheat genotypes, revealed in greenhouse experiments, offers an opportunity to breed for better nutrient use efficiency through conventional approaches. While the arbuscular mycorrhizal (AM) symbiosis is way to improve crop nutrient use efficiency and mitigate the impact of the World food crisis, AM surveys using molecular methods of detection and identification revealed important degradation of AM fungal communities in cultivated fields of the Canadian Prairie. Tools for the successful management of AM fungal bioresources are being developed for land managers based on the consideration of the plant and fungal genotypes, and of the conditions of the soil environment. A survey of cultivated Prairie fields revealed the relationship between soil types and AM fungal species distribution. This relationship opens the possibility of using modelling approaches to support AM fungal bioresources management in cultivated soils of different geographical areas. Models could predict the health or effectiveness of an AM fungal community in a given field, based on maps of AM fungal biodiversity and knowledge of the positive or negative impacts of the various cropping practices used. Healthier cropping practices could be selected, based on the information generated by such models. Cost-effective inoculation with superior AM strains based on adaptation to soil type could be practiced where models predict degraded AM fungal communities.

7.3 - Appropriate Host-Fungus selection: a key to success of Mass production and in depth comparison of cost economics.

Alok adholeya (Centre for mycorrhizal research, Biotechnology and Management of Bio-resource Division The Energy and Resources Institute, New Delhi, India)

Ultimate success of any technology lies with its end product acceptance by the end user; however, scientific prerequisite is to achieve optimum efficiency of the process. Host and fungus interaction have to be better understood in case of mycorrhizal symbiosis. The same, therefore, have to be further optimized in a process for achieving commercially viable product. Tripartite tools comprising of biological method, Process management software and cost effective formulation ultimately lead to a wider acceptability of technology. Mimicking nature's process under controlled condition is a major challenge and needs continuous efforts to find more effective product. How they have been achieved shall be discussed and elaborated.

Genome Structure, Plasticity and Evolution

8.1 - Non-mendelian inheritance of genomes in arbuscular mycorrhizal fungi

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A conventional tenet of classical genetics is that progeny inherit half their genome from each parent in sexual reproduction or a complete genome in an asexual process. The transmission of hereditary characteristics from parent organisms to their offspring can therefore be predictable, although, several exceptions are known. Heredity in microorganisms, however, can be very complex, and even unknown, as in arbuscular mycorrhizal (AM) fungi. This group of fungi are plant-root symbionts, ubiquitous in most ecosystems, that reproduce asexually via multinucleate spores. Here, we show that AM fungi have the ability, through the establishment of new symbioses, to pass hundreds of nuclei to subsequent generations. In addition, we discovered that spores are formed in the middle of the hyphae and not at its termini, as has been thought. More importantly, we observed surprising heterogeneity in nuclear content among sister spores and show that massive nuclear migration and mitosis are the mechanisms by which AM fungal spores are formed. In contrast to all other known organisms, in which all somatic nuclei derive from a single progenitor nucleus, we documented that AM fungi lack a single-nucleus stage during their life cycle, indicating that genome transmission by sporulation does not appear to constitute a strong genetic bottleneck. This previously undescribed, non-conventional process of heredity has fascinating implications for the diversity of modes of evolution in eukaryotes.

8.2 - Fatty acid metabolism during basidiosporogenesis in *Pisolithus microcarpus*

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Lipid metabolism is an important research topic to achieve a better understanding of the biology and physiology of mycorrhizal fungi. Our studies have focused on a poorly understood stage of the life cycle of *P. microcarpus*: spore formation. Little is known about the carbon flux during basidiosporogenesis in this fungus, but some recent results have shed some light on the importance of lipids during this process. Microscopical observations revealed the presence of abundant lipid bodies throughout basidiocarp development. Lipid accumulation takes place inside mature spores, occupying almost the whole space within them. Fatty acid composition analysis during the different developmental stages of the basidiocarps showed that the compound 18:2w6,9 is the most abundant at the

beginning of development and is almost inexistent at intermediate stages. The concentration of the fatty acid 18:1w9 increases at the final stages of basidiosporogenesis, being the most important compound in mature spores. Also, the concentration of total fatty acids increases as the basidiocarps mature. Finally, expression analyses of genes coding key enzymes of the fatty acids synthesis and oxidation pathways, glyoxylate cycle, and gluconeogenesis have contributed to a better understanding of the fate of lipids during spore formation. Together, our results present a new picture of lipid metabolism during the development of *P. microcarpus* basidiospores. Financial support: FAPEMIG and CNPq.

8.3 - Genetic exchange and recombination in AMF and their consequences on plant growth

Croll, D. (Department of Ecology & Evolution, University of Lausanne), Angelard, C. (Department of Ecology & Evolution, University of Lausanne), Sanders, I.R. (Department of Ecology & Evolution, University of Lausanne)

Arbuscular mycorrhizal fungi (AMF) are thought to be vegetatively incompatible and to reproduce strictly clonally. Combined with the morphological stasis of AMF over several hundred millions of years, this led to the suggestion of AMF being 'ancient asexuals'.

We used genetically different isolates of *Glomus intraradices* from one field to study contact zones of hyphae from germinating spores. Based on genetic markers, we found evidence for exchange of nuclei between genetically different AMF. The progeny were viable and established the symbiosis with plant roots. Phenotypic traits of the progeny were significantly different from the parental isolates. A greenhouse experiments using two different host plant species showed that the occurrence of genetic exchange among isolates had significant effects on the symbiosis with plants.

Exchange of nuclei among genetically different isolates provides the potential for recombination to occur. We tested for evidence of recombination among 18 genotypes identified within a field population. Five statistical tests predicted multiple recombinant regions in the genome of a core set of isolates. Our analyses refute the assumption of 'ancient asexuality' in AMF.

Taken together, these findings have important consequences for understanding AMF evolution and the co-evolution of AMF with plants.

8.4 - Population biology and evolutionary genetics of the ubiquitous ectomycorrhizal fungus *Cenococcum geophilum*: species complex or complex species?

Greg W. Douhan (Department of Plant Pathology and Microbiology, University of California, Riverside, CA, USA)

Cenococcum geophilum is perhaps the most widely distributed and most recognized ectomycorrhizal fungus based primarily on tip morphology. However, a significant amount of diversity has been found, despite the fact that *C. geophilum* is not thought to reproduce by meiotic or mitotic spores, but several recent studies based on both fine scale and global sampling has revealed that *C. geophilum* should actually be thought as a species complex. Based on primarily fine scale sampling and the analysis of four loci, three phylogenetically distinct lineages, or cryptic species, of *C. geophilum* were detected at the scale of a single soil sample and between sampling populations 8 meters apart. Further analysis of 10 loci from 44 isolates from one of these lineages, which were subdivided into two well supported subclades, found phylogenetic incongruence between the loci and recombination analyses using six different methods was consistent with recombination. However, most of the incongruence was caused by an apparently reciprocal recombination event between the actin locus and the other loci studied. Lastly, a more global sampling of isolates revealed that phylogenetic resolution of the previously found three lineages broke down and that two isolates used in previous studies were in fact phylogenetically distinct from all of the other isolates based on the analysis of two loci. In conclusion, these results are significant and demonstrate that inferences of population structure are highly dependent upon how *C. geophilum* species are interpreted based both on morphology and genetic data. This potential problem also has implications for other fungal taxa since cryptic species have routinely been found in recent years based on molecular data.

Mycorrhizosphere Interactions

9.1 - Carbon costs and benefits in the mycorrhizosphere

Thomas W. Kuyper (Department of Soil Quality, Wageningen University, PO Box 47, 6700 AA Wageningen, The Netherlands)

Plants spend up to 10-20% of their photosynthates on mycorrhizal symbioses in order to increase uptake of limiting nutrients. The question arises whether mycorrhizal plants are more carbon-limited than non-mycorrhizal plants and under which conditions this may result in lower plant fitness. If mycorrhizal plants are more C-limited than non-mycorrhizal plants, they should respond more strongly to elevated CO₂. The question also arises whether the mycorrhizal fungi are carbon-limited. If so, how does elevated CO₂ change fungal fitness? How would changes in fungal C-capture via the plant feed back to plant performance? After discussing the issue of C-limitation, I will

look at C-costs and C-benefits in the (mycor-)rhizosphere. Mycorrhizal fungi affect carbon fluxes in the (mycor-)rhizosphere in various ways. Both qualitative effects (signaling molecules; strigolactones) and quantitative effects (exudation of organic anions or acids; also repression of plant exudation of these compounds) will be discussed, with an emphasis on feed backs between plant and fungus. Even though mycorrhizal plants are not C-limited under normal conditions, the impact of mycorrhizal fungi on C-fluxes could reduce plant fitness.

9.2 - Correlation among fine roots morphology, rhizosphere microbial population and nutrient availability in ecosystems under different successional vegetation status in Southern Brazil

Galdino Andrade (Universidade Estadual de Londrina, Brazil)

Four areas with vegetation under different successional status: 1- Grass in degraded soil; 2- Grass and pioneer woody trees; 3- secondary forest with pioneer, early, and late secondary woody trees; and 4- mature forest with late secondary and climax woody trees were assessed. The parcels in each area were 100 x 100 m and samplings were made four times during one year in each season. In each parcel were collected fine roots, rhizospheric soil (adhered on the fine roots), and bulk soil. The nutrient level, microbial population, arbuscular mycorrhizal (AM) colonization and spore density, C and N microbial biomasses, and acid phosphatase activity were assessed. AM colonization and spore density decreased through the successional status (area 1 to area 4), and in the dry and cool seasons, when the plant growth is slow. Root length correlated positively with AM colonization and spore density. On the other hand, root dry weight and root tissue density showed negative correlation with both AM parameters. The rhizosphere soil from areas 1 and 2 were poorer in nutrients than in areas 3 and 4. Microbial activity and population increased in spring and summer time in all studied area, but decreased in areas 1 and 2 when compared with area 3 and 4, suggesting that plants at initial levels of succession rely more on AM for nutrient acquisition than plants at late successional stages, which rely more on other microbial processes for nutrient uptake from rhizosphere than on AM.

9.3 - Co-inoculation of AM fungi and PGPR for helping plant developments in either low-nutrient or heavy metal contaminated soils

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Arbuscular mycorrhizal (AM) fungi are known to act in co-operation with other soil microorganisms in the mycorrhizosphere, particularly those involving the so-called plant growth promoting rhizobacteria (PGPR), involved in nutrient cycling or the biocontrol of plant pathogens. Co-inoculation of AM fungi and PGPR to establish a tailored mycorrhizosphere has been investigated in our Department with emphasis on those interactions involved either in plant acquisition of P and N (by using ¹⁵N and ³²P-based approaches) or addressed to increase plant tolerance to heavy metals (HM). The role of a tailored mycorrhizosphere in phytoremediation was studied by: (i) isolation and characterization of microorganisms from a target HM contaminated site; (ii) developing phytoremediation experiments; and (iii) analysing the mechanisms involved. Autochthonous metal-adapted AM fungi and or PGPR were compared to microorganisms in the same taxa from culture collection, therefore non-adapted to the tested HM. In conclusion, it was demonstrated an effect of mycorrhizosphere interactions on "phytostabilization", but a role on "phytoextraction" was also shown. Therefore, a selected HM-adapted mycorrhizosphere can be tailored to improve plant tolerance to HMs and to benefit bioremediation of HM-contaminated soils. The ecological, biochemical and molecular bases of the AM role in increasing plant tolerance to HM, and the reasons supporting the AM fungi x PGPR interactions found will be discussed.

Taxonomy and Phylogeny of Glomeromycetes

10.1 - Systematics of *Glomeromycota* - phylogeny based taxa for modern research

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The systematics of *Glomeromycota* (arbuscular mycorrhizal fungi, AMF) is driven by morphology based descriptions and by aspects based on phylogenetics and evolutionary implications. As morphological characterization often is error prone, more validated sequence data are needed to improve species and higher taxa concepts, and for AMF community analyses. Recently, new AMF specific PCR primers were designed, which will allow easier DNA analyses from plant roots and from AMF spores (Krüger et al. 2009, New Phytol. 183).

Problems in species description and identification in the *Glomeromycota* are numerous and may be exemplified by the AMF called '*Glomus intraradices*' DAOM197198. This model fungus is, most likely, not con-specific with *G. intraradices* (Stockinger et al. 2009, New Phytol. in press). Well defined cultures are another base for the investigation of the closely related AMF. For example, *Diversispora celata* described from Switzerland (Gamper et

al. 2009, New Phytol. 182) could be shown, based on sequence data, to most likely occur in Africa and North America, and isolates are available from the BEG collection for further research.

Regarding higher taxa, several families have recently been described in the *Glomeromycota*. However, they obviously do not reflect a natural system of comparable taxonomic units, which should be the goal of any biologist taking into account the impact of a "natural systematic" for evolutionary, ecological, and functional implications.

10.2 - The fairytale of the difficult morphological identification of arbuscular mycorrhizal fungi – recent advances in the area

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The progresses in species identification through molecular analyses have changed the taxonomy in all known phyla during the last 20 years. This is particularly true for the fungal phylum *Glomeromycota*. To date this phylum comprises 13 families, 19 genera and about 220 arbuscular mycorrhizal fungal (AMF) species, but it is well accepted by now that a much larger number of AMF species remains unexplored. Simultaneously with the progresses in the molecular analyses, an opinion was build up and distributed that AM fungi can hardly be identified by spore morphological means, since there might not be enough characters to distinguish between many similar species. This situation discouraged young researchers to enter the field, but forced others to examine more precisely the available morphological features of spores and other AMF fungal structures, to search for characters that so far had not sufficiently been considered, and to understand which characters are congruent with the phylogeny of these fungi. We present the recent progresses in the morphological identification of AMF families, genera and species, and discuss them in the phylogenetic context. Our conclusion is the same as forty, twenty and ten years ago: the morphological tools need still to be further developed since we do not yet consider all morphological characters of glomerospores eventually available. Without any doubt, there were several important advances in the area in the recent past, not only in the molecular but also in the morphological identification of AM fungi, which hopefully encourage many young researchers to go straight ahead with both morphological and molecular analyses on AM fungi – for a better understanding of the fairytale about AMF identification.

10.3 - Basic principles of morphological identification of arbuscular mycorrhizal fungi forming glomoid spores

Janusz Błazkowski, Department of Plant Protection, West Pomeranian University of Technology (Szczecin, Słowackiego 17, PL-71434 Szczecin, Poland)

At present, the *Glomeromycota* comprises ca. 210 species. Unfortunately, most of them were described many years ago and their descriptions generally are incomplete, do not inform of the taxonomically most important phenotypic and biochemical properties of the components of their spores and mycorrhizae or are completely erroneous. Moreover, most species were described from field-collected spores, which usually are highly changed in colour, subcellular structure, and lack many structures needed to their correct identification, because they are short-lived and sensitive to the influences of harmful abio- and biotic stresses, including hyperparasites. Additionally, in the literature there is no clear system of grouping of most (ca. 62%) described species of AMF, i. e., species forming glomoid spores, based on their morphology. The difficulties in identification of glomoid spores also results from their relatively least morphological diversity. The arguments listed above undoubtedly decided of, e. g., the common erroneous identification of first *Glomus fasciculatum* and then *G. intraradices*.

Therefore, the aim of my presentation is to show the basic steps in grouping and identification of AMF forming glomoid spores. The modes of spore formation, their main morphological characters (spore colour and size), the components of subcellular structure of spores and their phenotypic and biochemical properties, as well as those of their subtending hyphae will be characterized and compared in different morphologically closely related species.

10.4 - Integration Of Morphological and Genetic data to revise hypotheses of Evolution in *Glomeromycota*

Joseph Morton and Zola Msiska (West Virginia University, 1090 Agricultural Sciences Building, Morgantown, WV 26506-6108, USA)

Analysis of 25S rRNA and β -tubulin gene sequences provided strong support for clades resolved by other workers

using the 18S rRNA gene. Relationships between clades at deeper nodes were not supported, rendering them ambiguous. Topology was highly sensitive to proportion of taxa per clade and number of taxa in the tree. Family-level relationships were resolved only by using a suite of morphological characters tested *a priori* for homology. The resultant phylogeny restored congruence at different scales to link evolution with functional traits. At higher nodes in the tree, morphology was less informative than genetic data, especially among species with intergrading or ambiguous phenotypes. In these cases, phenotype did not always correlate with genotype. An irreversible albino mutant of *Scutellospora heterogama*, whose juvenile state was arrested during spore ontogenesis to become the mature phenotype, was so unique that the fungus could be described as a new species. Genetic data grouped albino and wild-type phenotypes into one highly supported clade. In *Gigaspora*, 30 clones of five morphospecies grouped genetically into only two highly supported clades. Over 100 clones of multiple isolates of species in *Glomus* group B produced a similar result. These results indicated morphology and evolution of 25S and β -tubulin genes are asynchronous in some clades, so that both data sets are essential in resolving speciation events and clarifying evolutionary patterns and processes.

Mechanisms of Toleranc to Biotic and enviromental Stresses

11.1 - Water relations and salt resistance of ectomycorrhizal plants

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The effects of ectomycorrhizal (ECM) associations on drought and salt resistance of plants has received considerable attention. Early reports attributed enhanced root hydraulic conductivity (Lpr) in ECM plants to the decrease in apoplastic resistance by the hyphae penetrating plant roots. However, there is presently strong evidence accumulating which points to the role of plant aquaporins (AQPs) in this process. In *Populus tremula x tremuloides*, ECM formation resulted in an increased transcript level for three of the AQP genes, two of which are the most prominently expressed AQPs in roots. Subsequent studies showed that the enhanced Lpr of ECM conifer seedlings is accompanied by a large increase in AQP-mediated cell-to-cell water transport in the root cortex. There is also indirect evidence pointing to the importance of chemical signaling which may explain why mycorrhizal associations do not always result in increased Lpr. Hyphal penetration of the root cortex cells by the fungus which formed ectendomycorrhizal associations with *Populus balsamifera* enhanced plant growth, but had little effect on Lpr. In contrast, ECM association of *P. balsamifera* with *Hebeloma crustuliniforme* triggered an increase in Lpr in the absence of growth effects. AQPs have been also implicated in salt resistance of ECM plants since the inhibition of water transport through AQPs is one of the most salt-sensitive processes in plants. In the root cortex of *Pinus banksiana*, cell hydraulic conductivity was rapidly and drastically reduced by the NaCl treatment and the reduction progressed over time. However, in ECM plants, the initial decline in cell hydraulic conductivity was followed by a quick recovery to the pre-treatment level. These and other possible mechanisms of salt resistance in ECM plants will be discussed.

11.2 - Alleviation of salt stress by *Scleroderma bermudense* in seagrape (*Coccoloba uvifera* L.) seedlings

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In Guadeloupe (Lesser Antilles), seagrape (*Coccoloba uvifera*, Polygonaceae), forms one of the most important stands along a gradient of salinity in littoral forest with an abundant seedling recruitment. In the areas with high salt levels, seagrape is often associated with the ectomycorrhizal (ECM) fungus, *Scleroderma bermudense*, which thus, may play a major role in the establishment and survival of seagrape seedlings under saline stress. The purpose of this study was first, to survey the *in vitro* growth, osmotica and NaCl contents of *S. bermudense* compared to those of two ECM fungi (*Scleroderma* sp. and *Melanogaster* sp.) isolated from the areas with low salt levels, and secondly, to test the capacity of *S. bermudense* to alleviate saline stress in seagrape seedlings over a range (0, 100, 200, 300 and 500 mM) of NaCl concentrations. After one month of *in vitro* mycelial growth, *S. bermudense* was shown to be more resistant to NaCl treatments and accumulated more K and proline than the other ECM fungi in response to increasing concentrations of NaCl. On the other hand, although root colonization by *S. bermudense* under greenhouse conditions was reduced with increasing NaCl levels, ECM dependency of seagrape seedlings increased after three months of growth. Moreover, ECM seedlings displayed higher K and proline concentrations in the leaves, suggesting a higher osmoregulating capacity of seagrape in association with *S. bermudense*. The reduction in Na and Cl uptake with concomitant increase of K and proline levels and a higher water status in ECM plants may be important salt-alleviating mechanisms for seedling recruitment in saline soils.

11.3 - Heavy metal tolerance mechanisms in the ectomycorrhizal fungus *Suillus luteus*.

Jan V. Colpaert (Hasselt University, Centre for Environmental Sciences, Environmental Biology Group, Agoralaan, Building D, 3590 Diepenbeek, Belgium)

Organisms trying to survive in heavy metal contaminated ecosystems are subjected to selective pressures for increased resistance to toxic metals. Evolutionary adaptation to heavy metal toxicity is a well-documented phenomenon in different groups of organisms including bacteria, higher plants, animals and fungi. However, such genetic modifications resulting in true tolerance occur in relatively few species. Surprisingly, on toxic soils some tree species can survive without acquiring additional heritable metal tolerance traits. The question arises whether these trees can colonize metal-polluted sites with the assistance of particular mycorrhizal fungi that have acquired increased metal tolerance.

The heavy metal tolerance in the ectomycorrhizal fungi from the Suilloid clade is now well documented and the importance of this tolerance for host plants exposed to toxic metal concentrations is evident. The specific zinc tolerance mechanisms in *Suillus luteus* were investigated. Zinc tolerance is based on an exclusion mechanism. Zn-tolerant *Suillus* genotypes accumulate less Zn than their sensitive counterparts. Flux studies were performed to unravel the patterns of Zn accumulation in these fungi and two Zn-transporter genes of the CDF-family were studied. Identification of the molecular determinants of the zinc homeostasis network in *Suillus* is essential to better understand the mechanisms responsible for the adaptive zinc tolerance in *S. luteus*.

11.4 - Impact of arbuscular mycorrhizal symbiosis on plant resistance to pathogens

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Protection against biotic stresses has been amply described in different mycorrhizal systems, but the underlying mechanisms remain largely unknown. Besides improved plant nutrition and competition phenomena, experimental evidence supports the involvement of plant defence mechanisms in the protection. During mycorrhiza establishment, modulation of plant defence responses occurs in order to achieve a functional symbiosis. This modulation is associated to changes in phytohormone levels and transcriptional reprogramming. As a consequence of these changes, a mild, but effective activation of the plant immune responses may occur. This activation leads to a primed state of the plant that allows a more efficient activation of defence mechanisms in response to the attack by potential enemies¹.

Jasmonates (JA) has been proposed as key regulators in the induction of resistance by beneficial microorganisms^{2,3}. We have analyzed the role of JA in Mycorrhiza Induced Resistance (MIR) using the pathosystem tomato-*Botrytis cinerea*, causal agent of gray mold on leaves. Disease severity and pathogen proliferation was significantly reduced in *Glomus mosseae* colonized plants, confirming the induction of MIR. Remarkably, the expression of JA-regulated defense genes in response to *Botrytis* infection is higher in mycorrhizal plants. Additionally, mycorrhizal plants display a potentiated response to exogenous application of JA. All together, our results support that MIR is associated to priming of JA-dependent responses.

1. Pozo & Azcón-Aguilar *Curr Opin Plant Biol* 2007

2. Pozo, Van Loon & Pieterse, *J Plant Growth Regul* 2004

3. Pozo et al. *New Phytol* 2008

Symposia

Fungal Communities

S2.1 - Structure and function of ectomycorrhizal communities in response to soil mechanical and chemical disturbances

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Ectomycorrhizas are central in mobilizing and absorbing nutrients by trees in temperate forests. At the same time, ectomycorrhizal community structure is very reactive to environmental changes, including silvicultural practices. It is therefore important to know to which extent man-made disturbances affect the functionality of ectomycorrhizal communities. Here, we investigated on the impact of a mechanical disturbance (soil compaction caused by heavy logging machines) and of a chemical disturbance (Ca-Mg amendment aimed at correcting soil acidification in a declining stand) in two beech (*Fagus sylvatica*) forests in central Europe. The ectomycorrhizal community structure was described by combining morphotyping and ITS direct sequencing in order to identify the

species of the fungal symbionts. In addition, eight secreted enzymatic activities involved in mobilizing nutrients from soil organic matter were measured in the dominant populations of ectomycorrhizas. In the control plots in both sites, the initial community was dominated by a few species common in beech forests on acid soils in the region. However, their response to the two types of disturbance was very different in terms of species that were reduced or favoured. Liming had very significantly enhanced laccase activity. Laccases are polyphenol oxidases involved in the first step of the degradation of ligno-cellulosic materials. Thus, liming had resulted in a better mobilisation of the nutrients entrapped in the abundant beech leaf litter. The response to soil compaction was not so clear-cut. These results will be discussed in terms of contribution of ectomycorrhizal populations to the overall activity of the community and of nutrient cycling in forest ecosystems.

S2.2 - Diversity of arbuscular mycorrhizal fungi in the Caatinga, a semiarid Brazilian biome

Maia, L.C., Silva, G.A., Yano-Melo, A.M., Goto, B.T., Fungal communities

The Caatinga, an exclusive Brazilian biome, is characterized by deciduous vegetation, mostly belonging to Fabaceae, Euphorbiaceae and Cactaceae, and high floristic endemism. From its 825,750 km², 24.4% is native forest and 38.4% open herbaceous forest with xerophytes, 36.3% is under anthropic impact and the remaining is covered by water; the climate is semiarid (23 - 27 °C, 500-750 mm annual rainfall). This biome is one of the richest semiarid areas in diversity and that of AMF is also high, with 75 species. This estimate is preliminary, considering that most of the Caatinga is still unexplored. The acknowledge diversity for this biome corresponds to 75.7% of the AMF species registered for Brazil (99) and to 36.6% of those globally described (205). The impacted areas have smaller number of species (33), comparing with agrosystems (59) and preserved areas (52). Most of the species belong to Glomeraceae (26) and Acaulosporaceae (18); to the present, only Pacisporaceae and Geosiphonaceae have not been found in Caatinga. *Glomus* and *Acaulospora* are the commonest genera. The low diversity of AMF in disturbed areas was confirmed; however, when re-vegetated, the community of AMF is re-established including species that differ from those found before the disturbance. These data reinforce the need for preservation of areas considered hotspots for biodiversity, such as the Caatinga, where species of AMF can be maintained *in situ*, protected from the impact caused by anthropic actions.

S2.3 - Symbiotic fungi inhabiting roots of Norway spruce seedlings and neighboring ericaceous dwarf shrubs in a temperate montane meadow

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ECM trees are often accompanied by ericaceous undergrowth, but outcomes of this symbiosis are not well known. Ericaceae may limit tree regeneration by affecting seed germination, shading seedlings and competing with tree roots. On the other hand, dense shrubs increase relative humidity, reduce soil erosion and protect seeds against predation. Not least, interactions mediated by shared mycobionts are likely to occur. Our locality is a temperate montane meadow with a neighboring spruce forest, where most spruce seedlings regenerate inside or in the vicinity of patchy ericaceous vegetation. This may have several reasons; we are interested in roles of symbiotic fungi. Fungi from surface sterilized roots of spruce seedlings and neighboring Ericaceae were isolated using different media and determined. The ectomycorrhizae were dominated by *Tylospora* and *Amphinema*, but also regularly yielded *Meliniomyces* species and fungi from the *Phialocephala fortinii*-*Acephala applanata* complex (PAC). Ericaceous roots were dominated by REA and PAC members, ectomycorrhizal fungi were absent. Despite the limitations of the culture-dependent approach, it so far seems that the PAC and REA (excl. *Cadophora finlandica* and *Meliniomyces bicolor*) members are the only shared mycobionts and that EcM fungi ignore ericaceous roots. Though PAC and REA members are reported as common inhabitants of EcM tips, the significance of this phenomenon for ectomycorrhizal plants remains speculative.

S2.4 - Biodiversity and ecological functions of AMF and DSE in an ancient Pb/Zn mine tailing plot in Southwest China

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Community and ecological functions of arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) colonizing in 110 natural recovery plants (34 species, 20 families) in an ancient Pb-Zn mine tailing of Yunnan Province, China were investigated. About 56% and 79% plants were colonized by AMF and DSE, with an average colonization of 12.3±2.3% for DSE, and 19.8±3.30% for AMF respectively. AMF spores in the rhizosphere soils were wet-sieved and spore density varied largely from 17 to 3968 per 100 g soil, with an average of 515±115. *Glomus* and *Acaulospora* were the dominant genera. Twenty-five endophytic fungi were isolated and identified by their morphology and ITS sequences. *Exophiala*, *Phialophora*, *Cladosporium* and *Thysanorea* were the most common

genera. Pot culture experiments showed that the biomasses of maize inoculated with AMF (*G. mosseae* and *G. sp*) or DSE (*Exophiala pisciphila* H93 and *Thysanorea sp* H125) were significantly higher than the non-inoculated maize in single or complex heavy metal (HM) (Pb, Zn, Cd) contaminated soils. Importantly, inoculation significantly reduced the translocation of HM from roots to shoots compared to the non-inoculated maize, thus potentially protected hosts from toxicity of HM in heavily contaminated soils.

Mechanisms of Tolerance to Stresses

S3.1 - Functional changes in the soil-mycorrhizosphere-plant system due to ozone and pathogen stress

Pritsch, Karin, Ernst, Jana; Esperschuetz, Juergen; Schloter, Michael

The mycorrhizosphere (MR) of forest trees is a complex habitat influenced by plant roots, soil, and associated microorganisms namely the ectomycorrhizal mycobionts (ECM). MR functions are closely connected with soil-plant nutrient cycles and thus may be impaired as a result of plant stress. Stress factors such as elevated ozone and pathogens can alter carbon metabolism in plants resulting in qualitatively and quantitatively altered carbon supply to the MR thus indirectly affecting MR functions such as extracellular enzymatic processes involved in carbon and nutrient cycling in the MR.

Results are reported from experiments with elevated atmospheric ozone and stress imposed by the soil-born pathogen *Phytophthora citricola*. Model tree species were *Fagus sylvatica* L. and *Picea abies* (L.) Karst. As important MR functions related to nutrient cycling, extracellular enzyme activities (phosphatase, cellulase, chitinase) were studied on individual mycorrhizal tips and in mycorrhizosphere soil samples at different experimental scales from small seedlings to adult trees.

Enzyme activities in MR soil samples were strongly influenced by plant species and showed stress responses due to ozone and *P. citricola*, although the results were less clear in more complex systems. In addition, ECM species showed species specific enzyme activity patterns with a high redundancy of functions.

S3.2 - Mycorrhizas enhancing the capability of phytoremediation

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This paper deals with a comprehensive review of a series studies on the role of mycorrhiza in phytoremediation carried out by the Lab for Environmental Control and Bio-Technology in Peking University. Under stress of excessive heavy metal, Mycorrhizal association alleviates phytotoxicity by immobilizing the excessive heavy metal in soil and underground tissues. Sequestration of heavy metal was observed both in root tissues and mycelium. Furthermore, the physical and chemical characteristics of rhizospheral soil have been changed in terms of speciation of heavy metals that severely limited the bioavailability of excessive heavy metals and prevented it absorbed by plant. The glumaline, a protein improving aggregation of soil, was found to be exudated by mycorrhizal mycelia and implied that mycorrhizal fungal might also increase special component in root exudates to enhance the combination of heavy metal with soil aggregation. Incubated with DDT, ectomycorrhizal mycelium was found have capacity to decompose and even minimize DDT. Furthermore, mycorrhizal association, both ectomycorrhizal and arbuscular mycorrhizal enhanced the ratio to and extended the function area of DDT decomposition in rhizosphere. The key enzymes relevant to DDT composition, polyhydroxybenzene oxidase and laccase, were reserved that the activities of the enzymes induced by DDT.

S3.3 - Micro-XRF imaging using the SPring-8 synchrotron shows cadmium sequestration in intraradical structures of arbuscular mycorrhiza

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Cadmium (Cd) is a trace element in environment and to some extent organisms accumulate it in their tissues. Cd pollution in soils has been a food-safety matter in agriculture; on the other hand, hyperaccumulating plants of Cd have attracted considerable attention for decontamination of it from polluted soils. Mycorrhizal fungi colonizing root extend hyphae into soils, and participate in root functions, such as absorbing mineral nutrients. Mycorrhizal plants often show a higher metal tolerance compared to non-inoculated control plants. An objective of this study is to evaluate the role of arbuscular mycorrhizal fungi in Cd accumulation in the host plants. Resin embedded sections of mycorrhizal roots and the hyphae treated with Cd prepared with freezing techniques were examined with the SPring-8 synchrotron facility to localize Cd. When entire mycorrhizas were exposed, Cd was localized in cell walls of the fungi and the host plant and also in vacuoles of extraradical and intraradical hyphae, which was co-

localized with polyphosphate. A compartment experiment where only extraradical hyphae were exposed to Cd showed that in mycorrhizal roots the element was mainly localized in intraradical hyphae but not in plant cell wall. These results suggest that in mycorrhizal roots Cd originated from extraradical hyphal uptake is sequestered in intraradical fungal structures and contribution to the host Cd uptake is very low; these agreed with the result of a stable isotope-tracing experiment.

53.4 - Soluble protein and acid phosphatase exuded by ectomycorrhizal fungi and seedlings in response to excessive Cu and Cd

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Fungi and symbiosis can alleviate heavy metal stress by exudation soluble protein and enzyme. However the exudation mechanism of ectomycorrhizal fungi is less studied. The objective of this study was examining the role of soluble protein and acid phosphatase (AP-ase), which is related to P uptake, in *Xerocomus chrysenteron* and seedlings under excessive Cu and Cd. Growth types of fungi were assessed. The exudates from isolated fungi non-inoculated seedlings and inoculated seedlings were tested for soluble protein and AP-ase. All experiments were in Cu and Cd stresses both. The growth types of *X. chrysenteron* in Cu and Cd did not change, and it showed high tolerance to Cu and Cd. *X. chrysenteron* grew well at 80 mg•L⁻¹ Cu, and the EC₅₀ on Cd was 17.82 mg•L⁻¹. *X. chrysenteron* enhanced soluble protein exudation in both isolated and inoculated cultivation and alleviated the influence of Cu and Cd on exudation. However soluble protein exudate behaved different under Cu and Cd stress in isolates. In the medium with Cu, soluble protein exudates increased with concentration. In the medium with Cd, it increased to the comparative level at all concentrations. It demonstrated soluble protein was related to heavy metal tolerance, but different ions played different roles. AP-ase activity in exudates of fungi and seedlings decreased in both Cu and Cd stress compared to control, but in seedlings were maintained by inoculation. It showed *X. chrysenteron* helped plant to maintain the normal nutrient uptake to protect it from heavy metal toxicity.

Mixotrophic and Mycoheterotrophic Interactions

54.1 - The myco-heterotroph, *Arachnitis uniflora*, has a unique association with arbuscular mycorrhizal fungi

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Achlorophyllous plants dependent on an association with fungi linked to photosynthetic plants for their carbon source are known as myco-heterotrophs. *Arachnitis uniflora*, a monotypic member of the family Corsiaceae, fits this category relying on a glomalean fungus belonging to *Glomus* Group A for carbon acquisition. Previous observations showed the presence of intracellular hyphal coils, the development of branched structures with terminal swellings, and the occurrence of a zone in the inner root cortex in which degradation of fungal structures takes place. New information has been obtained using a variety of microscopical and cytochemical procedures. Arbuscules were never observed, the 'vesicles in bundles' reported earlier are unlike typical glomalean vesicles in that they form in clusters and have a complex multi-layered wall, the thick inner layer of which consists primarily of β -1,3 glucans and the thin outer layer of chitin. Each structure is surrounded by host cell wall material and a plasma membrane. Internally, a complex of small β -1,3 glucan-containing vacuoles, lipid bodies, endobacteria, and many nuclei are present. These structures enlarge to occupy most of the cortical cell volume and then degrade releasing lipids and other materials into the host cell. We suggest that these structures should not be equated with typical glomalean vesicles but are unique structures and perhaps, along with the hyphal coils, are involved in nutrient acquisition by the host.

54.2 - Seasonal changes and associating fungi of *Pyrola japonica* mycorrhizas growing under different light environment

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We monitored the mycorrhizal status of *Pyrola japonica* in an oak dominating forest at bimonthly from January to November. At samplings, three plants were collected at sun conditions, while other three were at shade conditions. Light microscopy of excised roots was conducted at distal, middle and proximal parts of root systems and the frequency of hyphal coils was calculated as mycorrhizal formations. We extracted DNAs from *P. japonica* roots and sequenced the fungal derived rDNA ITS region. *Pyrola japonica* roots possessed both hyphal coils and Hartig

nets, but no fungal mantles suggesting arbutoid mycorrhizas. Mycorrhizal formations were found at all plants and observed more frequently at distal positions than at middle or proximal positions. The contrasting response of the formation rates between light conditions was detected at the beginning of the coverage of tree crowns. For both the light conditions, the rate of mycorrhizal formations tended to increase at relatively darker environments. DNA phylotypes detected were mostly the same taxon as ectomycorrhizal groups and the *Russula* was the most frequently encountered. These results indicate that *P. japonica* might harbor abundant mycorrhizal fungi at the distal part of root systems and change mycorrhizal dependency responding to light conditions. Since the detected fungal groups are ectomycorrhizal, *P. japonica* may physically connect via extraradical mycelia with neighboring oak trees and thus can be a mixotrophic plant.

54.3 - Orchid mycorrhiza diversity in *Coppensia doniana*, a widespread Oncidiinae from Campos do Jordão, Brazil

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In nature, orchids are fully dependent on mycorrhizal fungi for germination. These fungi can penetrate root cells and form pelotons, which digestion provides simple sugars for embryo. During achlorophyllous seedling stage, orchids are obligatory dependent on the fungi, and some species remain so through life, while others become facultative responsive to fungal infection. The aim of this study was to identify how many fungal clades can establish mycorrhizal associations with *Coppensia doniana*, a widespread orchid from Campos do Jordão–Brazil, and to demonstrate how their morphological features, analyzed with multivariate statistics, can be useful for classification. Ten plants were sampled in a forest near Campos do Jordão. Fungi were isolated by transferring surface disinfected root segments containing pelotons to PDA culture medium. Three main fungal clades were formed by qualitative and quantitative morphological data. They were identified as two morphotypes of *Ceratohiza* (anamorphic stage of *Ceratobasidium*) and one uni-nucleated Rhizoctonia. The ITS sequencing corroborate it by showing the same three clades. All sequences were highly correlated to *Ceratobasidium* ITS data deposited at the Genbank, suggesting a high affinity between this *Oncidiinae* and *Ceratobasidium*. It also could be shown that morphological data associated with multivariate statistics can be a useful tool in fungal multi-level taxonomy.

Signal Perception and Metabolism

55.1 - *In situ* analysis of anastomosis in all genera of glomeromycotan fungi using a unique rhizohyphatron design.

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Vegetative compatibility in glomeromycotan fungi generally is studied from germinating spores or root explants. For the latter, only a limited number of species are culturable and artificial conditions might alter expression of critical genes. A "rhizohyphatron" was designed to optimize expression of vegetative compatibility genes and assess natural hyphal behavior originating from intact plants. It consists of four interconnected parts: (i) a compartment containing mycorrhizal plants (ii) a horizontal tube housing 1% agar-coated glass slides with attached coverslip, (iii) nylon mesh separating plant from slides, and (iv) a cap sealing the slide tube. Plants in the rhizohyphatron were grown for four weeks to allow a hyphal front to develop on the glass slides. At the half-way point, coverslips were collected and hyphal intersects counted. For each hyphal contact, interactions were categorized based on signs of compatibility or incompatibility. Vegetative self-compatibility was not detected in species of *Scutellospora* and *Gigaspora* or in *Archaeospora leptoticha*. Compatible hyphal fusions were observed in *Glomus clarum* and *G. intraradices* at mean frequencies of less than 20%. A broader range of fungal species is under analysis. The method developed here will expand knowledge of the biology and genetics of vegetative compatibility and further elucidate Glomeromycotan life history strategies.

55.2 - Root exudates and their effect on uptake and metabolic processes during presymbiotic growth of arbuscular mycorrhizal fungal spores

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Arbuscular mycorrhizal fungi are obligate symbionts that are not able to complete their life cycle without the association to a host plant. The reason for the obligate mycotrophy of these fungi, however, and the metabolic pathways that may be deficient in germinating spores are still unknown. We examined carbon and nitrogen uptake and metabolism and related gene expression during the presymbiotic growth of the arbuscular mycorrhizal fungus *Glomus intraradices* and analyzed whether the presence of a host root affects these metabolic processes in

germinating spores. Germinating spores are able to use internal and exogenous carbon and nitrogen sources and the results demonstrate that the ability to synthesize amino acids does not limit presymbiotic growth of arbuscular mycorrhizal fungal spores. Root exudates increase the uptake of exogenous carbon sources by germinating spores and stimulate the catabolic carbon and nitrogen metabolism in germinating spores. The regulation of these processes by root exudates enables the fungus to utilize internal stores more efficiently and prevents its carbon and nitrogen reserves from being compromised when no host roots are present. The results will be discussed in relation to the catabolic and anabolic metabolism of spores during the presymbiotic growth phase and the effects of plant signals on spore metabolism.

55.3 - Polyphosphate is responsible for the rapid and massive accumulation of phosphorus in arbuscular mycorrhizal fungi

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Evidence that AM fungi accumulate polyphosphate (polyP) in hyphae was first obtained more than three decades ago, but the significance in P-metabolism and -translocation in AM associations has yet to be elucidated. The cellular localization of polyP and enzyme activity involved in polyP-synthesis were investigated in relation to the dynamics of polyP. *Glomus* sp. HR1 was grown in the mesh bag culture system under P-deficient conditions using *Lotus japonicus* or *Tagetes patula* as host plants. Pi solution was applied to the hyphal compartment prior to harvest, and then extraradical hyphae were collected from the outside of the mesh bags. Total P and polyP levels in the hyphae increased synchronously after the Pi application, and the level of polyP reached 64% of total P 5 h after the Pi application. Strong signals of DAPI-polyP complex were observed in vacuoles 4 h after the Pi application. The hyphal cell obtained 4 h after the Pi application was fractionated by density gradient centrifugation, and the fraction with a density of 1.06-1.09 g mL⁻¹ showed polyP-synthesizing activity in the presence of ATP. The cellular capacity for polyP accumulation was much greater than those reported previously and comparable for those of polyP-hyperaccumulators. The nature of AM fungal cell that is occupied by numerous vacuoles might be responsible for such a great capacity for polyP accumulation. Polyphosphate-synthesizing activity was demonstrated for the first time in filamentous fungi.

55.4 - Importance of short molecular peptides to axenic culture of arbuscular mycorrhizal fungi

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Currently, alginate oligosaccharide (Ishii et al., 2000), 5'-deoxy-5'-methylamino-adenosine (Kuwada et al., 2006), ethylene (Ishii et al., 1996; Geil and Guinel, 2002), flavonoids (Carr et al., 1985; Gianinazzi-Pearson et al., 1989; Nair et al., 1991; Ishii et al., 1997), polyamines (El Ghachtouli et al., 1996; Ishii et al., 2000) and sesquiterpenes (Akiyama et al., 2005) have been known to influence markedly the growth of arbuscular mycorrhizal fungi (AMF). Alginate oligosaccharide and 5'-deoxy-5'-methylamino-adenosine are generally contained in some algae. We have developed commercial AMF growth stimulants made from a brown seaweed, *Laminaria japonica* Areschoug, and special liquid fertilizers containing the AMF growth stimulants. Although host plants naturally produce ethylene, flavonoids, polyamines and sesquiterpenes, these compounds have no effects on AMF spore formation. By using 25 % MeOH eluates of bahiagrass (*Paspalum notatum* Flügg.) roots fractionated by flash chromatography, however, we succeeded in producing new spores in the axenic culture of some kinds of AMF by red and blue lighting (Ishii et al., 2003). And very important peptides for the spore formation, Trp-Trp and Leu-Pro, were identified from the 25 % MeOH eluates. By using these peptides, we first developed the axenic culture of AMF without host roots or root exudates.

Mycorrhizal Fungal Structure in the Field

56.1 - Changes in AM fungi community structure in fragments of a tropical rain forest at Los Tuxtlas, Veracruz, Mexico

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Fragmented landscape is a common feature of tropical rain forest in Mexico, one of the main consequences of this fragmentation is changes in microenvironmental conditions, which explain losses of native species richness and invasion of exotic ones. As an outcome, modifications in aboveground community structure and ecosystem function occur. However, very few studies have taken into account belowground communities, even when their impacts on

nutrient cycling, maintenance of plant diversity, plant performance, etc., are well known. Arbuscular mycorrhiza is the most conspicuous mutualistic association in humid tropics. Nevertheless, we ignore if fragmentation has altered arbuscular mycorrhizal fungi (AMF) community structure, one hypothesis points out that small fragments will retain less species, altering fungi-plant relationships. Seven fragments of different size were chosen, and 12 compound soil samples were collected per patch. AMF spores were separated and counted. We found 28 species with a total of 3068 live spores. Mean estimated species richness and diversity were higher in small fragments, than large ones. No direct relationship between AMF species richness and plant diversity or species richness was found, but CCA multivariate analysis showed total nitrogen concentration, pH, soil texture, and plant species as the most important factors that explain AMF composition. These results can be related to stability and resilience of AMF community.

S6.2 - Depth differential colonization and population structure of mycorrhizal fungi in prairie grasses

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The colonization and community composition of AMF was studied at different soil depths in pure stands of the grasses crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), switchgrass (*Panicum virgatum* L.), green needlegrass (*Stipa viridula* Trin.) and western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Love), growing in southwest Saskatchewan. The pattern of root colonization at 3 to 15, 15 to 30 and 30 to 45 cm soil depths was studied in stained roots. The community composition of AMF was described in roots from 3 to 15 cm, and 30 to 45 cm depth using phylogenetic and molecular tools. Soil depth affected AMF colonization and community composition. Root colonization by two types of mycelia decreased with soil depth. Six AMF phylotypes of *Glomus* dominated the overall AMF community. Three AMF phylotypes were common colonizers and three were preferentially associated to some grasses. AMF communities at different depths differed from each other in all plant stands, and diversity and richness of AMF phylotypes was higher in shallow root samples, except in roots of *S. viridula* that showed higher richness of AMF at deeper depth. We conclude that although some AMF are general colonizers, some AMF show a strong host preference. Our results also suggest the existence of niche specialization in AMF along the soil profile; which is influenced by the host plant, and a strong effect of soil depth on AMF phylotype distribution and colonization.

S6.3 - Local adaptation in mycorrhizal symbioses maximizes nutrient uptake and plant fitness

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Our study tested two hypotheses: (1) Mycorrhizas improve plant fitness by increasing the uptake of either phosphorus or nitrogen, whichever nutrient is most limiting to plant growth; (2) AM fungi adapt to each other and to their local soil such that more beneficial mutualisms should occur in local (home) combinations of plants, fungi and soil than in non-local (away) combinations. Ecotypes of *Andropogon gerardii*, a dominant grass of Tallgrass Prairie, were obtained from three representative sites spanning much of the geographical range of this species. These sites included phosphorus-limited and nitrogen-limited soils. Ecotypes were grown with all possible home and away combinations of soils and AM fungal communities. Grasses grown in home soil and inoculated with home AM fungi produced more arbuscules and extraradical hyphae than those grown in away combinations. Extramatrical hyphae (EMH) and arbuscules were strongly positively associated with tissue nitrogen at Cedar Creek - the nitrogen limited site; but weakly correlated at Fermi or Konza - the phosphorus limited sites. In contrast, EMH and arbuscules were strongly related to tissue phosphorus at Fermi and Konza but not at Cedar Creek. These results indicate that AM fungi increase uptake of the nutrient that is most limiting in the soil. Further, we show that *Andropogon* ecotypes adapt to their local soil and to AM fungal communities such that mycorrhizal resource exchange of the most limiting resource is maximized.

S6.4 - Impact of wildfire frequency on the ectomycorrhizal resistant propagules communities in a Mediterranean open forest

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Ectomycorrhizal fungi (ECM), and in particular spore bank and other resistant propagules, play an important role in secondary succession processes facilitating regeneration following disturbance events. In this study, the effect of wildfire frequency on the resistant propagules communities (RPC) of a Mediterranean open pine forest was examined. Soil samples were collected in four mountain sites affected by different fire frequencies and used to test ectomycorrhiza development in two hosts, *Pinus pinaster* and *Quercus suber*. The RPC were characterized using PCR and DNA sequencing. A total of 18 ECM species were detected forming ectomycorrhizas in the bioassay. The most frequent fungi were *Cenococcum geophilum*, *Inocybe jacobii*, *Thelephora terrestris*, *Tomentella ellisii*,

Rhizopogon luteolus, and *Rhizopogon roseolus*. Only the first four fungal species (*C. geophilum*, *I. jacobi*, *T. terrestris*, and *T. ellisii*) were shared by both hosts. Fire occurrence affected the frequency, abundance, and species composition of RPC on bioassay seedlings, promoting in the high-frequency fire sites species like *R. roseolus*, and influencing negatively species like *R. luteolus*. Other species such as *I. jacobi* were instead positively affected by moderate-frequency fire sites but decreased significantly with recurrent fires. The effect of fire frequency on ectomycorrhiza formation differed between both hosts being more severe for *Q. suber* than for *P. pinaster*. Alterations induced by frequent fires on the structure and composition of the aboveground vegetation and on the associated belowground ECM community could compromise the resilience of the ecosystem and change the succession process towards a degradation cycle.

Mycorrhizas and Plant Nutrition

57.1 - Is C allocation to the fungus costly to the plant?

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The balance between C cost and nutrient gain is traditionally considered to determine if the plant benefits or not from mycorrhization. Benefit is most frequently evaluated through growth differences between M and NM plants. Although growth can be limited by factors other than C, namely nutrient availability, differences in growth between M and NM plants are consistently considered to be due to differences in belowground C expenditure, either directly, or due to differences in cost efficiency, i.e., amount of nutrients acquired per C expended. We analysed published works on ECM where at least one of the following was quantified: i) C allocation; ii) N gain and growth. In almost every reported case M plants allocated more C belowground than their NM controls. However, we found a similar number of cases of negative, null or positive effects of ECM on growth. C allocation and growth were therefore not correlated. In contrast, a very good correlation was found between N gain and plant growth. This leads to the question: is C allocation to the fungus in fact costly to the plant? We propose that under nutrient limited conditions C is an excess, rather than a costly, resource. ECM may always be more C expensive than NM roots, but this has no consequence in terms of growth. The outcome of the symbiosis depends only on whether mycorrhizae result in increased or decreased nutrient acquisition when compared with NM plants, and not on cost efficiency differences between M and NM plants.

57.2 - First evidence of mycorrhizal-mediated phosphorus transfer in a liverwort

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For over 30 years it has been hypothesised that the colonisation of land by plants some 450 million years ago was facilitated by an ancient symbiosis with arbuscular mycorrhizal fungi. However, this important hypothesis remains untested and so lacks experimental evidence supporting the notion that the relationship between 'lower' land plants and their fungal endophyte is mutualistic and analogous in function to mycorrhizal associations of higher plants. Here, we present the first experimental evidence of mycorrhizal-mediated nutrient transfer in a liverwort, an extant basal lineage of embryophytes representative of the earliest terrestrial plants. Molecular analysis identified that the endophyte of *Marchantia paleacea*, a thalloid liverwort, as a member of *Glomus* group A. Infected and non-infected controls of *M. paleacea* were planted in specially designed cylinders with a compartment containing ³³P labelled orthophosphate accessible exclusively to fungal hyphae. We report data from a series of experiments using this system that demonstrates ³³P transfer to liverwort thalli via the fungal symbiont. We will report further experimental results deploying bi-directional carbon isotope experiments as a novel quantitative cost/benefit approach to understanding the nature of the mycorrhizal symbiotic relationship.

57.3 - Tit for Tat? *G. intraradices* accumulates P under low plant C availability - C-P trade revealed by PIXE and isotope probing

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We investigated the effects of nutrient availability on elemental composition of *G. intraradices* in compartmented root organ cultures, using particle induced X-ray emission (PIXE). PIXE is a powerful tool to investigate the effects of different nutrient regimes of delicate tissue as AM fungal hyphae and spores without the need of sectioning. It does not only disclose concentrations of the different elements but also their spatial distribution down to a scale of 5µm.

In different studies, we either changed nutrient availability in the fungal compartment, or changed carbon (C) availability in the root compartment. A reduction of 50% C availability to the roots led to a strong (7- to 9- fold) accumulation of P and also other nutrients in the fungus. A 100-fold increase of P availability to the fungal mycelium also led to an accumulation of P and other nutrients in the fungus, but less pronounced than by the change of roots C status.

In an isotope probing experiment on a model plant population of root organ cultures connected with a common mycelium network we wanted to investigate the actual transfer of C and P between the symbionts. The mycelium associated with low C status plants received a disproportionately high contingent of C from other plants. We could confirm that there is little P transfer to those plants with low C status. We suggest the existence of strong fungal control mechanisms for P transfer in the C-P exchange between the fungal and plant symbiont.

57.4 - Nitrogen uptake by mycorrhizal roots: a space-related cost-benefit approach on mature trees.

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Below-ground competitiveness of the two important European tree species *Fagus sylvatica* (beech) and *Picea abies* (spruce) was quantified in a 60 year old mixed stand by determining space-related cost-benefit ratios. In particular, the uptake of nitrogen was investigated per root biomass, per associated ectomycorrhiza (EM), and per occupied space. Fine roots were enclosed in rhizotrons (32x22.5x1 cm) during the growing season while still attached to the trees. After four months, a $^{15}\text{NH}_4^{15}\text{NO}_3^-$ solution was applied via micro-suction cups (4 d). The ^{15}N was followed in root fractions, EM and soil at different distances from the application points. Space occupation by roots and mycorrhizae (mycelia excluded) was up to 12 % of surface area while mycelia occupied up to 83 % depending strongly on the exploration type of EM (according to Agerer 2001). ^{15}N enrichment was highest in EM and root fractions within a radius of 5 cm around the application area. In contrast, recovery of the ^{15}N -label was 10 ± 3 % SE (beech) and 16 ± 5 % (spruce) in fine roots, and 2 ± 1 % (beech) and 3 ± 1 % (spruce) in EM, respectively, while most of the label remained in the soil or was transferred to coarse roots. In spruce, root N uptake correlated with root biomass, whereas in beech, root N uptake seemed to be more related to EM biomass and both, EM and root biomass of beech were strongly positive correlated. These findings suggest different strategies of N acquisition between the two tree species associated with EM.

Inoculum Technology Application and Management of Mycorrhizal Fungi

58.1 - Ectomycorrhizal inoculations of seedlings: A biological approach for successful reclamation of the Canadian oil sands disturbed lands

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Surface mining of Canadian oil sand produces massive areas of disturbed lands. Reclamation of these lands is challenging because of the prevailing harsh conditions in areas affected by oil sands byproducts. Mining activity can affect soil nutrients, pH, toxicity, bulk density, biological activity, and soil moisture. Although several amending materials have been used to amend these disturbed lands, low levels of mycorrhizal fungi in these reconstructed soils and its saline-sodic nature frequently delayed in successful reclamation of the land. Mycorrhizal fungi can play a critical role in plant nutrient uptake, water relations, buffering environmental stresses, ecosystem establishment and plant productivity. Jack pine (*Pinus banksiana* Lamb.) and white spruce (*Picea glauca* (Moench) Voss) seedlings were pre-inoculated with ectomycorrhizal (ECM) fungal species and outplanted in Alberta oil sands reclamation sites to evaluate the field performances. After four growing seasons, inoculation of both white spruce and jack pine seedlings demonstrate positive responses compared to non-inoculated control seedlings when outplanted on two different reclaimed sites. On reclaimed cell 46 site, ECM inoculated seedlings outperformed control seedlings by enhancing plot volume index (PVI) at 58.5% for jack pine and 124% for white spruce. Similarly, the PVI of inoculated seedlings significantly increased by 56% for jack pine and 101% for white spruce compared to control seedlings at reclaimed W2 site. ECM inoculation significantly enhanced nutrient uptake of seedlings planted on the both reclaimed sites. The nitrogen content (mg N per seedling) had significantly increased by 64% (white spruce), 117% (jack pine) on cell 46, and 93% (white spruce), 89% (jack pine) on W2 site. Our study demonstrates the importance of nursery inoculation with selected ECM fungi that may support early growth and establishment, and improved nutrient uptake of seedlings in the reclaimed sites. The use of mycorrhizal biotechnology for improved reclamation of oil sands disturbed lands appears promising.

58.2 - The Triple-W model for a novel approach enhancing application of mycorrhizal fungi

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Awareness on mycorrhizal fungi has significantly increased over the last decades. As a consequence interest in the

application of mycorrhizal fungi has grown and commercial production of mycorrhizal products is increasing rapidly. This has resulted in availability of mycorrhizal fungal inocula on the market. Still, application of the mycorrhizal products can be increased as the EU-funded network COST Action 870 is aiming for. Application of mycorrhizal fungi takes generally place in human influenced environmental systems. These comprise urban and agricultural systems including horticultural and forest systems. The urban systems and large parts of the agricultural systems, particularly those in the western world, usually have highly disturbed and nutrient-enriched soils. The remaining parts of agricultural systems are located at soils with low availability of plant nutrients. Considering enhancing application of mycorrhizal fungi in the human influenced environmental systems, a fit-to-purpose approach is needed. Therefore, we have developed a model, the Triple-W-model, for affective application of mycorrhizal fungi. The model focuses on the added value in cash flows by the use of mycorrhizal fungal products tailored to meet the needs of specific species of plants, trees and crops, soil environments and management regimes. The costs of the mycorrhizal products are related to the economic benefits for the users in the different human-influenced environmental systems. Also, the Triple-W-model makes the economic benefits of application of mycorrhizal fungi clear for various ecosystem services such as water retention and carbon uptake. We will demonstrate by examples that application of the Triple-W-model enhances affective application of mycorrhizal fungi for plants, trees, and crops, and increases economic benefits of various ecosystem services.

58.3 - News, "déjà vus" and challenges of mycorrhiza production, application and marketing

Miroslav Vosatka, Jana Albrechtova

There has been a considerable boom of mycorrhizal inoculum producers since the last ICOM 5 and consequently development of international marketing in the field of mycorrhizal industry. There are though long lasting problems of inoculum producers such as lack of certainty if to regard the mycorrhizal products to be biofertilizers, plant/soil helping agents or biopesticides. There is still lack of suitable techniques for quality assurance and field tracing in particular for arbuscular mycorrhiza. There is insufficient pool of field data related to mycorrhiza cost and benefits in plant production. However, there are few new challenges in recently acquired scientific knowledge, which could potentially become important unique selling points for commercial applications of mycorrhizal inocula. Among these are true capability of mycorrhizal fungi to sequester C in soil, to affect essential oil production of pharmaceutical and biofuel plants and potential effects of mycorrhiza on production of antioxidants and other desired compounds in food crop. These challenges can only be fulfilled in close collaboration between science and industry, though both sometimes speaking different languages. The examples are given of successful large scale applications of mycorrhiza in different segments of plant production. Needs, potentials and reality of International or European bodies for quality certification of mycorrhizal products will be outlined. Funded by project 1M0571 (Ministry of Education of the Czech Republic).

58.4 - Parlor palm response to arbuscular mycorrhizal fungus (AMF) inoculation in a commercial nursery scale.

Trejo, D, Lara, L, Reyes, G. M.J., Rojas, Z, M., Díaz, M. E., Zulueta, R. R y Moreira, A, C. (Universidad Veracruzana)

Effect of arbuscular mycorrhizal fungus (AMF) in two grower-demonstration plots was assessed in collaboration with parlor palm farmers at commercial nursery scale by comparing traditional technology and addition of AMF. Four treatments were carried on by using a randomized complete block design: fumigated soil and non-fumigated soil plus AMF (SFCM and SNFCM), and, fumigated soil and non-fumigated soil plus grower technology (SFTP and SNFTP). Parlor palm seeds were inoculated with AMF and 210 days after inoculation significant differences were found among treatments in plant height, number of leaves and leaf diameter. Percentage of colonization, biomass and number of spores were also measured. SFCM resulted in the best treatment for both plots showing that inoculation can fit well in native soils. It was observed that adding AMF shortens the stay of parlor palms in the nursery up to 150 days reducing costs on one hand, and achieving more vigorous plants that performs better when transplanting to the field on the other.

Genome Structure, Plasticity and Evolution

59.1 - Expansion of signal pathways in *Laccaria bicolor* – evolution of protein kinase and RAS small GTPase gene families

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The ectomycorrhizal fungus *Laccaria bicolor* has the largest genome of all fungi yet sequenced. The large genome size is partly due to an expansion of gene family sizes. Among the largest gene families are protein kinases and RAS small GTPases, which are key components of signal transduction pathways. Comparative genomics and phylogenetic

analyses were used to examine the evolution of the two largest families of protein kinases and RAS small GTPases in *L. bicolor*. Expression levels in various tissues and growth conditions were inferred from microarray data. The two families had a large number of young duplicates (paralogs) that have arisen in the *Laccaria* lineage following the separation from the saprophyte *Coprinopsis cinerea*. The protein kinase paralogs were dispersed in many, small clades and a majority of them were pseudogenes. In contrast, the RAS paralogs were found in three large groups of RAS1-, RAS2- and RHO1-like GTPases with few pseudogenes. Duplicates of protein kinases and RAS small GTPase have either retained, gained or lost motifs found in the coding regions of their ancestors. Frequent outcomes during evolution are the formation of pseudogenes (nonfunctionalization) or proteins with novel structures and expression patterns (neofunctionalization).

S9.2 - Alteration of AMF genotypes and phenotypes caused by segregation and by change in host species

Angelard, C. (Lausanne University), Croll, D. (Lausanne University), Sanders, I.R. (Lausanne University)

Arbuscular mycorrhizal fungi (AMF) are eukaryotic fungi, known to harbour genetically different nuclei. We have recently found that single spores of AMF do not necessarily inherit the same genetic material due to unequal segregation of nuclei during spore formation. Moreover, this genetic drift results in genetic and phenotypic divergence between single spore lines. The aim of this study was to determine whether following segregation, AMF lines respond differently to a change of host. We produced segregated lines of AMF from single spores of "parental lines" and we cultivated these lines on different host species. After several generations, we compared the phenotypes and genotypes of segregated and parental lines. We show that segregation in AMF results in different phenotypes and genotypes on different hosts. Interestingly, we observed strong interactions between the AMF lines and host species. The effects of this type of segregation could have important consequences for the symbiosis and for the co-evolution of the plants and AMF.

S9.3 - Diversity and adaptation of metabolism in *Laccaria bicolor*

Dag Åhrén, Okawa, S. (Microbial Ecology, Department of Ecology, Lund University, Sweden), Åhrén, D. (Microbial Ecology, Department of Ecology, Lund University, Sweden)

Ectomycorrhizal fungi have adapted to an environment where the plant is providing easily metabolized carbon whereas non-symbiotic fungi degrade complex carbon sources, such as dead organic matter. By comparing symbiotic, parasitic and non-symbiotic fungi, genetic differences relating to adaptation in the metabolic network, i.e. gene loss, gene gain or differential gene expression, can be detected. In this study, the metabolic architecture of *Laccaria bicolor* was compared to six other basidiomycete fungi whose complete genomes are now available, *Coprinopsis cinerea*, *Cryptococcus neoformans* var. *grubii*, *Malassezia globosa*, *Phanerochaete chrysosporium*, *Puccinia graminis* sp. *tritici* and *Ustilago maydis*. The fungi with a plant host, *L. bicolor* and *U. maydis* contained the most complete setup of metabolic pathways, despite large differences in both genome size and phylogenetic distance. In all the studied basidiomycetes, pathways involved in catabolism are more evolutionarily conserved than anabolic pathways. The impact of expansion of protein families involved in carbon and nitrogen metabolism is discussed.

S9.4 - Mitochondrial large ribosomal subunit gene evolution in the *Glomus* group A lineage of arbuscular mycorrhizal fungi

Thiéry, O. , Boerstler, B. , Redecker, D. (Institute of Botany, University of Basel, Hebelstrasse 1, CH-4056 Basel, Switzerland)

The only molecular markers available covering all families of arbuscular mycorrhizal fungi (AMF) are the nuclear-encoded ribosomal genes. However, these genes show numerous variants within a single fungal isolate. The mitochondrial large subunit rDNA (mtLSU) has been shown to be homogeneous within isolates of *Glomus*, but the sequence data were limited to *G. intraradices* and *G. proliferum*. By using previously-designed and new specific primers, partial mtLSU was amplified and sequenced from five other species of *Glomus* group A, a *Glomus* group B member and two species of *Scutellospora*. Although clearly showing a phylogenetic relationship, the mtLSU sequences of *S. verrucosa* and *S. castanea* were distinct from *Glomus* in their exon sequence and did not contain introns. Sequence variation among the *Glomus* group A clade was mainly limited to presence/absence of introns. No sequence heterogeneity could be detected among four isolates of *G. mosseae* and two isolates of *G. clarum*. All *Glomus* group A members contained some homologous introns. In *G. mosseae*, *G. coronatum* and *G. caledonium* additional introns were found, some of them containing an ORF for LAGLIDADG homing endonucleases. Interestingly, *G. caledonium* possessed two copies of the conserved amino acid motif LAGLIDADG in the putative protein, which may have resulted from gene duplication. This could be an adaptive response of endonucleases to invade more divergent target sites from other species in the fungal lineage.

Mycorrhizal Fungal Structure in the Field

S6.5 - Impact of long-term manure and inorganic nitrogen fertilization on the community composition of arbuscular mycorrhizal fungi using PCR-DGGE analyses

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The long-term effect of manure or mineral NH₄ fertilizer application on the arbuscular mycorrhizal (AM) fungal community was analysed. Soil and grass roots were harvested from a forage field experiment treated for 12 yrs with equivalent doses of (i) NH₄ or (ii) dairy manure slurry or (iii) no fertilization. Sunflower plants were grown in unfumigated soil + grass roots (high level of native AM inoculum), fumigated soil + grass roots (low inoculum level), or fumigated soil + sterilized roots (AM control). Four months after sowing, roots and rhizosphere soil were separately harvested and the total genomic DNA was directly extracted from samples and subjected to PCR-DGGE and sequencing approaches targeting an 18S rRNA gene fragment. Seven AM fungal taxa were detected within roots and in soils and were identified as *Glomus intraradices*, *G. sinuosum*, *G. mosseae*, three taxa of uncultured *Glomus* sp., and *Acaulospora scrobiculata*. Under high inoculum levels, the percentage of root length bearing mycorrhizal colonization was significantly higher in plants grown in soil from NH₄-treated plots than in those with manure or no fertilization treatments. However, under low inoculum levels, root colonization was significantly lower in the NH₄-treated soil compared with other treatments. Total plant dry weight was significantly higher on manure-treated soil compared to NH₄ and no fertilization treatments. In addition, plant dry weight was significantly higher under low-inoculum manure than under high-inoculum manure. However, there was no significant difference in the total plant dry weight under low-inoculum manure and non-inoculated manure control treatments. Similarly, the low-inoculum or non-inoculated treatments caused a significant increase, compared with the high-inoculum treatment, to the total dry weight of plants grown on the NH₄ or non-fertilized soils. We found that *G. sinuosum*, *G. mosseae*, and uncultured *Glomus* sp. #1 and #2 were the most frequent taxa in roots under high inoculum levels. Uncultured *Glomus* sp. #3 was the most abundant taxon under low inoculum levels in the roots of plants grown on NH₄-fertilized and control treatments, while *G. intraradices* was most frequently found under low inoculum levels in the roots of plants grown in manure-treated soil. *G. mosseae* was frequently found in NH₄-fertilized soil, while *G. intraradices*, *G. sinuosum*, and uncultured *Glomus* sp. #1 were abundant in manure-fertilized soil. Our results showed that the manure-treated soil produced the highest increase in total plant dry weight under low inoculum levels or without inoculation, and that *G. intraradices* was the most frequently detected AM fungal taxon under these conditions.

S6.6 - Molecular diversity in the genus *Tuber* indicates the correlation with the postglacial migration routes of their symbiont trees in W and E Europe

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The area of SE Europe has only recently been more extensively explored for the presence and diversity of hypogeous fungi and substantial collection of hypogeous fungi from the genus *Tuber* were obtained. We attempted to characterize collections at the molecular level and compare the obtained genotypes in correlation to postglacial migration routes of their symbiont trees. The ML phylogenetic tree applied in the phylogeography approach showed a differentiation within the following species: *T. excavatum*, *T. fulgens*, *T. aestivum*, *T. brumale*, and *T. rufum*. We managed to identify clear correlation of sporocarp origin with Apennine, Balkan or Pyrenean last glaciation pools and subsequent postglacial migration routes for *T. excavatum*, *T. fulgens*, *T. aestivum* and *T. brumale*. The intensively studied area was a large part of Slovenia, which turned out to be an interesting crossing or meeting point of different genotypes of several different *Tuber* species. In these both post-glacial migration routes, from the south of Apennine peninsula and from the Balkan peninsula, meet and mix. The mixture of the two vegetation migration routes was not clearly proven by previous papers, based on the diversity of the studied ectomycorrhizal plants, but is clear if correlated to the ectomycorrhizal partner diversity.

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S6.7 - Diversity of mitochondrial large subunit rDNA (mtLSU) haplotypes of *Glomus intraradices* in different environments

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Glomus intraradices is one of the most widespread species of arbuscular mycorrhizal fungi (AMF). It has been detected in most studies of AMF diversity in field sites. However, the possible presence of specialized ecotypes of *G. intraradices* in different environments could not be studied so far as no marker was available allowing the detection of intraspecies groups within plant roots. Recently, sequence types (haplotypes) were distinguished in isolates of *G. intraradices*, using a nested PCR approach for the mitochondrial rRNA large subunit gene (mtLSU). Based on these results, a restriction fragment length polymorphism (RFLP) approach for mtLSU PCR products was developed as easy-to-use genotyping system for field studies. This molecular tool was applied in two agricultural field sites and two semi-natural grasslands. Each field site was dominated by one or two frequently found RFLP types of *G. intraradices*. RFLP types differed strongly between the agricultural sites and semi-natural grasslands and between the two agricultural sites. Phylogenetic analyses of completely sequenced representatives of each RFLP type separated the respective haplotypes from semi-natural and agricultural sites. Diagnostic mtLSU regions from the RFLP types were further characterized by sequencing. This is the first study providing insights into populations of AMF species in field-collected roots.

56.8 - Phylogenetic analyses of the Glomeromycota with species level resolution, based on a 3.3 kb fragment in the rDNA region

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AM fungi are associated with 70-90% of all vascular land plants. Their ecological and economical importance is obvious, by this number. On the contrary, to their crucial role in nearly all terrestrial ecosystems, they are yet poorly understood in respect of biodiversity and functional traits. The currently 214 described AMF species are only a small proportion of the AMF community present in the field, and appropriate methods were missing for community analyses. Using our new, AMF specific primer set (Krüger et al. 2009) we can resolve AM fungi down to the species level in sequence based community analyses. These primers amplify partially the small subunit (SSU), the whole internal transcribed spacer (ITS) region and a large part of the large subunit (LSU) rDNA (1.8 kb). In addition, we amplify a 1.8 kb fragment of the SSU rDNA region for sub-genus level resolution. Combining these two 1.8 kb fragments, which overlap in the SSU (approx. 250 bp) we can use a 3.3 kb fragment including SSU, ITS and LSU rDNA, for phylogenetic analyses. With these three rDNA marker reliable phylogenies can be inferred. This is the baseline for AMF community analyses on species level based on regions in the LSU or ITS region rDNA and a reliable picture of the AMF biodiversity becomes feasible.

Mycorrhizosphere Interactions

S10.1 - Bacteria living inside and/or on surface of *Gigaspora margarita* spores and their effects on soil-borne plant diseases and P solubilization

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This research was aimed to isolate some bacteria species (endobacteria) from *Gigaspora margarita* spores by osmotic techniques and a thin needle. For the endobacteria, around 500 spores were surface-sterilized, and then their internal cytosol was extracted using a 2000xg centrifugation. This modulation is associated to changes in phytohormone levels and transcriptional reprogramming. As a consequence of these changes, a mild, but effective activation of the plant immune responses may occur. This activation leads to a primed state of the plant that allows a more efficient activation of defence mechanisms in response to the attack by potential enemies¹.

Jasmonates (JA) has been proposed as key regulators in the induction of resistance by beneficial microorganisms^{2,3}. We have analyzed the role of JA in Mycorrhiza Induced Resistance (MIR) using the pathosystem tomato-Botrytis cinerea, causal agent of gray mold on leaves. Disease severity and pathogen proliferation was significantly reduced in *Glomus mosseae* colonized plants, confirming the induction of MIR. Remarkably, the expression of JA-regulated defense genes in response to Botrytis infection is higher in mycorrhizal plants. Additionally, mycorrhizal plants display a potentiated response to exogenous application of JA. All together, our results support that MIR is associated to priming of JA-dependent responses.

1. Pozo & Azcón-Aguilar Curr Opin Plant Biol 2007
2. Pozo, Van Loon & Pieterse, J Plant Growth Regul 2004
3. Pozo et al. New Phytol 2008

S10.2 - *Kalaharituber pfeilii* and associated microbial interactions

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 Adeleke, R.A. (Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, South Africa)

Investigating the host plants of *Kalaharituber pfeilii* through mycorrhizal synthesis experiments have been unsuccessful to date. In order to gain an understanding of the biology of this fungus, bacterial isolates associated with the fungal fruiting body were investigated. A total of 17 bacterial isolates were obtained from the fruiting bodies of *K. pfeilii* and these were examined for their various functional activities. In order to determine whether the bacterial isolates influenced mycelial growth of *K. pfeilii* *in vitro* dual cultures were conducted. Isolates were also tested for other characteristics such as indole production and phosphate solubilising capabilities. Three bacterial isolates showed potential to stimulate mycelial growth, four isolates produced indole and one was able to solubilise CaHPO₄ *in vitro*. DNA was extracted from bacterial isolates showing potential interactions using a phenol/chloroform method. The 16S rDNA region was amplified using GM5F and R907 primers and PCR products were aligned, sequenced and submitted to GenBank for identification. The most promising bacterial isolate was identified as a *Paenibacillus* species that stimulated mycelial growth. Other bacterial isolates that showed potential interactions were identified as a *Bacillus* sp. and two different strains of *Rhizobium tropici*. This study has contributed to the existing knowledge about the biology of *Kalaharituber pfeilii*, which may be useful in further investigations.

S10.3 - Biotic weathering: influences of plants and ectomycorrhizas

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Ectomycorrhizal (EM) fungi, living in symbiosis with tree roots release organic acids and chelators into the soil solution. These weathering agents are most concentrated around hyphal tips and act on minerals to mobilise nutrients which they exchange with their photosynthetic hosts for fixed carbon. We set up a microcosm experiment with mycorrhizal pine and birch seedlings to investigate whether an EM fungus alters its growth and carbon allocation to selectively weather different minerals and rocks. The microcosms were designed so that the EM fungus, but not the roots, could colonize minerals under sterile, controlled environment conditions. EM fungal colonization of apatite, basalt and quartz were compared. The seedlings were supplied with ¹⁴C₂O₂ and organic carbon flux from the plant to fungus and into patches of the different rocks and minerals were measured. Plant-to-fungus carbon flux and fungal biomass were significantly elevated in patches of basalt grains, followed by apatite, with the least C allocation to quartz. EM fungus intensively, but selectively, colonized mineral grains of the same particle size, with preferential colonisation of basalt, which may provide a range of essential elements for the fungus and its plant partner. The specific targeting of basalt grains by EM has implications for climate modelling since weathering of basalt and other Ca rich silicates results in significant drawdown of atmospheric CO₂, as a component of the geochemical C cycle.

S10.4 - What determines the abundance of AMF and other soil microbes under different land use and management in a tropical dry ecosystem?

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Conservation and sustainable management in tropical dry regions requires thorough knowledge of the most critical factors affecting the distribution and abundance of soil organisms. We investigated the effects of land use, seasonality, soil aggregation and soil fertility on the abundance of arbuscular mycorrhizal fungi (AMF) in 1) primary vegetation, 2) pastures originated by slash-and-burn conversion, and 3) secondary vegetation, in the Chamela region of the Pacific Coast of Mexico. Fatty acid biomarkers for AM fungi, some microbial groups (bacteria, actinomycetes, saprotrophic fungi) and microbial ratios such as fungi/bacteria, AMF/saprotrophic fungi, were measured in order to understand if AMF are influenced by the same factors as other rhizospheric microorganisms. We found that seasonality was an important factor for fungal abundance and land use for bacterial abundance. Soil-aggregate fraction was relevant only for fungal abundance, but more so for saprotrophic fungi than for AMF. Soil fertility influenced only AMF and, as expected, AMF were less abundant in the more fertile sites. Altogether the results suggest that bacterial and fungal abundance are influenced by different factors and that within fungi, saprotrophic and AM fungi are also quite different. Saprotrophic fungi seem more responsive than AMF and bacteria to the strong seasonality and to land use in this tropical dry ecosystem. Implications for sustainable management are discussed.

Poster Session

Cell Biology and Development of Micorrhizas

P1.1 - Efficient gene silencing in the ectomycorrhizal fungus *Laccaria bicolor* by ihpRNA-triggering via *Agrobacterium*-mediated transformation

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The pSILBA silencing vector was constructed for efficient RNA silencing triggering in the ectomycorrhizal fungus *Laccaria bicolor*. This plasmid carries the *Agaricus bisporus* gpdII-promoter, two multiple cloning sites separated by a *L. bicolor* nitrate reductase intron and the *Aspergillus nidulans* trpC terminator. pSILBA allows an easy cloning of hairpin sequences for expression in basidiomycetes. By a further cloning step into pHg (pCAMBIA1300 based binary vector) carrying a hygromycin resistance cassette, the pHg/pSILBA plasmid is compatible with *Agrobacterium*-mediated transformation. The pHg/pSILBA -system results mostly in single integrations of RNA silencing triggering T-DNAs in *Laccaria* genome and these integration sites can be resolved by plasmid rescue. The pSILBA construct and two other pSILBA plasmid variants (pSILBA and pSILBAa) were evaluated for their capacity to silence *Laccaria* nitrate reductase gene. While all pSILBA-variants tested resulted in 65-76 % of transformants with reduced growth on nitrate, pSILBA produced the highest number (65 %) of strongly affected transgenic strains. The strongly silenced phenotype was proved to correlate with T-DNA integration in transcriptionally active genomic sites. pHg/pSILBA was shown to produce T-DNAs with a minimum CpG methylation in transgene promoter regions which assures the maximum silencing trigger production. CpG methylation of the target endogene was minimal in RNA silenced strains carrying an intronic spacer hairpin sequence construct. The silencing capacity of the pHg/pSILBA was further tested with *Laccaria* inositol-1,4,5-triphosphate 5-phosphatase gene. Besides its use in silencing triggering, the herein described plasmid system can also be used for transgene expression in *Laccaria*. Even though the pHg/pSILBA silencing system is optimized for *L. bicolor* it should also result highly useful in other homobasidiomycetes due to the wide recognition of the heterologous *A. bisporus* gpdII promoter.

P1.2 -Identification of differentially expressed genes during the pre-symbiotic interaction between *Hydnangium* sp. and *Eucalyptus grandis*

Irene da Silva Coelho, Queiroz, M.V.(Universidade Federal de Viçosa), Costa, M.D.(Universidade Federal de Viçosa), Kasuya, M.C.M.(Universidade Federal de Viçosa), Araújo, E.F.(Universidade Federal de Viçosa)

The pre-symbiotic stage, which precedes the physical contact between the symbionts, is crucial to determine the compatibility between ectomycorrhizal partners. A suppressive subtractive cDNA library of the fungus *Hydnangium* sp. during the pre-symbiotic stage was constructed using the fungal mycelium obtained by an *in vitro* mycorrhization technique. A total of 274 cDNAs fragments were characterized and 131 *expressed sequence tags* (ESTs) were compared with sequences deposited in the NCBI database. Among the genes differentially expressed in the pre-symbiotic stage, genes that encode proteins related to carbohydrate and energy metabolism, transcription and synthesis of protein, cell communication, and response to stress were identified. Thirty-six percent of the amino acid sequences showed similarity to proteins of *Laccaria bicolor*, a fungus also within Hydnangiaceae. The expression of genes that encode acetyl-CoA acetyltransferase, ATP synthase, pyruvate dehydrogenase, a hydrophobin, and a voltage-dependent ion-selective channel were also present, confirming the activation of these genes at the pre-symbiotic stage. The identification of genes through suppressive subtractive hybridization of *Hydnangium* sp. will allow the study of different genes related to mycorrhization, facilitating the understanding of the molecular mechanisms involved in the ectomycorrhizal association.

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P1.3 - The ectomycorrhizal morphotype *Pinirhiza sclerotia* is formed by *Acephala macrosclerotiorum* sp. nov. ✓

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Relatively few ectomycorrhizal fungal species are known to form sclerotia. Usually sclerotia are initiated at the extra-radical mycelium. Here, we present anatomical evidence for the formation of sclerotia directly in the hyphal mantle of the mycorrhizal morphotype *Pinirhiza sclerotia*. A dark-pigmented fungal strain was isolated from *Pinirhiza sclerotia* and identified by molecular tools as *Acephala macrosclerotiorum* sp. nov., a close relative of *Phialocephala fortinii* s.l. *Resyntheses* with *Pinus sylvestris* and *A. macrosclerotiorum* were performed under axenic

conditions. *A. macrosclerotiorum* formed true ectomycorrhizas that were anatomically identical with ectomycorrhizas collected from the field. Mycorrhizas were characterized by a thin hyphal mantle that bore sclerotia in a later ontogenetical state. Hartig net was well developed and grew up to the endodermis. To our knowledge, this is the first evidence at the anatomical level that a close relative of *Phialocephala fortinii* s.l. forms true ectomycorrhizas with a coniferous host.

P1.4 - Cellular organization in germ tube tips of *Gigaspora*.

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The fine structure of fungal hyphal tips often is phylogenetically informative. In particular, morphology of the Spitzenkörper varies among higher taxa. To date, no one has thoroughly characterized the hyphal tips of members of the phylum Glomeromycota. This is partly due to difficulty growing and manipulating living hyphae of these obligate symbionts. We observed growing germ tubes of *Gigaspora gigantea*, *G. margarita*, and *G. rosea*, using a combination of light microscopy (LM) and transmission electron microscopy (TEM). We used a variety of optics for LM (phase contrast, differential interference contrast, and confocal laser scanning), using both transmitted and fluorescent light with the lipophilic dye FM4-64. For TEM, we used both chemical fixation and freeze-substitution. Spores were surface disinfested and germinated on cellophane sheets overlaying water agar prior to processing and viewing. We also germinated spores in agar in inverted cover glass culture chambers. To date, we have not detected evidence of an organized Spitzenkörper, either using LM or TEM techniques. Germ tubes of all species were extremely sensitive to manipulation. Healthy germ tubes often showed rapid bidirectional cytoplasmic streaming, whereas germ tubes that had been disturbed showed reduced or no cytoplasmic movement. Actively growing germ tubes contain a cluster of 10-20 large (0.5 μ m to 1.0 μ m dia.) spherical bodies approximately 3-8 μ m behind the apex. The bodies, which appear to be lipid bodies, move rapidly in healthy germ tubes. These bodies disappear immediately after any cellular perturbation. Freeze-substituted cells showed superior preservation of the two-layered cell wall when compared to cells that had been chemically fixed. Freeze-substitution also revealed at least three distinct types of putative vesicles near the tip. Our work emphasizes the ephemeral nature of cellular organization, and the need for as little manipulation as possible to observe germ tube structure accurately.

P1.5 - Role of a transcription factor, GintSTE, from *Glomus intraradices* in the establishment of arbuscular mycorrhiza

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Arbuscular mycorrhizal fungi (Glomeromycota) are ancient obligate symbionts which cannot be transformed in a stable manner. Thus the genetic programme involved in their mutualistic interaction with plant roots remains obscure. STE12-like proteins are homeodomain-containing fungal transcription factors (TF) that regulate different morphological processes, including invasive growth of yeast and infectivity of hemibiotrophic plant pathogens. We have identified the first STE12 homologue, GintSTE, from an AM fungus (*Glomus intraradices*). Similar to other STE12 from filamentous fungi, it possesses a double zinc finger in its carboxy terminal part. Expression of GintSTE is induced in extraradical fungal structures upon penetration of the rhizodermis by *G. intraradices*. Moreover, GintSTE can restore penetration of host tissues by the *clste12* mutant of the hemibiotrophic plant pathogen *Colletotrichum lindemuthianum*. These results suggest an implication of GintSTE in the control of the early morphogenetic events of root penetration by AM fungi. To support this hypothesis, we have started searching for GintSTE-targeted genes. Short genomic sequences putatively recognized by GintSTE have been identified using a reverse one-hybrid yeast approach. They contain regulating elements closely related to yeast PRE (Pheromone Responsive Element) and interact with a recombinant GintSTE protein in vitro. Identification of proximal coding regions by genome walking is under way.

P1.6 - Structure-function relationships of GiAGL proteins at plant-fungal interfaces in arbuscular mycorrhiza symbiosis

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The ability of arbuscular mycorrhizal (AM) fungi to colonise the root apoplast, and in co-ordination with the plant develop specialized plant-fungal interfaces, is key to successful symbioses. The availability of expressed sequence tags (EST) of the model legume, *Medicago truncatula*, and AM fungus, *Glomus intraradices*, permits identification of genes required for development of symbiotic interfaces. The *Medicago* EST database was searched to identify cell surface arabinogalactan-proteins (AGPs) expressed in mycorrhizal roots. Candidate genes were

characterised and gene expression tested using reverse-transcription-PCR and promoter:reporter gene fusions. Genes encoding one plant AGP and three AGP-like (AGL) proteins (from *G. intraradices*), were identified. AGL proteins encoded by two genes from *G. intraradices* (GiAGLs) represent a new structural class of AGPs not found in non-AM fungi or plants. These two GiAGLs differ from plant AGPs by containing charged repeats. Structural modelling shows that GiAGL1 can form a polyproline II helix with separate positively and negatively charged faces, whereas GiAGL3 is charged on all three faces. Bacterially expressed AGL proteins are being tested for their ability to form polyproline II helices and self assemble using circular dichroism spectroscopy. The unique structural properties of the newly discovered AGLs suggests they could assist the formation of symbiotic interfaces through self-assembly and interactions with plant cell surfaces.

P1.7 - Pea genes modulate symbiosis-associated fungal and plant molecular responses during late stages of the mycorrhizal symbiosis with *G. intraradices*

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The arbuscular mycorrhiza association results from a successful interaction between genomes of the two symbiotic partners. In this study, we analysed the effect of inactivation of late stage symbiosis-related pea genes on symbiosis-associated fungal and plant molecular responses in order to gain insight into their role in the functional mycorrhizal association. The expression of a subset of ten fungal and eight plant genes, previously reported to be activated during mycorrhiza development, was compared in *Glomus intraradices*-inoculated wild type and isogenic genotypes of pea mutated for the PsSYM36 and PsSYM40 genes where arbuscule formation is inhibited or fungal turnover modulated, respectively. Direct fluorescent in situ RT-PCR and microdissection were used to corroborate fungal structure and fungal gene expression. Molecular responses varied between pea genotypes and with fungal development. Most of the fungal genes were down-regulated when arbuscule formation was defective, and upregulated with more rapid fungal development. Some of the plant genes were also affected in their responses to fungal development but in a more time-dependent way by inactivation of the PsSYM36 and PsSYM40 genes. Results indicate a role of the late stage symbiosis-related pea genes not only in mycorrhiza development but also in the symbiotic functioning of arbuscule-containing cells.

P1.8 - Genetic exchange in AMF alters plant gene expression

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Arbuscular mycorrhizal fungi (AMF) are obligate symbiotic fungi associated with the majority of terrestrial plant species. They improve plant nutrition and promote their diversity and in exchange, plant supplied carbon to the fungi. Although AMF were thought to be ancient asexuals, we have recently been able to make crosses between "parental" isolates and test the influence of mixed genotypes on phenotypic and genetic traits. The crossed isolates are heterokaryotes and exhibit a variable bi-parental inheritance. Interestingly, this variability in nuclear composition of the cytoplasm may have a great influence on phenotypic traits of the associated plants, such as their growth, but its influence on gene expression remained poorly known. A recent study on *Oriza sativa* found 18 genes exclusively expressed in presence of AMF, suggesting specific interactions between genomes of AMF and host plants. Here, we used real-time PCR to test the expression of 12 of these genes on the host *Oriza sativa*. Specifically, we investigated potential variation in gene expression of parental and crossed isolates of AMF at 6, 9 and 12 weeks. We found that gene expression was significantly influenced by the type of isolate colonized. Four genes are differently expressed between isolates at different time, and three of them show a difference between crosses and parents, suggesting an effect of mixed fungi genotype on plant genotype. These results showed the potential effect of the AMF on plants and open new perspectives for the agriculture.

P1.9 - Common SYM gene NUP85 affects pollen tube growth in *Lotus japonicus*

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Leguminous plants have a common signaling pathway for nodule and arbuscular mycorrhizal (AM) formation, which is called the common symbiosis (SYM) pathway. Recently, one of common SYM genes, *NUP85*, was identified in *Lotus japonicus*. The *NUP85* gene encodes a nucleoporin protein which is the components of a nuclear pore complex. The *nup85* mutants showed temperature dependent defects during rhizobial and AM fungal infection. The defect was more pronounced at 22°C than at 18°C growth temperature. Furthermore, the *nup85* mutants showed pleiotropic defects in pollen tube growth as well as nodule and mycorrhizal formation. In this study, we observed pollen tube growth of *nup85* mutant grown at 22°C and 18°C to elucidate the involvement of *NUP85* gene in plant reproduction.

L. japonicus B-129 was used as a wild type. *nup85-2* is allele affecting AM and nodule symbioses in mutants isolated from EMS-mutagenized plants. Pollen grains were collected and put on a thin layer of pollen germination medium prepared on a microscope slide. Samples were incubated in Petri dishes and incubated at 22°C or 18°C. Pollen tubes were fixed with 2% formalin solution. Pollen tube length was measured using a differential interference microscopy. Pollen tubes were also stained with DAPI to be observed nucleus location with an epifluorescence microscope.

Pollen tube length of *nup85* mutant was shorter than that of wild type at 22°C, whereas at 18°C pollen tube length between both lines was almost the same. Growth rate of pollen tube in *nup85* mutant was reduced compared with the wild type. Location of pollen tube nucleus of *nup85* mutant did not differ from wild type. *NUP85* affect pollen tube growth as well as nodule and mycorrhizal formation in a temperature dependent manner.

P1.10 - Legume genetics of formation and function of arbuscular mycorrhiza: fundamentals and application

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The main object of investigations is pea (*Pisum sativum* L.) that is not only an important legume crop but also a highly convenient model to study mutually beneficial plant-microbe interactions. It forms at least two endosymbioses in the roots: arbuscular mycorrhiza (AM) and nitrogen-fixing nodules, which are notable for high level of partner's genetic systems integration and, to a great extent, are controlled by the host plant. There are three approaches to identify pea genes controlling AM formation and function: (1) among those implicated in nodulation, so-called 'common' genes (as a rule single copy genes, regulator *Sym* genes); (2) differential expression of gene products (as a rule gene families, so-called "mycorrhizins", or "symbiosins"); (3) direct screening of AM mutants (till recently it was used only for non-legumes). Existence of common pea genes implicated in interactions with different beneficial soil microbes including AM fungi creates a new theoretical basis for exploitation of plant-microbe interactions in sustainable agriculture. It was demonstrated for pea that it is possible and necessary to do plant breeding to improve its symbiotic effectiveness using complex microbial inoculants. This work was supported by the grants of RFBR (07-04-01171, 07-04-01558, 09-04-91054, 09-04-91293), NWO (047.018.001), Russian Ministry of Science and Education (5399.2008.4, 02.512.11.2254, 02.512.11.2280, 02.740.11.0276).

P1.11 - Mycelium growth *in vitro* cultures and morphologic characteristics of three ectomycorrhizae fungi strains from temperate forests in Mexico

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The study of morphologic characteristics and growth analysis of ectomycorrhizae (EM) mycelia *in vitro* conditions, are very important to define physiology behavior, and can be use as taxonomic character. Macro and micro characteristics of three EM fungi from a temperate forest of a basin in central Mexico were analyzed. MAE, PDA and BAF cultures were used, with different sugar concentrations (10, 20, 30, 40 g/L) and pH (3, 5,7). The strains were incubated for 30 days to 25 oC in dark conditions. Biomass, diameter, rate growth and biomass/area ratio were measured. *suillus brevipes* had yellow-gray colonies, smooth margins, velvety texture, hyalines exudates to dark brown, thin wall hyphae and rugose, some oleiferous; medium concentrations of glucose show significant differences (230 mg, 0.1300 mg/ mm), with BAF and PDA cultures (4.5 mm/day) and acid pH (50-75 mg). In *Boletus pinophilus* colonies were yellow, of texture pruinose, with margin fimbriate, green-gray hyphae, thin wall and smooth, some oleiferous; significant higher growth was observed in low glucose concentrations (150 mg), in BAF and EMA (2.9 mm/day); pH was not a determinant factor. Yellow-gray to dark gray colonies in *Illyocybe aff. splendens* were observed, slightly dense texture, presence of micelial cords and hyalines exudates; the hyphae were green-gray with high cytoplasm content, sinuoses, thin and smooth walls; growth was higher in enriched cultures (0.050 mg/mm), acid pH (0.090 mg/mm) and there were not differences for glucose concentrations. These EM strains have different growth strategies according to their capacity for explore and efficient use of resources availability.

P1-12 - Expression profiling of the arbuscular mycorrhiza-specific blue copper proteins in *Medicago truncatula*

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Specific induction of members of the blue copper protein family were identified by previous studies at the proteomic and nucleotidic level in *Medicago truncatula* colonized by *Glomus* species. In order to get a better understanding of the function of these proteins, expression profiling of MtBcp1 and of a homologous AM-specific blue copper-binding gene (MtBcp2) was performed by real-time quantitative PCR, in *M. truncatula* Jemalong J5 during the mycorrhizal development with *G. intraradices*, up to seven weeks. In parallel, AM colonisation of *M. truncatula* mutants was investigated in Myc- mutants TRV25 C and D (dmi3, Mtsym13), Myc-/+ mutants TR25 (dmi2, Mtsym2) and TR89 (dmi2, Mtsym2), Myc++ mutant TRV17 (sunn, Mtsym12) and Jemalong J5 as control. Time-course analysis showed that colonisation intensity, arbuscule content and expression of MtPT4, MtBcp1 and 2 increased continuously. No expression of MtBcp1 or 2 was detected in the Myc- mutants which are stopped at the appressoria formation stage. On the other hand, TR25, TR89, Jemalong J5 and TRV17 showed a gradual increase of MtBcp1 and 2 expression both in 3 and 7-week old plants, similar to the increase of the colonisation intensity and the arbuscule number. The strong correlations between the expression level of AM-specific blue-copper encoding genes and the AM colonisation may imply a basic role of both bcps in symbiotic functioning and their presence in the arbuscules. RNAi experiments are under way to test this hypothesis.

P1.13 - Transformation of ectomycorrhizal fungus *Scleroderma* sp. mediated by *Agrobacterium tumefaciens*

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Scleroderma sp. is an ectomycorrhizal fungus found in association with *Eucalyptus* plantation in Brazil. Obtention of mutants can facilitate to assess the functional genes involved in mycorrhization and understanding the mycorrhizal formation process. So, transformation system mediated by *Agrobacterium tumefaciens* was developed. The strain of *A. tumefaciens* AGL-1 containing the vector pBGgHg was co-cultivated with the *Scleroderma* sp. mycelium. Three independent experiments were performed and in each, 120 discs of mycelium were used. It was observed an average of 7 transformants that represents an efficiency of 5.8 %. To detect the presence of hph gene, the transformants genomic DNA was amplified, generating a 690 bp fragment, confirming its presence into the genome. The transformants are being tested for mycorrhization, using the *in vitro* mycorrhization technique, to select mutants unable to form mycorrhiza in order to isolate genes involved in fungus-plant interaction.

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P1.14 - The full length of fungal PtSRR1 mRNA encodes a protein that is a potential controller of ectomycorrhiza formation

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PtSRR1 EST (*Pisolithus tinctorius* symbiosis related receptor 1) was previously identified in the first hours of *Pisolithus tinctorius* / *Castanea sativa* interaction and was partially characterized as a potential fungal controller/marker gene of the ectomycorrhizal symbiosis formation. The 5' RACE (rapid amplification of cDNA ends) strategy was performed to obtain the PtSRR1 mRNA 5' portion and assembly all mRNA sequence using bioinformatics tools. Complete cDNA sequence (636 pb) revealed 5' upstream and 3' downstream untranslated regions (UTR). The Kozak's sequence (ccc aag ATG A) was observed in the 5' UTR portion. The PtSRR1 ORF codifies a 127 amino acid peptide which revealed, after *in silico* analysis, two N-glycosylation and two phosphorylation sites. The protein is composed by one hydrophobic transmembrane alpha helix and at least six hydrophilic beta-strands. Computational analysis also indicated a putative cleavage site close to the membrane anchor. There is as yet no similar crystallized structure deposited in the protein databank, thus we were unable to obtain a tridimensional model for PtSRR1. Taken together, our results account to further studies of the PtSRR1 as a possible controller/marker of the fungal development during the initial stages of ectomycorrhiza and suggest that protein PtSRR1 could act as a fungal membrane receptor. The possibility of it to be a secreted peptide involved in early steps of ectomycorrhizal formation has also been considered.

P1.15 - Localization of fungal gene expression at different stages of arbuscular mycorrhizal symbiosis : development of an in situ RT-PCR method

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The Glomeromycota which establish arbuscular mycorrhiza (AM) with the roots of most land plants are non-transformable, obligate symbionts. For this reason most of the genes involved in the development of these fungi remain unknown. We have developed a new methodology, called fluorescent in situ RT-PCR, to directly localize of

gene expression in fungal structures associated with plant tissues, by combining transcript amplification with specific Texas red-labelled primers and confocal microscopy. Using this technique, expression of the LSU-25S reference gene and that of other fungal genes involved in lipid (stearyl-CoA desaturase, DESAT) and protein (peptidyl-prolyl-isomerase, PEPISOM) metabolism, stress response (superoxide dismutase, SOD) and transcription (transcription factor STE12) was detected in extraradicular (spores, extraradicular mycelium, appressoria) as well as intraradicular (vesicles and arbuscules) fungal structures at different stages of interactions of *Glomus intraradices* with *Medicago truncatula* roots. The SOD gene showed a spatially differential expression pattern suggesting specialization of intraradicular fungal tissues. The use of fluorescent in situ RT-PCR to link gene expression to fungal structures provides a novel tool to identify molecular mechanisms involved in morphofunctional processes in the AM symbiosis.

P1-16 - Cellular and subcellular studies of the arbuscular mycorrhizal symbiosis in *Medicago truncatula*: a proteomic survey

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Proteomics has emerged as a powerful tool to study cellular and subcellular protein composition together with protein abundance modifications of *Medicago truncatula* roots colonized by arbuscular mycorrhizal (AM) fungi. Overlaps in response to *G. mosseae* and *G. intraradices* were studied through comparative proteomics allowing identification of several plant and fungal proteins.

At the subcellular level, the control root plastidome was surveyed allowing the identification of 266 protein candidates whose functional distribution mostly resembled that of wheat amyloplasts and tobacco proplastids, while displaying major differences with those of chloroplasts. Most of the identified proteins have a role in nucleic acid-related processes, carbohydrate and N/S metabolisms and stress response mechanisms. BLAST searches against proteins reported in different plastidomes identify 30 putative plastid proteins for which homologues were previously unsuspected as plastid-located. Most of them display putative roles in responses against abiotic/biotic stresses. Some proteins were previously reported as AM-related on the basis of transcript and/or protein profiling. Moreover, preliminary AM-plastid proteomics, allowed identification of several plastid proteins induced or up-accumulated. The results are supported by electronic northern. Together, the data provide new insights into the functioning of root cell compartments and reinforce the idea of their important role in plant mycorrhization.

Fungal Communities

✓ P2-1 - P doses on the prevalence of native arbuscular mycorrhizal fungi

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A greenhouse experiment was installed with bait cultures, to extract the AMF species present in a rhizosphere soil sample of a native *Araucaria angustifolia* forest in Campos do Jordão, Brazil. The experimental design was completely randomized, with four increasing phosphorus doses (0, 20, 50, and 150 mg kg⁻¹, as triple superphosphate), with five replicates, and the bait plant was *A. angustifolia*. After six months the spores were extracted, counted and identified, and the percent root colonization was also determined. When taking all four P doses into account, eleven FMA species could be identified: *Acaulospora bireticulata*, *A. morrowiae*, *Acaulospora* sp.1, *Entrophospora colombiana*, *Gigaspora margarita*, *Glomus diaphanum*, *G. etunicatum*, *G. macrocarpum*, *Scutellospora calospora*, *S. gilmorei*, and *S. pellucida*. There was no effect of the P dose on the total amount of spores neither on the percent root colonization. However, a correspondence analysis showed that the different AMF species were selectively associated mostly to one or another P dose.

✓ P2.2 - Responses of arbuscular mycorrhizal fungal community to the zonal distribution of coastal sand dune vegetation

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Coastal sand dune vegetation is rich in rare species and forms a unique ecosystem. Dune soil is constantly disturbed and poor in nutrients, and thus arbuscular mycorrhizal (AM) fungi may play important roles in plant survival and establishment in the ecosystem. In the present study, the community structures of AM fungi were investigated with respect to the zonal distribution of coastal vegetation in Ishikari sand dune in northern Japan. The seaward slope of the dune is unstable and dominated by *Elymus mollis* (Poaceae), while the landward slope is rather stable and dominated by *Miscanthus sinensis* (Poaceae). The roots and rhizosphere soils of *E. mollis* and *M. sinensis* were collected, and AM fungi in the rhizosphere soils were proliferated by *M. sinensis* seedlings in a

greenhouse (trap culture). DNA was extracted both from the field-collected roots of the two species and the *M. sinensis* trap culture roots, then AM fungal LSU rDNA were amplified, cloned and sequenced for community analysis. In total, 22 AM fungal phylotypes across five families were detected. The diversity of AM fungi in the field was much higher in *E. mollis* than in *M. sinensis*, although several phylotypes were shared between the two species. The detrended correspondence analysis on the community data of the field and trap culture suggested that the host preference of AM fungi and soil disturbance are likely to be the main driving forces for structuring the communities.

P2.3 - The effect of weed and cover crop management on mycorrhizae of grapevine (*Vitis vinifera*) roots

Kendra Baumgartner

Phillip Fujiyoshi (U.S. Department of Agriculture, Agricultural Research Service, Davis, California), Fujiyoshi, P.T.(U.S. Department of Agriculture, Agricultural Research Service, Davis, California), Smith, R.F.(University of California)

There is evidence that increased plant community diversity is associated with increased arbuscular mycorrhizal fungi AMF diversity and spore production, and that host species preference occurs among AMF. We investigated whether manipulation of herbaceous vegetation communities in a perennial agroecosystem would lead to changes in AMF colonization or diversity in the roots of a woody crop plant. In a commercial vineyard three weed control treatments – cultivation, post-emergence herbicide, and pre-emergence herbicide – and three interrow cover crop treatments – rye (*Secale cereale*), triticale (*X Triticosecale*), and none – were established in a split-block design, and in the 4th through 6th years of treatment we collected grapevine roots for determination of percent AMF colonization and analysis of AMF diversity using polymerase chain reaction. Cover crops had no effect on weed community, but weed control method caused significant shifts in the community, including the frequency and diversity of mycorrhizal weeds in the first year. However, weed diversity had no effect on AMF diversity or percent colonization in grapevine roots. Furthermore, there was no consistent trend in the effect of weed control method on percent AMF colonization of the grapevines, although colonization increased significantly over the three years. Colonization level and AMF species were either specific to the grapevine host, or treatments were not extreme enough to show grapevine differences.

P2.4 - Community diversity and structure of arbuscular mycorrhizal fungi across distinct land uses in Western Brazilian Amazon

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Species diversity and community structure of arbuscular mycorrhizal fungi (AMF) were analyzed in distinct land uses in the Amazon region in Brazil. The benchmark area included pristine forest and areas converted to pasture, crops, agroforestry, young and old secondary forest via the slash and burn practice. A total of 63 AMF morphotypes were recovered and 39% of them could not be attributed to known species. Community was dominated by the genus *Glomus* when species number was considered and by *Acaulospora* in terms of sporulation. *Acaulospora* "gd", *A. foveata*, *A. spinosa*, *A. tuberculata*, *Glomus corymbiforme*, *Glomus* "rod shaped", *Scutellospora pellucida* and *Archaeospora trappei* were detected in all land use. Total spore numbers were highly variable among land uses and mean species richness average was twice as high in Crop, Agroforestry, Young and Old Secondary Forest as compared to Forest and Pasture. Fungal communities were dominated by two or three species which accounted for 48% to 63% of all spores, except in Young Secondary Forest. Our study showed that this region of Amazon can be considered a hotspot for AMF diversity and that land uses influenced AMF community in (i) AMF species frequency of occurrence, (ii) mean species diversity, and (iii) relative abundance of the most abundant species. We concluded that conversion of pristine forest into distinct land uses does not reduce AMF diversity and cultural practices adopted in this region maintain a high diversity of AMF

P2.5 - Ectomycorrhizal communities of *Eucalyptus urograndis* monocultures and ectomycorrhizal colonization of *Psidium guajava*.

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Eucalyptus plantations are well known for their mycorrhizal diversity, which may vary along with soil type, companion plants, tree age, species and management. The first objective of this research project was to compare the ectomycorrhizal communities on two different sites planted with eucalypt hybrid *urograndis*. The two sites are clonal plantations managed by the company CENIBRA and were selected based on their contrasting altitudes (average altitudes of 802 m and 236 m) and soil types (cambiosol and neosol). The second objective was to assess the ectomycorrhizas present on other Myrtaceae trees of the Mata Atlantica forest nearby the plantations. The efforts were mainly focused on *Psidium guajava*. Guava. The guava is known to be associated with arbuscular

mycorrhizas. We have also found for the first time associated ectomycorrhizas. At least two different ectomycorrhizal morphotypes were found and will be identified by molecular analysis (rDNA PCR-RFLP, coupled with sequencing). A traditional method of morphotyping is used and molecular analyses will be done in the future to identify the ectomycorrhizal fungi at the species level. The percentage of root colonization was also assessed, for both research objectives. Guava and Eucalypt both showed a high percentage of mycorrhizal colonization, arbuscular mycorrhiza being dominant on the guava and ectomycorrhizas on eucalypt.

P2.6 - Diversity of arbuscular mycorrhizal fungi and glomalin

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In this study, ten soil samples were collected from organic and conventional farms in Goesan-gun, Chungbuk and communities of arbuscular mycorrhizal fungi(AMF) and glomalin, a glycoprotein produced by AMF were analysed. Spores of *Glomus mosseae*, *Acaulospora longula*, *Scutellospora heterogama*, *Paraglomus occultum*, *Archaospora leptotica* were identified in the study sites, using morphological and molecular characteristics. While *Acaulospora longula* was the most dominant species in soils of organic farms, *Paraglomus occultum* was dominant species in the conventional farms. Species diversity and evenness of AMF communities were not significantly different in both sites. However, species richness of AMF communities in soils from organic farms was significantly higher than in soils from conventional farms. Also, glomalin was extracted from the soil samples collected from organic and conventional farms and analysed by enzyme-linked immunosorbent assay. The contents of glomalin in soils from organic farms were significantly higher than soils from conventional farms. The results suggest that agricultural practices significantly influence AM fungal community structure and glomalin contents.

P2.7 - Diversity of Glomeromycota in El Palmar National Park (Entre Ríos, Argentina)

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The Palmar National Park (58° 17' W, 31° 50' S) is one of the most floristically diverse national parks in Argentina. With over 700 vascular plant species, the park shelters an important concentration of palm trees *Butia yatay* (Mart.) Becc., that represents both a characteristic edaphic community and one of the last *B. yatay* remnants in the region. Such a protected area results a propitious place to conserve floristic biodiversity and associated microorganisms. The purpose of this study was to determine the effect of different plant communities on mycorrhizal fungal communities. The samples were collected from five plant communities at The Palmar: gallery jungle (GF), grassland (GRA), marsh (MAR), palm forest (PF) and scrubland (SCR). Spores of arbuscular fungal species were isolated from soil by wet sieving and decanting and sucrose-gradient centrifugation. Species abundance was calculated and used for a two-way indicator species analysis. Forty six taxa of AMF were identified. This analysis indicated that the AMF community from GRA are closely related to PF -Group I- and GF, MAR and SCR constitute Group II. The species selected by cluster were *Gigaspora candida* for the samples in Group I and *Glomus claroideum* for Group II. The strong AM fungal community differentiation is in response to an ecosystem heterogeneity. To our knowledge, this study constitutes the first report of arbuscular mycorrhizal fungi in a protected area in Argentina.

P2.8 - Linking coexistence with genetic distance in arbuscular mycorrhizal fungi

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Ecological interactions among AMF in natural communities is a topic that is poorly understood. Moreover, interactions among AMF at the intraspecific level have been little studied. *Glomus intraradices* is an interesting model species on which to conduct experiments on among AMF interactions as it shows large variation between isolates at the genetic, morphologic and ecological level. Furthermore, genetic differences among isolates lead to differential effects on plant growth. We propose a green house experiment using *G. intraradices* that should shed light on the interactions between isolates from one field. The main question is whether genetically closely related isolates are more likely to co-exist or whether they strongly compete similar to different species competing for the same niche. We will use three different host plant species (*Allium porum*, *Oryza sativa* and *Plantago lanceolata*) and inoculate them with pairs of AMF differing in genetic distance. Based on real-time PCR, we aim to measure the relative abundance of each isolate in the plant roots. Our experimental design should also give information on several additional points such as: Existence of host preference, dynamic of the colonization and correlation between plant growth and genetic diversity of AMF. The way in which genetic distance between isolates alter their coexistence and plant growth, should provide new insight into AMF diversity and its consequences for ecosystems.

P2.9 - Forest management and ectomycorrhizal communities of cork oak stands

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Most of the cork oak woods in Sardinia (Italy) are included in agro-sylvo-pastoral systems in which the microbial communities associated to plants can be subjected to strong alterations. For this reason, a research was carried out in order to verify the variations both in the composition of ectomycorrhizal fungal (*ecm*) community, and in the density of ectomycorrhizal tips of cork oak plants living in 3 different stands, respectively subjected to shrub clearing, grazing and undisturbed.

In each cork oak stand, 15 soil cores of 4.5 cm diameter positioned 1 m from each other were collected along a transept. The ectomycorrhizas isolated from soil cores were characterised under the morpho-anatomical profile and identified on genetic basis by sequencing of the ITS region of fungal rDNA.

The variations in species composition were detected through the Constrained Analysis of Principal Coordinates (CAP) and those related to the ectomycorrhizal tips density by ANOVA and Tukey's test. The results show that all the 3 cork oak stands differ for the composition in species of the *ecm* community, and that 2 of them, i. e. the undisturbed and the shrub cleared, differ also for the density of ectomycorrhizal tips. It is very likely that these variations are the consequence of the reduced vegetal diversity and soil modifications in the most anthropized cork oak stands.

P2.10 - The effects of long-term fertilization and rotation cropping regimes on the AMF community in the Loess Plateau of northwestern China

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The Loess Plateau of northwestern China is a region where the agriculture production was extremely low due to the infertile soil and drought. Thus, how to improve the yield of crops meanwhile maintain the soil fertility is essential for the agriculture in this region. A long-term fertilization experimental station which has six treatments including of N (inorganic N), NP (inorganic N and P), SNP (straw, inorganic N and P), M (farmyard manure), MNP (farmyard manure, inorganic N and P) and CK (no fertilization), was established in 1979 at Gaoping village of Gansu province, being used for estimate the long-term effects of different fertilization and maize-wheat rotation regimes on the rain-fed agriculture production. Although some previous studies showed that the yield of crops was significantly improved by some fertilization treatments in this station, some important soil microorganisms, like arbuscular mycorrhizal fungi (AMF), which highly related to crops growth and soil fertility has not been investigated. For fully understanding the response of AMF to the long-term fertilization and crop rotation regimes, the communities of AMF were investigated using PCR-DGGE, cloning and sequencing techniques in different growth stage of maize (2006; July and September) and wheat (2008; April, May and June). Additionally, the soil properties were also analyzed for elucidating the soil fertilities which caused by different fertilization treatments. A total of nine AMF phylotypes all belonging to the genus of *Glomus* were identified in the two-year experiment, but no more than five phylotypes was detected in each year. An AMF phylotype, highly related to the *G. mosseae* sequence, was the only one that occurred in all samples, whereas the other phylotypes occasionally occurred in samples. The AMF community compositions were significantly different between the roots of maize and wheat, but had no or little difference among the fertilization treatments. Also, we found no significant but distinct shift of AMF community composition through the growth stage of the two crops. In order to characterize the soil fertilities, a principle component analysis (PCA) was carried out using the soil properties and generated two principle components (PC1 and PC2) which can successful explaining the variation of soil properties. The loading of axes showed that the PC1 reflects the soil fertility which including the total N, total C, organic C and available P status of soil and the PC2 reflects the soil moisture. The pattern of soil PCA showed that the samples of M and MNP were distinctly divergent from the others and the soil fertility ordination seemly like this: MNPMSNPNPCK (from the axis of PC1). However, the ordination of canonical correspondence analysis (CCA) using the scores of PC1, PC2 and the data of AMF community showed that the AMF communities distinctly separated by the sampling time and the factor of PC2 was the most determining factor in constructing the AMF community composition. The result of CCA suggested that the fertilization regime was not an important factor for AMF but the soil moisture was. This study showed that the long-term fertilization including of inorganic and organic fertilization regimes had no or little influence on AMF community, but the soil moisture and rotation cropping regime take effects. We speculated that the agro-ecosystem functions of AMF were limited and need to be further estimated in the rain-fed farming fields of Loess Plateau of northwestern China. Acknowledgements We are thankful for financial support by National Natural Science Foundation of China (30870438, 30570270), New Century Talent Plan of MOE (NCET-07-0390).

P2.11 - ITS TRFLP profiling limitations: problematic fungal genera

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TRFLP profiling of the ITS rDNA of unknown fungal communities is currently unsupported by a broad range

enzyme choosing rationale. An *in silico* study of terminal fragment size diversity was therefore performed following the virtual digestion of all published fungal ITS sequences putatively annealing to primers ITS1 and ITS4 using a set of commercially available 135 type IIP restriction endonucleases. Enzyme set size was optimized by comparing the cumulative TRFLP richness and the congeneric vs. non-congeneric shared ribotypes ratio in 1-6 enzymes sets. The fungal genera presenting more difficulties to be profiled with this optimized TRFLP enzyme set were identified and analyzed. Three major groups were identified. Some genera presented multiple TRFLP profiles, each one being shared by few species (e.g. *Cortinarius*). Some others had multiple species sharing their TRFLP profiles owing to the absence of one or more enzyme targets (e.g. *Alternaria*). Finally, some genera presented too low genetic diversity between their species or were subject to improper species definition or sample identification (e.g. *Penicillium*, *Eurotium*).

P2.12 - The ectomycorrhizal community and cork oaks' state of health

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In the last decades the main oak Mediterranean areas have been interested by a progressive diffusion of the oak decline syndrome with serious damages to plants and the consequent imbalance of the microbial communities associated. In this study, the variation in terms of difference and composition of the ectomycorrhizal community (*ecm*) was evaluated according to the degree of deterioration of *Quercus suber* in a forest located in the North Sardinia (Italy). In order to detect the *ecm* community, 8 cores were collected (2 for each cardinal point) near 20 cork oak plants equally divided in 4 homogeneous groups depending on the disease intensity. The isolated *ecm* were characterised under the morpho-anatomical profile and identified on genetic basis by sequencing of the ITS region of fungal rDNA. The variations of *ecm* communities in terms of species richness, diversity (Shannon index) and evenness (Pielou index) were analysed by the Kruskal-Wallis test, and those related to the species composition through the *adonis* test and the Non-Metric Multidimensional Scaling. The results show that the plants' state of health influences the evenness and the species composition of the *ecm* communities. It is probable that the low photosynthetic efficiency of declining plants could have favored the development of *ecm* species less exigent in nutritional terms.

P2.13 - Arbuscular mycorrhizal fungi, a tool to access the side effects of pesticides used in grapevine production

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Grapevine is a crop receiving amongst the highest number of pesticide and fungicide treatments. In France, an average of 20 treatments are performed annually, depending on atmospheric conditions, representing respectively up to 20% and 30% of the total amount of pesticides and fungicides used in French agriculture. A part of these compounds accumulates in the soil where they represent a non-targeted risk for the soil microflora and especially for arbuscular mycorrhizal (AM) fungi. Soil being a non-renewable resource, evaluation of its biological activity is an important factor to consider for the sustainable production of grapevine.

In this context, we have studied possible side effects of a mixture of 2 fungicides and an insecticide on AM fungi in vineyard soils submitted to different agricultural practices. The survival in soil of the mycorrhizal fungi was evaluated 30 days after pesticide application by quantifying the active propagule population by the MPN test. The AM fungal community composition was also analysed using molecular methods. For this purpose, DNA was extracted from soil and the 5' end of the LSU rDNA of the Glomeromycota was amplified. Sequences were grouped into ribotypes, for which specific primers were designed and used to quantify modifications in the composition of the AM fungal community after pesticide treatment.

Work, initiated by G. Soulas (INRA, Bordeaux, F), was supported by the MEDD (F), UAM Xochimilco and Conacyt, Mexico (fellowship to FRB).

P2.14 - Arbuscular mycorrhizal fungi in the Sete Cidades National Park (Piauí, Brazil)

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Considering the ecological importance of the arbuscular mycorrhizal fungi for maintenance of natural ecosystems, a survey of species was performed at the Sete Cidades National Park – SCNP (State of Piauí). Located in the Northeastern region, the park, with > 6000 ha, has a warm tropical climate and is characterized as an ecotone with a mixture of Savanna, Caatinga and Forest vegetation. Soil samples were randomly collected in four areas: (a) grassland; (b) savanna; (c) tropical forest (evergreen broad-leaved woodland); (d) tropical semi-deciduous forest. Glomerospores were extracted from soil and morphologically identified. Twenty species were registered in the

genera: *Acaulospora* (2), *Ambispora* (1), *Cetraspora* (1), *Dentiscutata* (2), *Fuscutata* (1), *Gigaspora* (2), *Glomus* (8), *Paraglomus* (1), *Racocetra* (1), and *Scutellospora* (1). Higher number of species (10) was found in area "c". The communities of AMF from the savanna and the tropical forest were more similar (44.4%) than the other areas probably because many plant species present in the savanna were also found in the evergreen tropical forest. In contrast, the AMF of the savanna area presented low similarity (13.3%) with those of the other forest type. Comparing with other investigations performed in the Brazilian Northeast, the diversity of AMF was low in the studied areas and further studies, including more collections should be performed to confirm the data.

P2.15 - New data on *Tuber pseudohimalayense* Chinese truffle

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Tuber species studies from China collected in European markets began 14 years ago. *T. pseudohimalayense* G. Moreno, Manjón, Díez & García-Montero was proposed as a new species, but this species has been questioned. This work evaluates the taxonomic validity of the *T. pseudohimalayense* species studying type collections, genetic studies and by comparing the morphology of *T. pseudohimalayense*, *T. pseudoexcavatum* and *T. indicum ectomycorrhizae*. It also provides the corrected taxonomical name and additional molecular data on the *T. pseudoexcavatum* typus collection.

P2.16 - Ectomycorrhizal fungal community of *Pinus thunbergii* in a coastal pine forest and a plantation in east coast of Korea

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The status of ectomycorrhizal (ECM) fungal colonization in *Pinus thunbergii* in a coastal pine forest and a plantation was investigated on the east coast of Korea. At *P. thunbergii* coastal forests in Samcheok, fifty soil blocks (5 x 5 x 15 cm) and ten *P. thunbergii* seedlings of 5-10 years old were sampled from each of two 20 x 20 m plots. At a *P. thunbergii* plantation in Gangneung, lateral roots from 10 *P. thunbergii* 8 years old seedlings were sampled from each of three 10 x 10 m plots. ECMs were classified into morphological groups and the number of root tips of each morphotype was counted. Fungal taxa that form each morphotype were identified by sequencing of the internal transcribed spacer of the nuclear rDNA. A total of 15, 23 and 8 ECM morphotypes were observed from seedlings and soil samples in coastal pine forests and seedlings from the plantation, respectively. *Cenococcum geophilum* was dominant and accounted for more than 50 % abundance with 100 % frequency in seedlings and soil samples in the coastal pine forest. *Russula* sp., *Sebacina* sp. and *Cortinarius* sp. were also relatively abundant in seedlings and/or soil samples. On the other hands, in the plantation, *Suillus granulatus* was the most abundant in all plots, followed by *Tomentella ellisii* and Atheliaceae sp.. These 3 fungal taxa accounted for almost all of the ECM abundance in the plantation. ECM fungal communities differed largely between that of seedlings in the coastal pine forest and plantation.

P2.17 - Arbuscular mycorrhizal fungi community structure at the Colombian Amazon region

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Colombian Amazon region is 477.274 Km², representing 41.7% of Colombia. Although phosphorus is the limited element for plant nutrition and arbuscular mycorrhization occurred in 90% of the plant species of the region, the arbuscular mycorrhizal fungi (AMF) community structure has been poorly studied in a regional scale. During 7 years, samples of topsoil (source of AMF spores) and fine roots from natural and secondary forests, and itinerant agriculture and introduced pastures plots were collected and used to describe AMF community structure in relation to geographical, biological and physicochemical issues. Percentage of AMF colonization varies randomly into the region, however in a same place, colonization between different plants is not quite different, suggesting a no specific AMF colonization. AMF sporulation is affected negatively by low pH, clayed texture and low phosphate availability at soil. According to spore occurrence, *Glomus* dominates the community with 64.5% of the total spore, *Acaulospora* and *Scutellospora* is the 16.2% and 9.7% respectively and *Entrophospora*, *Archaeospora* and *Gigaspora* represent only 3.2%. Although *Glomus rubiforme* and *Acaulospora foveata* have a broad distribution into the region, any estimation about their potential use has been performed. AMF spores are commonly attacked by soil microorganisms, however dark and ornamented spores of *Glomus* and *Acaulospora* are less susceptible than big, clear and non-ornamented spores of *Gigaspora*.

P2.18 - Ectomycorrhizae fungi richness from Conifers forests of Central Mexico

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The Magdalena River Basin (MRB) is the most important forest ecosystem of the Distrito Federal, Mexico; its

conifers forests have been disturbed by several factors such as fire, pollution and soil use changes. One promising for his recovery is the use of conifers seedlings inoculated with ectomycorrhizae fungi (EM). However, first is necessary to know which ectomycorrhizae fungi species grown in each one the different vegetation types of the basin. This will permit to select those EM with potential to produce native inocula. The objective of the study was to estimate the ectomycorrhizae fungi richness in the *Pinus hartwegii* and *Abies religiosa* forests of the MRB. Nine field surveys were made during the rainy season of 2006 and 2007 in order to explore the area for collecting sporomes to the basidiomycetes of ectomycorrhizae fungi; 291 specimens were collected in both forests types. The sporomes were described, herborized and taxonomic identified. These specimens belong to 12 families, 15 genus and 38 species. In the *iAbies/i* forest there were registered 28 species, while in the *iPinus/i* forest there were 24 species only. Species richness for the most abundant genus was: *Russula* (12), *Amanita* (5), *Inocybe* (5), *Lactarius* (5) and *Boletus* (3). Although the species richness of EM from the MRB is low in relation to other similar vegetation types in Mexico, there are several promising EM species that can be useful for forests restoration, such as *Suillus brevipes*, *Inocybe splendens* and *Laccaria trichodermophora*. These species show a great potential for inoculum production due to their easy propagation and because form ectomycorrhizal association in early succession steps.

P2.19 - Diversity of ectomycorrhizal fungi (Basidiomycotina) in Brazil. A brief study at the Parque das Dunas de Natal, RN.

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Studies about ectomycorrhizal fungi (ECM) that grow in Brazil are rare. Although ECM are known to occur in tropical ecosystems, few researchers have investigated the occurrence of these fungi in Brazil. Singer was one of the few mycologists to cite ECM from Brazil, which he collected in the Amazon during the 1980s; however, very little is known about the ecology and hosts of these species.

The goal of this work was to generate a list of the genera of fungi from Brazil that are assumed to be ectomycorrhizal. Data was gathered from the literature, herbaria, and field observations. The genera were georeferenced and distribution maps were created.

As an example field study, basidiome collections from Parque das Dunas de Natal, a forest growing on white sand dunes, were correlated with the trees growing in the park. The collections were made in 2008 during the rainy season. The trees were identified and the roots were analyzed for the presence of mantle and Hartig net.

The results of this work indicate the presence of genera of ECM in Brazil. In order to better understand these putative relationships, ectotrophic forests in Brazil, such as Parque das Dunas de Natal, need to be studied in more detail. Further, the occurrence of cryptic ECM species from different regions of Brazil reinforces the need to perform phylogenetic and molecular studies that include Brazilian fungi.

P2.20 - Diversity of arbuscular mycorrhizal fungi in the Caatinga, a semiarid Brazilian biome

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The Caatinga, an exclusive Brazilian biome, is characterized by deciduous vegetation, mostly belonging to Fabaceae, Euphorbiaceae and Cactaceae, and high floristic endemism. From its 825,750 km², 24.4% is native forest and 38.4% open herbaceous forest with xerophytes, 36.3% is under anthropic impact and the remaining is covered by water; the climate is semiarid (23 - 27 °C, 500-750 mm annual rainfall). This biome is one of the richest semiarid areas in diversity and that of AMF is also high, with 75 species. This estimate is preliminary, considering that most of the Caatinga is still unexplored. The acknowledge diversity for this biome corresponds to 75.7% of the AMF species registered for Brazil (99) and to 36.6% of those globally described (205). The impacted areas have smaller number of species (33), comparing with agrosystems (59) and preserved areas (52). Most of the species belong to Glomeraceae (26) and Acaulosporaceae (18); to the present, only Pacisporaceae and Geosiphonaceae have not been found in Caatinga. *Glomus* and *Acaulospora* are the commonest genera. The low diversity of AMF in disturbed areas was confirmed; however, when re-vegetated, the community of AMF is re-established including species that differ from those found before the disturbance. These data reinforce the need for preservation of areas considered hotspots for biodiversity, such as the Caatinga, where species of AMF can be maintained in situ, protected from the impact caused by anthropic actions.

P2.21 - Arbuscular mycorrhizal fungi in Tropical Atlantic Forests of Brazil with different regeneration periods

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In the Northeast of Brazil the Atlantic Forest has been largely replaced by sugarcane plantations and in some abandoned areas the vegetation is slowly and naturally recovered. Considering the importance of the AMF for plant establishment, its diversity was investigated at four sites in Igarassu, PE, Northeast Brazil: one forest with preserved vegetation, one sugarcane plantation, and two sites in different stages of recovering (10 and 20 years of regeneration after agricultural land use). Soil samples were collected in December 2007 from the rhizosphere (0-20 cm depth) of several native plant species characteristic of the Atlantic Forest. Glomerospores were extracted from soil and identified based on spore morphology. Up to now 16 species were identified (*Acaulospora foveata*, *A. laevis*, *A. mellea*, *A. morrowiae*, *A. scrobiculata*, *A. spinosa*, *Cetraspora pellucida*, *Dentiscutata scutata*, *Gigaspora ramisporophora*, *Glomus claroideum*, *G. glomerulatum*, *G. intraradices*, *G. macrocarpum*, *Paraglomus occultum*, *Scutellospora aurigloba* e *S. pernambucana*), most of them in the oldest regeneration area. Among the species, only *C. pellucida*, *S. aurigloba* e *S. pernambucana* were not found in both regeneration forest fragments. *Acaulospora* was the most representative genus in number of species, followed by *Glomus*, what suggest higher adaptability of these fungi at the study sites.

P2.22 - Arbuscular Mycorrhizal fungi diversity from timber plantations of *Gmelina arborea* and *Pachira quinata* in the Colombian caribbean region

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Arbuscular mycorrhizal fungi (AMF) establish association with 90% of plants and are components of most terrestrial ecosystems and agroecosystems. Knowledge regarding the diversity and variability of AMF in specific ecosystems is the basis for pre-selection in application programmes to optimize the symbiosis. The objective of the present work was to evaluate the diversity of AMF in soils of *G. arborea* and *P. quinata* plantations, as well as the natural root colonization, in a transect along the Caribbean area where the edapho-climatic differences are quite high. A total of 95 soil and root samples were collected from timber plantations during dry and rainy seasons. Soil chemical and physical analyses were performed in order to establish the relationships between soil characteristics and AMF diversity. In addition, spore abundance, presence of ecotypes, and root colonization were recorded. The spores were morphologically classified. The results showed that natural colonization by AMF in two forest species was between 0 and 67%. Four AMF genera (*Glomus*, *Acaulospora*, *Scutellospora* and *Gigaspora*) and 20 ecotypes were identified from the area of study, reflecting the high diversity present in tropical forest areas as already reported. There was observed that spore abundance ranged between 1 and 121/g of soil, with predominance of the *Glomus* species not only in terms of abundance, but also in diversity. This result confirms *Glomus* plasticity and adaptability to different environments

P2.23 - Glomeromycota fungi diversity in agricultural systems of Central Venezuela

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Soil micro-biota diversity and richness are related to physical and chemical conditions. Microbial composition may vary due to soil management and plant species. We determined variations on Glomeromycota fungi populations associated to some agroecological transition systems of Central region of Venezuela. We studied an organic coffee farm, two soil restoration plots with Vetiver and an agroecological system under ecological management with standing crops and crop rotation; the main crop being sorghum. In each case four rhizospheric soil samples were collected and analyzed. Spores were sieved, decanted and centrifuged to be counted and classified through morphological characteristics. To compare fungal diversity between each system, ecological diversity indexes were calculated. In the coffee cultivar, Shannon's Index was 0,966; in the Vetiver plots of 3 and 5 years old a difference was found at all the indexes, Shannon's Index were 1,098 (3 years) and 1,608 (5 years). Agroecological sorghum plots were less diverse, with Shannon's Indexes between 1,571 and 0,686. Our results indicate that agroecological systems showed more diversity, than those under conventional management. Increasing biodiversity is one of the aims of sustainable agriculture; ecological implications of our results are discussed.

P2.24 - Management of soil microbial quality using chickpea cultivars and endophytic inoculation to improve the productivity of subsequent wheat crops

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Chickpea has the ability to bring free N into cropping systems, but is only a fair rotation crop, leading to approximately 10% lower yield in following wheat crops, as compared to pea or lentil. Residue-free soil previously growing the unifoliolate Kabuli chickpea strongly inhibited durum seed germination suggesting an effect on the soil microbial community. We hypothesized that the productivity of chickpea and subsequent crops could be promoted

through: (1) The inoculation with indigenous endophytic fungi and (2) The use of chickpea cultivars that favour the growth of beneficial microbial populations. A greenhouse experiment was conducted. It had three factors: chickpea cultivars (2 Kabuli and 2 Desi), levels of irrigation (sufficient and insufficient water) and inoculation treatments (presence and absence of endophytes). After 8 weeks, plant materials were mixed with the soil in designated pots. The soil of half of the pots that had grown Kabuli chickpea varieties was sieved to remove plant debris with the aim of assessing the effect of root exudates. FAME analysis results showed that the structure of the soil microbial community is influenced by chickpea cultivars. Cultivars could influence the performance of the subsequent wheat crop. Inoculation with indigenous endophytes increased drought tolerance and grain yield of the unifoliate Kabuli chickpea. Inoculation of Kabuli chickpea with indigenous endophytes improved wheat seeds germination in Kabuli tissue amended soil.

P2.25 - Diversity of arbuscular mycorrhizal fungi in semiarid areas with Caatinga vegetation in Brazil

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Knowledge about the presence and diversity of arbuscular mycorrhizal fungi (AMF) in a specific area is an essential first step for utilizing fungi in any application. However, the works with AMF in undisturbed Caatinga areas are still incipient and the studies concentrated in regions altered by human activities. The diversity and infectivity of AMF were investigated in three areas on native vegetation in the semiarid of Pernambuco, northeastern Brazil. Soil samples were collected in December/2005 and October/2006 in Caruaru and August/2005 and August/2006 in Serra Talhada and Araripina. The AMF community composition and structure varied among the areas. Twenty-nine AMF taxa were identified in the soil samples collected, twelve belonged to *Acaulospora*, seven to *Glomus*, four to *Scutellospora*, two to *Gigaspora* and one of each to *Ambispora*, *Cetraspora*, *Entrophospora* and *Kuklospora*. The spore density and root colonization by AMF in Caruaru were higher than observed for the others areas, presumably influenced by condition of higher humidity and diversity of plants of this area. The most probable number (MPN) of infective propagules of AMF was low (42 to 158 propagules 100 mL⁻¹ of soil) and possibly influenced by root colonized fragments and extraradical mycorrhizal mycelium evidencing an importance this propagules from colonization of root in this environment.

P2.26 - Impacts of 90-year-incorporation of chemical fertilizer on the community structure of arbuscular mycorrhizal fungi

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Overloading of chemical fertilizer in modern agriculture has serious impacts on environment. Enhancing nutrient cycling via manipulating rhizosphere microorganisms such as arbuscular mycorrhizal (AM) fungi could be one breakthrough for sustainable agriculture. It has been demonstrated that increases in soil P-level reduced the effects of AM symbiosis on the P-uptake of the hosts through suppressing the colonization. The impacts of P-fertilizer on the community structure of AM fungi, however, are not well understood. The roots of *Glycine max* and soils were collected from two low-P (no history of P-fertilization) and two high-P (100 kg ha⁻¹ y⁻¹) plots in the long-term experimental field of Hokkaido University established in 1914. *Lotus japonicus* were grown on these soils in a greenhouse for 8 weeks. DNA was extracted from the roots of the field-grown *G. max* and from those of the *L. japonicus* trap culture, and AM fungal large subunit ribosomal RNA gene were amplified, cloned and sequenced for community analysis. The diversities of AM fungi in the low-P soils were relatively higher than those in the high-P soils. Several AM fungal phylotypes were found only from the low-P soils, while no high-P specific phylotype was observed. Most of the phylotypes, however, were shared among the low- and high-P soils. Our results suggest that long-term incorporation of P-fertilizer slightly decreased the diversity but may not be accompanied by drastic changes in the community compositions.

Mechanism of Tolerance to Biotic and Environmental Stresses

P3.1 - Proteome analysis of a Cd-tolerant and a Cd-sensitive *Suillus luteus* after cadmium exposure

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Heavy metals are important environmental pollutants and can cause serious problems to organisms. Still, some micro-organisms show a genetic adaptation to heavy metals and could be useful in the revegetation of contaminated sites. A promising organism is the ectomycorrhizal fungus *Suillus luteus*, a common root symbiont of *Pinus* species. *S. luteus* populations thriving in pioneer forests that colonize sites severely contaminated by Zn smelters in NE-Belgium, have undergone a genetic adaptation to toxic cadmium (Cd) concentrations. However, the exact mechanisms of metal tolerance in ectomycorrhizal fungi are largely unknown. The aim of this study was therefore to identify fungal proteins involved in the mechanism sustaining the adaptive cadmium tolerance in *S. luteus*. Using the high-tech 2D-DIGE technology, a comparison of the proteome under Cd stress (0 μ M, 9 μ M and 45 μ M) was performed for two genotypes of *S. luteus*, one Cd-tolerant isolate originated from a contaminated soil and one Cd-sensitive isolate from a control soil. In this time-response experiment (0, 24, 48 hrs), fungal proteins were phenol extracted and afterwards precipitated with methanol/ammonium acetate. Resulting protein mixtures were labelled with fluorescent dyes (Cy2, Cy3 and Cy5) and analysed by two-dimensional gel electrophoresis. Proteins that showed statistically significant changes between both isolates will further be analysed with mass spectrometry.

P3.2 - Proteomic analysis of *Glomus mosseae* colonized tomato plants during early infection of the plant-parasitic nematode *Meloidogyne incognita*

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Arbuscular mycorrhizal fungi (AMF) like *Glomus mosseae* can protect their host plant from the highly damaging plant-parasitic root-knot nematode *Meloidogyne incognita*. However, the mechanisms involved have rarely been investigated for AMF-nematode interactions. Therefore, the AMF-plant-nematode interaction is monitored during the different stages of nematode infection, both at the biological and proteomic level.

In this study, tomato plants (*Lycopersicon esculentum* cv. Marmande) were grown under controlled environmental conditions, considering 4 treatments: control; AMF-colonized; nematode-infected; AMF-colonized and nematode-infected plants. AMF inoculum was added at sowing, after 6 weeks plants were infected with nematodes and harvested 12 days later to proceed with phenolic extraction of the selected plant roots, then proteins were separated by 2-D gelelectrophoresis followed by Coomassie blue staining.

On average 700 spots were detected, out of which 52 spots with a significant differential expression were selected and identified by MALDI-TOF/TOF. Nematodes had a more profound effect on the root proteome than AMF (29 vs. 12 differential proteins), but the presence of both together resulted in the greatest impact (42 differential proteins), including upregulation of plant defense proteins that had no differential expression in any of the other treatments. The differential proteins involved in the reaction of AMF-colonized plants to nematode infection will be discussed.

P3.3 - Biocontrol mode of action of AM fungi against *Pythium* root diseases

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Biocontrol mode of action of AM fungi against *Pythium* root diseases in various plants (cucumber, tomatoes and clover) was examined in pot experiments. The pathogens *Pythium ultimum* and *P. aphanidermatum* were included and inoculated in terms of pre-infected cucumber discs. Pathogen quantification was performed in terms of their plant growth suppressive effects and/or in terms of ELISA, recovery on selective media and numbers of oospores quantified using microscopy. The AM fungi *Glomus intraradices*, *G. mosseae* and *G. claroideum* were included and were quantified in terms of AM fungus root colonization and the external mycelium was quantified using the biomarker fatty acid 16:1 5. In all experiments *Pythium* biocontrol was achieved at least with one of the tested AM fungi. However, the biocontrol efficacy depended on the AM fungus and plant in question. Dual inoculation of AM fungi with other biocontrol agents such as the bacterium *Burkholderia cepacia* and the fungus *Clonostachys rosea* did not increase the biocontrol efficacy. The influence of AM fungi on plant defence reactions, in terms of productions of phytoalexins (flavonoids) and expression of pathogenesis-related genes (PR1) were limited and does not seem to be important in *Pythium* biocontrol, whereas antagonistic effects of mycorrhiza associated bacteria from the genus *Paenibacillus* against *Pythium*, indicate that these bacteria may play an active role in the biocontrol of AM fungi against root diseases caused by *Pythium*.

P3.4 - *In vitro* study of the combined effects of elevated CO₂ and temperature on arbuscular mycorrhizal associations

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Climate change is having tremendous effects on ecosystem functioning, including arbuscular mycorrhizal (AM) associations. The combined effects of elevated CO₂ and temperature were investigated on the intraradical (IRM) and extraradical mycelium (ERM) of an AM fungus (i.e. *Glomus intraradices*) associated to *Medicago truncatula* under

autotrophic in vitro conditions. Colonized plants were placed in standard (380 ppm CO₂ and 22/18°C day/night temperatures) or elevated (600 ppm CO₂ and +3°C) growth conditions. A set of plants was subsequently harvested after 4, 8, 12 or 16 days to assess root colonization, while another was followed for 8 weeks to investigate ERM development. Root length colonisation, and abundance of arbuscules (%A) and vesicles (%V) did not differ between the two treatments during the 16-days experiment. However, %A was significantly higher when the mycorrhizal plants were exposed to elevated CO₂ and temperature conditions for 8 weeks. The ERM was also affected under these conditions. At week 8, the extraradical hyphal length was 75% higher under elevated conditions as compared to the standard conditions. Spore production was affected by the treatments, but only at week 1 and 2 where the number of spores was significantly higher in the elevated conditions. This study showed that both IRM and ERM were promoted by elevated CO₂ and temperature conditions. These observations could be related to a higher transfer of carbon from the plants to their symbiotic partners.

P3.5 - Plant accumulation of organic contaminants and their dissipation in soil as affected by arbuscular mycorrhizal (AM) fungus *Glomus etunicatum*

Zhang, S.Z.(Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences), Huang, H.L.(Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences), Wu, N.Y.(Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences)

Plant accumulation of organic contaminants and their dissipation in soil as affected by arbuscular mycorrhizal (AM) fungus *Glomus etunicatum* Shuzhen Zhang, Honglin Huang, Naiying Wu Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P. O. Box 2871, Beijing 100085, China. Plant uptake of organic contaminants (atrazine, DDT and phenanthrene) under the influence of an AM fungus *Glomus etunicatum* was investigated. Inoculation led to an increase in the accumulation of these contaminants in roots but a decrease in shoots. Furthermore, inoculation enhanced the contaminant dissipation in soil, which is consistent with the evidence that inoculation significantly increased bacterial and fungal counts and dehydrogenase activity in the soil. Mechanisms on the influence of inoculation on plant uptake of organic contaminants were investigated by using phenanthrene as the model compound. Studies on sorption and desorption of phenanthrene by roots and characterization of heterogeneity of mycorrhizal and non-mycorrhizal roots using solid-state ¹³C NMR spectroscopy demonstrated that increased aromatic components due to inoculation resulted in enhanced phenanthrene uptake by the roots but lower translocation to the shoots. Direct visualization using two-photon excitation microscopy (TPEM) also revealed higher phenanthrene accumulation in epidermal cells of roots and lower transport into the root interior and stem in mycorrhizal plants than in non-mycorrhizal controls.

P3.6 - Arbuscular mycorrhiza alters coffee seedlings response to increasing Zn concentrations in soil

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The role of mycorrhiza as causing physiological changes which improve plant performance under metal stress conditions has been recognized. The objective was to evaluate the physiological response of mycorrhizal *Coffea arabica* seedlings to increasing soil Zn concentrations. The experiment consisted in a 2x4 factorial scheme: non-inoculation or inoculation of an AMF mixture (*Glomus clarum*, *Gigaspora margarita* and *Acaulospora* sp.) X addition of four Zn rates to soil (0, 100, 300 and 900 mg kg⁻¹ of Zn). Growth parameters; catalase and glutathione reductase activities; free amino acid composition, sucrose, proline, lipid peroxidation, total phenol and amino acid contents in leaves and nutrients contents were analyzed. Mycorrhizal plants showed a better growth in all soil Zn concentrations except for the maximum concentration. Increasing Zn concentrations caused decrease in K, P, Mg shoot concentrations and increase in Cu, Mn, and S in shoots and roots. P:Zn ratios in shoots were significantly higher in mycorrhizal plants. Sucrose contents were higher, but total phenol and free amino acid contents in leaves were significantly lower in mycorrhizal plants. Increasing concentrations of Zn induced foliar catalase activity only in mycorrhizal plants. Free amino acid composition in coffee leaves was influenced by the added metal and AMF inoculation. Increasing Zn concentrations increased contents of glutamine, asparagine and glutamic acid in mycorrhizal plants and decreased lysine content.

P3.7 - Inoculation of arbuscular mycorrhizal fungi and arsenic uptake by plants: implication for As bioremediation

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Arsenic contamination is widespread in soils and waters due to anthropogenic activities or from geogenic sources. In recent years there has been increasing contamination of water, soil and crops by this metalloid in many regions

of the world, particularly in some countries of southern Asia. Experiments were carried out to study the role of inoculation of arbuscular mycorrhizal fungi (AMF) in arsenic uptake by both As hyperaccumulator plant of Chinese brake fern (*Pteris vittata*) and non-hyperaccumulator plants (*Medicago truncatula*, *Zea mays* and *Lycopersicon esculentum*). Our results indicate that AM fungus was more tolerant than plants to arsenate. There were positive effects of arbuscular mycorrhiza inoculation on plant growth and As uptake in *Pteris vittata*. Increased As accumulation in *P. vittata* due to AMF is closely linked with AMF mediated enhanced plant P uptake and the consequent P/As ratios. Mycorrhizal inoculation increased plant growth by enhancing P nutrition and lowering shoot and root As concentrations in maize plants compared with uninoculated plants. Additionally, deposition of As in external mycelium indicates a possible role of mycorrhizal fungi in the detoxification of As in the host plants. In conclusion, our results show that the interactions between AMF and As uptake may follow multiple mechanisms, and more work are required to elucidate the underlying mechanisms. The AMF-mediated effect on As uptake and accumulation in plants may vary with plant species, soil conditions, As supply level or fungal isolates inoculated. AMF may have great potential for clean-up of As contaminated soil by using hyperaccumulators such as *P. vittata*, or for phytoextraction of As from moderately contaminated soils or phytostabilization of more highly polluted sites by non-hyperaccumulator plants.

P3.8 - Sex-specific responses to mycorrhiza in a dioecious species

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In dioecious species, female and male plants allocate resources differently. Females often invest relatively more resources into defense and reproduction, whereas males allocate more resources into vegetative growth. Most plants grow in symbiosis with arbuscular mycorrhizal (AM) fungi. AM are beneficial for the plants as they increase the supply of nutrients and water, but they also represent a cost in terms of the carbon allocated to the fungus.

Plant sexes frequently differ in drought tolerance and AM fungal colonisation has been shown to provide higher resistance to drought. We examined in a greenhouse experiment whether female and male *Antennaria dioica* (Asteraceae) plants have a different relationship with their AM symbiont (*Glomus claroideum*) under two water regimes.

We found that the sexes did not differ in AM colonisation levels. Overall, AM symbiosis was beneficial for both sexes. Phosphorus acquisition was increased due to inoculation and both sexes accumulated similar amount. However, female plants benefited more from AM than males under well-watered condition in terms of biomass accumulation, but both sexes benefited equally when water level was lowered. Therefore, we conclude that the two plant sexes seem to provide equal amounts of carbon to their fungal root symbiont. Furthermore, plants may gain sex-specific benefits from AM symbiosis, which nevertheless may depend on water availability.

P3.9 - Field inoculation Studies of Arbuscular mycorrhizal fungi

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Field inoculation Studies of Arbuscular mycorrhizal fungi Kandru, A.M. ANU, India Arbuscular mycorrhizal fungi are ubiquitous in nature and have been reported from all the soils round the globe. For the past 25 years, I am working on mycorrhizas and brought out the widespread occurrence of a number of AM fungi from different soils and in the root system of crop plants and grasses. The growth responses of AM fungi on a number of crop plants particularly, grasses, cereals and millets were reported. In the present study field inoculation experiments were conducted to evaluate the potential of AM fungi as a biofertilizer. The impact of field inoculation with *Glomus epigaeum* and *G. monosporum* on jowar and pearl millet was studied at different Phosphorus levels in the Botanical garden soil. Of the two endophytes, *G. monosporum* showed highest level of root colonization at 50Kg/ha. Of the two hosts jowar showed greater response than pearl millet. The results will be discussed at length.

P3.10 - Corn (*Zea mays* L.) growth as affected by soil compaction and arbuscular mycorrhiza

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Using agricultural machinery in the field, especially at a high soil moisture, results in soil compaction and hence alteration of soil properties. Evaluating appropriate methods of alleviating the stress of soil compaction on plant growth is of great economical and environmental significance. Data regarding the effects of biological methods such as using arbuscular mycorrhiza (AM) on corn (*Zea mays* L.) growth under compaction is rare; hence this research work was performed under greenhouse conditions. The objectives were to evaluate: 1) the effects of soil compaction on corn growth, 2) if using different species of AM under non-sterilized and sterilized conditions can alleviate the stress of soil compaction on corn growth. The collected field top soil was sieved and half of it was sterilized using autoclave. The soil was then compacted in 20x20 cm pots using 2-kg weights, planted with corn seeds and inoculated with different species of AM species including *Glomus etunicatum*, *G. mosseae*, and *G. intraradices*. Soil and plant parameters including soil resistance to penetrometer and soil bulk density as well as corn root and leaf growth were determined. While high levels of soil compaction decreased corn growth, AM inoculation

significantly enhanced root growth and hence plant growth under compaction. These very important complementary results to the previously rare documented results regarding the topic are of great agricultural and ecological significance.

P3.11 - Extraradical mycelium of arbuscular mycorrhizal fungi decreases cadmium toxicity in the rhizosphere of mycorrhizal plants

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The extraradical mycelium (ERM) of arbuscular mycorrhizal (AM) fungi has a high heavy metal sorption capacity. However, it is unclear whether sorption to ERM may explain higher heavy metal tolerance of mycorrhizal plants. This can also result from mycorrhiza-induced changes in plant growth, nutritional status and root exudation. The aim of the presented study was to confirm that cadmium (Cd) bioavailability is lower in the rhizosphere of AM plants and to distinguish between the contribution of ERM and plant-mediated effects.

Mycorrhizal or non-mycorrhizal tobacco (*Nicotiana tabacum*) was cultivated in quartz sand and the resulting rhizosphere substrates were amended with a range of Cd concentrations. Cd toxicity was evaluated by a biotest. Compartments separated by a nylon mesh, which were accessed by ERM but not by roots, and split-root plants inoculated only at one half of the root system were used to differentiate between the effects of ERM and plant-mediated effects of mycorrhiza.

Cd toxicity was significantly lower in mycorrhizal than in non-mycorrhizal rhizosphere. In the rhizosphere of split-root plants, Cd was more effectively immobilized at the inoculated part of the root system, which indicates that ERM was the dominant factor decreasing Cd toxicity. The results of the biotests were consistent with differences in rhizosphere pH, which could be also attributed to the presence of ERM.

P3.12 - Arbuscular mycorrhizal fungi contribute to the phytoremediation of a highly alkaline anthropogenic sediment through pH amelioration

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Interest in the use of plant-microbe based systems for the restoration of degraded soils and sediments has been increasing. Black alder (*Alnus glutinosa*) can grow and improve soil fertility in disturbed sites. Arbuscular mycorrhizal fungi (AMF) may reduce the negative effects of stresses caused by lack of nutrients, adverse soil structure and extreme pHs. The aim of the present work was to investigate the feasibility of phytoremediation strategies using *A. glutinosa* inoculated with native AMF. The study site was a 10-ha sedimentation pond located in the industrial complex of Estarreja, Northern Portugal, into which 300 000 ton of solid waste residues from the production of acetylene and PVC had been deposited. The pH values of the sediment were found to be very high (between 11.8 and 12.6). Extreme alkalinity, high salinity and low nutrient levels appeared to be the main stresses for plants. A greenhouse experiment was conducted using sediment with *A. glutinosa* seedlings inoculated singly with four different native AMF. After a six-month growth period, plants inoculated with different AMF had significantly greater growth and P concentration. In the treatments of the AMF with greater extraradical mycelium lengths the rhizosphere pH was significantly less alkaline. Inoculation with native AMF resulted not only in improved plant growth and nutrition, but also in the amelioration of the high pH of the alkaline sediment, contributing to its restoration.

P3.13 - Impact of DDT stress on laccase

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The ectomycorrhizal fungus *Xerocomus chrysenteron* (*X. chrysenteron*) in our lab, has showed high tolerance to DDT, 52% degrading efficient within 45 days. Therefore *X. chrysenteron* was chosen in this study as a model fungus for determining the potential key enzyme in the DDT degradation process.

Four oxidative enzymes, which were concerned as key enzymes in DDT biodegradation of fungi, were tested after 15 days cultivation in the solid culture. Three of these enzymes, laccase, polyphenol oxidase and peroxidase were observed in all the cultures. *X. chrysenteron* showed very strong laccase activity in both control and DDT treated cultures. The laccase activity increased along with raised DDT concentration, this result indicates laccase as a potential key enzyme during DDT biodegradation by *X. chrysenteron*.

To study DDT's impact on laccase production, different amount of DDT was added to the liquid culture media on

the 10th day. After 32 days exposure, activity of laccase was significantly higher than control and showed good correlation with DDT concentration. Native-PAGE staining with ABTS illustrated the laccase isozyme patterns after 32 days exposure to DDT. It showed two laccase isoforms in both control and DDT treated cultures, while DDT-treated cultures also showed a third isoform. This result indicated the impact of DDT or its metabolites on laccase production and that laccase might be an important enzyme in DDT's biodegradation by *X. chrysenteron*.

P3.14 - Mycorrhizal colonization in plants growing in lead contaminated soil

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Mycorrhizal fungi can increase both the tolerance to stress induced by high concentrations of metals and protective effects on colonized plant. Lead occurs naturally in plants, but can be harmful on high concentrations, resulting in low development of plant. In order to evaluate the percentage of mycorrhizal colonization, samples of root system. From the surrounding areas of METAIS PB LTDA industry, located in Rio Tinto, Paraíba, Brazil, of four species were collected: vetiver [*Chrysopogon zizanioides* (L.)], jureminha (*Desmanthus virgatus*), grasses (*Poaceae*), cashew (*Anacardium occidentale*). This company operates in the recycling of electrical automotive batteries. To evaluate mycorrhizal colonization it was used the method of plate grid intersection after clearing with KOH and staining with Trypan blue. All species and 100 % of the samples showed mycorrhizal colonization, being jureminha the one that present greatest colonization (83 %) and grass presented the lowest colonization (48 %). The remaining species showed about 55 % of colonization. The mycorrhizal association may be contributing to the greater tolerance of plants growing in the lead contaminated soils. (Financial support: CAPES / FAPEMIG / CNPq)

P3.15 - Studies on arbuscular mycorrhizal profiles in tsunami affected coastal soils : a case study from India

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Studies on Arbuscular Mycorrhizal Profiles in Tsunami Affected Coastal Soils : A Case Study from India K. SAMBANDAN P.G. Department of Plant Science, Avvaiyar Govt. College of Women, Karaikal - 609 602, U.T.of Puducherry, India sambandhan@gmail.com The earthquake triggered tsunami of December 2004 in the Indian Ocean caused unprecedented damage to the coastal environment of peninsular India. Tsunamis are reached the shore with tremendous amounts of energy and stripped beaches of sand together with coastal vegetation. Increased turbidity in the wake of the tsunami was suffocated large coastal areas, killing many organisms that will make the serious consequence on ecosystem. Salination on the lands affected by tsunami is deep and widespread and posing high risks to plants survival and regeneration. The exploitation of soil microbes for utilizing salt stressed lands and saline water is of great importance. Plants and soil organisms will have to cope with this additional intensity of stress in the tsunami affected soil environment. An understanding of how plants may respond to a rising sea level is incomplete without considering role of plant-associated soil organisms. Mutualistic associations between plants and soil fungi, such as arbuscular mycorrhizae have an important role in improving the plant tolerance and growth in stressful environments. However, the role of mycorrhizal fungi in sudden salt stress, particularly tsunami affected soils completely unknown. The present work was investigated the mycorrhizal status in tsunami affected soils of Karaikal District. A total of 35 species of vascular plants screened for mycorrhizal colonization and 14 mycorrhizal fungal species were recorded in the rhizosphere soils based on seasonal field surveys. Physio-chemical analyses of rhizosphere soils showed that all the study sites were alkaline, high Electrical Conductivity and ESP levels. Among the mycorrhizal genus, *Glomus* and *Scutellospora* populations were dominant in this ecosystem. The potential adaptation of this dominant indigenous AM fungi and their ability to colonize saline tolerant plant communities in the coastal regions indicate that mycorrhizal biofertilizers will help to remediate the tsunami affected ecosystem with suitable host plants.

P3.16 - Growth response of ornamental fountain grass (*Pennisetum setaceum*) to mycorrhizae in compost amended media contaminated with NaCl, Na₂CO₃ or CaCl₂.

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This study was conducted in order to determine if inoculation with arbuscular mycorrhizal fungi (AMF) and soil amendment with compost of urban waste (consisting of decomposed grass clippings, leaves, and wood chips) would enhance the tolerance of fountain grass to high level of salinity and alkalinity found in urban soils. Fountain grass is used as bedding plant in urban area very frequently polluted with NaCl, CaCl₂ or Na₂CO₃ – all detrimental for plant growth. AMF-inoculated and non-inoculated plants were planted in growing substrates non-amended and amended with compost and irrigated with water containing 3.3 g NaCl dm⁻³, 10.5 g Na₂CO₃ dm⁻³, or 6.3 g CaCl₂ dm⁻³. Electrical conductivity (EC) of each watering solution was 6.3 S m⁻¹. Plants were watered with salty waters every

week until visual injury of leaves were observed. Mycorrhization improved visual quality ratings of plants grown in substrates polluted with all salt tested. Mycorrhization and compost amendment enhanced dry weight, height and number of inflorescences of plants grown in non-polluted substrates. AMF-inoculation had a non-significant effect on plant growth and flowering in polluted substrates. At experiment termination, total AMF colonization between 0 and 33% was observed in non-AMF plants. Treatment with NaCl and Na₂CO₃ decreased total colonization. Treatment with CaCl₂ did not affect total AMF colonization.

P3.17 - Protective effect of *Glomus clarum* of the root-knot nematode *Meloidogyne incognita* in micropropagated banana plant

Lydice Sant'Anna Meira-Hadad

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The interaction between the *Glomus clarum* and the root-knot nematode *Meloidogyne incognita* and their effects on the growth of micropropagated banana plants was studied. In the first experiment, eggs of nematode were applied to soil in pots in cv. Prata-Anã and FHIA-01 previously colonized by the AMF to evaluate the effect of mycorrhiza of the nematode. In the second experiment, split-roots system was used in which, AMF and nematode eggs were inoculated in the same or different compartments to assess the role of AMF in the induction of resistance to nematodes was localized or systemic. At last split-roots system was used to study the activity of peroxidase (PO) and phenylalanine ammonia lyase (PAL) in the root in different compartments, before (0), 4 and 7 days after inoculation of nematode. AMF promoted the growth of banana's cultivars and reduces the incidence of galls and eggs in the root system. The effect of *G. clarum* is located, compensating the damage cause by nematode infection. The enzymatic activities are higher in the presence of the fungus and, or nematode. So, it can be concluded that AMF promote the growth of micropropagated banana plants, protect them against nematodes with localized effect, and the effect of the enzymes related to stress may contribute to a higher localized protection effect.

P3.18 - Systemic resistance to the nematode *Xiphinema index* induced by a mycorrhizal fungus in grapevine

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The dagger nematode (*Xiphinema index*) can cause severe damage to the root system of grapevines. A glasshouse pot experiment was conducted to investigate the impact of inoculation with *Glomus intraradices* BEG141 of the commonly used grapevine rootstock SO4 (*Vitis berlandieri* x *V. riparia*) at the repotting stage on (i) plant growth and (ii) incidence of *X. index* inoculated 21 days after the arbuscular mycorrhizal (AM) fungus. Mycorrhiza development promoted plant growth and significantly reduced both nematode numbers in the soil and gall number in the root system. Observations from a split-root experiment showed decreased nematode development in both mycorrhizal and nonmycorrhizal parts of mycorrhizal root systems. These data represent the first report of systemic induced resistance to nematodes in AM root systems, and concord with that previously described against fungal pathogens (Cordier et al. MPMI 1998). In order to gain insight into the molecular mechanisms underlying bioprotection by *G. intraradices* against *X. index*, transcription accumulation of general defence and nematode resistance-related plant genes was monitored during interactions in roots. Gene expression levels indicate that bioprotection involves processes related both to general defence and to nematode-specific mechanisms.

P3.19 - Vacuolar zinc storage in zinc sensitive and adaptive tolerant *Suillus luteus* (L.:Fr.) isolates

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Zinc is an essential micronutrient for all organisms but can become toxic when present in excess. The amount of free zinc in the cell has to be precisely controlled to assure growth and reproduction in all circumstances, in spite of the potential toxic nature of the element. Fungi evolved a zinc homeostasis network storing zinc in the vacuole when present in excess and releasing it again in case of deficiency. Zinc sensitive and adaptive tolerant isolates of the ectomycorrhizal basidiomycete, *Suillus luteus*, show variations in their capacity to store free zinc. We isolated two transporters of the CDF-family by genome walking, both are zinc specific and localised to the vacuolar membrane as shown by heterologous complementation assays and visualization of GFP-fusion proteins in yeast. A search of the TRANSFAC v6.0 database identified some general stress and heat shock responsive elements in the

promoter sequences. Transcription factor binding sites unique for one of the promoter regions are also found. Real-time PCR analysis of gene expression revealed differences in the expression of both transporters. Comparing zinc sensitive and adaptive tolerant *S. luteus* isolates, distinct basic transcription levels are found. Identification of the (epi)genetic factor causing variations in vacuolar zinc storage gives insight into the functioning of the zinc homeostasis network and contributes to a better understanding of the mechanisms responsible for adaptive zinc tolerance in *S. luteus*.

P3.20 - Mycorrhiza and copper toxicity: proline accumulation and changes in amino acid composition in leaves of *Coffea arabica* seedlings

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Plant association with arbuscular mycorrhizal fungi (AMF) has shown to attenuate metal stress reducing uptake or deleterious effects of metals on plants. Some amino acids may accumulate in plants under metal stress, suggesting a protective or a regulatory role. An experiment was carried out to study the proline accumulation and alteration in the amino acid profile in *Coffea arabica* seedlings inoculated or not with AMF (*Glomus clarum*, *Gigaspora margarita* and *Acaulospora* sp.) in response to increasing Cu concentration in soil (50, 150 and 450 mg kg⁻¹). At harvest, biomass production, mycorrhizal colonization, foliar Cu, proline contents and amino acid composition in leaves were determined. Mycorrhiza greatly promoted seedlings growth indicating a mycorrhizal dependence of coffee seedlings. Foliar Cu concentrations increased with Cu in soil only in non-mycorrhizal (NM) plants, reaching a threshold of 30 mg kg⁻¹ Cu, considered in the critical level for phytotoxicity. Proline content in leaves increased linearly with Cu in soil with twice proline content in the highest Cu dose in relation to control plants. Mycorrhizal plants growing in high Cu soil showed 20% higher proline content than NM ones. Free amino acids composition in leaves showed significant differences in plants associated or not to AMF. This may be a cause of the different nutrient status of plants or to specific physiological changes during symbiosis. N metabolism was significantly influenced by both mycorrhiza and Cu in soil.

P3.21 - Environmental plasticity of AM fungi with herbaceous plant species

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Arbuscular mycorrhizal (AM) symbiosis is fundamental in terrestrial environments. However, many of its interactions remain to be explored. Firstly, we built a meta-analysis to show that AM symbiosis contributes to toxic metal root acquisition and plant tolerance by two cross-linked processes, the hyphal enhanced uptake and metal-binding. We then tested this model in a carrot root-organ culture experiment which shows that both processes occur independently whether metal levels were low or high. These findings demonstrate that biological processes involved in AM symbiosis can be adjusted to changing environmental conditions. In this context, we would expect the existence of factors influencing the establishment of AM interactions. Strigolactones that are produced in plants act as hyphal branching factors, but still is unknown whether soil salinity triggers their synthesis. Therefore, a second set of greenhouse experiments are underway and further in vitro studies will test spore and hyphal development. Thirdly, the effect of azoxystrobin, a broad-spectrum crop fungicide with the risk of leaching in the environment, was tested on non-target native plants. We postulated that azoxystrobin exerts direct or indirect detrimental effects on plants, depending on their ability to establish AM symbiosis. To test this hypothesis, mycotrophic and non-mycotrophic plant species were grown alone or together under greenhouse conditions. The significance of our results will be discussed.

P3.22 - Arbuscular mycorrhiza on plant growth and protection against heavy metals toxicity in contaminated soil

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We report the results of several experiments conducted in our lab dealing with the effects of several arbuscular mycorrhiza (AM) fungi on growth and protection of different plant species against heavy metal toxicity. A soil from a zinc smelter duping site contaminated by Cd, Cu, Pb and Zn was used in all studies. It was found that contamination adversely affects colonization, but some fungal isolates from contaminated sites were more efficient in promoting plant protection and growth than fungi from non-contaminated soil. In most cases AM beneficial effects were related to reduction in metal concentration in plant shoots. Because of the enhanced plant growth, AM increased metal phytoextraction by 845, 142, 68 and 54% for Cu, Pb, Zn and Cd, respectively, but metals are mostly retained in roots. Application of phosphate to contaminated soil exhibited complementary effect with AM

in alleviating the metal toxicity, but AM effects were not related to increase in P uptake suggesting these factors act independently. We also show that application of formononetin stimulates colonization and reduces metal toxicity for corn. Ongoing researches are evaluating AM applications in land rehabilitation.

P3.23 - Beneficial effect of arbuscular mycorrhizal fungi and indigenous filamentous fungi on chromium accumulation by plants grown on tannery sludge

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Arbuscular Mycorrhizal Fungi (AMF) are known to enhance the metal accumulation in plants. Some authors have also confirmed the existence of synergistic effects of filamentous fungi on the effectiveness of AMF in plant resistance to heavy metals in soils. Therefore, a study was undertaken to find out the response of indigenous AMF trap culture, *Glomus intraradices* and two indigenous filamentous fungi (*Aspergillus niger* and *A. flavus*) separately and in different combinations towards the growth and heavy metal accumulation by five different plants (*Aloe vera*, *Vetiver zizanoides*, *Jatropha curcus*, *Melia azadirachta* and *Dalbergia sissoo*) grown in Chromium (Cr) sludge. The analysis of results revealed that all the plant species showed variations towards metal accumulation. In *Vetiver*, *Jatropha* and *Melia*, filamentous fungi significantly enhance % root colonization through indigenous AMF and *G. intraradices*. Highest accumulation of Cr was found in the plants inoculated with indigenous AMF+A. *niger*, Indigenous AMF and *G. intraradices*. Whereas *Vetiver* and *Aloe vera* inoculated with indigenous AMF +A *niger* and indigenous AMF respectively showed higher accumulation of Cr than other plant species. The application of indigenous AMF + *A. niger* to *Vetiver* and Indigenous AMF to *Aloe vera* found to be most promising solution for phyto-bioremediation in Cr contaminated sites.

P3.24 - Effect of soil salinity on growth and P uptake by *T. alexandrinum* L.

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In a pot experiment, different sources of soluble salts (NaCl , Na_2SO_4 , MgCl_2 and MgSO_4) at 3 levels of salinity were added to a sterilized soil to obtain soil electrical conductivity of 2.2, 5.0 and 10.0 dSm^{-1} . Six clover seedlings (*T. alexandrinum* L.) were transplanted into each pot and grown for 8 weeks. Treatments were 4 kinds of salts \times 3 levels of salinity \times 2 mycorrhiza (with and without *G. intraradices*). The results showed that increasing soil salinity by each above salts decreased shoot d.wt. of both mycorrhizal (M) and non-mycorrhizal (NM) plants, although shoot d.wt. of M plant was greater than that of NM plant. A similar trend was observed for root growth and plant height. The detrimental effects of NaCl on plant growth were much higher than those of MgCl_2 , Na_2SO_4 and MgSO_4 as salinity was increased. The detrimental effects of added salts on plant growth was as $\text{NaCl} > \text{MgCl}_2 > \text{Na}_2\text{SO}_4 > \text{MgSO}_4$. Results of this study, with respect to the specific effects of ions, show that clover plants as a sensitive plant to soil salinity considerably affected by sodium and chloride than by magnesium and sulphate. Increasing soil salinity decreased the percentage of root colonization. This led to a decline in P uptake. Decline in P uptake can be attributed to the reduction in root colonization due to the increase in soil salinity. In fact, the decline in root colonization with increasing soil salinity leads to a decline in surface area of interface for nutrients transfer to the host plant.

P3.25 - Proteomics of *Vitis* root cuttings: a step forward to understanding molecular mechanisms involved in mycorrhizal symbiosis of a woody plant species

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Grapevine vineyard surfaces are increasing every year in Brazil. Due to *Phylloxera* attacks, *Vitis vinifera* has to be grafted onto resistant rootstocks. The rootstock SO_4 (*V. berlandieri* x *V. riparia*) is largely utilized, though it can be attacked by other pathogens. The implementation of arbuscular mycorrhizal fungi (AMF) for controlling root diseases is a promising approach. However, better knowledge of molecular mechanisms involved in symbiotic interactions in woody plants is required. In this context, we have applied canonical 2-DE-based proteomics on total proteins to SO_4 root cuttings colonized or not by *Glomus mosseae* or *G. intraradices*. This has resulted in the largest "mycorrhizal proteome" so far identified in a woody plant species. Many plant proteins were commonly regulated in response to both fungi and a few proteins responded to one or the other.

No fungal proteins could be detected by datamining of available databases. However, about ten *G. intraradices* proteins were identified in the *Glomus* EST database. These proteins belong mainly to stress-induced protein families. We have also undertaken investigations performed in the Brazilian Northeast, the diversity of AMF was low in the studied areas and further studies, including more collections should be performed to confirm the data.

Mixotrophic and Mycoheterotrophic Interactions

P4.1 - Mycorrhizal association on seed germination and seedling acclimatization of *Cyrtopodium cardiochilum* (Orchidaceae)

Pereira, M.C., Guimarães, F.A.R., Oliveira, S.F., Torres, D.P., Felício, C.S., Bocayuva, M., Liparini, O.P., Kasuya, M.C.M. (Universidade Federal de Viçosa, Brazil)

In nature, orchid's seeds need mycorrhizal association for germinating. *Cyrtopodium cardiochilum* is a Brazilian orchid that has high commercial and medicinal value. Xaxim is one of the substrates most frequently used in orchid production, but is threatened for extinction and prohibited to collect in the field. Therefore, the aim of this study was compare seed germination promoted by *Epulorhiza* sp., a mycorrhizal fungus growth, in OMA medium and in asymbiotic media (Knudson C, MS and UFV), and to evaluate seedlings surviving during acclimatization in xaxim, pinus peel, eucalypt peel, sawdust, and corn cob substrates. After 70 d, in the symbiotic medium higher seeds germination and protocorms development was observed, producing seedlings with leaves and roots. Whereas the asymbiotic medium UFV shows better results among asymbiotic media, but just inducing first leaf elongation beginning. Seedlings with 130 d old, produced by symbiotic method, were transferred to the substrates. After 5 months, the best developed of plants (weight, shoot and root) were observed in xaxim, followed by corn cob, pinus peel, and eucalypt peels substrates. However, the greater mycorrhizal colonization was observed in corn cob. It can be concluded that the symbiotic method, and corn cob is an appropriate combination to *C. cardiochilum* seedlings production (Financial support: CNPq and FAPEMIG).

P4.2 - *Epidendrum* (Orchidaceae) and *Epulorhiza* (mycorrhizal fungus) compatibility

Torres, D. P. Pereira, M.C., Guimarães, F.A.R., Oliveira, S.F.O., Silva, C.F., Bocayuva, M. Pereira, O.L., Kasuya, M.C.M. (Unirvesidade Federal de Viçosa, Brazil)

Epidendrum secundum growing in areas of "campo de altitude" of Serra do Brigadeiro State Park (PESB/MG) is frequently found associated to *Epulorhiza* spp., a mycorrhizal fungus, which, based on ITS of rDNA sequence analysis, formed three different groups. The objective of this work was to evaluate the compatibility between *Epulorhiza* isolates and *Epidendrum*, evaluating seed germination ability. Representative isolates from the three groups (6 belong to group1, 2 to group2 and 2 to group3) were co-inoculated with seeds from two populations of *E. secundum* (population1 and 2) and *Epidendrum* sp. (population3 and 4), growing in PESB. After 33 d, seeds from the population1 (grass field, near a scrub) germinated when associated to almost all *Epulorhiza* isolates of group1, and among them, two promoted the development of first leaf. However, seeds from population2 (grass field) didn't germinate when co-inoculated with fungi isolated from the same population, and the maximum stage reached was the shoot initiation, when in the presence of one isolate belonging to the group1. Isolates from group3 were able only to induce the rupture of testa by enlarging embrion of seed of population1. No isolate was able to induce seeds germination of any *Epidendrum* sp. (population3 and 4). *Epulorhiza* isolates belonging to group1 are the most compatible to *E. secundum*, that coincide to the most frequently group found in association with that orchid species in PESB (financial support: Fapemig and CNPq).

P4.3 - Characterization of mycorrhizal fungi of *Epidendrum secundum* (Orchidaceae)

Pereira, O.L.; Pereira, M.C.; Coelho, I.S.; Torres, D.P.; Guimarães, F.A.R. Oliveira, S.F.; Araújo, E.F.; Kasuya, M.C.M. (Universidade Federal de Viçosa, Brazil)

Mycorrhizal fungi diversity of Brazilian orchids has been studied to comprehend the ecology of this symbiosis, which is essential to survival of this plant in the nature, and to develop the strategies to conserve the orchid, the mycorrhizal fungi and their habitats. Morphological and molecular characteristic of twenty isolates, being sixteen *Epulorhiza* spp., isolated from three populations of *Epidendrum secundum*, two *Rhizoctonia* sp., isolated from *Oncidium barbaceniae*, one *Ceratohiza* sp. and one *Rhizoctonia solani*. Multivariate analysis of morphological characteristics shows results similar to molecular characteristics, both ITS-rDNA sequence analysis and ITS-RFLP analysis, distinguishing the three selected genus. Symbionts of *O. pirarense* were grouped with *Ceratohiza* sp. and show ITS sequence similar to *Ceratobasidium* species. Homogeneity was observed among *Epulorhiza* spp. isolates from same population or close populations, however, variability was observed among isolates from large distant locations. *E. secundum* isolates show sequence similarity to *Tulasnella* spp. and were separated in two groups, suggesting that they represents two different species of genus *Epulorhiza*. (Support: CNPq and FAPEMIG).

P4.4 - Uninucleate *Ceratobasidium* sp. is a mycorrhizal fungi of native Brazilian orchid *Oncidium pirarense*

Feliciano, S.O., Pereira, M.C., Torres, D.P., Guimarães, F.A.R., Coelho, I.S., Bocayuva, M., Pereira, O.L., Kasuya, M.C.M. (Universidade Federal de Viçosa, Brazil)

Oncidium pirarense, an orchid that grows in the soil or on the rock, is frequently observed in Campos de Altitude of the Serra do Brigadeiro State Park (PESB), MG. It was previously observed that the root system of these plants was colonized by mycorrhizal fungi. So, the objective of this work was isolating these fungi, to characterize them morphologically, to identify them based on ITS rDNA sequence analysis and to evaluate them according to the capacity to induce the germination of host seeds. Ten mycorrhizal fungi were obtained from intact pelotons observed in roots of *O. pirarense*, which were sampled in the PESB. These fungi presented barrel shaped monilioid cells, abundant aerial mycelia with immersed edge and one nucleus by cell, and were identified as uninucleate *Rhizoctonia* sp. The diameter of the colony and the growth rates, determined in the medium BDA and CMA, and the dimensions of the monilioid cells revealed high variability among the isolates. Based on ITS sequence analysis, the symbionts were identified as belonging to *Ceratobasidium* genus. Representatives fungi isolates tested had the capacity to stimulate *O. pirarense* seed germination. This show that uninucleate *Ceratobasidium* is mycorrhizal fungi of *O. pirarense*.

(Financial support: CNPq and FAPEMIG).

Signal Perception and Metabolism

P5.1 - Root exudates of Annatto in the pre-infection stage of *Glomus clarum*

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In arbuscular mycorrhizal symbiosis, root exudates have an important signaling function in pre-infection stage, releasing factors capable of inducing spore germination and growth of fungus hyphae. The production of these factors can be regulated by the availability of phosphorus (P) in the soil. Our objective were to evaluate the effect of root exudates produced by transformed roots of annatto (*Bixa orellana* L.) to induce the spores germination and to stimulate the hyphal growing assessed by radius and branching of hyphae (total hyphal length) of *G. clarum* in vitro condition. MS culture media, where the annatto transformed roots were cultivated, in the presence or absence of P, during 15d, and was used as root exudates solution, which were distributed onto the agar-water at 1x, 0,66x, and 0,33x, in Petri dishes. The additional treatment using only water was used as control. Spores were arranged in the dishes and incubated for 7d. The highest percentage of spores germination occurred in medium containing 0,33x and the medium under P starvation. In contrast, the lowest percentage of germination was found in water, showing that root exudates are capable of stimulating germination of spores of *G. clarum*. However, no difference on the radius and total hyphal length was observed, which can be associated to high values of standard deviation, the heterogeneity of the spores and the P pattern concentration necessary to root growth (financial support: CNPq, Fapemig and PESCO).

P5.2 - Characterization of GintGLT, a gene encoding a glutamate synthase in *Glomus intraradices*

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Extraradical hyphae of arbuscular mycorrhizal (AM) fungi are able to take up and assimilate both nitrate and ammonium. The primary products of ammonium assimilation in the extraradical hyphae are glutamine, glutamate, asparagine and arginine. Although there is increasing evidence that AM fungi assimilate the absorbed nitrogen through the glutamate synthase/glutamine synthetase, asparagine synthase and the urea cycle, nothing is known about the regulation of glutamate synthase in AM fungi. Glutamate synthase, also known as glutamine:2-oxoglutarate aminotransferase (GOGAT or GLT), is a key enzyme involved in the *de novo* synthesis of glutamate, a molecule that plays a central role in amino acid metabolism. The aim of the present work was to get some insights into the role of glutamate in nitrogen metabolism in AM fungi by studying a *Glomus intraradices* gene putatively encoding a glutamate synthase (*GintGLT*). The full-length cDNA of *GintGLT* is 6524 bp long and was isolated from the extraradical mycelia by using different PCR strategies. The predicted protein has 2099 amino acid residues and is closely related to the GLT homologs of other organisms. Gene expression analyses revealed that *GintGLT* is transcriptionally regulated by nitrogen and carbon. The role of *GintGLT* in carbon and nitrogen metabolism in AM fungi will be presented and discussed.

P5.3 - Metabolomic analysis of arbuscular mycorrhizal symbiosis

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Our goal is to compare the metabolomes of mycorrhizal and non mycorrhizal root exudates and root tissues. We wish to identify the primary and secondary metabolic pathways modified or triggered during the symbiosis. By

examining in parallel the primary and secondary metabolic profiles we also wish to identify some metabolites with potential symbiotic activity.

Hairy roots of *Medicago truncatula* and the AM fungus *Glomus intraradices* (DAOM 197198) are used as models. Exudates or ground tissues of mycorrhizal and non mycorrhizal roots are produced at different times. Organic and water extracts of exudates and ground tissues are prepared by standard methods. Analyses of extracts are carried out by ultra performance liquid chromatography (UPLC) coupled with mass spectrometry (Q-TOF) and data are processed using the comparative softwares markerlynx and metabolynx. Chemical identification of the ions selected by the softwares, showing intensity differences between the two conditions, are predicted using different chemical databases.

P5.4 - Evolutive origin of strigolactones

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Strigolactones have recently been shown to be essential for the establishment of AM symbiosis and to control the development of buds in flowering plants¹. These molecules have been detected in all plants tested so far, even in non mycotrophic plants like *Arabidopsis thaliana*. However, we have no data concerning the strigolactone content in other plants like Gymnosperms, ferns and mosses. Are strigolactones a specific trait of the most recent plant lineage? Were they present in the first green organisms? To answer to this question and to evaluate the role of these molecules in the colonization of the first green plants by ancestors of AM fungi, we investigate strigolactone content from in vitro cultures of green alga (*Chlamydomonas reinhardtii*) and bryophyta (*Physcomitrella patens*, *Lunularia cruciata*). From the culture medium of each organism, we perform classical ethyl acetate extraction and C18 purification. Then we test the purified extracts for their activity on hyphal branching of *Gigaspora rosea* and for the actual presence of strigolactones by Mass spectrometry. Preliminary results indicate that strigolactones are not present in non mycotrophic green algae, but occur in the green lineage up to the bryophyta, which are mycotrophic but do not present axillary buds. These results suggest that the first role of strigolactones could have been to facilitate AM colonization. Strigolactones could have been conserved and positively selected throughout terrestrial plant evolution and more recently used as hormone to control the aerial architecture of plants. ¹ Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pagès V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Bécard G, Beveridge CA, Rameau C, Rochange SF. Strigolactone inhibition of shoot branching. *Nature*. 2008 Sep 11;455(7210):176-7.

P5.5 - Dynamics of inorganic and organic cations during polyphosphate-hyperaccumulation in an arbuscular mycorrhizal fungus

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Arbuscular mycorrhizal (AM) fungi promote the growth of the host plants through enhanced uptake of phosphate (Pi). The fungi take up Pi quite rapidly and accumulate a large amount of polyphosphate (polyP), linear polymers of Pi, as a translocation form through hyphae. Since the massive accumulation of polyP is accompanied by accumulation of a great amount of negative charge in cell, it is hypothesized that the fungi possess a regulatory mechanism that balances intracellular charge. In the present study, the dynamics of inorganic cations and basic amino acid during the polyP-hyperaccumulation phase of an AM fungus were investigated. *Glomus* sp. HR1 was grown with *Lotus japonicus* in the two-compartment culture system separated by a 37 micro nylon mesh bag under P-deficient conditions for 8 weeks. Extraradical hyphae were collected from the hyphal compartment at 1 h intervals after 1 mM Pi application. Na, K, Ca and Mg were increased significantly and synchronously with polyP during the polyP accumulation phase, and the total positive charge of the four cations was quantitatively comparable to the negative charge of polyP all through the accumulation phase. The arginine pool was largest among those of free amino acids, but the levels were constant and far below those of polyP during the accumulation phase. These results suggest that synchronous uptakes of inorganic mono- and divalent cations may be involved in the neutralization of the large amount of negative charge of polyP.

P5.6 - Optimizing extractant concentration for glomalin removal from soil

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Glomalin, a glycoprotein produced by glomeromycotan fungi, is insoluble in water and typically is extracted by autoclaving soil in alkaline solutions of sodium salts. Wright and Upadhyaya (1996, *Soil Sci.* 161:575) introduced a sodium citrate protocol for glomalin extraction that has been used widely and has become a *de facto* standard, even though they suggested that extraction conditions should be optimized for different soils. We examined the effects of extractant concentration on the amount of glomalin removed from soils. We found different optima for

maximum glomalin extraction from different soils by fitting third-order polynomials to amounts of protein removed by a range of extractant concentrations. For example, 250 mM sodium citrate maximally extracted Bradford-reactive substances from a Miami, Florida soil with a slightly basic soil pH. This contrasted markedly with the 50 mM sodium citrate selected by Wright and Upadhyaya, who developed their original protocol with an acid soil. By fitting a third-order polynomial to their data, we predicted that the optimum extractant concentration for their soil should have been approximately 75 mM. Our results affirm Wright and Upadhyaya's suggestion that extractant concentration should be optimized for each different soil in order to assess total glomalin, for example, for the purpose of gauging potential carbon storage or immobilization of heavy-metal pollutants.

P5.7 - Correlates of glomalin abundance and extractability

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In *Soil Biol. & Biochem.* 40:728, we suggested that to estimate total glomalin in soil the Michaelis-Menten equation can be fitted to glomalin concentrations accumulated over successive extraction cycles, and the equation's asymptote used. Additionally, the equation's curvature parameter inversely reflects "extractability", i.e., how readily a soil releases glomalin under a particular extraction protocol. In the current study, we examined the equation's goodness of fit for both Bradford-reactive substances (BRS) and immunoreactive protein (IRP) for 100 different soil samples from 36 sites ranging from the arctic to the tropics. We also examined potential biotic and abiotic correlates of the equation parameters. We found that the equation could be fitted with high coefficients of determination to BRS data for all 100 soils, but could not be fitted to IRP data for 19 soils that had little immunoreactive protein. BRS and IRP extractability diminished with increasing pH among soils and with increasing soil moisture deficit among sites. Asymptotic BRS was significantly correlated with soil organic carbon, but asymptotic IRP was not. Neither site average nor site maximum asymptotic IRP was correlated with latitude, mean annual temperature, mean annual precipitation, or soil moisture deficit, somewhat in contrast to the relationship between IRP and net primary production reported by Treseder and Turner (*Soil Sci. Soc. Am. J.* 71:1257).

P5.8 - A *Medicago truncatula* mutant hyper-responsive to mycorrhiza and defective for nodulation

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One key strategy for the identification of plant genes required for mycorrhizal development is the use of plant mutants affected in mycorrhizal colonisation. We report a new *Medicago truncatula* mutant defective for nodulation but hypermycorrhizal for symbiosis development and response. This mutant, called B9, presents a poor shoot and especially root development with short laterals. Inoculation with *Glomus intraradices* results in significantly higher root colonisation of the mutant than the wild type genotype A17 (+ 20% for total root length, + 16% for arbuscule frequency in the colonised part of the root, + 39% for arbuscule frequency in the total root system). Mycorrhizal effects on shoot and root biomass of B9 plants are about 2 fold greater than in the wild type genotype. The B9 mutant of *M. truncatula* is characterised by considerably higher root concentrations of the phytoestrogen coumestrol and by the novel synthesis of the coumestrol conjugate malonyl glycoside, absent from roots of wild type plants. In conclusion, this is the first time that a hypermycorrhizal plant mutant affected negatively for nodulation (Myc++, Nod -/+ phenotype) is reported. This mutant represents a new tool for the study of plant genes differentially regulating mycorrhiza and nodulation symbioses, in particular those related to autoregulation mechanisms

P5.9 - fHANT-AC gene expression and regulation in the ectomycorrhizal fungus *Laccaria bicolor*

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The expression of the fHANT-AC cluster, nitrate transporter (*Lbnrt*), nitrate reductase (*Lbnr*) and nitrite reductase (*Lbnir*), responsible for nitrate utilization in *Laccaria bicolor*, was studied under different N source conditions. The three genes were shown to be under a common regulation. They were repressed by the presence of ammonium while in nitrate resulted in a high transcript accumulation. The presence of nitrate was shown not to be indispensable for activation of *Laccaria* fHANT-AC because N starvation and growth on urea and L-asparagine also resulted in high transcript levels of these genes. Equally high expression of *Laccaria* fHANT-AC genes was also detected in mycelia grown with different concentrations of L-glutamine. This finding shows that in *L. bicolor* N

metabolite repression of fHANT-AC genes is not mediated by L-glutamine like in filamentous ascomycetes. The expression profile of *Lbnrt* and *Lbnir* was also studied in a nitrate reductase-silenced *Laccaria* strain. No differences were observed with respect to the N source regulation or the degree of transcript accumulation of *Lbnrt* and *Lbnir* genes indicating that the presence of a high activity of nitrate reductase is not essential for *L. bicolor* fHANT-AC expression. The simultaneous utilization of nitrate and organic N sources, suspected by the high transcript levels of *Laccaria* fHANT-AC genes on organic N sources, was indirectly demonstrated by culture medium alkalization resulting from active nitrate uptake.

Mycorrhizal Fungal Population Structure in the Field

P6.1 - ARBUSCULAR MYCORRHIZAL FUNGI (AMF) IN NATIVE PITAHAYA (*Hylocereus undatus* BRITT & ROSE) PLANTS UNDER THREE ENVIRONMENTAL CONDITIONS OF THE DRY TROPIC

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Arbuscular mycorrhizal fungi (AMF) develop symbiotic associations with the majority of vascular plants in natural environment and cultivated plants, what provide a broad range of benefits. Their function is important for the water obtaining and mineral nutrients, nevertheless, it also helps in the protection against the drought or attack of pathogens. The pitahaya is a native cactus of the regions of Mexico and South America that grow in arid and semi arid. The objective of this study was to determine root colonization percentage by AMF in native plants pitahaya under three different environmental conditions in the dry tropic. The sites studied in the field are located in Colima State. Soil and root samples were collected between February and March 2008. Twenty-one samples were pooled into three different areas together according to height above sea level (A: 400 to 800, B: 900 to 1300 and C: 1400 a 1800 m). It was demonstrated an important variation levels of root colonization in native plants pitahaya in each site, however, was much higher and constant in the lower area with higher temperatures and increased water stress (A = 69 to 79%). This means that when the height increases, the rate of colonization decreases (B: 48 to 67, C: is 42 to 53%). These results suggest a strong effect on the height above sea level and environmental conditions on the rate root colonization in plants pitahaya.

P6.2 - Arbuscular mycorrhiza fungi and glomalin as soil quality indicators for the rehabilitation of a riparian forest of Velhas river in Brazil

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A disturbed riparian area of Velhas river was found without vegetation, high soil pH (>7.0), high soil compactation, low organic matter content, low aggregation index and high microporosity. The rehabilitation of this area was performed using native tree species under mycorrhizal inoculation. Its success has been evaluated through plant growth and some soil quality indicators as arbuscular mycorrhizal fungi (AMF) populations, glomalin production, soil organic matter content, soil aggregation and soil porosity. However, this area was reached by fire 18 months after the transplantation and therefore the state before and after the fire was considered as treatments. The morphological and DGGE analyses of AMF communities showed a dominance of the *Glomus* followed by *Acaulospora* species and the plant species presented a selective rizosphere effect over AMF populations. *Mimosa bimucronata* was able to improve significantly the AMF population in their rhizosphere in comparison to degraded area. After 18 months of transplantation there was a significant improvement in the soil organic matter, glomalin-related soil protein (GRSP) concentration, macro and micro aggregates content with a concomitant reduction of microporosity. When the fire reached this area all these parameters were decreased except microporosity that was significantly increased which achieved the same values of degraded area. The presented data confirm the role of AMF and glomalin as soil quality indicators for rehabilitation of degraded riparian areas.

P6.3 - Survey of arbuscular mycorrhizas in preserved and impacted riparian environments

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The rapid decline of riparian areas in the southeast of Brazil due to cattle and human impact has as a consequence increasing risk of flooding. Although riparian zones are complex and important ecosystems and the associated arbuscular mycorrhizal fungi (AMF) have an important role in this vegetation, information about factors affecting AMF is scarce. In the present study, we determined the AM fungal occurrence, glomalin content and soil aggregate stability in three riparian sites differing from each other in terms of more or less natural or impacted river. The "Natural" site was a natural forest (at least 25-30 years old); the restored site was rich in leguminous trees (2 years old); and the degraded site presented vegetation dominated by herbs (Poaceae, Asteraceae). Cattle and human

activities decreased AMF in the disturbed areas compared to the preserved site. Low AMF spore diversity was found in degraded sites and the highest genus richness was found in the preserved area. Among the AMF, *Glomus macrocarpum*, *G. constrictum* and *G. etunicatum* were the most commonly found. Higher AMF sporulation occurred in the rainy period in the disturbed and preserved sites while in the restored sites this occurred in both seasons. Moreover, cattle and human activities reduced production of glomalin and aggregate stability compared to the values registered in the native vegetation, suggesting that they may represent useful parameters for the assessment of soil fertility in riparian areas. Key words: arbuscular mycorrhizas, riparian areas, native species, weeds, spores, glomalin Financial support: Minas Gerais State Agency for Research and Development (FAPEMIG) Process 311/07, Brazil.

P6.4 - Occurrence of arbuscular mycorrhizal fungi in avocado orchards in Mexico

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Mexico is the world's leading producer and exporter of avocados and the state of Michoacan contributes with 93% of the country production, although it is found under seven climatic types and various soil units. The aim of this study was to determine the species diversity of arbuscular mycorrhizal fungi (AMF) in avocado orchards from Michoacan, Mexico. Samples of rhizospheric soil (4 kg) were taken from 13 orchards with and without irrigation. From each sample, a 100 g soil aliquot was used to recover and identify AMF species, and the remaining soil was used to establish trap cultures with corn as the host plant. 49 morphospecies of AMF pertaining to eight different genera were identified: *Acaulospora* (16), *Ambispora* (2), *Archaeospora* (1), *Entrophosphora* (1), *Gigaspora* (3), *Glomus* (13), *Pacispora* (1) and *Scutellospora* (12). Twenty two were found only in trap cultures, six only in the field soils and 21 in both. Considering species abundance and presence and absence, the cluster analysis showed that 77 % of the orchards have similarity index higher than 50%. The most frequently species found under trap culture (in 70 % of orchards) were *Glomus aurantium*, *Glomus mosseae*, *Glomus sp*, *Gigaspora decipiens* and *Scutellospora gregaria*. Our study demonstrated that avocado orchards harbor a high diversity of AMF and to the best of our knowledge, this is the most complete and descriptive document of the AMF found in the orchards of the avocado producing region of Michoacan, Mexico.

P6.5 - Arbuscular mycorrhizal fungi associated with *Aniba perutilis*

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Aniba perutilis or colombian Laurel cumin is one of the most recognized lumber species in the international market for its resistant wood, its long duration and its excellent quality in the construction industry. As a consequence of these conditions, there is overexploitation, that summed to the difficulty of its propagation, conduce it to be in danger of extinction. This tree grows very slowly and in an individual way, in cloudy primary forest or rain forest conditions; but sometimes they also grow in groups over the edges of the mountains, where they have established a vital correlation with their ecosystem. There is few information about the mycorrhizosphere of this native species, for this reason we opt to isolate, identify, propagate and conserve the AMF associated to *A. perutilis*. In this study, soil samples and roots associated with this plant were collected and analyzed. The samples were extracted from forest located in the footpaths of Casa de Zinc and La Miranda, Santiago Perez, Tolima, and the Nirvana Reservation, Pradera, Valle del Cauca. In this work were isolated NM genus *Glomus*, *Gipaspora*, *Acaulospora*, *Entrophosphora*, *Scutellospora*. The analysis showed that from the AMF, *Glomus* was the most abundant genus with the 75.37%, followed by *Acaulospora* with 20.1%.

P6.6 - Effects of botanical and synthetic pesticides on the structure and function of arbuscular mycorrhizal fungi

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In agricultural systems with limited inputs the use of arbuscular mycorrhizal fungi (AMF) is promising. Pesticides of botanical origin are allowed and considered safe in such systems, however, their effects on non target organisms, such as AMF, are not known. We conducted a pot experiment to investigate the effects of azadirachtin, spinosad, pyrethrum, and plant-derived terpenes, on the ability of a mixed AMF inoculum to colonize the roots of pepper plants. The fungicide carbendazim and non-pesticide treated controls with or without AMF were also included. Pesticides were soil applied prior to inoculating with AMF. Plant growth and AMF root colonization were measured 20 and 40 days post inoculation. Effects of pesticides on the structure of the AMF community were determined via PCR-DGGE in plant roots. AMF inoculation did not significantly promote plant growth compared to non AM controls. Application of carbendazim completely inhibited AM fungal root colonization, while this was not

associated with an inhibition of plant growth; terpenes had a transient inhibitory effect on root colonization, although this coincided with a significant promotion of plant growth. In contrast, pyrethrum and spinosad resulted in a late inhibition of root colonization. PCR-DGGE analysis showed that more AMF colonized the roots with time, and that azadirachtin hampered colonization by a member of the AMF community, compared to the other botanicals which did not have any effect.

P6.7 - Arbuscular mycorrhizal fungi in agricultural soil under disposal of tannery sludge

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The impact of tannery sludge application in agricultural areas on the arbuscular mycorrhizal fungi (AMF) was evaluated. The field experiment received increasing doses of tannery sludge application (0; 2,3; 9,0; 15,8 and 22,6 Mg ha⁻¹). Three months after superficial application, the sludge was incorporated by ploughing, followed by the sowing of corn. The soils were sampled 12, 138 and 271 days afterwards. AMF spores were counted and identified after extraction by wet sieving of the soil sample. The mycorrhizal colonization was estimated by the gridline intersect method after clearing and staining of roots. The root colonization rate by AMF was high (~60%), but there was no difference among the treatments. The spore density was low (between 1 and 50 spores 50 g⁻¹), and always decreased with the increase of the doses. Seventeen AMF species were identified.

P6.8 - Mycorrhization of teak (*Tectona grandis* L. F) in the Caribbean region of Colombia

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The mycorrhiza of Teak was studied on La Gloria plantation in Magdalena department, Colombia. The effect of the age, the soil and the climate period on the mycorrhization of teak were determinate. We work with trees of one, three, five and 17 years old, established in high soils (143-168 m.o.s.l) and low soils (151-216 m.o.s.l), on dry and wet periods. Mycorrhization percentages (%F), mycorrhizal intensity in the radical system (%M), intensity in the mycorrhizae colonization on radical fragments (%m), abundance of vesicles in the radical system (%A), abundance of vesicles on colonized fragments (% a), length of external mycelium (ME), abundance of spores (ESP) and its relation with organic matter concentration (%MO), available (Pd) and soluble phosphorus (Ps), total nitrogen (%N) and pH were evaluated. The five years old individuals, located in high soils and evaluated in the rainy period presented the highest values for %M, meanwhile the trees of 17 years old, located in high soils and evaluated in the rainy period, presented the highest values for the mycorrhiza in the radical fragments (%m) and for the vesicles abundance in the radical fragments (%A). It was found negative correlation between pH and ESP (-0,574; p= 0,0016) and positive correlation, high and significative, between the percentages of mycorrhization. No correlation was found between the variables of mycorrhiza and %CO, Pd nor Ps. The mycorrhizal variables, external mycelium and spores had seasonal variation. Several morphotypes was found in teak soils, *Acaulospora mellea*, *Acaulospora delicata*, *Acaulospora sp.*, *Acaulospora rehmi*, *Acaulospora sp.*, *Glomus sp.*, *Glomus coremioides*, *Glomus sinuosum*, *Diversispora spurca*, *Cf. Glomus*, *Cf. Pacispora*. Find mycorrhizae with the described characteristics suggest the necessity of association management on plant material of *T. grandis* L.F. from the initial stage of establishment in greenhouse and plantation establishment of in the studied soils.

P6.9 - Arbuscular mycorrhizal fungi in desertification nucleus (Cabrobó, PE, Brazil)

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The various nuclei of desertification in Brazil has never been studied in regard to the presence of arbuscular mycorrhizal fungi (AMF). However, knowledge about AMF in degraded areas is needed considering their potential application in management programs. Thus, the diversity of AMF in a semiarid area in process of desertification in Northeast Brazil (PE) was assessed. Soil samples were collected in three areas, two of them already degraded: (a) low degree of desertification; (b) high degree of desertification; and one (c) preserved, without signs of desertification. Glomerospores were extracted from soil and morphologically identified. Forty-four species were registered in the following genera: *Acaulospora* (10), *Ambispora* (2), *Cetranspora* (1), *Dentiscutata* (1), *Entrophospora* (1), *Fuscutata* (2), *Gigaspora* (2), *Glomus* (16), *Kuklospora* (1), *Pacispora* (1), *Paraglomus* (1), *Racocetra* (2) and *Scutellospora* (4). Areas with high and low degrees of desertification presented higher number of species (27 and 23, respectively) than the preserved area, where only 12 taxa were found. Sporulation usually is higher under stress conditions, as those of the desertification areas, increasing the availability of propagules for study. Moreover, the high levels of P (150 mg/dm³) found in the preserved area, opposite to the degraded areas (maximum 12 mg/dm³), is probably affecting the occurrence, diversity, and distribution of the arbuscular mycorrhizal fungi in these soils.

P6.10 - A COST 870 workshop on molecular ecology of AM fungi

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The COST Action 870 workshop (held at INRA, Dijon, May 2009) provided a unique opportunity by which to discover the variation and distribution of arbuscular mycorrhizal (AM) fungi across Europe and how this is affected by soil physiochemical properties. Participants in the workshop from 17 countries provided *Plantago lanceolata* root tissue and soil samples from three different agricultural or natural sites within their local area. Root samples were initially stained for AM fungi colonisation, with the degree of colonisation for each locality recorded. This showed that all the root samples were colonised by AM fungi but the variability of the intensity of root colonisation was high between the different countries. AM fungi present within root samples were identified using nested PCR targeting ITS and LSU rDNA nuclear sequences using primers specific for different genera or different species. In addition, a novel combination of nested PCR/RFLP analysis was used for the discrimination of *Glomus intraradices* isolates using mtLSU sequences. This study indicated that *Glomus* species like *G. intraradices* were present in all the samples whilst the distribution of other AM fungal genera and species was more variable. Statistical analyses will be carried out to determine the link between soil physico-chemical properties, root colonisation by AM fungi and AM fungal biodiversity.

P6.11 - Diversity and density of spores of arbuscular mycorrhizal fungi under different land use systems

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This work aimed to investigate arbuscular mycorrhizal fungi (AMF) species diversity and spore density in a low-fertility Oxisol under six different land uses at southern Brazil. Soil was sampled in November 2007 (0-10 cm) in native forest (NF) (Atlantic forest with *Araucaria angustifolia*); reforestation with *A. angustifolia* (AR) or *Pinus eliottii* (PI), secondary forest (SF), clear cutting of *P. eliottii* (CT), and an agricultural site with annual crops (AG). Spores were extracted by wet sieving and flotation in sucrose, and counted under stereomicroscope at 40x. Spores morphologically similar were fixed on slides with polyvinyl lactic acid (PVA) and Melzer reagent for taxonomic identification. The relative and absolute frequencies for each AMF species and the Shannon index (H') of diversity were calculated. Four families and 21 species were found, from which 19 were in NF, 20 in AR, SF and CT, 15 in PI, and 14 in AG. The largest spore density occurred in PI (155 spores/g soil), while the lowest were in NF (45) and AG (28). The sites with *A. angustifolia*, either native or reforested, had the highest Shannon index ($H' = 2.25$ and 2.24 for NF and AR, respectively), in contrast with PI or AG. Acaulosporaceae predominated in the forest sites, while Glomeraceae predominated in AG. Gigasporaceae was the least predominant family and was absent in NF and PI. We concluded that AMF species diversity was more impacted by land uses with anthropogenic influence than spore abundance.

P6.12 - Ectomycorrhizal fungi: the genus occurring associated to *Eucalyptus* spp. in Mato Grosso State, Brazil

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The genus *Eucalyptus* includes many species and has high economic importance to Brazil. Many species of ectomycorrhizal fungi have been found associated to *Eucalyptus* spp. in other regions of Brazil. The aim of this work was to evaluate the ectomycorrhizal fungi (ECMF) occurring in commercial plantations of *Eucalyptus* spp. in the Central region of Mato Grosso State, Brazil, which present sandy soil. *Scleroderma* spp. was the unique genus found. Some species of this genus has high specificity with *Eucalyptus* and can form hypogeous, sub-epigeous, or epigeous mushroom. All basidiocarps presented yellow color and were 4-6 x 6-16 mm in size and form epigeous mushroom. The morphological characterization of ectomycorrhizal root tips also indicated low diversity and distribution of ECMF and all analyzed ectomycorrhizas seem to belong to *Scleroderma* spp. genus.

P6.13 - Effect of agricultural practices on growth and diversity of arbuscular mycorrhizal fungus in lily (*Lilium longiflorum* Thunb) cropping.

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Growth and diversity of arbuscular mycorrhizal fungus (AMF) in a lily cropping in Veracruz, Mexico was assessed under seven treatments with three replicates in a randomized block design: a rotation with jicama (*Pachyrhizus erosus*) and inoculation of native AMF; a rotation with mucuna (*Mucuna pruriens*) and inoculation of native AMF;

a rotation with jicama; a rotation with mucuna; solarized soil plus manure addition; chemical fertilizer; and the control. Root colonization was measured in the treatments with legumes in rotation two months after inoculation. Root colonization in lilies, spores number, Shannon and Simpson diversity index of AMF were determined for all seven treatments five months after lily sowing. Percentage of root colonization in the inoculated rotation treatments was statistically different (55.53% for jicama and 52.27% for mucuna) than the non-inoculated ones (20.7 % and 23.04% respectively). A similar trend was observed for lily roots in the same treatments (84.1% 81.6% in comparison to 21.43% and 21.29%). Eleven morphospecies were identified pooling all treatments with significant differences for Simpson index. Spores number highest value was found in the control (1058/50 g soil). Results show that inoculation throughout legumes in rotation with lilies might be an alternative to the reintroduction of AMF to disturbed soils.

P6.14 - Abundance and diversity of ectomycorrhizas in native and nursery cultivated *Nothofagus nervosa* specimens

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The establishment, survival and growth of different tree species depend on ectomycorrhizas (ECM). *Nothofagus nervosa* is a Patagonian species that was overexploited and its populations were reduced, determining the implementation of domestication programs. ECM can be important for the successful establishment of outplanted seedlings, so they must be considered in these programs.

The aim of the project is to analyze the abundance and diversity of ECM in *N. Nervosa* and to select strains which would have significant application in domestication processes. The first step was to analyze the percentage of ECM and to compare it among plants of different ages belonging to 4 categories: seedlings, young and old native individuals and young nursery cultivated plants (NCP). Then, ECM were classified in different groups according to its macromorphology, so as to evaluate if the ECM composition changed among plant categories.

In autumn 2008, 5 trees of each category were selected from a native forest and from two field trials: one established within a native forest and the other beneath a *Pinus* plantation. Seedlings were collected with complete root systems and the rest of the plants were sampled by taking 3 soil cores per tree. Roots were sieved from the soil cores and ECM were quantified and classified.

Every sample had ECM. In the native forest, more than 90% of the root tips examined per plant were colonized and 22 morphological groups were described, 18 of them were present in the NCP. Young NCP established under the exotic plantation showed a significantly lower colonization (~67%) and only 3 ECM groups were observed. ECM abundance and composition varies between these places, being the percentage values and the biodiversity much higher in the native forest.

P6.15 - Diverse tomato cropping systems affect arbuscular mycorrhizal fungal community diversity and structure

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In conventional agricultural systems, AMF are greatly affected by factors including soil disruption, intermittent lack of host root tissue, and plant inhibition of AMF colonization due to high soil fertilization. To determine the effect of diverse agricultural land management and crop production practices on the AMF community structure and diversity, five tomato crop production systems consisting of bahiagrass pasture cover, conventional, continuous removal of vegetation (disk fallow), organic, and undisturbed (weed fallow) were initiated. The plots were adjusted to the new management regime, except for conventional, for three or four years followed by one or two years of tomato cropping. Soil DNA samples were taken in the off season, at planting, and after harvest. Phylogenetic analysis of AMF 18S rDNA sequence combined with multivariate statistical analysis using PRIMER-E was used to compare community structure and diversity. Initial analysis shows that bahiagrass, weed fallow, and organic land management practices support different, diverse AMF communities, while disk fallow and conventional practices greatly reduced detection of AMF sequences. Tomato cropping caused the emergence of common sequences for the *Glomus mosseae* group, in all cropping systems. Bahiagrass and weed fallow diversity were unaffected by the emergence of the *G. mosseae* group, while organic, conventional and disk fallow all converge on a low diversity community dominated by the *G. mosseae* group. Current analyses will determine if the shift in AMF community caused by tomato cropping in organic and bahiagrass plots is seasonal or persistent, and if other factors such as soil fertility and disease incidence correlate to these community changes.

P6.16 - Mycorrhizal community of a relict Balkan shrub species from the upper forest border

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At the upper forest border several shrub species can influence soil aggregate stability and prevent soil erosion. We have concentrated our studies on a relict species from the Dinaric Alps (*Sibiraea altaiensis* var. *croatica* (Degen) Beck.) from the mountain region SW from Mostar in Bosnia and Herzegovina, growing in abandoned pastures invaded by several native pine species. Morphological and anatomical characteristics of mycorrhiza have been studied and identifications were done through single tip (PCR-ITS-RFLP and sequencing) and community structure (DGGE and sequencing) analysis. In non-ectomycorrhizal roots also AM staining procedures were applied. On roots and in the rhizosphere of *Sibiraea* we found ectomycorrhizal and ericoid mycorrhizal isolates, some reported previously as endophytic (including dark-septate endophytes), 'root-associated fungi' and as orchidaceous fungi. The array of root-associated and mycorrhizal fungi of *Sibiraea* (*Rosaceae*) can be linked to its pioneer characteristics, stabilizing the pastures and ski slopes at the upper forest zone.

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P6.17 - Diversity of arbuscular mycorrhizal fungi in natural and disturbed *Polylepis australis* forests in Central Argentina

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Forests dominated by *Polylepis* belong to one of the most endangered tropical and subtropical high mountain forest ecosystems of the world. *Polylepis australis* is the southernmost species of the genus and is endemic to Northern and Central Argentina. One of the most important groups of microorganisms in ecosystems for its implication on plant-soil processes and restoration of degraded soils, are the arbuscular mycorrhizal fungi (AMF) belonging to the Phylum Glomeromycota. AMF diversity is severely affected by disturbance to which some AMF are particularly sensitive. We evaluate the diversity of AMF of *P. australis* in three forests: a semi-natural forest which is relatively undisturbed (Quebrada del Condorito National Park) and two disturbed forests areas (Los Gigantes). Soil samples collected during winter show that *Glomus* spp. (*G. brohultii*; *G. mosseae*) predominate in disturbed forests; meanwhile *Acaulospora* spp. (*A. bireticulata*; *A. mellea*) in semi-natural forests. Considering the predominance of AMF in *P. australis* woodlands and the potential benefits that these associations may bring to plant establishment, AMF should receive special attention in reforestation projects for these woodlands.

P6.18 - Spatial patterns of mycorrhizal fungal genets in the field: evidence of vertical niche partitioning by two closely related species of *Rhizopogon*

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There is mounting evidence that the mycelia of fungal genets colonize and link the roots of multiple trees, forming a mycorrhizal network through which resources may be transferred. However, there is little known about the extent and continuity of fungal genets over space and time. We determined the fine-scale spatial distribution of *Rhizopogon vesiculosus* and *R. vinicolor* genets in a mixed-age interior Douglas-fir forest using multi-locus microsatellite DNA analysis. Three-dimensional patterns of 20 cm³ soil blocks were sampled to determine the resolution and scale at which individual genets existed. In each soil block, *Rhizopogon*-like hyphae or rhizomorphs and tuberculate ectomycorrhizas were isolated and subjected to DNA analysis so as to identify and map genets. *Rhizopogon vesiculosus* genets were encountered significantly deeper than *R. vinicolor* genets. We observed spatial overlap between the two *Rhizopogon* species, but not between different genets of the same species. The vertical partitioning exhibited by these closely related species may explain their co-occurrence within Douglas-fir forests despite their shared morphology and ecology. While *R. vesiculosus* was highly continuous across space, the occurrence of *R. vinicolor* appeared more sporadic, thus likely representing a collection of fractioned ramets rather than continuous genets.

P6.19 - Diversity and function of the arbuscular mycorrhiza in a fifty year old long term experiment

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The structural and functional diversity of arbuscular mycorrhizas depend on their response to both environmental and soil attributes and to anthropogenic stressors particularly management practices and land use. The possible functional role provided by mycorrhizal biodiversity is increasingly replaced by chemical and mechanical inputs as tillage practices intensify. The extent of these anthropogenic inputs and the subsequent response by AM to changing habitat can translate into a continuum of changes in the composition of the mycorrhizal populations.

We compared the diversity and function of arbuscular mycorrhiza in monoculture and corn legume rotation plots established in 1956 on Brookston clay loam, a poorly drained lacustrine soil located at Woodslee, Ontario. Over a period of five years we used morphological-molecular- and biochemical methods for characterization of the AM population. The most frequent morphotypes in both treatments were the small white to yellow spores, identified after nested PCR and sequencing to *Glomus intraradices*. They made more than 2/3 of the spore population. Besides *G. mosseae* the second frequent species, we detected a noticeable high abundance of *Gigaspora gigantea*.

The influence of the different AM communities on photosynthetic and root enzyme activity of corn were tested in climate chamber experiments.

P6.20 - Effects of irrigation on the functional and structural diversity of ectomycorrhizal fungi

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Several thousand fungal species live in ectomycorrhizal symbiosis with trees. What is the functional significance of this diversity for the host plant? It has been shown that the community structure of ectomycorrhizal fungi (ECM) can change when exposed to drought. What is the functional significance of this change for the host tree? To study these questions, various root samples are collected in the context of an irrigation experiment which is conducted in one of the driest valleys in Switzerland. The ECM community structure is investigated by morphotyping and ITS sequencing. Furthermore, the activities of exoenzymes on these ECMs are analyzed. A microplate fluorimetric assay which can be applied on individual ectomycorrhizal tips is used. First results indicate that the ECM community differs between spring and fall, but not between the treatments. The enzyme activities vary among the different species. E.g., *Cantharellus lutescens* shows a strong increase on the Chitinase activities for the irrigated sites, whereas *Lactarius deliciosus* exhibits a decrease on this enzyme. The differences regarding the total activities when comparing control and irrigated sites are dependent on the enzymes: For instance Phosphatase activities are significantly decreased in the irrigated sites. In contrast, Chitinase activities show an increase. Thus, the functional structure of the ectomycorrhizal community as well as the community itself seems to change.

P6.21 - Morphological and molecular characterization of ectomycorrhizas occurring in commercial plantations of *Eucalyptus grandis*, in Minas Gerais, Brazil

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The ectomycorrhizal fungi species composition has been hardly studied in many forest ecosystems and in many cases, these studies have revealed that composition communities of ectomycorrhizal differ from the sporocarps. Thus, to elucidate the ectomycorrhizal fungi ecology it is necessary to study not only the potential sporocarp of ectomycorrhizal fungi, but also the vegetative structure, like the ectomycorrhiza and mycelia in the soil. Molecular markers based on DNA polymorphism are useful in studies of fungi molecular taxonomy, facilitating their typing and identification into genera and species. The sporocarps and ectomycorrhizas were collected in plantations of *Eucalyptus grandis* and were characterized macroscopically. Fungi genera were observed, such as *Hydnangium*, *Laccaria*, *Pisolithus*, *Thelephora* and with higher frequency, *Scleroderma*, both hypogeous and epigeous habit. Collected ectomycorrhizal samples shows the typical structures like mantle and Hartig net. The basidiocarps and ectomycorrhizas rDNA ITS region was amplified. All samples present a fragment of 650 pb, which was cleaved with restriction enzymes: *EcoRI*, *XhoI*, *EcoRV* and *EcoRI / RsaI*, the same pattern of epigeous ectomycorrhizal fungi of *Scleroderma* sp., showing the dominance of this fungus colonizing eucalypt in the commercial eucalypt plantation of Minas Gerais, Brazil.

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P6.22 - Arbuscular Mycorrhizal Fungi in natural and disturbed *Polylepis australis* forests in Central Argentina

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Forests dominated by *Polylepis* belong to one of the most endangered tropical and subtropical high mountain forest ecosystems of the world. One of the most important groups of microorganisms in ecosystems for its

implication on plant-soil processes and restoration of degraded soils, are the arbuscular mycorrhizal fungi (AMF) (Glomeromycota). AMF diversity is severely affected by disturbance to which some AMF are particularly sensitive to human activities. We evaluate the diversity of AMF of *P. australis* in three forests: a semi-natural forest which is relatively undisturbed (Quebrada del Condorito National Park) and two disturbed forests areas (Los Gigantes). An inventory of AMF spores was done taking samples during winter. Results show that there is a high AMF diversity in the undisturbed semi-natural forest. Results also pointed out that spores belonging to *Glomus* spp. (*G. brohultii*; *G. mosseae*) predominate in disturbed forests (75%); meanwhile *Acaulospora* spp. (*A. bireticulata*; *A. mellea*) in semi-natural forests (60%). Considering the predominance of AMF in *P. australis* woodlands and the potential benefits that these associations may bring to plant establishment, AMF should receive special attention in reforestation projects for these woodlands.

P6.23 - Comparative functional diversity of ectomycorrhizal fungal communities in contrasted physico-chemical environments

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Do species assemble because of their diversity in functions, and can most of concepts in community ecology classically based on taxonomic diversity be extended towards functional diversity? Ectomycorrhizal fungal ones, may be a "guinea pig" for most needed advance in a better understanding of interactions between functional and taxonomic diversity. Therefore, an experiment has been implemented, with 8 locations within three countries of Western Europe (France, Germany, Switzerland, Mediterranean, Oceanic and Continental climate), and several controlled modalities of modifications of physico-chemical environment: irrigation, water exclusion, fertilization, compaction, ozone deposition and host effect. Complete inventory of taxonomic diversity and abundances of ECM communities has been performed (morphotyping and molecular identification), and activities of eight enzymes have been measured on root tips with similar protocols. We will present the first results of this work, supported by multivariate statistical analysis, on three aspects: first, how does a change in a physical environment change the activity of a species, or of a community; second, how activities of different enzymes of the same species in the same environment are correlated or not, and how this varies in time (up to six campaigns); third, having those results in mind, how is it possible to define and measure a functional activity at the community level, taking into account taxonomic diversity, abundances and specific enzymatic activities.

P6.24 - Arbuscular mycorrhizal diversity and root colonizaton potential of agricultural soils from Doukkala regions, Morocco

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The diversity of the arbuscular mycorrhizae flora in semi-arid agricultural soils of the Doukkala region, Morocco and the impact of soil physico-chemical factors on their root-colonization potential has been studied. Both selected sites: Ouled Ghanem (sandy soil) and Sidi Bennour (muddy soil), possess neutral to alkaline substrates, with relatively low levels of organic matter and low to medium available P contents. The comparative analysis of the arbuscular mycorrhizal flora reveal a certain amount of shared species and is characterized in sandy soils by a preponderance of Diversisporales: *Scutellospora calospora*, *Acaulospora cavernata*, *Entrophospora infrequens*, *Gigaspora margarita* whereas in the muddy soils a majority of Glomerales (*Glomus* spp) is found. Correlation between physico-chemical and biological soil data enlightened the negative impact of alkalinity and available phosphorus on the soil root colonization potential. The data analyses bring in a fresh look at mycorrhizal fungi biodiversity as being influenced by soil edaphic factors and, in return, having a potential impact on soil root colonization potential.

P6.25 - Diversity of AMF isolated from *Araucaria angustifolia* forest soils using *Mimosa scabrella* and *M. bimucronata* as trap plants

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Araucaria angustifolia is under the risk of extinction and its reforestation in consortium with legumes as *Mimosa scabrella* Benth and *Mimosa bimucronata* (de Candolle) Otto Kuntze may stimulate the regeneration of Brazil Pine forests. These leguminous trees fix nitrogen in symbiosis with β -rhizobia, however, little is known about their association with arbuscular mycorrhizal fungi (AMF). The objective of this study was to identify the AMF associated with these plants in the *Araucaria* ecosystem. In a greenhouse experiment both *Mimosa* species were used as trap

plants, growing in soils sampled from araucaria forests in three different preservation stages: Preserved native Forest (FN), reforested area (R), and reforested and burned (RQ). Considering all treatments, the recuperated spores could be classified as: *Acaulospora laevis/koskei* (the most abundant species), *A. morrowiae*, *A. foveata*, *A. sp1*, *Archaeospora leptoticha*, *Gigaspora sp1*, *Glomus sp1*, *G. sp2*, *Paraglomus occultum*, *Scutellospora sp1*, *S. calospora*, *S. verrucosa/persica*. AMF richness varied according to the trap plant and to the preservation stage of the forest. *M. scabrella* recuperated a lower AMF species number (FN 4, R 2, RQ 1) than *M. bimucronata* (FN 6, R 4, RQ 3). Thus, the greatest richness was found in FN, followed by R and finally by RQ. This result indicates that diversity of AMF was reduced in degraded areas, probably because of reduction of plant diversity.

P6.26 - Density and diversity of arbuscular mycorrhizal fungus an oxisol of Cerrado (Savannah) under different system of management of the soil

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The present work aimed at to evaluate the diversity of arbuscular mycorrhizal fungus (AMFs) in different systems of management of the soil, in a Oxisol in a low-fertility of Cerrado (Savannah), located, in the experimental farm of the Federal University of Goiás (UFG) - Campus Jataí, Brazil. Ten samples were collected in three systems of cultivations (coffee plantation, no till and pasture) and native Savannah. They were recovered a total of 42 species of the AMFs, being 18 *Acaulospora*, 10 *Scutellospora*, 07 *Glomus*, 06 *Gigaspora* and 01 *Paraglomus*. Of all of the found species, 10 could not be identified, what can be an indicative of potentials new species. The species *Gigaspora decipiens* and *Gigaspora margarita* were found in all of the areas. The systems of coffee presented smaller diversity of species, while the areas of direct planting and pasture were the ones that presented larger diversity. Regarding the index of Shannon-Weiner, it was obtained larger value in the systems pasture and a better distribution of the species in the samples. We can say, then that, as larger the intensity of management of the soil, minor will be the diversity of AMFs, could influence in the behavior of the vegetable species, presents in the area.

P6.27 - Arbuscular mycorrhizal fungi in different ecosystems on Tibetan Plateau

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The Tibetan Plateau is a unique biogeographic region where various landscapes, altitudinal belts, alpine ecosystems, and endangered and endemic species have developed. An investigation on the diversity of arbuscular mycorrhizal fungi (AMF) was carried out on the Tibet Plateau began in 2003. The distribution and community composition of AMF was compared among various ecosystems including grasslands, mountain areas, wetlands and agricultural lands. Arbuscular mycorrhiza occurred in plant roots in all the ecosystems investigated, including highland wetlands in Qomolangma, Lhalu wetland, Sejila Mountain. Percentage root length colonized and arbuscule abundance were higher in Rosaceae and Compositae, and plant species belonging to plant families typically considered to be non-mycorrhizal such as Cyperaceae, Juncaceae, Polygonaceae and Caryophyllacea were found to be mycorrhizal to some extent. 70 AMF species were found and *Glomus* was the dominant genus across all the ecosystems examined. However, species richness and spore density differed among different ecosystems, following the sequence: mountain areas wet grasslands agricultural land. Some AMF genera and species were found only in certain areas or at certain altitudes, indicating high adaptation to specific habitat.

P6.28 - Long-term phosphorus fertilization impacts on AM fungi and other soil microbial communities in alfalfa rhizosphere

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Soil function may be affected by cropping practices impacting the soil microbial community. The effect of different phosphorus (P) fertilization rates on soil microbial diversity was studied in 8-year-old alfalfa monocultures. The hypothesis that P fertilization modifies soil microbial community was tested using denaturing gradient gel electrophoresis (DGGE) and phospholipids fatty acid (PLFA) profiling to describe soil bacteria, fungi and arbuscular mycorrhizal (AM) fungi diversity. Soil parameters related to fertility were also monitored and related to soil microbial ribotype profiles. Change in soil P fertility with the application of fertilizer did not modify crop yield but was associated with shifts in the composition of fungal and bacterial communities without affecting their richness, as evidenced by the absence of effect on the average number of ribotypes detected. However, variation in soil P level created by a history of differential fertilization did not significantly influence AM fungi ribotype assemblages. Fertilization increased P flux and soil soluble P level but reduced soil microbial activity, as revealed by

dehydrogenase assay. Results suggest that soil P fertility management could influence soil processes involving soil microorganisms. Seasonal variations were also recorded in microbial activity, soil soluble P level as well as in the abundance of specific bacterial and fungal PLFA indicator of soil microbial biomass.

P6.29 - Arbuscular mycorrhizal fungi diversity in conventional and organic vineyards of Central Chile

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The study and management of microbial interactions in the soil-plant interfaces play a key role in low-input-based viticulture. The objectives of this study were to study and characterize indigenous arbuscular mycorrhizal (AM) fungal communities in six conventional and organic vineyards in Central Chile. The study was carried out between 2007 and 2008 at three localities: Cauquenes 35° 54' S; 72° 04' W, Sagrada Familia S 35° 02' S; 71° 16' W and Chimbarongo 34° 43' S; 71° 02' W. Wine grape cultivars Cabernet Sauvignon, Chardonnay, País, Carménère and Torontel were utilized. Rhizosphere soil and roots were sampled and analyzed in December 2007, April and October 2008. Spores were extracted using centrifugation methods. Mycorrhizal root colonization and soil chemical analysis were conducted. None of the vineyards surveyed lacked spores, but genus composition differed among the vineyards. Most of the fungi were in the genus *Glomus*; *Acaulospora* and *Scutellospora*. Spore diversity and quantity and mycorrhizal root colonization were greater in organic than in conventional vineyards. This study was supported by the Research project No "81927", Mycorrhizal symbiosis in *Vitis vinifera* which is financed by the Universidad Católica del Maule-Chile.

P6.30 - Plant invasion-induced biogeographical shift in the arbuscular mycorrhizal communities: a morpho-molecular approach

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Whilst the impact of plant invasions on above-ground biodiversity has received recent attention, no continental-scale comparisons have been made of such impacts on arbuscular mycorrhizas (AM). Here we present, for the first time, invasion-induced biogeographical-scale changes in AM communities using a morpho-molecular approach. The study was conducted to evaluate the impact of *Conyza canadensis* (L.) Cronq., native to North America and invasive in the Kashmir Himalaya, on rhizospheric AMF communities both in its native and invaded regions. We first compared the AMF species composition and spore density of the *C. canadensis* invaded soils with nearby uninvaded sites in the Kashmir Himalaya, India and found reduced species richness in invaded than uninvaded sites. However, AM spore density was higher in the invaded than uninvaded soils. Subsequently, soil samples were collected from rhizosphere of *C. canadensis*, both from North America and Kashmir Himalaya, and DNA extracted from isolated AMF spores was amplified targeting AM partial SSU rRNA gene. The PCR products were cloned and for each sample 120 putative cloned products were randomly selected and amplified by PCR. These PCR products were then digested independently with restriction. The DNA fingerprint analysis showed significant difference in AMF community composition in native and invaded habitats, thereby suggesting a controlling influence of plant invasions on AMF biogeography with likely implications for diversity and distribution pattern of their host plants.

P6.31 - Are AMF more resilient under agricultural practices of severe disturbance and intensive nutrient inputs than previously thought?

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To test the resiliency of AMF, their incidence and infectivity were evaluated for 5 divergent land management systems in a 5-year study of tomato cropping systems. For the first 3 years, 2 of the treatments were practices considered highly detrimental to AMF including tillage-mediated elimination of vegetation (disk-fallow) and soil fumigation. The remaining treatments were considered more conducive for AMF, including organic production methods, bahiagrass (*Paspalum notatum*) pasture and undisturbed weed fallow. After tomato cropping commenced in years 4 and 5, tomato, weed and cover crop roots and rhizosphere soil were collected. Roots were evaluated for AMF colonization and soil assayed for infective inoculum and AMF spore morphotypes. In year 5, tomato roots and soil were also analyzed for the AMF biomarker fatty acid 16:1 ω 5c. Soil disturbance and phosphorus (P) had the greatest influences on AMF infectivity. All plots had high levels of available P, resulting in low levels of colonization across all treatments. Bahiagrass pasture, the only treatment without repeated, intensive soil mixing, had the highest level of tomato root colonization. Flooding and a shortened growing season likely contributed to reduced infection potentials in all treatments except for organic plots in year 5 compared to year 4. Areas of highest disturbance from frequent tillage had the lowest levels of primary inoculum, but recovery to levels comparable to

less disturbed treatments occurred after a single season of tomato cropping. Diversity of AMF morphotypes was typical of agricultural fields, with at least 10 spore morphotypes dominated by *Glomus* spp. Hence, AMF were persistent and active under highly stressful conditions of the intensively managed systems.

P6.32 - Mycorrhizal fungi for sustainable production of Christmas trees in the French Morvan region

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Abies nordmanianna L. which is the main produced Christmas tree in France is genetically very variable and grows heterogeneously. Producers from the Morvan region, which is the most important production area, want to optimise tree growth in plantations whilst using agricultural practices which are respectful of the ecosystem and ecosystem services.

Ectomycorrhizal fungi are generally present in plantation soils but poor symbiosis establishment can lead to bad tree growth in the field. Diversity of ectomycorrhizal fungi is therefore being investigated in different Christmas tree plantations in order to isolate fungi efficient in promoting plant growth and health. This combines the analysis of ITS rDNA sequences as well as isolation of fungi on culture media. Potentially interesting mycorrhizal fungi include *Laccaria amethystina*, *Tricholoma* sp. and *Hebeloma* sp. as well as *Tuber borchii* or ericoid mycorrhizal fungi. The effect of these fungi on growth of *A. nordmanianna* seedlings is currently under investigation in order to select isolates adapted to local soil and environmental conditions.

In addition, as Christmas tree producers are starting to grow grass in plantations and to use sheep for grazing in order to reduce water contamination by herbicides, root colonisation by indigenous AM fungi and their diversity are being investigated in these systems as a marker of good soil ecological quality.

P6.33 - Nickel tolerance and population structure of *Pisolithus albus* in New Caledonia

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The worldwide genus *Pisolithus* is common in New Caledonia, mainly *P. albus* associated with a range of native species: *Acacia spirorbis*, *Babingtonia* spp., *Tristaniopsis* spp., *Melaleuca* spp. This species is present in volcano-sedimentary and ultramafic soils. Ultramafic soils are characterized by high iron (up to 85%), high heavy metal (Ni, Cr, Mn, Co), low nutrient (N, P, K) contents and an unbalanced Ca/Mg ratio; they represent one third of the total surface of New Caledonia. Nickel is a highly toxic heavy metal reaching very high total and bio-available concentrations in ultramafic soils. We hypothesised that Ni tolerance is a key factor in the structuration of *P. albus* populations. For that purpose, we investigate Ni tolerance (EC50) on 28 strains from volcano-sedimentary and ultramafic soils. Average EC50 is about 30 μ M of NiCl₂ for strains isolated from volcano-sedimentary soils and range from 400 to 850 μ M for strains from ultramafic soils. Notwithstanding, on ultramafic soils three classes of EC50 have to be distinguished: - hyper-tolerant strains with EC50 above 600 μ M, - tolerant strains with average EC50 around 100 μ M and sensitive strains with average EC50 of 30 μ M. The paradoxical occurrence of Ni sensitive strains in ultramafic soils suggests that *P. albus* developed several strategies against Ni, this phenotype being not reliable in understanding structuration of *P. albus* populations.

P6.34 - Response of arbuscular mycorrhizal fungi and soil enzyme activities to *Eupatorium adenophorum* invasion

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Eupatorium adenophorum is one of the most destructive invasive plants in China. In order to understand the response of AM fungi and soil enzyme activities to *E. adenophorum* invasion, Three blocks with different cover levels of *E. adenophorum*, i.e. 0 (N), 30-50% (M) and 80% (H), were selected in Xishan of Yunnan province, south China. Three plots (20m \times 20m for each) were randomly constructed in each block. Rhizosphere soil samples were collected in each plot. The results indicated that the roots of *E. adenophorum* was infected by AM fungal structures, i.e. arbuscules, hyphal coils, vesicles and non-septate hyphae, but colonisation rate not significantly different between M and H blocks. A total of 29 AM fungi belonging to six genera were found based on spores isolated, of these four belonged to *Acaulospora*, two to *Paraglomus*, one to *Entrophospora*, one to *Gigaspora*, 20 to *Glomus*, and one to *Scutellospora*. *Glomus etunicatum* was the dominant species in the three blocks, and *G. geosporum* and

G. aggregatum were the second dominant species in M and H blocks. The mean spore density of AM fungi was significantly higher in N than in M and H blocks. The mean species richness was not significantly different in the three blocks. The biomass of AM fungi was significantly higher in N (1.188 µg/ml) than in M (0.154 µg/ml) and H (0.152 µg/ml) blocks assessed by PLFA profiles. Soil enzyme activities of cellulose, polyphenol oxidase and catalase significantly increased with the increasing cover levels of *E. adenophorum*, except for cellulose between M and N blocks. However, there was no significant difference of the activities of urease, invertase, β-glucosidase, acid phosphatase, neutral phosphatase, and alkaline phosphatase among the three blocks. It suggests that AM fungal composition and biomass and some soil enzyme activities have different response to *E. adenophorum* invasion.

P6.35 - Illuminating the hyphal world: mapping fungi to soil phosphatase activity

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Though ectomycorrhizal fungi (EMF) play a critical role in tree nutrition, it remains a challenge to study the functional role of EMF in the field due to the difficulty in determining the species of fungal hyphae growing in soil and associating these species with soil processes. We used a soil imprinting technique to visualize phosphomonoesterase activity at root windows installed in birch/Douglas-fir stands in the southern interior of British Columbia. These sites comprise a chronosequence from four stages of development, with three replicates per age. Shortly after imprinting, small soil samples (0.25 g) were collected from three locations where enzyme activity was detected, and three where it was not detected, in each organic and mineral horizon at each window. Using terminal restriction fragment length polymorphisms, we compared the fungal assemblages associated with the two types of functional soil micro-sites, between organic and mineral horizons, and across stand age. Multi-response permutation procedure detected broad differences in the total fungal assemblages among stand ages, but variability among sites made fine-scale effects more difficult to interpret. Differences in EMF assemblages associated with high and low phosphatase areas were found in 4 of 9 older stands but not in the younger stands, indicating that some EMF species associated with older trees may play an active role in organic phosphorus mobilization.

Mycorrhizas and Plant Nutrition

P7.1 - Influences of improved fallow systems and phosphorus application on arbuscular mycorrhizal fungi symbiosis in maize in western Kenya

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A field study was carried out in six-year-old on-farm field trial during long-rains season (April-August) 2003 to investigate the effect of improved fallow (*Crotalaria grahamiana* and *Tithonia diversifolia*) systems and phosphorus application on Arbuscular Mycorrhiza fungi (AMF) symbiosis in maize. The experiment was randomized complete block design with three cropping (continuous maize, *Crotalaria* and *Tithonia* fallow) systems and two phosphorus levels (0 and 50 kg P ha⁻¹). AMF colonization in maize roots, maize yield and macro-nutrients uptake were recorded. Phosphorus applications improved ($p < 0.05$) early (<8 weeks old maize) AMF colonization, nutrient uptake and maize yield in improved fallow systems. Greater differences due to phosphorus application were noted in maize in *Tithonia* fallow than in *Crotalaria* fallow. A positive relationship existed between early AMF colonization and maize yield ($R=0.38$), phosphorus and nitrogen uptake ($R=0.40$ and $R=0.43$, respectively), demonstrating the importance of phosphorus fertilization in enhancing low-input technologies (improved fallows systems) in phosphorus deficient and acidic soils of western Kenya.

P7.2 - Responses of fungal root colonization to long-term exposure of elevated atmospheric CO₂ and warming in a subarctic birch forest understory

Olsrud, M., Michelsen, A., Svensson, B., Carlsson, B., Melillo, J.M.

Responses of the mycorrhizal fungal community in terrestrial ecosystems to global change factors are not well understood. In this study we investigated for the first time how ericoid mycorrhiza (ErM), fine endophytes (FE) and dark septate endophytes (DSE) in roots responded to elevated atmospheric CO₂ concentration and warming in the dwarf shrub understory of a birch forest in the subarctic region of northern Sweden. The ErM colonization in ericaceous dwarf shrubs increased under elevated atmospheric CO₂ concentrations, but did not respond to warming following 6 years of treatment. This suggests that the higher ErM colonization under enhanced atmospheric CO₂ concentrations might be due to increased transport of carbon below-ground to acquire limiting resources such as nitrogen. FE colonization in grass roots decreased under enhanced atmospheric CO₂ concentrations and under warming, which might be due to increased root growth, to which the FE fungi could not keep up, resulting in

proportionally lower colonization. DSE hyphal colonization in grass roots significantly increased under warmer conditions, but did not respond to elevated atmospheric CO₂ concentrations. This complex set of responses by mycorrhizal and other root-associated fungi to global change factors of all the fungal types studied could have broad implications for plant community structure and carbon and nitrogen biogeochemistry of subarctic ecosystems.

P7.3 - Fe nutrition in mycorrhizal *Pinus sylvestris*

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Weathering of soil minerals accounts for the primary source of most essential elements for organisms and thus contributes to soil fertility. Although abiotic, physical weathering is important, biotic weathering by soil organisms greatly influences the availability of essential elements.

In recent years, there has been a lot of interest in weathering of mineral particles by ectomycorrhizal fungi. Overall, there is compelling evidence that these fungi increase mineral weathering by CO₂ release in respiration and by secreting protons and low molecular weight organic acids. Although the mineral dissolution of macronutrients such as P receives the most attention, the availability of micronutrients is also essential.

Fe is one of the most abundant elements on earth, but its bioavailability is generally low as it forms insoluble minerals. Therefore organisms have evolved different strategies to maintain iron homeostasis; e.g. secretion of siderophores by ectomycorrhizal fungi.

We identified two ectomycorrhizal *Suillus* species with remarkable characteristics concerning Fe nutrition. One of them accumulates Fe in its sporocarps while the other one consistently enhances the Fe status of its host *Pinus sylvestris*. In pot experiments, seedlings were inoculated with these fungi and grown in a variety of substrates. The dissolution of iron from these substrates was measured and the consequences for the nutrient status of the host plants were assessed.

P7.4 - Effect of *Glomus intradices* and nitrogen source on growth and fruit production of strawberry plants.

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In México, nitrogen (N) fertilizers application in strawberry production systems exceeds plants requirements, involving plant physiological disorders, environmental pollution and increasing the production costs. The aim of this work was to evaluate the effect of inoculation with *Glomus intradices* and N forms [nitrate (NO₃⁻) and ammonium (NH₄⁺)] on growth and fruit production of strawberry plants. One experiment was carried out in a glasshouse with strawberry plants grown on black polyethylene bags, containing a sterilizer mixture coconut fiber/agrolita (1/4 v/v). Before planting plant roots were disinfected with a solution of sodium hypochlorite (1%) and two weeks after planting the half of the plants were inoculated with around 120 spores of *G. intradices* per plant. Two weeks after inoculation each group was separated in four sub-groups, according with the N proportions forms (NH₄⁺:NO₃⁻) treatments: 0:4, 1:3, 2:2 and 3:1. All plants were fertirrigated with a complete nutrient solution. The eight treatments were replicated four times and distributed in a completely randomized experimental design. Plants were harvested at two phenological stages: flowering and fruit production, 147 and 267 days after planting (dap), respectively. The results show that at 147 dap, the non-inoculated plants were greater in shoot fresh weight, leaf area, photosynthesis and N content, as compared to inoculated ones. However, at 267 dap, the inoculated plants showed a significant increase on fruit production and root colonization. NO₃⁻ promoted root colonization, fruit production and plant growth.

P7.5 - Short-chain polyphosphate in arbuscular mycorrhizal roots colonized by *Glomus* spp.: a possible phosphate pool for host plants

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Inorganic phosphate (Pi) absorbed from soil by the extraradical hyphae of arbuscular mycorrhizal (AM) fungi is considered to be transformed into polyphosphate (poly P), which is then translocated into the intraradical hyphae in mycorrhizal roots. Although, it has been suggested that poly P in the mycorrhizal roots could be used as an index of Pi supply to the plant, information about plant growth and poly P accumulation is limited. In the present study, the relationship between plant growth and poly P contents in roots colonized by *Glomus* species was investigated.

Welsh onion (*Allium fistulosum*) was grown with or without AM fungal inoculation at four levels of phosphorus

addition (0, 131, 262, or 437 mg P kg⁻¹ dry soil). Two fungal species, *Glomus* sp. R10 (Idemitsu Kosan) and *Glomus etunicatum* were used. Poly P was quantified by two enzymatic assays: long-chain poly P (>~20 Pi residues) with polyphosphate kinase, and total poly P with polyphosphate exopolyphosphatase. The difference between these values was designated as short-chain poly P (<~20 Pi residues).

The content of long-chain poly P in mycorrhizal roots and plant growth was poorly correlated or not, depending in fungal species. However, the content of short-chain poly P and plant growth was highly correlated in both species. This difference suggests that short-chain poly P in mycorrhizal roots colonized by *Glomus* spp. functions as a pool of Pi for the plant.

P7.6 - AM symbiosis enhances the salinity tolerance of *Pistacia vera*

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Pistacia vera (Pistaciaceae) is a very important horticultural plant in Iran. Studies have shown that salinity stress is one of the most important abiotic stresses for this plant. Since there are some records showing AM symbiosis increases host resistance to salinity stress, this study has been conducted to survey whether AM symbiosis could improve the tolerance of *P. vera* to salinity or not. Seedlings of *P. vera* were inoculated in pots with 200gr inoculum of *Glomus* sp and were treated with two different salinity levels. After three months the amounts of chlorophyll, proline, fresh and dry weight of whole plants and shoot length were measured. Result showed that mycorrhiza inoculated plants have more proline and chlorophyll content compared to non inoculated plants, also fresh and dry weights and shoot length were higher in AM infected plants. The results suggest that AM symbiosis improve salinity tolerance in *Pistacia vera*. Affiliations 2 and 3 of the first author: Semiarid Prairie Agricultural Research Station, Airport Road, Swift Current, Saskatchewan, Canada. Department of Biology University of Zabol, Iran.

P7.7 - The effect of symbiotic association between arbuscular mycorrhizal fungi and *Physalis peruviana* in crop nutrition

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Physalis peruviana is a tropical fruit (gooseberry) original from South America, used by Precolombian cultures. This plant is well adapted to climate conditions of the Andean Mountains, but the soils have low pH and low available P for gooseberry plants. Colombia is one of the main producers of *Physalis* fruit in the world. This research is focused on determining the effect of symbiotic association of arbuscular mycorrhizal fungi (AMF) in *Physalis peruviana*. The experiment was developed in a research center, Tibaitatá, under controlled conditions. A random complete blocks design was used with a total of seven treatments and eight repetitions: two controls (no inoculation) one without fertilization (T0) and one with 100% fertilization (T100) -104 g/plant of triple superphosphate (TSF) - and four treatments with 54 g fertilizer/plant (TSF) and inoculated with single AMF genus -*Gigaspora*, *Glomus*, *Scutellospora* and *Entrophospora*- and a mixed inoculum containing -*Glomus*, *Entrophospora* and *Acaulospora* spores-.The results confirmed the mycotrophic condition of *Physalis*, which indicated the benefits of inoculation with AMF, especially with both treatments, the mixed inoculum and *Gigaspora* sp. The increments were in terms of biomass levels and nutrient absorption (N, P, K, Mg y S) and inoculation with mixed inoculum and *Gigaspora* sp replaced 50% of Phosphate application for crop production.

P7.8 - Evaluation of arbuscular mycorrhizal fungi isolates associated with two forest species (*Gmelina arborea* and *Pachira quinata*) in the Colombian

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The Caribbean region suffers high levels of deforestation and soil degradation. Timber plantations are an alternative for reforestation and land recovery for reducing pressure on natural forests, improving wood provision, as well as yield profitability for producers. One of the problems for timber production is the high losses in seedling in breeding nursery and also in transplant (30-50%) because of low adaptation to the environmental conditions and low nutrition at early growing stages. Handling of arbuscular mycorrhizal fungi (AMF) from seedling stage, improves nutrients and water uptake as well as plant tolerance to biotic and abiotic stresses, which results in better seedling survival and reduction of chemical fertilization. The experiments were set at the breeding nursery of Monterrey Forest Station, in Zambrano district, in order to evaluate the effects of association between *Gmelina arborea* and *Pachira quinata* with 6 mixed isolates of AMF (4 native and 2 introduced + 50% of fertilizers), compared with controls with different doses of fertilization, 10, 50 and 100%, in a complete blocks random design with three

repetitions. Forest species showed high dependency of association with AMF and differential response linked with AMF identity (isolate). The AM association improved plant growth and reduced seedling with a consequent reduction in chemical fertilizers application 50% and production costs

P7.9 - Overcoming growth depressions in arbuscular mycorrhizal symbiosis: Can we make the mycorrhizal and direct P uptake pathways additive?

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Mycorrhiza-induced growth depressions occur in a wide variety of host plants including cereals. Growth depressions are typically attributed to C drain to the fungus in the absence of host benefits from increased nutrient supply. However, this explanation does not hold in all cases. Our data suggests that growth depressions in arbuscular mycorrhizal (AM) cereals are linked to reduced P uptake rather than C demand (also see FA Smith *et al.* this ICOM). Although considerable growth depressions were observed in AM barley, ³²P radiotracer studies demonstrated that the AM pathway of P uptake was functional, contributing 50% of total plant P. In contrast P uptake via the direct pathway was significantly reduced (Grace *et al.*, 2009). We have taken a transgenic approach to further understand the functionality of this relationship. We are investigating P uptake in AM barley overexpressing transporters involved in the direct or AM P uptake pathways with the ultimate aim of maintaining direct P uptake during AM colonisation. We hypothesise that if P uptake via the direct pathway is maintained then the direct and AM uptake pathways will become additive and growth depressions will be alleviated.

Grace EJ, Cotsaftis O, Tester M, Smith FA, Smith SE. 2009. Arbuscular Mycorrhizal Inhibition of Growth in Barley Cannot Be Attributed to Extent of Colonization, Fungal Phosphorus Uptake or Effects on Expression of Plant Phosphate Transporter Genes. *New Phyt.* 181(4): 938-949.

P7.10 - The tripartite symbiosis between diazotrophic bacteria, arbuscular mycorrhizal fungi, and *Araucaria angustifolia* seedlings

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Araucaria angustifolia is a species of socio-economic and environmental importance in Brazil and today the remaining area of the Brazil Pine forest comprises less than 2% of the original area. The diversity of microorganisms is important for the restoration of plant ecosystems, especially the diazotrophic bacteria (DB) and arbuscular mycorrhizal fungi (AMF). The DBs fix nitrogen and the AMF stimulate nutrient absorption. To evaluate the effect of diazotrophic bacteria and their interaction with AMF in *A. angustifolia* seedlings, a greenhouse experiment was installed with four DB isolates, with and without AMF. During the eight months growth period the plant height was measured biweekly and the stem diameter monthly. After harvest, the shoot (SDW) and the root (RDW) dry weight, AMF root colonization and nitrogen and phosphorus contents of plants were evaluated. No difference among inoculated and non-inoculated plants and no interaction between AMF and DB could be detected. Since the diazotrophic bacteria did not affect plant growth, all 12 replicates with AMF were compared to the other 12 without AMF. Now the stem diameter, SDW and RDW of the AMF plants were statistically superior. However, not even one plant showed signs of mycorrhizal root colonization, although an increase in AMF spores occurred in the rhizosphere of all AMF-inoculated plants.

P7.11 - P inflow and mycorrhizal growth response of two sorghum cultivars as affected by drought stress

Nadian Ghomsheh, H.(Associate Professor of Soil Science),Jafari, S.(Assistant Professor of Soil Science)

Two *Sorghum bicolor* L. Cultivars including speed feed cultivar with long root and high root proliferation and KFS2 cultivar with low root proliferation were chosen and their response to growth and phosphorous (P) inflow (P uptake per unit root length per unit time) with and without mycorrhizal colonization under drought stress were studied. Seedlings of both cultivars were colonized by *G. intrradices*. The pots were watered when 80, 60, 40 and 20 percent of soil available water consumed. The experiment had a randomized complete block design and treatments (2 mycorrhiza*4 drought stress*2 sorghum cultivars) were arranged in a factorial combination. Results showed that shoot d. wt. of both mycorrhizal and non-mycorrhizal plants decreased with increasing drought stress. However, shoot d. wt. of mycorrhizal sorghum was greater than that of non-mycorrhizal sorghum at all drought stress levels. Mycorrhizal growth response of KFS2 was greater than that of speed feed. The greater growth of mycorrhizal KFS2 than mycorrhizal speed feed was contributed to the greater percentage of root colonization of KFS2. P inflow to both mycorrhizal plants was greater than that of non-mycorrhizal plants at all the treatments. Although P

accumulation per plant of mycorrhizal KFS2 was less than that of mycorrhizal speed feed, P inflow to mycorrhizal KFS2 was greater than that of mycorrhizal speed feed. This shows that KFS2 was more depended to mycorrhizal fungus than speed feed.

P7.12 - Influence of exogenous treatment by plant growth regulators on development of *Medicago lupulina* with mycorrhizal fungus *Glomus intraradices*

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Spring black medic cv. VIK32 (*Medicago lupulina* L., self-pollinating species, diploid) was used as a model plant, characterized by: 1) dwarf symptoms under low phosphorus (P) level and without arbuscular mycorrhiza (AM) fungi in soil; 2) positive growth response to AM. We analyzed influences of exogenous treatments by plant growth regulators – PGR (3-indolylacetic, abscisic, gibberellic A3 (GA), salicylic (SA), 1-aminocyclopropane-1-carboxylic acids, and 6-benzylaminopurine (BAP) in different concentrations) on mycorrhization, development of plants with/without AM fungus *Glomus intraradices*, P and nitrogen accumulation in shoots and roots under low P level in soil.

The analysis of plants was provided at 30th day after sowing. GA depressed AM development. Similar data were obtained in *Pisum sativum* L. by Ghachtouli et al. (1996). In our test GA negatively influenced on P uptake. As a result P accumulation was low both in plants with and without AM in comparison with control (-AM). BAP treatments suppressed plant growth, but didn't decrease mycorrhization and P accumulation by plants. Otherwise SA treatments increased plant growth (with/without AM) and AM development. This phenomenon may be used as technological method of AM fungi cultivation. All other PGR didn't essentially influence on analyzed characteristics.

In black medic we obtained 4 myc—mutants, selected with dwarf symptoms. Efficiency of ethyl methanesulfonate mutagenesis was considerable (~1.3%).

P7.13 - Survival and growth of micropropagated physic nut (*Jatropha curcas* L.) Plantlets associated to *Glomus clarum*

Folli, M.S.,Rodrigues, A.L.,Meira, L.S.,Lani, E.R.G.,Otoni, W.C.,Silva, L.C., Kasuya, M.C.M. (Universidade Federal de Viçosa, Brazil)

The association of micropropagated plants with arbuscular mycorrhizal fungi (AMF) can improve the survival rate during the acclimatization, besides increasing the tolerance of the plants to adverse environmental conditions. The aim of this work was to evaluate the effect of *Glomus clarum* inoculation on the survival, growth and nutrients absorption by micropropagated *Jatropha curcas*. Micropropagated plantlets at 0, 14 or 21 d of growth in rooting medium, added or not with IBA, were transferred to a substrate composed by sand:soil:vermiculite (1:1/2:1, v:v:v). After 2 weeks, the survival rates throughout the acclimatization conditions were 100 % for plants maintained during 0 or 14 d and 93 % for those maintained for 21 d in rooting medium. The period of time in rooting medium and the addition of IBA did not affect the growth of the plants. The colonization ranged from 70 to 93 % and the stimulatory effects of AMF were observed in all the characteristics related to the growth, except to the height, that was very similar among them presenting average of 7.97 cm tall. The plants inoculated with *G. clarum* efficiently absorbed nutrients, mainly P, with average of 0.13 dag Kg⁻¹ in the dry matter. Micropropagated plants of *J. curcas* may not require *in vitro* rooting phase, and inoculation with AMF in the beginning of the acclimatization process can benefit the survival and growth of this species.

P7.14 - Do arbuscular mycorrhizal fungi influence caesium uptake by *Medicago truncatula*?

Lea Wiesel, Martin R. Broadley, Philip J. White

Radiocaesium (Cs) contamination of soils is a worldwide problem that has arisen from human activities, such as accidents at nuclear power plants and radioactive fallout from nuclear weapons tests. The contamination of soils by Cs is of serious concern because of the long half-lives of the radionuclides (¹³⁴Cs = 2yr, ¹³⁷Cs = 30yr) and the emission of harmful β and γ radiation during decay. Radiocaesium enters the food chain through vegetation and therefore has an impact on human health. Plants acquire Cs from the rhizosphere and Cs is transported symplastically to the xylem. Because of the chemical similarity of Cs and potassium (K), K transport proteins contribute to Cs uptake by roots and the delivery of Cs to the shoot. Arbuscular mycorrhizal (AM) fungi play an important role in plant nutrition. Since AM fungi deliver nutrients to plant roots it has been suggested that they might also affect Cs uptake by plants. To investigate the influence of AM fungi on Cs uptake by plants, *Medicago truncatula* was grown in association with *Glomus* sp. under *in vitro* conditions. Under K-deficient conditions, neither Cs in the media nor mycorrhizal infections influenced fresh weight of *M. truncatula*. The colonization of roots with *Glomus* sp. did not change K or phosphorus (P) concentrations in plant tissues under these assay conditions. Neither the Cs concentration in shoots nor the Cs concentration in roots were affected by mycorrhizal infection.

P7.15 - Fe nutrition in mycorrhizal *Pinus sylvestris*

Wevers, J.H.(Universiteit Hasselt, Environmental Biology, Agoralaan Gebouw D 3590 Diepenbeek Belgium), Colpaert, J.V.,Vangronsveld, J. (Universiteit Hasselt, Environmental Biology, Agoralaan Gebouw D 3590 Diepenbeek Belgium),

Weathering of soil minerals accounts for the primary source of most essential elements for organisms and thus contributes to soil fertility. Although abiotic, physical weathering is important, biotic weathering by soil organisms greatly influences the availability of essential elements.

In recent years, there has been a lot of interest in weathering of mineral particles by ectomycorrhizal fungi. Overall, there is compelling evidence that these fungi increase mineral weathering by CO₂ release in respiration and by secreting protons and low molecular weight organic acids. Although the mineral dissolution of macronutrients such as P receives the most attention, the availability of micronutrients is also essential.

Fe is one of the most abundant elements on earth, but its bioavailability is generally low as it forms insoluble minerals. Therefore organisms have evolved different strategies to maintain iron homeostasis; e.g. secretion of siderophores by ectomycorrhizal fungi.

We identified two ectomycorrhizal *Suillus* species with remarkable characteristics concerning Fe nutrition. One of them accumulates Fe in its sporocarps while the other one consistently enhances the Fe status of its host *Pinus sylvestris*. In pot experiments, seedlings were inoculated with these fungi and grown in a variety of substrates. The dissolution of iron from these substrates was measured and the consequences for the nutrient status of the host plants were assessed.

P7.16 - Estimate of symbiotic efficacy for *Acacia mangium* inoculated with different mycorrhizal fungi species

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This study aimed to estimate values for the symbiotic efficacy of seedlings of *Acacia mangium* Willd inoculated with different mycorrhizal fungi. These values were estimated using units of symbiotic efficacy (UES) calculated through the following equation: $UES = \{[(\text{dry matter of the inoculated plant} / \text{dry matter of the non-inoculated plant}) * 100] / \text{percentage of root colonization}\} - 100$, where UES corresponds to the percentage of dry matter increment per unit of percentage of root colonization. Treatments consisted of inoculations with single mycorrhizal species (*Acaulospora morrowiae*, *Scutellospora calospora*, *Scutellospora heterogama*, and the ectomycorrhizal isolate O64-ITA6), and all possible species combinations. Experimental design was randomized blocks with 4 replicates, and conducted under greenhouse conditions until the plants achieved four months of age. The symbiotic efficacy index was capable of selecting promising combinations of mycorrhizal inoculants in terms of plant growth promotion. For instance, plants inoculated with the combinations of *S. calospora* + *S. heterogama* + O64-ITA6, and of *A. morrowiae* + *S. calospora* + *S. heterogama* presented the highest symbiotic efficacy, with UES values of 170 and 180, respectively (increase of up to 180% in shoot dry matter for every 1% of root colonization). In contrast, plants inoculated with single inoculants of *Acaulospora morrowiae* and O64-ITA6 or composed of *A.morrowiae*+O64-ITA6 which showed the lowest values of UES (< 5).

P7.17 - Enhancement of plant growth by arbuscular mycorrhizal fungi under N-limited conditions

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It is now well recognized that arbuscular mycorrhizal (AM) fungi can supply not only P but also N to host plant. In contrast to phosphorus, mineral N in soil is not strongly adsorbed with soil particles and moves with mass flow of water. Therefore, it has been recognized that N supply through AM fungi may not much increase plant growth under usual arable conditions. In the present work, we examined if AM fungi can enhance plant growth through their N supply to plant. We used a split compartment system composed of root and hyphal compartments (Tanaka and Yano 2005). In the system, the compartments were separated with fine nylon mesh and air gap so that neither mineral nitrogen moved nor roots elongated across the mesh. A 30 days seedling of Welsh onion (*Allium fistulosum*) was transplanted and inoculated with *Glomus* sp. R10 (Idemitsu Kosan Co.) in the root compartment (RC). After transplanting, ¹⁵N labeled ammonium nitrate was added to either hyphal compartment (HC) or RC. "N addition to RC" treatment increased almost all plant and fungal growth parameters. "N addition to HC" treatment increased plant dry weight, shoot N and root ¹⁵N concentrations, and P uptake. These indicate that, when movement of soil mineral N to plant roots is limited, AM fungi can enhance plant growth by supplying N to plant.

P7.18 - Arbuscular mycorrhiza and cattle manure in the establishment of *Tocoyena selloana* Schum. in dune areas

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In dune mining areas in the north coast of Paraiba, Brazil, revegetation with manured seedlings of native species is a common practice. The hypothesis that mycorrhization can effectively contribute for the establishment of *Tocoyena selloana* was tested in greenhouse and field experiments. In the greenhouse, seedlings were grown in substrates with 5 doses of cattle manure (0, 5, 10, 15 and 20% v/v) and 3 AMF treatments: inoculated with *Acaulospora longula*, with a mixture of native AMF, and non inoculated. The inoculated seedlings responded significantly to the mycorrhizal association with biomass increments of 30 and 39%, respectively, for treatments with *A. longula* and native AMF. In all treatments growth was limited in the absence of manure, however, growth increased with fertilizing doses $\geq 10\%$. For the field experiment, seedlings were grown in substrate with 16.5% manure (usual practice) and inoculated with *A. longula* or *Gigaspora albida* during their transfer to the field. After 13 months, the inoculated plants were significantly more colonized than those non-inoculated and the plants associated with *G. albida* were taller than the others. The effectiveness of inoculation with *A. longula*, compared to the control, in aboveground biomass increment (0.29), and P (0.16) and N (0.31) content was positive, although statistically non significant. Finally, AMF inoculation is promising to enhancing production of *T. selloana* seedling. Key words: AMF, symbiotic efficiency, seedlings, revegetation

P7-19 - Absorption of stable forms of phosphorus by arbuscular mycorrhizal maize

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The low availability of phosphorus (P) for plants in tropical soils is mainly due to the formation of stable complexes of P with surfaces of Fe and Al oxides. The objective of the present study was to investigate the accessibility of this stable P to maize (*Zea mays*) inoculated with arbuscular mycorrhizal fungi (a mixture of *Glomus* sp.). We used a technique of a double pot (one pot with soil and one with nutrient solution without P and double compartment (one compartment accessible by mycorrhizal roots and one only by mycorrhiza). We used two soil horizons (Umbric Epipedon and Oxic) with or without mycorrhiza. Eighty-seven percent of the mycorrhizal roots were colonized. The plant cells showed an intense colonization by an unidentified fungus that produced large amounts of microspores, and the fungus did not cause any apparent deleterious effects to the plants. For both soils, the P concentration was higher in the mycorrhizal plants than in the non-mycorrhizal plants. Ninety days after planting, the mycorrhizal plants had absorbed 60 (Oxic Horizon) and 67 (Epipedon umbric) mg of P kg⁻¹ of soil. These values are larger than was present in the labile P pool (on average, 3.6 mg P kg⁻¹ of soil) indicating that they accessed part of the more stable P. However, the sequential P extraction method was not sensitive enough to identify the more stable compartment from which mycorrhiza absorbed part of the P.

P7.20 - Mycorrhizal colonization in sugarcane after herbicide application

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The objective of this work was to evaluate the impact of herbicides on the mycorrhizal colonization of the sugarcane cultivars RB86-7515 and SP80-1816. The experiment was conducted in the field in a conventional tillage system, with 1.4 m spacing between plants and 18 buds m⁻¹. A randomized block design was used following a 2 x 5 factorial, with four replications, in which the first factor corresponded to two sugarcane cultivars (RB867515 and SP80-1816) and the second to four herbicides (ametryn, 2,000 g ha⁻¹; trifloxysulfuron-sodium, 22.5 g ha⁻¹; ametryn+trifloxysulfuron-sodium, 1,463 + 37.5 g ha⁻¹; sulfentrazone, 750 g ha⁻¹). A control treatment without herbicide application was also included. Herbicide application was done when sugarcane plants presented three to four leaves. The root system was collected for the evaluation of mycorrhizal colonization at 7, 14, and 28 days after herbicide application (DHA). RB897515 presented higher mycorrhizal colonization, 22.5 and 27.0 %, compared to the control treatment at 7 and 14 DHA, respectively. After 7 DHA, the lowest mycorrhizal colonization, 11.5%, was recorded for the cultivar RB867515 treated with trifloxysulfuron-sodium + ametryn. Trifloxysulfuron-sodium stimulated mycorrhizal colonization in the cultivar SP80-1816 at 14 DHA (46.3 %). Both cultivars presented higher values of mycorrhizal colonization in the treatments with ametryn at 28 DHA. Mycorrhizal colonization was distinct among the cultivars and herbicides tested.

P7.21 - Rhizosphere and root mycorrhizal diversity of maize genotypes contrasting for phosphorus efficiency

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Acid soils and low phosphorus (P) availability limit plant growth in many soils of the tropics. One of the strategies

developed by plants in order to improve P acquisition efficiency is the symbiotic association with arbuscular mycorrhizal fungi (AMF). In this work, the soil AMF natural community associated with two maize genotypes contrasting for phosphorus efficiency was evaluated, in soils with low P levels. Mycorrhizal population DNA library was generated from the roots and rhizosphere of the maize lines: L3 (P efficient) and L22 (P inefficient) by cloning rDNA fragments amplified by AMF specific primers. Direct sequencing of these fragments indicated that differences in the genetic diversity of the microbial community of the root was reduced when compared to the rhizosphere, and there were some differences in the AM fungi species between the two genotypes. Sequencing analysis revealed that *Scutellospora* was the dominant genus found in the efficient genotype. Visualization of mycorrhizal colonization using stereomicroscope analysis demonstrated greater colonization in roots of the L3, compared to L22. The number of AMF spores found in the rhizosphere was similar in both genotypes but the mycorrhizal infection was significantly reduced in the roots of the P inefficient genotype. These results provide strong evidence that that some mycorrhizal groups were stimulated by P efficient maize genotypes.

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P7.22 - Contribution of arbuscular mycorrhizal fungi inoculation to the reduction of N fertilization on forage grasses

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Two groups of experiments were carried out in Rodic Nitisol and Calcic Cambisol soils to evaluate the effects of inoculating the arbuscular mycorrhizal fungi (AMF) strains *Glomus hoi* "like" and *G. intraradices*, selected in previous essays for their efficiency in both soil, on the response of *Brachiaria decumbens* cv. Basilik, *B. Brizantha* cv. Marandú and *B. hybrid* cv. Mulato (CIAT 36061) to nitrogen fertilization. In both groups of experiments, applications of 100, 200 and 300 kg N ha⁻¹ year⁻¹ plus a control without N, alone and combined with the inoculation of the corresponding AMF strain, were assessed. The grasses were sown with a dosage of 8 kg ha⁻¹ of total seed and AMF were applied at sowing by the method of seed coating. During the first year of grasses establishment, the application of 200 kg N ha⁻¹ combined with the inoculation of the above mentioned AMF strains produced the highest (P0.05) mycorrhizal colonization percentages, as well as dry matter yields and crude protein contents, which were similar to those obtained with 300 kg N ha⁻¹ without inoculation. In the Rodic Nitisol soil, application of 100 and 200 kg N ha⁻¹ contributed to extend until the second year after its inoculation, the effect of the AMF strain in the grasses. It is concluded that the inclusion of AMF inoculation in the fertilization systems of forage grasses could be an ecological and effective way for reducing the high nitrogen fertilizer levels required by these crops without affecting their biomass production and nutritive value.

P7.23 - Effect of *G. intraradices* on the growth and yield of garlic (*Allium sativum*) and the usage of its oil extract as antimicrobial agent

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Garlic, *Allium sativum* L. has been used as common foods and for the treatment of many diseases. Garlic has a broad antimicrobial spectrum. Calcareous soils occupy wide areas in the North African countries such as Egypt. The soils have high percentage of carbonate. The availability of macro and micronutrients is not enough in these soils. In order to increase the productivity of these soils, addition of nutrients is recommended. Biological fertilizer (AM) was used to increase the availability of the nutrients. This study was carried out in two field experiments during two winter seasons using two garlic varieties. The aim was to study (1) the positive effect of AM fungus under different levels of mineral phosphorus fertilizer (zero, 75 and 100% of the recommended dose) on the growth, yield and garlic oil extract under calcareous soil. (2) The suitable quantity of P mineral fertilizer for garlic in the presence of AM. (3) Antimicrobial effects of garlic oil extract. There was no significant increase between inoculated plants in the presence of 75% and uninoculated with 100% of the recommended dose of P fertilizer. The average dry weight of the inoculated plants was increased by 12.6 and 17.0%, while the bulb weight in gm was increased by 21.0 and 15.0% for both varieties. Inoculated Egyptian variety yielded 6.3 in comparison with 5.5 ton/ha in the uninoculated plants. The total oil extract showed an increase of 23.0 and 21.0% for both varieties. Oil extract inhibited the growth of *Salmonella*, *Staphylococcus*, *Fusarium*, *Pythium* and *Candida*.

P7.24 - Arbuscular mycorrhizal fungi in two mangroves of south China

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The symbiosis of arbuscular mycorrhizal fungi (AMF) and mangrove plant species were investigated in two mangroves of south China. AMF, mainly in the forms of hyphae, were found to be commonly present in all investigated species. Seven AMF species belonging to genera of *Glomus* or *Acaulospora* were identified from both mangrove sites. The multiple step-wise linear regression analyses showed that hydrological condition and phosphorus levels in rhizosphere soil had significant effects on AMF colonization in different mangrove plant species at both Futian (FT) and Zhuhai mangroves (ZH); while soil nitrogen and organic matter levels also significantly influenced the colonization in ZH. A greenhouse experiment was conducted to evaluate the effects of AMF inoculation on growth and nutrient uptake of an introduced true mangrove plant species, *Sonneratia apetala* B.Ham. The AMF inoculation significantly improved growth, including plant height, diameter at ground level and plant biomass, as well as absorption of nitrogen, phosphorus and potassium. These findings suggest that AMF can play an important role in mangrove ecosystems.

P7.25 - Limiting factors for the growth of four woody legumes in an Oxisoil

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Nodulating woody legumes can play an important role in reforestation and restoration of degraded lands. The aim of this study was to evaluate the effects of different factors on the growth of four woody legumes species, *Albizia lebeck* (L.) Benth., *Enterolobium contortisiliquum* (Vell.) Morong,, *Leucaena leucocephala* (Lam.) de Wit e *Sesbania virgata* (Cav.) Pers., in a low-fertility soil. The experiment was conducted in a glasshouse at the Soil Science Department of UFLA in 4 kg plastic pots, in a completely randomized design with eight replications. Treatments were as following: 1) Complete fertilization-CF(NPK+micronutrients+liming) + MR (inoculation with mycorrhizal fungi and rhizobia); 2) CF minus N+MR; 3) CF minus N + M; 4) CF minus N + R; 5) CF minus N and P + MR; 6) CF minus N and lime + MR; 7) CF minus N and micronutrients + MR; 8) Control with no fertilizers. Plants were allowed to grow for four months when plant growth, nodulation, mycorrhizal colonization and nutrient contents were assessed. Phosphorus was the most limiting nutrient for plant growth, followed by nitrogen. *L. leucocephala* and *S. virgata* were the most responsive plants to micronutrients addition and liming, with increases in nutrient contents, plant height, and root and shoot dry matter. When compared to single inoculation, dual inoculation resulted in higher plant heights for all plants, except *A. lebeck*, which did not respond to either rhizobium or mycorrhizal fungi inoculation.

P7.26 - Phosphorus efficiencies and responses of maize (*Zea mays* L.) to arbuscular mycorrhizal fungus *Glomus caledonium* in an experimental field as affected by long-term fertilizer management

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The P efficiencies and responses of maize to AMF *Glomus caledonium* were tested in an experimental field as affected by 18-year fertilizer management. The experiment included five treatments: organic manure (OM), half organic manure plus half fertilizer (1/2OMN), fertilizer NPK, fertilizer NK, and the control (without fertilization). AMF inoculation impacts on plant growth and P-uptake were estimated by comparing plants inoculated with *Glomus caledonium*, with plants grown in untreated soil. Both biomass and P content of the young maize plant were greatly increased ($P < 0.05$) by the application of OM, 1/2OMN, and NPK, respectively, except for NK. On the contrary, all maize plants were colonized by indigenous AMF, while undiminished AMF colonization was only observed in the case of the NK treatment. However, *Glomus caledonium* inoculation accelerated AMF colonization significantly ($P < 0.05$) in all treatments, and increased P content of maize significantly ($P < 0.05$) in the case of balanced fertilization, including NPK, 1/2OMN, and OM. Nevertheless, *Glomus caledonium* inoculation resulted in higher ($P < 0.05$) responsivenesses of soil alkaline phosphatase activity, plant biomass, and P-uptake of maize in NK-fertilized soils than in balanced-fertilized soils, suggesting that AMF inoculation is more efficient in long-term P-deficiency-fertilized soils. Our results also showed the different effects of OM versus fertilizer on AMF inoculation responsivenesses in arable soils. Keywords: Alkaline phosphatase activity; Mineral fertilizer; Organic manure; P-deficiency fertilization; P uptake

P7.27 - Response of mycorrhizal wheat genotypes to saline soil under field conditions

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In this research the hypothesis was that, with respect to the great abilities of AM fungi under different stress conditions, they are able to alleviate salinity stress under field conditions. Three AM fungi species including *Glomus*

etunicatum, *G.mosseae* and *G.intraradices* and wheat genotypes of Roshan, Kavir and a mutated line of Tabasi were tested in the soil with 7.41 ds/m salinity. The electrical conductivity of irrigation water was 13.87 ds/m. Under this condition, AM species significantly increased the growth and nutrient uptake of the mutated Tabasi line compared with then other genotypes. The mycorrhizal Tabasi genotype resulted in the lowest concentrations of Na⁺ and Cl⁻.The influence of different species of AM on enhancing plant growth under salinity was observed in the following order: *G.etunicatum* *G.mosseae* *G.intraradices*.The symbiosis of *G.etunicatum* and *G.intraradices* with the Tabasi mutated line resulted in the highest (42.08%) and the lowest (7.55%) increases in wheat dry weight, respectively. The highest (38.3%) and the lowest (4.5%) grain yield increases were related to the symbiosis of Tabasi mutated line with *G.etunicatum* and *G.intraradices*, respectively. Although different species of AM can be very beneficial to wheat plants under salinity stress, it is obvious from the results of this research that *G.etunicatum* can perform more efficiency under such conditions compared with other AM species. This indicates the great importance of selecting the right combination of AM species and host plant to make cultivation under salinity even more likely. Introduction Soil salinity decreases crop yield through increasing osmotic stress on the plant. Mycorrhizal symbiosis is actually a specialized network of hypha , enhancing the uptake and translocation of nutrients to the plant, compared with plant roots (Marschner and Dell,1994). Hence, under soil stresses ,mycorrhizal plants are ,due to the higher soil exploration by hypha, able to adsorb more water and nutrients and hence increase their tolerance to the stresses (Miransari et al.,2007). Other researchers have noted that AM can alleviate the stress of salinity on plant growth through inhibiting high uptake of Na⁺ and Cl⁻ and their transfer to the plant shoots (Giri and Mukerji, 2004; Scheloske et al.,2004; Al-Karaki, 2006). Materials and Methods This experiment has been done in a saline soil at the Karaj area(Tehran province)-Iran on 2006 with executive support of Agriculture, Medicine and Industrial Research School .The inoculum of AM species included *Glomus etunicatum*,*G.mosseae* and *G.intraradices* isolated from saline soils (Aliasgharzadeh et al., 2001) were produced over four month period on sorghum plants under greenhouse conditions using sterilized sands(Miransari et al.,2007). Two wheat varieties (Roshan and Kavir) and a Tabasi mutated line(T-65-7-1) were used. The experiment was a factorial design (including two factors) on the basis of complete randomized block design in four replicates. In each plot, the AM inoculum was applied underneath each seed at 200 active propagules. To measure nutrient concentrations and yield components, 10 plants were randomly selected from middle rows of each plot. Root colonization by AM was determined according to the method of Philips and Hayman (1970), and root stained using the gridline intersect method (Giovannetti and Mosse,1980). Results While cultivar significantly affected the concentration of P,K,Cl and the K/Na ratio, the AM species exerted a significant effect on concentrations of all nutrients . Results showed that the symbiosis of Tabasi with more efficient AM species including *Glomus etunicatum* and *G.mosseae* under such conditions can be very beneficial in salty soils. Tabasi shows important characteristics required for salt stress alleviation on plant growth under field saline conditions including the height and efficient symbiosis ability with AM and morphological and physiological characters (grain yield, shoot dry weight, ear length, number of grains per ear ,number of ears per square meter, number of tiller per plant and harvest index). Higher root colonization by *G.etunicatum* and *G.mosseae* relative to *G.intraradices* resulted in increased nutrient uptake and less Na⁺ and Cl⁻ adsorption, and hence, increased plant growth under salinity. The right combination of AM species and host plant can partially completely alleviate stress of salinity and make the use of saline soil and water for cultivation of crop plants even more likely than before. 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P7.28 - Response of the neem (*Azadirachta indica* A. Juss.) to mycorrhizal inoculation

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Neem is an Indian native plant that grows in dry, nutrient poor soils. Besides its use for timber, the plant has been studied for its fungicide, insecticide and nematicide properties, as well as the potential of its subproducts (neem cake and leaves) as fertilizer and substrate for vermicomposting. The AMF can improve plant growth thus a greenhouse experiment was carried out to investigate whether neem plants would also benefit from mycorrhizal association. The experiment was an entirely randomised 10 replicates set with three inoculation treatments: *Acaulospora longula*, *Glomus etunicatum* and uninoculated control. The plants were cultivated in 2 Kg pots filled with disinfested soil (pH 4.7; 4 mg P dm³). During transplanting, the seedlings were inoculated with 400 glomerospores. Height and shoot diameter were evaluated every 30 days and after 300 days the fresh and dry biomass of leaves, stems and roots were also measured. Seedlings associated with *A. longula* presented greater height than other treatments from the 30th day on. ANOVA showed great significance between treatments. Neem is highly responsive to inoculation (p<0.01) with *A. longula*, with increments of 42% in stem diameter, 101%, 253% and 450%, respectively, in leaves, stems, and roots dry mass. In conclusion, plants of neem are benefited from AMF association but this depends on the isolate and further studies should investigate the viability of using these fungi for increasing seedlings production.

P7.29 - Synergic interaction between arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* and its effect on plant phosphorus uptake

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One of the main goals in modern agriculture is to improve our understanding on soil microbial interactions to optimize plant-microbe relationships as potential and effective applications for sustainable crop production. The ability of fluorescent pseudomonads and arbuscular mycorrhizal fungi (AMF) to promote plant growth is well documented. It is, therefore, fundamental to investigate on the functionality (i.e. plant phosphorus concentration) of the relationship between these two important soil microorganisms if a biotechnological application is planned. Strains of *Pseudomonas fluorescens*, phosphate solubilizers, were isolated from rhizosphere of potato crops in Andisols from Colombia, to study their interaction with AMF measured as plant phosphate uptake. Our results show that bacterial strains relate differentially with the extraradical AM hyphae, and that this is reflected on the effectiveness of the interaction, regarding plant phosphorus uptake. In addition it is demonstrated that bacterial strains and the AMF in combination, synergistically increased plant growth and phosphorus concentration in roots, compared with no-inoculated plants. Therefore, this work clearly shows that specific interaction plant-AMF-bacteria can be effective in agricultural systems. Finally, monoxenic culture is a good methodological approach to evaluate microbial interactions of this kind, as our results from both monoxenic and soil assays show high correlation. **Keywords:** AMF-bacterial interactions, extraradical hyphae, phosphorus solubilization bacteria. Corresponding autor. Tel:+51 3165000 Ext. 19028 E-mail address: aliarodriguez@hotmail.com

P7.30 - The opportunistic behaviour of *Glomus intraradices* in relation to phosphorus acquisition

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The arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* is ubiquitous in natural and agricultural systems, including systems that have high phosphorus (P) concentrations. *G. intraradices* was associated to autotrophic *Medicago truncatula* plants grown in vitro in Petri plates. Plants and AMF grew in the root compartment, while in the hyphal compartment (HC) only the fungus was allowed to develop. Three P treatments were considered for the HC, consisting of two different P levels and absence of P. Upon P addition and at week 2, 4 and 6 after P addition, the treatments were harvested. Plant and fungal development and metabolic activity (succinate dehydrogenase, acid and alkaline phosphatase) of the extraradical mycelium (ERM) were determined. The content of total P in roots and the proportion of ERM containing poly-phosphate were analysed. Increased root colonisation and extensive ERM development were observed for treatments containing P in the HC. The proportion of ERM showing metabolic activity and vitality was larger for high P than for normal P treatment, indicating that AMF was actively taking up P from the medium. Poly-phosphate present in the ERM confirmed that the fungus had an active P uptake strategy and reallocated P within its mycelium. *G. intraradices* readily took up P and transported it towards the host plant, thus increasing plant life span and vitality. We demonstrated that *G. intraradices* showed an opportunistic behaviour towards P acquisition.

P7.31 - Physiological parameters influenced by artificial inoculation of arbuscular mycorrhizal fungi *Glomus clarum* to the achievement of the rubber rootstock

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The artificial inoculation of arbuscular mycorrhizal fungi in heveiculture is a technique that has been little used and may be an alternative to increase the formation of early and well nourished rootstocks. The study aimed the evaluation of the influence of fungi inoculation on growth and biophysical, biochemical and anatomical characteristics of rubber seedlings. The treatments consisted of inoculated plants fertilized with 50 ppm of phosphorus (mic+50P), not inoculated plants fertilized with 50 ppm of phosphorus (s/mic+50P) and not inoculated plants fertilized with 500 ppm of phosphorus (s/mic+500P). It was evaluated biophysical characteristics, the colonization rate, dry matter production, the nitrate reductase enzyme activity and root and leaves anatomical characteristics. The results showed that, even in the low root colonization of 18%, the mycorrhizal plants showed total and aerial part dry matter accumulation and stomatal density similar to the plants s/mic+500P. Furthermore, these plants presented higher root dry matter accumulation, transpiration rate and nitrate reductase enzyme activity, and lower stomatal resistance when compared to other treatments. The root anatomic analysis revealed vascular tissue changes, with increase in the xylem poles number in the inoculated roots.

P7.32 - Influence of *Phosphorus* homeopathic medicine on growth and root colonization by AMF native in sorghum

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The main goal of this study was to evaluate the influence of homeopathy *Phosphorus* in the growth of grain sorghum and root colonization by AMF. The experimental design was completely randomized, with 5 levels of dynamizations (0, 6, 12, 18 and 30 CH) and 4 replications. The variables evaluated were: stem diameter (DIAM), plant chlorophyll content (CLOR), height (HEIG), shoot dry matter (SDM), root dry matter (RDM), concentration of P in shoots (CPS), accumulation of phosphorus in the shoot (APS), P extracted by Melich 1 (P) and root colonization by AMF (RC). All variables of the plant growth responded positively to increased dynamizations of *Phosphorus*, while root colonization showed response according to a fourth degree polynomial function. The root colonization was significantly reduced by homeopathy *Phosphorus* 12 CH, and was negatively correlated with the variables: CLOR, CPS and APS in the treatment *Phosphorus* 6 CH, DMAP in the treatment *Phosphorus* 18 CH, and P in the treatment *Phosphorus* 30 CH. It was found that the dynamizations 6 CH and 12 CH promoted the plant growth, but there was competition between the symbionts, with limitation of root colonization by AMF. The highest dynamizations (18 CH and 30 CH) produced better results of growth for the plant, which favored the colonization by AMF, reducing competition between them.

P7.33 - Do ectomycorrhizal fungi have a role in the formation of Ca stores as oxalate crystals inside eucalypt roots?

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Eucalypt plants are efficient at taking up Ca from the soil, however, little is known about the contribution of ectomycorrhizas and of organic acids to this process. The accumulation of Ca oxalate crystals (CaOx) was evaluated in two sites planted with *Eucalyptus* spp. Abundant accumulation of Ca as druses or grains in the cells of the root cortex was observed in the fine lateral roots. In one of the sites, the presence of CaOx was observed in 56.2 % of the ectomycorrhizas and in 17.5 % of the nonmycorrhizal fine lateral roots, evidencing the role of the ectomycorrhizal association in the storage of Ca in the roots of *Eucalyptus* sp. In the ectomycorrhizas, druses were the predominant CaOx form, while in nonmycorrhizal roots crystalline grains were the most frequent. The ectomycorrhizal morphotypes observed varied as to the content and morphology of CaOx in the root cortex, suggesting distinct capacities of each ectomycorrhizal fungal species to supply Ca to the host plant. The analysis of the mantle surface of different ectomycorrhizal morphotypes by scanning electron microscopy did not show the presence of CaOx in this structure, confirming that under the conditions evaluated, the accumulation of CaOx in the association is limited to the root cortex.

P7.34 - Field validation and in vitro culture and mass production of AMF with particular reference to low-input wheat-rice and wheat-pulse cropping systems

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This study aims to develop a complete package of practice using microbial bio inoculants with considerable economic or social return for their application in sustainable agriculture and environment and to increase their practical use in tune with existing agricultural practices by (i) *in vitro* culture and development of mass production

technology for selected arbuscular mycorrhizal fungal (AMF) isolate and (ii) conducting field validation trials and estimating cost benefit ratio using bio inoculants as biofertilizers and soil stabilizers in low-input, sustainable agriculture in marginal soil conditions. Four Root Organ Culture (ROC) from LL2 consortia, the selected AMF for wheat based cropping system in marginal soils, were developed as a part of the study. To achieve this, monosporal cultures initiated from LL2 consortia trap cultures were used as a starter material for attempting *in vitro* based spore germination and mass production. The steps included: i) isolation and optimization of techniques suitable for spores surface sterilization for the *in vitro* raising of AM using Ri T-DNA transformed host root, ii) successful spore germination, iii) raising dual cultures of LL2 AMF *in vitro*. These inocula are mass multiplied and produced in bulk and being tested in one field. Field trials on black gram were augmented in Kharif season and on wheat in rabi at Palwal, Haryana, India. The results showed that the joint inoculation of AMF with PGPR were found to be superior.

Inoculum Technology Application and Management of Mycorrhizal Fungi

P8.1 - Arbuscular mycorrhiza (AM) for reforestation

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Arbuscular mycorrhiza forming fungi (AMF) are obligate symbionts of more than 80% of all vascular land plants and many lower plants. Especially in the tropics, most tree species form AM, obtaining inorganic nutrients from the AMF and providing carbohydrates in exchange. From former researches in the South Ecuadorian rainforest ecosystem it is known that the vast majority of tree species (>98% of those investigated) form AM.

Our approach is, to apply AMF for a more efficient reforestation on abandoned pastures in South-Ecuador, with native tree species. It was shown before, that AMF from the studied ecosystem can improve the growth of nursery grown tree seedlings (Urgiles et al. 2009, DOI: 10.1007/s11056-009-9143-x). By an inoculation of tree species with a defined AMF cocktail, we aimed to find out which AMF species persist and have positive impact on certain tree species.

Therefore AMF from tree seedlings, native from Ecuador, were isolated and analysed at the morphological and molecular level (SSU, ITS, and LSU rDNA regions). From several attempts we established 10 AMF cultures, most of them single spore isolates, which were used as a mixture to inoculate seedlings from three different tree species. To address the question whether inoculation with AMF improves performance of these tree seedlings, plant growth parameters are measured and AMF are detected in roots. This is done in the nursery phase, and in future also after out-planting in the field.

P8.2 - Misleading mycorrhiza?

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Pinus pinea L. is an important Mediterranean forest species, mostly due to its edible seeds. Those seeds constitute a relevant resource for the Portuguese economy. To enhance *in vitro* rhizogenesis of *P. pinea* microshoots a co-culture system with ectomycorrhiza-derived fungi was developed. Plant and fungi were grown in double-layer WPM medium (with ½ the macronutrients and 0.2% of sucrose). Acclimation was made in pots containing mixed substrates, or in peat rhizotrons. Furthermore, axenic root cultures were prepared with roots excised from microshoots previously induced with auxin treatment. Observations were made monthly and root samples were taken from co-culture system and axenic cultures for further histological analysis. Structures similar to pine ectomycorrhiza (fine dichotomous and coralloid branching) occurred not only in co-cultures but in uninoculated controls, continuing to develop in the acclimation phase even in the absence of any symbiosis, and also in the axenic root cultures. The latter observation shows that axenic cultures may mimic the signaling from ectomycorrhizal fungi that is believed to induce the characteristic branching. This can be exploited in order to understand how plants perceive their mutualistic partners. Other anatomical and histological differences were found between mycorrhiza-like and fungal-induced structures.

P8.3 - Growth of the ectomycorrhizal fungus *Pisolithus microcarpus* on different nutritional conditions

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The exploitation of the ectomycorrhizas to improve plant productivity requires fungal inoculants in a commercial level. To develop such technology it is necessary to define the optimal composition of the culture media for each fungus to be cultivated. The studies already performed have only dealt with biomass production. However, other variables, such as biomass yield and culture productivity, are essential to process scale up and to the designing of

bioreactors. The effect of the composition of the culture medium on biomass yield and productivity of the fungus *Pisolithus microcarpus* was studied. Two culture media were modified to obtain several values of C/N ratio. The modification that promoted maximum biomass was employed to determine the minimal concentration of the more expensive nutrients, still able to allow a maximum growth. A 23 factorial design was employed to study the contribution of glucose, peptone and yeast extract to the efficiency of the cultivation process. The relation between biomass and substrate (glucose) was used to obtain the biomass yield where the final biomass was related to the initial glucose content instead of to the amount of glucose consumed. Results showed that glucose concentration should be increased up to 40% in order to promote higher biomass yield. Peptone had a positive effect whereas yeast extract had a negative effect. These results will contribute towards the development of fungal inoculants for commercial forestry programmes.

P8.4 - Ectomycorrhizal inoculum viability for *Nothofagus* species from the Patagonian forest of Argentina

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The increasing concern to recover degraded areas of *Nothofagus* spp. forests poses the challenge to produce ectomycorrhizal seedlings prepared to survive under unfavorable conditions. Starting an inoculation program requires selecting the best fungal species, adjusting the technology of inoculum conservancy and running nursery inoculation trials. To do that, species of ECM fungi were classified according to their aptitude to be used as spore inoculums, and their conservation tested. Taxa were classified according to the ease to be found, abundance, frequency of occurrence and spore density. 6 ECM species were selected and stored at 5°C as spore slurries and dried sporocaps with 4 repetitions each. Viability was measured along 270 days using Thiazolyl Blue Tetrazolium Bromide. *Cortinarius xiphidipus* and *Thaxterogaster* sp.nov. had 3200 and 3492 spores/mm³, *Austropaxillus statuum* and *Setchelliogaster fragilis* 5820 and 6060 spores/mm³ and *H. purpurea* 27046 spores/mm³. After 270 days *Thaxterogaster* sp.nov., *C. xiphidipus* and *S. fragilis* showed the highest viability as slurries (25.9, 19.1 and 18.8 % viable spores) while *S. fragilis* and *A. statuum* had better values conserved dried (26.3 and 21.6 % viable spores). *Hallingea purpureus* showed a drastic viability loss under both methods (8-11 % at 270 days). Better inoculum conservation method seems to depend on species. *A. statuum* and *S. fragilis* presented the better combination of characteristics to be used for inoculations.

P8.5 - Bioreactor for the production of ectomycorrhizal inoculants

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Ectomycorrhizal fungi (ECMf) are filamentous organisms with slow growth rates. The cultivation of these fungi in bioreactors requires minimal shear rates, and pneumatic bioreactors, such as airlift bioreactors, are quite adequate for that condition. A five-liter stainless steel airlift bioreactor was conceived and studied in terms of its hydrodynamics. It allowed the definition of important cultivation parameters such as the volumetric oxygen transfer coefficient. Further studies showed that the control of the oxygen transfer during the cultivation of a ECMf is essential for the productivity of the process. Afterwards, an electronic control system was installed to monitor the entire cultivation period, allowing to record the variation of air flow, dissolved oxygen, pH and temperature, with a specific software. A 120 h-controlled cultivation of *Rhizopogon nigrescens* presented a specific growth rate of 0.63 d⁻¹, and a productivity of 1.2 g.L⁻¹.d⁻¹. The control of the variables of the process confirmed that the oxygen availability is the most limitant factor to the growth of the fungus. Growth limitation occurred when the biomass reached 4 g.L⁻¹. At that moment the critical oxygen concentration was higher than 50% of the saturation due to the reduction of the rate of oxygen transfer caused by accumulation of compact mycelial pellets. Understanding the behaviour of cultivation variables is fundamental for the production of ECMf inoculants.

P8.6 - Mycorrhizae inoculated vegetable seedling production and use for field experiments

Ortas, I.

Mycorrhizae Inoculated Vegetable Seedling Production and Use for Field Experiments Ibrahim ORTAS, Cagdas AKPINAR Department of Soil Science, University of Çukurova, Faculty of Agriculture, Adana, Turkey iortas@cu.edu.tr Under semi arid soil conditions, since soils are poor in availability of nutrients because of its high clay and lime content and high pH, mycorrhizal inoculation is seem to be a good strategy for sustainable agriculture. Since it is still very difficult to produce large quantity of mycorrhizal inoculum for large area, it is sound to produce mycorrhizal inoculated seedling and then transplanting to the field. Green pepper, bell pepper, eggplant and

tomato seedling were produced in different growth medium which are made from different composting material with several mycorrhizal species. After several experiments selected seedling were used under field conditions. The experiments revealed that under field conditions, mycorrhiza inoculated seedlings are effectively established and given high response to plant growth and yield. The results showed that, mycorrhizal inoculated plants have high P and Zn content than not inoculated one. It has been concluded that seedling quality is much more depend on growth medium and mycorrhizal spore effectiveness. Also it is important to indicate using mycorrhiza inoculated seedling it is a good strategy for horticultural production.

P8.7 - *Ostryopsis davidiana* seedlings inoculated with ectomycorrhizal fungi facilitate formation of mycorrhizae on *Pinus tabulaeformis* seedlings

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Reforestation in China is important for reversing anthropogenic activities that degrade the environment. *Pinus tabulaeformis* is desired for these activities, but survival and growth of seedlings can be hampered by lack of ectomycorrhizae. When outplanted in association with *Ostryopsis davidiana* plants on reforestation sites, *P. tabulaeformis* seedlings become mycorrhizal and survival and growth are enhanced; without *O. davidiana*, pines often remain without mycorrhizae and performance is poorer. To better understand this relationship, we initiated an experiment using rhizoboxes that restricted root and tested the hypothesis that *O. davidiana* seedlings facilitated ectomycorrhizae formation on *P. tabulaeformis* seedlings through hyphal contact. We found that without *O. davidiana* seedlings, inocula of five indigenous ectomycorrhizal fungi were unable to grow and associate with *P. tabulaeformis* seedlings. Inocula placed alongside *O. davidiana* seedlings, however, resulted in enhanced growth and nutritional status of *O. davidiana* and *P. tabulaeformis* seedlings, and also altered rhizosphere pH and phosphatase activity. We speculate that these species form a common mycorrhizal network and this association enhances outplanting performance of *P. tabulaeformis* seedlings used for forest restoration.

P8.8 - Development and test of a counting equipment for *in-vitro* produced AM-spores

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In agriculture the use of mycorrhiza is gaining more and more importance. The AMYkor GmbH is able to produce *in-vitro* mycorrhiza spores on a large scale in a pilot plant. In order to formulate the propagules it is necessary to count the exact number of the spores. Therefore an equipment to determine the number was developed. It consists of a counting chamber with a constant flow rate and a CCD-camera. In this laminar flow the spores are detected by optical image processing. An adapted AxioVision software identifies the spores in the suspension as picture elements. Depending on feature and magnitude of the spots single spores, spore groups, hyphae and impurities - like root segments and air bubbles - can be identified. Thus, an exact and fast counting of the spores is possible. In extensive tests with a defined spore suspension counting results with a standard deviation of less than 5 % were achieved.

P8.9 - *Eucalyptus grandis* Hill ex Maiden essential oil biostimulation in ectomycorrhizal fungi isolates *in vitro*.

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Ectomycorrhizal fungi (fECM) contribute to the development of plants in degraded environments. A controlled mycorrhization of forest essences requires the production of inoculum of these fungi. Production of fECM inoculants have being increase used for searching the greatest efficiency of the final forest and plant products. The study on the phenolic compounds, which can be extracted from essential oils, may be involved in interactions between plants and microorganisms contributing to the highest plant adaptability to its environment. The present study aimed to evaluate the bioestimulating performance of the essential oil of *E. grandis* in the development of ectomycorrhizal isolates *in vitro*. Seven isolates of fECM were incubated in liquid culture medium containing an increasing concentration gradient of essential oil extracts of this substrate. At the end of the incubation period, it was determined the dry weight and hyphal morphometrical characters of mycelia. It was concluded that the eucalyptus essential oil addition in certain concentrations, significantly increases the *in vitro* mycelial growth. This oil could be an alternative for the ectomycorrhizal inoculum production optimization.

P8.10 - IBG: a tool for promoting ecosystem services in plant production systems

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Ecosystem services are components of nature that are directly enjoyed, consumed, or used to yield human well-being (www.rff.org/rff/Publications) and the UE Millennium Ecosystem Assessment demonstrated that services provided by ecosystems could reduce poverty, hunger and disease. Within this context, the importance and functional integration of AM fungi within agricultural, horticultural, forestry and revegetation systems makes them obvious tools for ecosystem services linked to plant production. The International Bank for the Glomeromycota (IBG; www.kent.ac.uk/bio/beg/) was created by COST 8.10, 8.21 and 8.38 European networks on mycorrhiza (www2.dijon.inra.fr/cost838), in order to register and provide a quality source of AM fungal germplasm. This service has been extremely useful for many studies and publications throughout the world. However, in the new context of ecosystem services, it is proposed that the IBG activity could expand and evolve towards providing innovating on request services with targets to: - exploit molecular technology to elaborate molecular probes to monitor fungal presence or for their identification (barcoding) - preserve commercial lines and assess inoculum quality for industrials and producers - provide technical information and training for industry and users By satisfying such new demands, IBG would participate to fulfilling requirements of ecosystem services created by a modern agriculture and to promoting innovations in plant production systems.

P8.11 - Responses of grain legumes to rhizobia and arbuscular mycorrhizal fungi: a meta-analysis in the perspective of photosynthate limitation of symbioses.

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Legumes, valuable as food, fodder, green manure and biofuel are prized for their grain protein and lipid mass fractions. Since legumes spend up to 20% of photosynthesis on each of the rhizobial and arbuscular mycorrhizal fungal (AMF) symbioses, positive responses in grain yield due to these symbioses are likely constrained by a photosynthate (C) limitation. We performed a meta-analysis of 348 data points with 12 legume species to test whether yield, harvest index (HI) and grain protein and lipid mass fractions are affected by symbioses. There was a significant increase in yield due to rhizobial inoculation (16%, field; 59%, pot). There were no responses of yield to AMF and rhizobial + AMF inoculations in the field (most probably because it is hard to ensure an AMF-free control), but significant responses in pots (45%, AMF; 44%, rhizobial + AMF). Rhizobial inoculation improved grain protein mass fraction by 7% in the field; AMF increased this parameter by 14% in pots. There were no effects of symbioses on grain lipid mass fraction. The responses of rhizobial symbioses in the field were most likely explained by increases in HI (+5%), meaning that photosynthetic rates were increased to compensate larger C sink strength. The AMF did not affect HI. In conclusion, increases in yield due to symbioses did not decrease grain protein and lipid mass fractions, indicating that legumes are not C-limited under symbiotic conditions.

P8.12 - Mycorrhiza as a component of organic approach in fruits and vegetables cultivation

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A European project within the frame of the Eureka programme E13375 was completed in 2008 focused on development of alternative organic management of cultivations of small fruits namely blueberries and raspberries. Conventional commercial cultivation in Southern Spain involves field grown fruits fertilised with chemical NPK with added phosphoric acid. Alternative approach consisted in inoculation of plants at planting with either ericoid or arbuscular mycorrhizal (AM) fungi and as a main source of nutrients organic matter (OM) input in a form of wood chips treated with selected white rot (WR) fungi to support faster decomposition. Pot and field experiments showed potential of this combined technology of mycorrhizal inoculation with WR fungi treated OM input to replace into major extent the input of agrochemicals in field cultivation. Testing of this technology on fruits is now being extended for organic growing of vegetables. Main target crops are tomato, leek, cucumber, peppers, strawberry and others. Results show potential synergism between AM fungi and WR fungi in promoting plant growth. Fungal inoculation seems to promote not only growth but also potentially improves target food health properties as content of antioxidant compounds. Support of the project Mycotech, funded by the Ministry of Education, Youth and Sports of the Czech Republic, grant No. OC09057 and Eurostars Project Microfruit E14366 is acknowledged.

P8.13 - Growth response of sago palm plantlets, *Metroxylon sagu* (Rottboll) to vesicular arbuscular mycorrhiza

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Sarawak, Malaysia produces clean, odourless and undegraded sago starch, ranking highly in the world. Sago palm, *Metroxylon sagu* (Rottboll) is cultivated in the world's first large-scale commercial plantations. However, sufficient planting material depends on micropropagation to convert sago palm from its present semi-wild status to modern clones of high yield, shorter gestation period and responsiveness to fertilizer. Although such technique has been successful, extensive use at the field has yet to be achieved due to poor growth in ex-vitro conditions. A study was conducted to determine whether a vesicular arbuscular mycorrhiza (VAM) isolated from wild sago palm roots can improve the growth of the sago plantlets using a Complete Randomized Block Design replicated three times with four treatments: non-inoculated without phosphate as control, non-inoculated with 5 gm phosphate, VAM without phosphate and VAM with 5 gm phosphate application. Results at the end of six months showed that the VAM inoculated plantlets without phosphate application showed the best growth in shoot height. In contrast, the control treatment produced the longest root length. Although there were no significant differences in the dry weight (gm) of the sago plantlets, the VAM and with combination of phosphate recorded heavier dry weight. This study illustrates the beneficial effect of VAM and concluded that VAM inoculation should form an integral component of the micropropagation process of sago palm.

P8.14 - Survival of *Castanea sativa* Mill. plants mycorrhizal with *Pisolithus tinctorius* after transplantation to soils infected with *Phytophthora cinnamomi*

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Several tree species, including the family of *Pinaceae* are dependent upon mycorrhizas. The use of pure cultures of selected ectomycorrhizal fungi is proven to be the best practice for improvement of tree survival and growth.

Mycorrhizal studies on sweet chestnut (*Castanea sativa*) led us to work on inoculum production and inoculation of chestnut plants in greenhouse and nursery to evaluate mycorrhizal effects under semi natural and natural conditions. The production of *Pisolithus tinctorius* mycorrhizal inoculum and the mycorrhization of seedlings in greenhouse were made by inoculation during germination stage. Three different methods were followed for plant transference to nursery and/or to the field with soils naturally infected with *Phytophthora cinnamomi*. We report the results obtained on survival and growth along the acclimatization process during three years time of nursery/field trials with chestnut tree seedlings mycorrhizal with *Pisolithus tinctorius*.

Mycorrhizal plants when transplanted to soils infected with *P. cinnamomi* revealed higher survival capacity than nonmycorrhizal plants. The survival revealed to be dependent on time since mycorrhization, being higher when plants were inoculated for a longer period before transference to the *P. cinnamomi* infected soils (6 months vs 1 month). Plant growth is influenced by mycorrhization, mainly in what concerns the height/stem diameter ratio of the plants.

P8.15 - Yield of Welsh onion (*Allium fistulosum* L.) inoculated with arbuscular mycorrhizal fungi at different soil phosphorus levels in the field

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The effect of inoculation of arbuscular mycorrhizal (AM) fungi on growth, P uptake and yield of Welsh onion (*Allium fistulosum* L.) was studied under non-sterile field conditions. Welsh onion was inoculated with or without (control) AM fungi *Glomus* R-10 and grown in a glasshouse for 58 days. Inoculated and non-inoculated seedlings were transplanted to field with four available soil P levels (30, 60, 100, and 150 mg P₂O₅ / 100 g) and grown for 109 days. Mycorrhizal colonization, shoot P concentration, shoot growth and yield were measured. Mycorrhizal colonization of inoculated plants was 94% at transplanting and ranged 60-77% at harvest. Non-inoculated plants were colonized with indigenous AM fungi. Shoot length and stem diameter of inoculated plants were higher than that of non-inoculated plants at 30 and 60 mg P₂O₅ levels. Shoot P content of inoculated plants was higher than that of non-inoculated plants at 30 and 60 mg P₂O₅ levels. Yield (shoot fresh weight) of non-inoculated plants was higher at 100 and 150 mg P₂O₅ levels than at 30 and 60 mg P₂O₅ levels. Yield of inoculated plants was not different among four P levels. Yield of inoculated plants at 30 mg P₂O₅ levels was same as that of non-inoculated plant at 100 mg P₂O₅ levels. These results indicate that inoculation of AM fungi can reduce application of P fertilizer to Welsh onion under field condition.

P8.16 - Effect of dual inoculation (*Glomus mosseae*-*Pseudomonas fluorescens*) on the fruit quality of tomato (*Lycopersicon esculentum* Mill)

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In this study the effect of inoculation of tomato (*Lycopersicon esculentum* Mill.) cv. Rio Fuego with *Glomus mosseae* and *Pseudomonas fluorescens* on greenhouse and fruit quality was evaluated. The treatments were fertilized plants (12-43-12) (F), inoculated (*P. fluorescens* Juv8 [CB], *G. mosseae* [CGm] y *P. fluorescens* Juv8 + *G. mosseae* [CB+ CGm]) and non-inoculated control (C). Although the responses of plant growth were variable, the largest percentage of degrees Brix (percent soluble solids, °Brix) was found in tomato fruits harvested from CB treatment at 101 days after transplantation (DAT), without appreciable changes at any sampling date (105, 112, 120 and 129 DAT). CGM treatment was higher only at 120 and 129 DAT with colonization rates of 26%. In CB + CGM it was 19.3% but also showed the highest number of CFU. These results demonstrated the importance to further evaluate these microorganisms for single and dual tomato inoculation in fruit quality attributes, particularly on °Brix.

P8.17 - Glomerospores density (*Glomus etunicatum*) for neem seedlings production.

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Neem subproducts are important in biotechnology with recognised application in agriculture, medicine and industry and, presently, there have been some interest in this plant for timber production. Neem is adapted to the arid and semiarid regions and to low fertility soils. The slow growth of seedlings in nurseries is a limiting factor and the AMF can contribute to speed the process. These fungi, especially *Glomus*, have been registered colonizing neem roots under natural conditions. The aim of this work was to evaluate the effect of the amount of *G. etunicatum* inoculum in the growth of neem seedlings. The experiment was carried out in greenhouse in entirely randomised set with 3 treatments (0, 60 and 230 glomerospores) and eight repetitions, in pots with disinfested soil containing 4 mg P dm³ and pH 4.7. The plantlets were obtained from seeds, transferred to pots and inoculated. The ANOVA showed a significant difference in stem diameter for all evaluations and, at the end (330 days), fresh and dry stem, fresh leaf mass and total aboveground mass were also significantly different. The inoculum constituted of 60 AMF glomerospores per plant contributed to increments of 20% in stem diameter, 23% and 30%, respectively, for fresh and dry stem mass, and 40% for total aerial fresh mass after 330 days. In conclusion, the addition of 60 glomerospores of *G. etunicatum*/plant can be recommended for neem seedling preparation as it stimulates growth in the early stages of development.

P8.18 - Arbuscular mycorrhizal colonization and soil enzyme activities early in the organic transition: effect of soil solarization and organic matter applied

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During the organic transition period, crop yields are often reportedly lower than in conventional system. Initially lower yields on organic farms have partly been attributed to the negative effects of conventional practices on the soil microorganisms that accelerate nutrient uptake by mineralizing soil organic matter. A field experiment was conducted to clarify the effect of some techniques used on Japanese organic farms on the soil microbial activities which may affect plant nutrient uptake.

Carrot was grown from Aug. to Dec. in 2008, and lettuce was grown from Feb. to Apr. in 2009 in 2 conventional and 4 organic systems. The 2 conventional systems, which received inorganic fertilizers, herbicides and pesticides, differed in compost application (with or without compost). The 4 organic systems, which received organic fertilizer, differed in compost application and soil solarization (with or without solarization).

Soil solarization significantly lowered beta-glucosidase, galactosidase, cellulase, saccharase, dehydrogenase activities, however, accelerated soil N mineralization. Soil solarization did not significantly lowered AM colonization of carrot and lettuce. Organic fertilizer application raised alpha-glucosidase, protease and dehydrogenase activities, but did not affect AM colonization. Compost application raised cellulase activities, but did not affect AM colonization. Further studies are needed to clarify microbial properties during the organic transition period.

P8.19 - Effect of arbuscular mycorrhizal fungi and green waste compost on growth and nutrient uptake of selected container grown ornamental nursery crops

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This study investigated the effects of commercially available arbuscular mycorrhizal fungi (AMF) and supplement

of green waste compost (0, 10 or 20%) to peat substrate medium on growth of ornamental plants under a nursery-container production system at recommended and reduced mineral fertilizer level (50% of recommended rate). Deciduous plants *Potentilla fruticosa* 'Gold Drop', *Physocarpus opulifolius* 'Diabolo', *Spiraea japonica* 'Pruhoniana' and conifers *Chamaecyparis lawsoniana* 'Golden Wonder', *Taxus 'media* 'Hicksii' and *Thuja occidentalis* 'Smaragd' were inoculated with commercial AMF Endorize-mix and Endo-mix or non-inoculated (NonAMF) during potting of one-year old plants to containers. Height and fresh weight of deciduous plants was significant lower at reduced mineral fertilizer level, but mycorrhizal fungi better colonized roots. The effect of AMF inoculation on plant growth varied according to species and AMF. Endo-mix inoculum stimulated growth of *Taxus* and *Potentilla* but negative affected the growth of *Chamaecyparis* and *Spiraea* when compared with the NonAMF control plants. Endorize-mix inoculum stimulated growth of *Potentilla* (height and fresh weight) and reduced growth of *Spiraea*. Growth of *Thuja* and *Physocarpus* was not affected by AMF. Phosphorus content was higher and calcium content was lower in broad-leaves plants inoculated with Endorize-mix compared to non-inoculated plants especially in potting medium supplemented with green waste compost (10%).

P8.20 - Native Glomeromycota biofertilization to improve maize development under agroecological management

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An agroecological experiment was in set acid soils savannas, as an alternative proposal to manage agricultural systems. Direct sowing and Glomeromycota native species inoculum were applied to maize crop in combination with a moderate dose of P as phosphate rock; *Centrosema macrocarpum* and *Urochloa dictioneura* were used as standing crops. Comparative treatments were established on the basis of chemical fertilizer in combination with standing crops: I0 (control without fertilization), IR (low dose of NPK, 50% P as rock phosphate and 50% as ammonium phosphate), RF (Dose NPK, 100% rock phosphate as source of phosphorus) and BF (25% of the dose of P as phosphate rock + inoculation with mycorrhiza). Maize was harvested to determine nutrient content in shoot; biometric parameters of maize were measured. IR treatment was more effective in maize development, when *C. macrocarpum* was applied. RF and BF treatments were second and third in maize yield, respectively, with slight differences. Considering IR as the best nutritional treatment for maize development, inoculation with Glomeromycota (BF) reached values of up to 88% of the production obtained in IR. Also BF application helped maize to overcome fallow disorder. Suitable biofertilizers stimulate 25-30% of the crop grown in optimal nutritional conditions (such as IR treatment). The application of Glomeromycota inoculum with native fungi looks promising to improve the productivity of acid soil savannas in central Venezuela.

P8.21 - Mass propagation of VAM inoculum

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Glomus mosseae spores were added to sterilized soil in a 15 cm diameter plastic pot at the beginning and sorghum (*Sorghum vulgare*) seeds were sown. After two months the shoots were removed, roots were cut and mixed with soil in the pot and used as an initial vesicular arbuscular mycorrhiza (VAM) inoculum. This procedure was repeated with *Glomus mosseae* inoculum and sterile soil in different ratios to reproduce the VAM inoculum having highest growth of sorghum in two months. *Sorghum* seeds sown in two parts of *Glomus mosseae* inoculum mixed with one part of sterilized soil gave highest growth of sorghum. Therefore this was used as a final VAM inoculum. Seed-beds were prepared and VAM inoculum collected from sorghum roots was spread out over the seed-bed at the beginning and finger millet (*Eleusine coracana*) seeds were sown. After two months the shoots were removed and 5 cm of the top soil in the seed-bed was collected and used as a mycorrhizal inoculum containing finger millet roots. Fresh root samples of sorghum and finger millet were examined under microscope for the presence of rhizomorphs. Root hairs were not visible in the inoculated plants. The hyphal growth in the inoculated roots was visible when stained with cotton blue. It was clearly visible in finger millet than in sorghum. Inoculated plants showed high rate of growth and it was highest in finger millet inoculum. Hence, VAM (*Glomus mosseae*) spores were found to be proliferated well in finger millet roots. Therefore finger millet roots containing VAM can be used as an inoculum for perennial crop nurseries.

P8.22 - Production of in vitro mycorrhized plants: learning of the nature

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Several scientific evidences reveal that it is possible that the first terrestrial fungi colonized land before plants did. Analysis of DNA-based phylogenies jointly with palaeobotanical and morphological studies confirm the coevolution of arbuscular mycorrhizal fungi and roots for around 400 million years. The obligate biotrophic condition of these organisms and its hypogeous nature has hindered and complicated the studies of the association; therefore

numerous attempts have been made to reproduce this symbiosis in vitro. During the last decade promising systems for in vitro mycorrhization of plants have been proposed, standing out the importance of those for physiological, biochemical and molecular studies; but in our opinion the significance of mycorrhizal vitroplants in the micropropagation industry is undervalued. In view of these unquestionable facts the possibility of introduce mycorrhizal fungi during plants propagation process should be seriously considered. The clear potential of these fungi for agriculture regarding plant productivity and reduction of different stresses associated with nutrition, water relations, pH and pathogens must be the principal reason; considering the artificial conditions in which plants develop, making them more susceptible to the transplant. Here we present some results obtained on mycorrhization of micropropagated potato plants with two of the most promising systems lately described. We compare both and we discuss some of its limitations and potentialities for micropropagation industry.

P8.23 - Desinfection efficiency of *Glomus* spores for *in vitro* culture

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The *Glomus* spores were desinfected by different methods before culturing them in vitro in order to obtain aseptical material for the experiments. In the desinfection process, the effects of the antibiotics were compared in four treatments, in the following way: treatment A, that is the one used in the laboratory of the Zaidin Station, and treatments B, C, D, that are the modifications proposed by the author for this exercise. Treatment A: two drops of Tween 20 + Chloramine T, washed with distilled water and then Streptomycin 0.01g/L (5, 10, 15 min.). Treatment B: two drops of Tween 80 + Chloramine T, washed with distilled water and then Amikacin 0.01g/L (5, 10, 15 min.). Treatment C: two drops of Tween 80 + Chloramine T, washed with distilled water and then Gentamicin 0.01g/L (5, 10, 15 min.). Treatment D: two drops of Tween 80 + Chloramine T, washed with distilled water and then Amikacin-Gentamicin 0.01g/L (5, 10, 15 min.). The analysis showed that the best treatment to desinfect the spore surface and that does not affect the viability of it, was the treatment B. According to this results, this protocol was implemented for the development of the monoxenic culture in the microbiology laboratory of the GEBIUT group.

Genome Structure, Plasticity and Evolution

P9.1 - Organization of the respiratory chain of *Glomus intraradices* deduced from genome analysis

Formey, D & Roux, C. (Laboratoire Surfaces Cellulaires et Signalisation Chez les Végétaux UMR5546 CNRS-université Paul Sabatier Equipe Symbiose Endomycorhizienne et Signalisation Cellulaire, France) - The *Glomus* Genome is a program from the US Dpt of Energy's Joint Genome Institute (JGI).

The *Glomus intraradices* genome consortium is in charge to annotate the genome of the strain DAOM 197198 (Martin et al., 2004). Our team is involved in the annotation of the mitochondrial genome and energetic metabolism genes. We here present the organization of the respiratory chain deduced from genome and EST analysis. The mitochondrial genome has a size of 70,783 bp and presents a classical structure with a standard core of mitochondrial genes. The comparison with the mitochondrial genome map of the strain 494 of *Glomus intraradices* (Lee and Young 2009) revealed a high sequence homology in coding regions and highly polymorphic islands present in non-coding regions. The structure of the respiratory chain of *Glomus intraradices* is typical from fungi. As previously suggested by a pharmacological approach (Besserer et al, 2009), AM fungi present a ramified respiratory chain which could participate to energy production during asymbiotic growth. The presence of a nuclear genome encoded alternative oxidase support this hypothesis.

Besserer A, Bécard G, Roux C, & Sejalón-Delmas N (2009). *Plant Signaling & Behavior* 4(1):1-3.

Lee, J. and J. P. Young (2009). *New Phytol.*

Martin F, et al. (2004). *New Phytologist* 161:330-335.

Damien Formey is granted by Agronutrition (France)

P9.2 - Glomeromycota (AM fungi) host a pan-global, monophyletic lineage of endobacteria

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Arbuscular mycorrhizal fungi (AMF) are obligate and ancestral land plant symbionts known to host two types of bacterium-like organisms (BLOs). One has been identified as *Burkholderia* related and was described as *Candidatus Glomeribacter gigasporarum*. The other was documented as coccoid with a distinct cell wall, but has a yet obscure phylogeny. We investigated endobacteria in spores from 28 AMF cultures originating from four continents morphologically by staining the cytoplasm with fluorescent dyes. Analyses of the BLO 16S rDNA revealed them to represent a clade in the Mollicutes. FISH with specific oligonucleotides allowed us to localize the AMF BLOs in the

spore cytoplasm. Our results indicate that BLOs are endobacteria that have been living in AMF for more than 400 My. The result is a vertically inherited, monophyletic and ancient population of uncultured endobacteria. The BLOs cluster within the Mollicutes, notwithstanding the presence of a distinct cell wall. They probably maintained this Gram-positive bacterial trait, whereas other Mollicutes lost it after divergence from Gram-positive ancestors.

P9.3 - Identification and expression analysis of isocitrate lyase and malate synthase genes in *Pisolithus microcarpus*

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In ectomycorrhizal fungi, mobilization of lipid reserves is shown to have a central role in processes such as basidiosporogenesis, spore germination, and symbiosis establishment. The glyoxylate cycle is an important pathway linking lipid mobilization to gluconeogenesis. The objective of this work was to study the glyoxylate cycle in the ectomycorrhizal fungi *P. microcarpus*. The genes coding the glyoxylate cycle enzymes, isocitrate lyase and malate synthase, were identified and the full length sequences were obtained by Genome Walking. Isocitrate lyase and malate synthase genes are interrupted by 5 introns in ORFs presenting 1897 and 1880 bp. The localization of isocitrate lyase is peroxisomal, while malate synthase localization could not be predicted based on amino acid sequences. In the vegetative mycelium, the expression of glyoxylate cycle genes was up-regulated in the presence of acetate and repressed by glucose. Also, the flavonoid rutin is capable of stimulating the expression of these genes. Other rhizospheric factors, as the flavonoid quercetin and citrate, and the presence of the host root did not induce changes in the expression of glyoxylate cycle genes. The identification of these genes opens the possibility of studying lipid metabolism throughout developmental processes, such as basidiosporogenesis and ectomycorrhiza formation in *P. microcarpus*. Financial support: FAPEMIG and CNPq.

P9.4 - Analysis of the microtranscriptome of *Medicago truncatula* during symbiotic interactions

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In plants, several works describe the role of microRNA (miRNAs) to mediate plant cell responses during microbial interactions (Navarro et al, 2008). During *Medicago truncatula/Sinorhizobium meliloti* symbiosis, it was demonstrated that the microRNA MtmiR169 is involved in the posttranscriptional regulation of a transcription factor required for nodule growth and development (Combiér et al., 2006). The partial overlapping of cell signalling pathways and host cell responses for arbuscular mycorrhizal (AM) symbiosis and nodulation suggest common and specific patterns of miRNA expression. We analyse the miRNA diversity of *Medicago truncatula* during AM and *S. meliloti* symbiosis. We used recent technical advances in miRNA isolation (kit Illumina®, Solexa® sequencing) and bioinformatic analysis (based on Noirot et al., 2008) to identify new miRNAs in *Medicago truncatula* and compare their presence during AM symbiosis and nodulation. (Financial support: Agronutrition and MIRMED program from Genoscope, France)

Combiér J., Frugier F., de Billy F., Boualem A., El-Yahyaoui F., Moreau S., Vernié T., Ott T., Gamas P., Crespi M. and Niebel A. (2006). *Genes & Dev* 20: 3084–3088

Navarro L, Jay F, Nomura K, He SY, Voinnet O. (2008). *Science*. 321 (5891):964–7.

Noirot C, Gaspin C, Schiex T and Gouzy J. LeARN. (2008) *BMC Bioinformatics*. 4(9) 21–.

P9.5 - ITS rDNA of six ectomycorrhizal fungi isolates

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Ectomycorrhiza is a type of symbiotic association between fungi and plants. In this type of mycorrhiza, there is a low diversity of plant hosts but a high diversity of the fungi involved. Most of the ectomycorrhizal fungi may be identified in the field or in the laboratory through the examination of their fruiting-bodies. However, molecular techniques as PCR and gene sequencing have been very helpful in the characterization of these organisms. The aim of this work was to characterize and to analyze the variability in the ITS rDNA region of ectomycorrhizal isolates from different locals and hosts, through the technique of PCR-sequencing. The DNA was extracted from mycelium produced in MNM liquid medium and the samples of genomic DNA were submitted to the Polymerase Chain Reaction (PCR) with the universal primers ITS1 and ITS4, and the product was sequenced. The amplified products of all isolates presented approximately 600 base pairs (bp). Their ITS regions showed differences in the nucleotide sequence making it possible to characterize and differentiate among isolates. Isolates of the genus *Pisolithus* formed a single cluster, whereas those belonging to *Scleroderma* spp. formed two separate clusters: one of the clusters was formed by the Brazilian isolates and the other by an Australian isolate. Finally, the fourth group was formed by one isolate of *Chondrogaster angustisporus*, a new species isolated from *E. dunnii* plantations in Santa Catarina, Brazil.

Mycorrhizosphere Interactions

P10.1 - Heat shock protein 60 (Hsp60 protein) from *Glomus intraradices*, sequence alignment and phylogenetic analysis

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Glomalin, an Hsp60 glycoproteinaceous product from arbuscular mycorrhizal (AM) fungi has been found to be beneficial for soil stability, and thus environmental sustainability and plant productivity. Amino acid sequence of Hsp60 from *Glomus intraradices* was aligned and phylogenetically analysed with Hsp60 sequences from organisms of six groupings using ClustalW2 BLAST programme. As Hsp60s are commonly found chaperone proteins in biological systems the relationship between glomalin and other Hsp60 sequences were investigated. The peptide chain of *G. intraradices* Hsp60 is 590 amino acid residues long. Alignment identities were with six bacterial species (54%), six yeast species (69%), 72% with 17 filamentous fungal species, 60% with eight insect species, 59% with 10 plant species, 58% with eight terrestrial animal species (including human, *Homo sapien*), and 60% for six water animal species. Ciliate protozoan *Tetrahymena thermophila* had 17% identity and a terrestrial animal European rabbit, *Oryctolagus cunicular*, had 13%. Phylogenetic analysis indicated *G. intraradices* Hsp60 to be closely related to *Neocallimastix patriciarum*, aerobic ruminal fungus that digest lignin, and *Piromyces* sp. E2, anaerobic fungus isolated from droppings of Indian elephant. Structural functional studies of glomalin will also be conducted. Knowledge of other Hsp60's may assist in increasing glomalin production in AM fungi, or in using other organisms to produce polymers offering benefits of glomalin.

P10.2 - Interaction between arbuscular mycorrhizal fungi (AMF) and phosphate solubilizing fungi. Effect on AMF hyphal growth and alkaline phosphatase activity

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Phosphorus (P) is one of the main nutrients. It occurs mainly as inorganic phosphate (iP) and as organic phosphate (oP) derivatives, unavailable to plants. iP is converted into plant-available forms by some saprophytic fungi (SF). Arbuscular mycorrhizal fungi (AMF) produce alkaline phosphatase enzymes (AP) that solubilize oP sources and improve nutrient absorption by plants by increasing the absorptive surface area of the roots. Exudates of some SF are considered germination modulators, since they either stimulate or inhibit hyphal growth, depending on their concentrations. In this work, we tested the effect of *Talaromyces flavus* exudates on *Gigaspora rosea* *in vitro* by studying the interaction between SF and AMF. The 1-3% and 0.5-1% SF exudate concentrations increased the hyphal length and the number of branches respectively, suggesting the presence of substances that improve the growth and ramification of the AMF mycelia in the exudates of SF. We also evaluated the effect of *T. flavus* on the hyphal AP of *Glomus intraradices* in green-house experiments. The AP activity percentage was higher in the presence of *T. flavus* with iP amendment. The results show that the presence of iP produces changes in *T. flavus* exudates that positively influence the activity of AP of *G. intraradices*.

P10.3 - Occurrence of ecto- and arbuscular mycorrhizas and nitrogen fixing bacteria in *Dimorphandra wilsonii*, a threatened species from the Brazilian Cerrado

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Dimorphandra wilsonii is a Caesalpinoideae leguminous tree, native to the Brazilian Cerrado (Savannas type) and is currently threatened of extinction. Nowadays, there are only eleven of these trees that have survived in this region of Paraopeba and Lagoa Santa in the Minas Gerais state. Mutualistic associations between plant and soil microorganisms are strategies closely linked to the adaptive success of plant species. This study aimed to evaluate the presence of symbionts in the roots of *D. wilsonii*. Surprisingly a concomitant occurrence of ectomycorrhizal, arbuscular mycorrhizal fungi (AMF) and nitrogen fixing bacteria (indeterminate growth nodules) were found. There was abundant ectomycorrhizae forming by a specific morphotype and the identification is being done by ITS DNA sequence. The plants presented about 22 % of root AMF colonization. Morphological analysis of AMF spores from rhizosphere soil revealed the dominance of *Acaulospora* species. The tripartite infection is a rare event, and may be related to the adaptive strategy for the survival of this species.

P10.4 - Spore of the mycorrhizal fungus *Glomus mosseae* host yeasts that solubilize phosphate and accumulate polyphosphates

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In addition to the well-known interactions between plants and fungi, mycorrhizal roots offer excellent ecological niches for other microbes. The present paper reports the presence of bacteria and yeasts tightly associated with spores of an isolate of *Glomus mosseae*. Healthy spores were surface disinfected by combining chloramines-T 5%, Tween-40, and cephalixin 2.5 g L⁻¹(CTCf). Macerates of these spores were incubated on agar media, microorganisms were isolated, and two yeasts were characterized (EndoGm1, EndoGm11). Both yeasts were able to solubilize low- soluble P sources (Ca and Fe phosphates) and accumulate polyphosphates (polyps). Sequence analysis of 18S ribosomal deoxyribonucleic acid showed that the yeasts belong to the genera *Rhodotorula* or *Rhodosporidium* (EndoGm1) and *Cryptococcus* (EndoGm11). Results from inoculation experiments showed an effect of the spore-associated yeasts on the root growth of rice, suggesting potential tripartite interactions with mycorrhizal fungi and plants.

P10.5 - Powering mutualism: Rhizosphere Protozoa and arbuscular mycorrhizal fungi cooperate for exploitation of soil nutrient patches

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Nitrogen limits plant growth in most terrestrial ecosystems. Plants interact with multiple symbionts of very different taxonomic affiliation to improve their acquisition of nitrogen. Previous studies focused on interactions between single symbionts and plants are documented e.g., root colonizing symbionts, such as arbuscular mycorrhizal (AM) fungi, enhance plant nitrogen acquisition by extending the absorptive surface of roots in exchange of photosynthetically fixed carbon. Free living protozoa, mobilize nitrogen locked up in bacterial biomass thereby rendering it available for plant uptake. In neither case the importance of AMF – protozoa interaction was considered. By examining interactions between these two ubiquitous rhizosphere biota for plant N acquisition and growth, we showed that protozoa and AM fungi complement each other. Thus, the interaction consists in protozoa mobilizing N from organic matter sources and AM fungi translocating it to the host plant. Our findings demonstrate that plant nitrogen acquisition and growth in terrestrial ecosystems can only be understood by including synergistic interactions among rhizosphere symbionts and heterogeneous resource distribution.

P10.6 - Mycoparasitism of arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal (AM) fungi and saprophytic fungi from the genus *Trichoderma* are well known to develop beneficial associations with plants. The combination of both appears attractive within the context of sustainable use of soil natural resources. *Trichoderma* spp. parasitizes a wide range of fungi and is likely to infect the extraradical (ERM) and intraradical (IRM) mycelium of AM fungi. Microcosms experiments allowing interactions between *Glomus intraradices* and *T. harzianum* were set up under *in vitro* autotrophic conditions using *Solanum tuberosum* as host. Microscope camera-imaging system coupled with enzyme analysis (i.e. succinate dehydrogenase) was used to assess the mycoparasitism in the ERM and IRM. The microscopic observations of the ERM showed an extensive mycoparasitism characterized by hyphae invasion, cytoplasm retraction and septa formation and total degradation of spore content causing bursting of spores. The scrupulous monitoring of the IRM revealed for the first time the presence of *T. harzianum* in intraradical hyphae and vesicles. Given that AM fungi are integral part of all terrestrial ecosystems, mycoparasitism of extra- and/or intraradical phases of AM fungal symbiosis could have important consequences for AM fungal communities and symbiotic efficiency, therefore affecting plant productivity and ecosystem functioning.

P10.7 - Development of in situ PCR for species-specific detection of ectomycorrhizal fungi colonising trees

Visnovsky, Sandra B., Wang, Y., Bulman, S.R., Guerin-Laguette, A., and Pitman, A.R.,

Molecular tools have been increasingly developed to track the establishment of ectomycorrhizal (ECM) associations on host plants. However, previous studies have demonstrated that a single ECM root tip can harbour more than one species of ECM fungi, resulting in multiple amplicons when using basidiomycete-specific primers. Furthermore, the detection of an ECM fungus on a root does not confirm that it has successfully formed a mycorrhizal association with that host. In this work, *in situ* PCR was developed for the unambiguous identification

of several economically important edible fungi forming ECMs on root tips of artificially inoculated trees. Species-specific PCR primers were designed or adapted from published work for the identification of *Boletus edulis*, *Lactarius deliciosus*, *Tuber melanosporum*, *Rhizopogon roseolus* and other edible fungi. Digoxigenin (DIG)-based in situ PCR was then performed on permeabilised and fixed tissue sections using the species-specific primers to incorporate DIG labelled nucleotides directly into the mantle and Hartig net of the fungi during PCR amplification. DIG-labelled PCR products in the sections were visualized by a colorimetric reaction resulting in a purple precipitate on the target DNA. Our results showed that in situ PCR can be used to detect species-specific ectomycorrhizal structures on the roots of a number of hosts, opening the way to differentiate multiple fungi associated with a single root tip.

P10.8 - Evaluation of actinomycete strains for key traits related with mycorrhiza helping activities

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Three actinomycete strains were isolated from *Trifolium repens* rhizosphere and identified by microscopic characteristics, biochemical tests and molecular techniques. They were tested for interactions with AM fungi. *Streptomyces* MCR9, *Thermobifida* MCR24 and *Streptomyces* MCR26 were selected for further experiments because MCR9, MCR24 and MCR26 produce siderophores, solubilize phosphates and grow in N-free medium. The inoculation of AM fungi improved early establishment of MCR9 in clover rhizosphere, and the late establishment of MCR24. The three strains improved spore germination and AM mycelia growth. Inoculation with either of the selected actinomycetes enhanced plant growth and AM formation. Co-inoculation produced synergic benefits on plant growth and N acquisition. The three selected actinomycetes improve AM formation by the inoculated *Glomus* sp.

P10.9 - Identification of AMF and associated bacteria isolated from P-fixing and non P-fixing sugarcane rhizospheres of KwaZulu-Natal, South Africa

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Arbuscular Mycorrhizal Fungi (AMF) and bacteria associated with AMF spores within the sugarcane mycorrhizal ecosystem, play an important role to improve the health and yield of crops. This study was aimed at the identification AMF spores indigenous to sugarcane plantations of the KwaZulu-natal region of South Africa as well as the bacteria associated with these indigenous spores. Spores were extracted from rhizospheres of two soil types i.e. P-Fixing (Klipp 3 in the midlands) and Non P-fixing (La-Mercy 2-2 on the coast). Spores were extracted using wet sieving and centrifugation and subjected to biochemical staining using polyvinyl lactoglycerol (PVLG) and/or Meltzer's reagent. Spore morphology was analyzed using fluorescent light microscopy and Environmental Scanning Electron Microscopy (ESEM). Results indicated the presence of five spore types viz.: *Scutellospora nigra* (abundant in P-fixing soils), *Glomus deserticola* (abundant in non P-fixing soil), *Glomus geosporum* (both soil types), *Gigaspora verucosa* (non P-fixing soil only) and *Acaulospora laevis* (non P-fixing soil only). Mycorrhizal associated bacteria were identified using the polymerase chain reaction (PCR), genomic DNA sub-cloning and sequencing. After identifying spores morphologically and surface disinfecting them using EDTA, spores were placed onto nutrient agar to encourage bacterial growth. Pure cultures enabled bacterial DNA extraction, amplification, sub-cloning and sequencing. Sequence identification indicated the following bacteria: *Brevibacillus reuszeri*, *Bacillus* spp. and *Stenotrophomonas maltophilia* to be associated with AMF spores: *Scutellospora nigra*, *Glomus deserticola* and *Glomus geosporum* respectively. These spores were present in both P-fixing and non P-fixing soils. *Bacillus cereus* ATCC14579 was isolated from white AM spores (species yet unidentified) and *Paenibacillus chitinolyticus* was isolated from *Acaulospora laevis* (non P-fixing soil only). Key words: arbuscular mycorrhizal fungi, identification, bacteria, P-fixing and non P-fixing.

P10.10 - Some associated shrub plants facilitating *Cupressus atlantica* introduction by improving soil biological and chemical fertility.

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Lavandula spp and *Thymus* spp are considered as representative shrub species associated with *Cupressus atlantica* G. in a degraded area in Morocco (Haut Atlas). These shrubs grow following a patchy distribution in these semiarid ecosystems and form "fertility islands" which could be involved in the development of native plant species. This vegetation type can improve its own environment and act as "nurse plants" through their positive impacts in the survival of other native plant species. The aims of our study were to asses the potential effects of these shrub species in revegetation strategies, by testing in field conditions the impact of dual cultivation of *Lavandula stoechas* and

Cupressus atlantica on the growth of this tree species and on microbial functional capabilities and more particularly on arbuscular mycorrhizal soil potential. The mycorrhizal status of several representative shrub species (*Lavandula* spp. and *Thymus satureioides*) was evaluated in-situ and in-vitro. As well their contribution to the soil mycorrhizal potential. Rhizosphere soils collected under these target species were tested for their influence on the growth of *Cupressus atlantica*, a tree species whose natural stands has declined in Morocco. The main effects of the association between *Lavandula* species and *Cupressus* on the soil microbial activities, on the growth and the mycorrhizal status of each plant species were assessed in a glasshouse experiment. The growth of *C. atlantica* seedlings was significantly higher in the shrub species soils than in the bare soil. *Lavandula* enhanced significantly the mycorrhizal soil infectivity, and when *Cupressus* and *Lavandula* were grown together, the growth and mineral contents of seedling were strongly enhanced. After 3 years in field conditions, benefits from the association between *Lavandula* and *Cupressus* on the *Cupressus* development and soil functioning is sustainably maintained in field conditions, especially plant mineral nutrition soil microbial activity. The higher development of external mycelium in the dual cultivation treatment has enhanced soil microbial activities (total and dehydrogenase activities). In addition to the quantitative external mycelium effect on fluorescent pseudomonad population, fluorescent pseudomonad strains isolated from soil are able to solubilize tricalcium orthophosphate. It suggests that the selective effect of external mycelium on soil microbiota can improve the phosphorus soil content around the hyphae and, consequently enhance the phosphorus uptake by the external mycelium that further transfer this nutrient to the host plant. These results show that multitrophic interactions take place between mycorrhizal fungi, mycorrhizosphere microbiota and host plants, and that these shrub species can be considered as nurse plants that could promote the establishment of tree species such as *Cupressus* in reforestation programs.

P10.11 - Development of an in vitro system to assess the effect of *Glomus intraradices* on indigenous South African sorghum plants

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In vitro systems are useful tools for investigating different agricultural plant-mycorrhiza interactions under sterile conditions. We aimed to investigate the effects of arbuscular mycorrhizal fungi associated with maize, sorghum and medicago plants. Different media viz. Modified Strullu Romand (MSR), MSR with no sucrose and no vitamins (MSR-S-V), were used to determine, growth, maintenance and contamination. Seeds were surface sterilized and then germinated for 4 days in MSR media containing neither sucrose nor vitamins, then introduced into HAM-P systems, before being associated with *Glomus intraradices* MUCL 4133. Plant-fungus interactions were then observed via electron microscopy and root staining techniques. After a four week period colonization of roots by AMF, maize and sorghum plants indicated better growth compared to non mycorrhizal plants. Sorghum proved to grow faster when associated with AMF than did *Medicago truncatula* in which growth was observed only after three months. AMF associated crops do indeed display better and faster root and shoot development, compared to non mycorrhizal plants. Keywords: in-vitro systems, arbuscular mycorrhizal fungi, maize, sorghum, plant growth

P10.12 - Effect of transgenic soybean on the functional groups of microorganisms in the rhizosphere

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The presence of new proteins produced by transgenic plants in the soil should influence microbial community in the rhizosphere. The objective of this work was to evaluate in a greenhouse, the influence of two transgenic soybeans resistant to glyphosate, on the functional groups of microorganisms including enzymes. The experimental design was in block completely randomized with five replicates. Two variety of transgenic soybean resistant of Glyphosate (GM), BRS - 244 - Londrina (RR EMBRAPA 59) and soybean Valiosa (RR Conquista), and their parental non-GM, EMBRAPA 59 and soybean Conquista - Uberaba were used. The plants were inoculated with *Glomus clarum* or *G. etunicatum* and *Bradyrhizobium japonicum* SEMIA 5080 and *B. elkanii* SEMIA 587. As bioindicator were evaluated a population of functional groups of microorganisms which participate of C cycling, N cycling and P cycling, and the activity of soil enzymes from C cycling (dehydrogenase and cellulase), N cycling (urease and asparaginase) and P cycling (acid phosphatase). Also the biomass of C, N and plant growth were estimated. The results showed that no significant differences were observed among GM plants and their parental non-GM, except for N biomass, AM colonization and cellulase activity. The presence of AM fungi had great influence on the functional groups of microorganisms while some enzymes activity decreased.

P10.13 - *Glomus mosseae*, *Glomus hoi* like and systemin in the control of roots and leaf pathogens in tomatoes

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Martínez, B.

Tomatoes is affected for several microorganisms, which produce many economic losses. The arbuscular mycorrhizal fungi (AMF) is an alternative with big possibilities to be employed in the crop protection to different roots and leaf pathogens. The influence of two AMF species and the elicitor systemin on the control to *Alternaria solani*, *Phytophthora nicotianae*, *P. Infestans* and *Oidium* sp., in the tomatoes (*Solanum lycopersicum* L.) variety Amalia, was studied. The application of AMF and systemin together produced a positive effect on the reduction of damage produced by the pathogenic agent. The elicitor and *G. mosseae* combination produced the reduction of early blight (*A. solani*) and later blight (*P. Infestans*) with respect to the control (1 and 1,5 lever). The combined of *Glomus "hoi like"* and the elicitor produced decrease the damage produced by *P. nicotianae* and *Oidium* sp. (decrease in 1,6 and 1,3 level). However, his action on the early and later blight was less significant. The systemin application produced an important effect on the control of *A. solani* (decreased in two levels), but not on the another patogenic agents.

P10.14 - Aluminum effects on root and *Glomus irregulare* external mycelium development in monoxenic root organ cultures

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Soils in the neighborhood of leather treatment factories in central Mexico are contaminated by aluminum from dumping of chemical residues. Crops and wild plants grow successfully though in these polluted environments. As part of a program to investigate the contribution of AMF to plant heavy metal tolerance and plant Al uptake in contaminated sites we examined the tolerance of roots and fungi to Al in *in vitro* cultures. We studied the development of carrot (*Daucus carota* L.) roots and the external mycelium of *Glomus irregulare* with increasing concentrations of Al. Plates were prepared to expose nonmycorrhizal roots, mycorrhizal roots and external mycelium to Al in order to evaluate the tolerance of roots and fungus separately and together. Nonmycorrhizal carrot roots showed lower biomass than mycorrhizal roots at all Al concentrations tested (50,100 and 200 μ M). Biomass of the external mycelium of *G. irregulare* was not reduced by Al. On the contrary, aluminum stimulated fungal growth at 200 μ M concentrations. When roots and fungus were grown and exposed together to Al, root biomass was less reduced than when roots were grown alone (as nonmycorrhizal). We conclude that although *G. irregulare* is not adapted to Al polluted soils; this fungus allowed carrot roots to develop better in the presence of Al. We are looking for native AMF isolates that show similar plant protection effects to promote their activity and assist bioremediation of Al polluted soils.

Taxonomy and Phylogeny of Glomeromycetes

P11.1 - Parallel between molecular and morphological species within *Glomus* genus (Glomeromycota, Fungi): the case of three protein-encoding genes.

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Ribosomal genes are routinely utilized in molecular identification of Fungi. The different copies of these genes within each *Glomus* species make them more or less useless. We have tested three basic metabolism protein-encoding genes: *EF1-a* (825bp), *F-ATPase β -subunit* (620bp) and *V-H⁺-ATPase VHA5* (1475bp). *F-ATPase β -subunit* has never been used for the *Glomeromycota* identification so far. Twelve distinct species and five strains of the same species (*Glomus intraradices*) were molecularly analysed for these genes as well as for rDNA (ITS1, 5.8S, ITS2). Morphological identifications were all done or confirmed by the same author to avoid any confusion. Ten species gave only one copy of each gene. Two species gave two copies of one gene. Cloning was necessary to analyse rDNA genes but they could not be used due to the intra-specific variation. Results indicate that molecular identifications confirm morphological specifications. Close morphological species show 99% nucleotide homology, while clearly distinct species show less than 94% for at least one gene. Strains of the same species show 100% molecular homologies for all three genes. More species will be tested to confirm these results.

P11.2 - Genetic diversity of dahongjun, the commercially important "big red mushroom" from southern China

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In southern China, a wild mushroom commonly called "dahongjun" or "big red mushroom", "true *Russula*" by the locals, has been harvested, consumed and/or exported as an exotic food for many years. The species complex were identified as *Russula vinosa* by previous mycologists. Although ecologically and economically important, very little is known about this mushroom, including its true phylogenetic position and basic ecology. In this study, we investigated the genetic diversity of dahongjun using sequences of the internal transcribed spacer (ITS) regions, the

gene spacer (IGS) regions, the second largest subunit of RNA polymerase II (RPB2) and the mitochondrial genome mtSSU ribosomal RNA gene region. We found that this species complex contained at least three divergent lineages, with one corresponding to the recently described *Russula griseocarnososa* and two others likely representing closely related novel species within the genus *Russula*. Both phylogenetic and population genetic analyses suggested that dahongjun populations were geographically structured at the regional level. Our study provides the first insights into the diversity and population biology of this endemic gourmet mushroom in southern China.

P11.3 - Morphological, biochemical, functional and molecular characterization of CMCC germplasm

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To conserve and exploit the diversity of arbuscular mycorrhizal fungi (AMF), the Centre for Culture Collection (CMCC), was established in 1993 at The Energy and Resources Institute (TERI), with the aid of Department of Biotechnology, Govt. of India. A total of 40 isolates from this repository were characterized at different levels. Spores were identified and then used for functional characterization, for fatty acid methyl ester (FAME) profiling and for sequence analysis of 18S-ITS region of rDNA gene as well as sequence analysis of LSU region of rDNA gene of AMF germplasm. Phylogenetic analysis of these isolates was then carried out. In the present study, the isolates were selected based on their performance at different levels of Phosphorus (low, moderate and high). All the spores analysed were found to contain marker fatty acid C 16:1(c-9). A total of twenty seven of different FAME were identified among the AMF spores surveyed in the study. It was observed C 14:0, C 16:0, C 16:1(c-9), C18:0, C18:1(c-9), C18:2(c-9,12) were common to all isolates. Certain unusual fatty acids were also reported from some isolates such as C16: 1(c-9), C17: 1(c-10), C18: 1(t-9), C18: 2(t-9, 12), C21:0 and C23:0. Neighbour-joining tree based on SSU -ITS and LSU rDNA sequences from CMCC Germplasm along with sequences retrieved from gene bank separated into three major clusters (cluster A, B and C) of *Glomus* group A.

P11.4 - Molecular phylogeny of several *Scutellospora* species indigenous of La Gran Sabana (Venezuela).

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La Gran Sabana is part of the "Canaima Nacional Park" located in the south-east region of Venezuela. This region, belonging to the Guayana Shield, has an important diversity of *Scutellospora* with unique characteristics that suggest their classification as new species. All of these species are difficult to grow in trap cultures. Some of them, with conspicuous spore ornamentation, have already been described (*S. spinosissima*, *S. crenulata* and *S. striata*). However, in other cases the number of healthy spores obtained from field samples is not enough to complete the morphologic description. The geographic distribution of these species is restricted to La Gran Sabana, which might be related to the particular biogeographical and ecological conditions of this tropical region. In this work we infer the phylogenetic relationship of *S. spinosissima*, *S. crenulata*, *S. striata* and two probable new species by means of sequencing 18S rRNA gen. Molecular data provides support to the status of new species suggested for the two new morphospecies. All the analyzed species were located in the most ancestral clade corresponding to *Scutellospora* genus (Fam. *Scutellosporaceae*), according to the recent restructuring of *Gigasporaceae* proposed by Oehl and collaborators. There was no conformity between germination shield morphology and the rRNA-based phylogeny. The importance of including a high diversity of species in this combined analysis is highlighted.

P11.5 - Some new species of arbuscular mycorrhizal fungi associated with sugar cane crop in Iran

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Ten species of AMF belonging to four genera including *Kuklospora colombiana*, *Glomus manihotis*, *G.eburneum*, *G. aggregatum*, *G. caledonium*, *G. coronatum*, *G. microcarpum*, *G. diaphanum*, *Paraglomus occultum* and *Pacispora scintilans* were identified. Among them five species of *Kuklospora colombiana*, *Glomus eburneum*, *G. manihotis*, *G. diaphanum*, *G. microcarpum* and *P. occultum* are reported for the first time from Iran; *Glomus aggregatum*, *G. caledonium*, *Glomus coronatum* and *P. scintilans* are new records for sugarcane mycoflora of Iran.

P11.6 - Combined morphological and molecular analysis to assess the community composition of glomeromycotan fungi in North American grasslands

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A project was undertaken to integrate morphological and molecular data sets for classifying glomeromycotan

species. Fungi were collected from remnant and old-field land-use sites in grasslands spanning the U.S. Fungi were trapped in pot cultures and sporulating species were identified by morphology and categorized as (i) described, (ii) ambiguous or (iii) undescribed. Sequences of the 25S rRNA gene were integrated into a 114 taxa phylogenetic tree. Preliminary analysis has been completed from three tallgrass (TG) and two shortgrass (SG) prairies. Thirty-seven species were identified. Widespread species were *Glomus intraradices*, *Glomus microaggregatum*, *Glomus mosseae*, *Glomus spurcum*, and *Paraglomus occultum*. Species richness was higher in TG than SG sites and in TG remnant compared to TG oldfield sites. The reverse was true in SG sites, with fewer species in remnant areas. *G. microaggregatum* and *P. occultum* were detected in both land-use types. *Glomus etunicatum* and *Entrophospora infrequens* were collected most frequently from old-field communities. Species clades reconstructed from LSU sequences correlated well with morphology, verifying described species, grouping ambiguous hyaline-spored types with known species, and clarifying boundaries for a number of species with intergrading phenotypes.

P11.7 - *Acaulospora* sp. a new species of arbuscular mycorrhizal fungus forming-Glomeromycetes with unique spore wall structure

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Species in *Acaulospora* (Acaulosporaceae) form glomerospores laterally to sporiferous sacule, with three spore walls, being one structural and two germinal components with "beaded" layer in the first layer of the second germinal wall. During studies in the Amazonia biome in Brazil and in the Savanna biome in Venezuela a new AMF with acaulosporoid spores presenting four walls, and differing from all other acaulosporoid species was found, presenting itself as a probable new taxon in Diversisporales. Acaulosporoid spores were extracted from field and trap culture soils by wet sieving and sucrose centrifugation. The glomerospores of the new species are characterized by four walls and ornamentation (spines) in the first middle wall layer. This feature is not found in glomerospores of other taxa. Spores of *Archaeospora* and *Otospora* have just two walls whereas *Acaulospora* and *Ambispora* are characterized by three spore walls. Spore development and an ornamented "beaded" layer indicate this species as a member of *Acaulospora*. In the future spore wall structure and germinal shield (orb) morphology could be useful to segregate genera from *Acaulospora*. It has been shown that the *Glomeromycota* phylum is a broad fungal group and new data about undescribed species will allow to establish new taxa and understand the actual diversity of these symbiotic and unique fungi.

P11.8 - Typological discrimination of spore wall layers in mycorrhizal Glomeromycetes

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The classical identification of arbuscular mycorrhizal fungi (AMF) is based on ontogeny and morphology of spores as well as phenotypic characters and histochemical reactions of their walls. There are classical purposes of diagrammatic representations for the wall structuration, by means of murographs and muronyms, which represent practical tools for recognizing the species. However, some inconsistencies were adopted in those purposes, and in this work they are discussed and a new model of typological characterization is shown. The mainspring for the formation of types consider three aspects, phenotype, consistency and persistence, which are treated separately. It's important to stress out this purpose is addressed to the identification of species and didn't have any intention or relation with the process of taxonomic classification.

Fungal Communities and Effects of Mycorrhizal Fungi Communities on Plant Communities

P12.1 - Effects of invasion of exotic plant *Eupatorium rugosum* on arbuscular mycorrhizal fungal community and plants growth in Korea

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The objective of this study was to investigate the effects of invasion of exotic plants on arbuscular mycorrhizal fungi (AMF) community and how these changes of AMF community affect growth of native plants. Soils and roots were collected at Mt. Inwang, Mt. Nam and Mt. Namhan, where were covered with exotic plants, *Eupatorium rugosum*. AMF communities in roots of exotic plant were compared with communities in roots of native plants sites. Also, we made pots using soil invaded by exotic plants and soil where native plants grew. Total biomass of plants, mycorrhizal root colonization, species diversity and richness of AMF were analysed after four month of growth. We found that AMF species composition of exotic plants were significantly different from those of native

plants. Biomass of plants grown in exotic plants soil was less than biomass of plants grown in native plant soil. Also, AMF species diversity and richness showed significant difference between roots grown exotic and native plant soils. Mycorrhizal root colonization rates of plants grown in exotic plants soil was lower than that grown in native plant soil. The results in this study suggest that the invasion of exotic plants changed AMF communities in the sites.

P12.2 - Communities of arbuscular mycorrhizal fungi in different landscapes, location 338 South, Pacajá-PA

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Arbuscular mycorrhizas (AMs) are involved in nutrients uptake by plants and play an important role in maintenance natural fertility of soils. The change of landscape by agricultural activities can affect the diversity of AM fungal, therefore the efficiency of the symbiosis and growth plant. Thus, the objective of this study was to evaluate the density of AM fungal spores, diversity and rate of root colonization of six kinds of landscapes in properties of family farmers. Soil and roots samples were collected in sixteen points from nine properties farmer family. The landscapes were forest, pasture, boskage, crop of cocoa, rice and beans. The spores of AM fungi were extracted from soil samples by wet sieving and centrifugation in sucrose, and then analyzed by microscopy. The roots have been stained with ink and the rate of colonization was evaluated. The density of AM fungi spores was higher in monocultures, but the diversity has not changed. Nevertheless was observed the increased dominance of AM fungi groups in monocultures. The diversity and density of spores found in forests and cocoa were similar. These results indicate that agricultural practices have influence on the behavior of AM fungi communities in soil and plant.

P12.3 - Ectomycorrhizal fungi improve early performance of *Pinus elliottii* var. *densa* seedlings in Florida pine flatwoods soil

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Pinus elliottii var. *densa*, a South Florida native pine, is locally endangered because of urban expansion and altered fire regimes. Such disturbances, by mechanisms that are incompletely understood, sometimes preclude *P. elliottii* var. *densa* regeneration. Pine seedlings must be able to establish successfully within vegetation matrices that may comprise both ectomycorrhizal (ECM) adult pines and oaks together with various arbuscular mycorrhizal (AM) host species. We investigated the effects of ECM on *P. elliottii* var. *densa* seedling performance in microcosms including an AM nurse plant. We grew pine seedlings with or without ECM root inoculum crossed with four AM fungus treatments (autoclaved AM roots, fresh AM roots, AM hyphae extending from the nurse plant, or nurse plant AM hyphae severed weekly). Seedlings without contact with AM hyphae extending from a *Tamarindus indica* nurse plant grew in intact Dee-pots, but those exposed to AM hyphae from the nurse plant grew in Dee-pots with a slot covered by GORE-TEX membrane. All treatments' Dee-pots were arranged around a central nurse plant and were embedded in soil. At harvest, eleven month-old seedlings in all treatments had ECM, and ECM abundance was positively correlated with seedling performance. None of the AM treatments detectably affected pine seedling performance. Therefore, in a competitive milieu, ECM can form early and might contribute to *P. elliottii* var. *densa* seedling regeneration.

P12.4 - Effect of ericaceous dwarf shrubs on ectomycorrhizal community on invasive *Pinus strobus* and native *Pinus sylvestris* from the Czech Switzerland NP

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Pinus strobus is native to North America; shortly after introduction to Czech Republic in 1812, it became an antagonist to autochthonous plants, e.g. *Pinus sylvestris* in the Czech Switzerland National Park. Invasive species are mostly facultatively mycorrhizal or nonmycorrhizal, some might be antagonistic to native mycorrhizal fungi; however, *P. strobus* is in its native habitats obligately ectomycorrhizal. Similarly to *P. sylvestris* or *Picea abies*, it often regenerates within ericaceous shrubs. Ericaceae form ericoid mycorrhiza, but some of their mycobionts are capable of forming ectomycorrhiza - thus, ericaceae might represent a source of inoculum for coniferous seedlings. Our presentation addresses the following questions: 1) Are there differences in ectomycobionts between *P. sylvestris* and *P. strobus*? 2) What is the influence of Ericaceae on ectomycobionts of both *Pinus* species? 3) Does the supposed Ericaceae effect differ between invasive *P. strobus* and native *P. sylvestris*? Answering these questions might help understanding spread mechanisms of *P. strobus* and its possible indirect interactions with *P. sylvestris* through Ericaceae and their mycobionts.

P12.5 - Arbuscular mycorrhiza and the fragmentation of the Atlantic Forest in Northeast, Brazil

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The Atlantic Forest is characterized by high biological diversity but anthropic activities such as deforestation has affected ecological processes. Arbuscular mycorrhiza are important for functioning and sustainability of the ecosystems, being necessary to increase the knowledge regarding this association in fragmented areas to suggest more efficient recovering practices. Thus, the effect of forest fragments size in the activity of arbuscular mycorrhizal fungi was investigated. Four compound soil samples were collected in three large fragments (~340 ha) and three small fragments (~10 ha) during the dry and raining seasons. Glomerospores number, mycorrhizal colonization and glomalin-related soil proteins (GRSP): [easily-extractable (EEG) and total (TG) fractions] were estimated. There was no effect of fragment size and sampling period for number of glomerospores and colonization. However, there was highly significant effect ($P < 0.001$) of these factors for the GRSP. Higher production of EEG and TG (3.15 and 4.5 mg g dry soil⁻¹, respectively) were obtained in soils from the largest fragments, during the dry period. Numbers of glomerospores and colonization are less affected by size of the fragments, considering that they are more depend on the associated plant species which were present in both types of fragments. The results suggest that the GRSP are more suitable to show the effects of fragmentation of the Atlantic Forest on soil microbial activity related with the AMF.

P12.6 - Ectomycorrhizal fungal diversity: updating the wheat and the chaff

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Estimates of global species richness of ECM fungi differ widely. Many genera have been proposed as being ECM, but evidence for the ECM habit is often lacking. Recently, we have collected evidence for the ECM habit of fungal species and for the identification of the mycobiont in ECM associations, using existing mycorrhiza literature (Rinaldi et al, Fungal Diversity 33: 1-45, 2008). The methods considered were morpho-anatomical characterization of natural ECMs, pure culture synthesis, molecular identification, isotopic evidence. Phylogenetic information was also considered as a relevant criterion to assess ECM habit. Our published survey indicated that for some 343 fungal genera an ECM status has been alleged, and for about two thirds of these supportive evidence on ECM status exists or can be at least hypothesized. For the remaining taxa, currently no indication exists as for their ECM nutritional habit, besides field observations of associations with putative hosts. On the basis of our literature search we conservatively estimated ECM species richness around 7750 species, but a final estimate of ECM species richness would likely be between 20000 and 25000. Here, we update our survey by reporting the new evidence that become available after our study was released.

P12.7 - Molecular diversity and community

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In some cases when forest plantations fail, such as when exotic pines have been introduced to tropical sites, a mismatch between the mycorrhizal guild need of the seedlings and that supplied by the site may be at fault. In our research, we are using a metagenomic approach to study the community composition and diversity of mycorrhizal fungi in two adjacent forest ecosystems: one dominated by arbuscular host species (western redcedar) and one dominated by ectomycorrhizal host species (western hemlock and amabilis fir). These ecosystems share the same site characteristics, but differ in their regenerative capacity for certain tree species. The objective of this project is to determine whether there are underlying differences in mycorrhizal fungal communities that potentially contribute to the performance of tree species that associate with different mycorrhizal classes. Twelve soil samples were collected from each of three replicate plots from the two ecosystems (72 soil samples total). DNA fragments of the ITS region of rDNA were PCR amplified and cloned from extracted total genomic DNA using fungal specific primers ITS1F and TW13. From each of the 72 random clone libraries, we are sequencing 96 clones (6912 clones total). To identify species composition in the metagenomic profiles from random clone libraries, we are combining DNA barcoding and DNA sequence homology matches. Preliminary results will be discussed in the context of fungal ecology and forest management.

P12.8 - Successional pattern of sporocarp production of ectomycorrhizal fungi: chronosequence studies in old fields afforested with Scots pine

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The ECM community of pine trees planted in old fields in Poland was investigated. Succession of fungi and relationships between the chronosequence of species, stand/habitat features, and development of mycorrhiza, were

assessed. Observations were carried out on permanent plots, 1000 m² each, established on 1 to 42 years old *Pinus sylvestris* first-rotation stands planted in formerly arable fields on poor soil. Fruitbodies and ectomycorrhizae in all plots were subject to analyses using classical and molecular methods. Here the successional pattern of sporocarp production is presented as a chronosequence of species reflecting forest development. Sporocarps of ca. 100 species of ECM fungi were found. A number of species increased with pine age up to 36 taxa in the 23-year-old plot. In the 42-year-old forest it remained over 30. First sporocarps of ECM fungi occurred under the seedlings 3 years after outplanting. Species of *Hebeloma*, *Inocybe*, *Laccaria* and *Suillus* were exclusive dominants at first. After the canopy closure and acceleration of litter accumulation *Cortinarius* spp. and *Tricholoma* spp occurred abundantly (8-12th year). The ECM species of the 42 years old pines were typical of mature forest. It mainly consisted of the genera *Russula*, *Cortinarius*, *Tricholoma*, *Lactarius*, *Amanita*, *Xerocomus* and *Paxillus*. Successional pattern of ECM sporocarp production reflects the changes in stand/habitat features and is typical of the first-rotation Scots pine communities.

P12.9 - Mycorrhizal status of invading *Pseudotsuga menziesii* seedlings in *Nothofagus* spp. forests from Patagonia, Argentina

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Pseudotsuga menziesii is one of the most widely planted conifers in the Patagonian Andes region of Argentina, and its invading characteristics have been widely reported. Invasion would be strongly conditioned by its autoecology, environmental variables and the establishment of shared mycorrhizal associations. In this study, the ecto- ectendo- and endomycorrhizae present in *P. menziesii* seedlings growing in *Nothofagus-P. menziesii* matrix were evaluated and analyzed in relation to environmental parameters. Three study sites were selected and characterized. One transect along the effective recruitment area was established at each site in order to wrench 25 seedlings along with a soil sample. Colonization percentage and richness of EM, ECM and VAM associations were evaluated and pH, total N, OM %, CEC (cationic exchange capacity), electrical conductivity and available P were determined from soils. The association between variables was analyzed using PCA. It was found that invading seedlings had >75% EM and ECM mycorrhization, and morphotype richness varied between sites, although *Rhizopogon*, *Wilcoxina* vel aff. and *Hebeloma* morphotypes were always dominant. VAM colonization varied between 25-40%, with different proportions of Arum and Paris types between sites, but with a constant presence of dark-septate endophytes. High mycorrhizal colonization was positively associated with soil OM, total N and electrical conductivity, and negatively with precipitation and Ca.

P12.10 - Mycorrhizae in *Quercus robur* L.: diversity after fire disturbance

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This work proposes to characterize the ectomycorrhizal fungi (EMF) communities associated with *Quercus robur* L. in a mountain area of Northern Portugal (Viana do Castelo) increasingly disturbed by fire. The recurrence of fires over the last decades has led to profound changes in the landscape, including the establishment and expansion of alien plant species. We aim to evaluate the impacts of fire on EMF community and to investigate its broader influence in the establishment of plant community. Field work was conducted in three areas of *Q. robur*, selected according to the temporal distance to fire and the similitude in edaphic and geographical conditions. Three sites were selected: Z1 (burnt in 2006), Z2 (burnt in 2002) and Z3 (un-burnt in last 30-years). Ten seedlings of *Q. robur* (2-years age) were collected per sampling site. EMF abundance and richness was analysed considering its distribution in two horizons. Flora data was georeferenced and analyzed in GIS (Geographic Information System). Results so far indicate that EMF are present in roots since early stages and that fire did affect communities above and below-ground, with a significant decrease immediately post-fire. We also noticed that *Q. robur* is amongst the plant species that germinates after fire and that the species *Cenococcum geophilum* and the genus *Tomentella* are the most common EMF after fire. We expect to gather scientific support to better conduct reforestation programs and prevent plant invasions.

P12.11 - Mycorrhizas in forestal plants (pine and eucalyptus tree), fruit plants (blueberry), horticultural plants (strawberry) and medicinal plants (yacón) in Tucumán, Argentina

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The aims of this project were: a) to detect mycorrhizas in *Pinus* sp and *Eucalyptus* sp., wild and introduced Ericáceas, strawberry (*Fragaria x ananassa* Duch), wild Asteraceas (*Smallanthus macroscyphus*) and introduced

Asteraceae (*Smallanthus sonchifolius*), medicinal plants; b) to isolated them; c) to identify them; and d) to multiply them in artificial mediums and/or in trap plants, to make inoculums to be used in a nursery. During March-April from 2007 to 2009, sporocarps were collected from pinus and eucalyptus, rootlet and samples of soils associate to *Pemetia*, blueberry, strawberry, and to yacon cultivated in gaps and wild yacon from Tucuman's Yunga. Ectomycorrhizic fungus were identified with keys. They were isolated and cultivated in culture media (MMN, APG, AEM); they were multiplied in a liquid medium and inoculated in *Pinus* and *Eucalyptus* seedlings in a greenhouse. Ericoid mycorrhizas were detected in *Pemetia* and only dark septates endophytes (DSE) in *Vaccinium corymbosum*. Commercial strawberry are spontaneously colonised with DSE and with arbuscular mycorrhizas (AM) in which *Glomus* predominated. Rootlet of *Smallanthus macroscyphus* are highly colonised with AM and DSE, and *Smallanthus sonchifolius* only with AM. *Scutellospora* sp., *Entrophospora infrequens*, *Acaulospora acrobiculata*, *Pacispora* sp. and *Glomus* spp. were identified in wet sieving of soil. The incidence of the mycorrhizas in these vegetal species and the liquid inoculums used will be evaluated.

P12.12 - Afforestation of degraded soils by mycorrhization of trees in Beijing (China)

Watzke, R., Stützer, M., Wang, X., Chen, J.

Since several years a green belt has been planted in the region of Beijing. However, ten thousands of trees are not grown or died due to inappropriate soil and climate conditions.

The German-Chinese project of the "Gesellschaft für Technische Zusammenarbeit (GTZ)" has the intention to prove that the application of mycorrhiza leads to a decrease of mortality rate, improvement of the growth rate and secures the sustainability.

90 000 trees (*Quercus variabilis*, *Pinus tabulaeformis*, *Pinus bungeana*, *Platycladus orientalis*, *Prunus davidiana* and *Cotinus coggygria*) were planted in the field in 3 different areas and in a nursery during the rainy season 2008. The trees were inoculated with AM-fungi or ectomycorrhiza respectively during the planting. The field areas were characterised by high aridity and an insufficient nutrient supply. During the duration of the project a rating of the growth rate, further growth parameters and the mycorrhization of the roots was performed. First results illustrate that by using mycorrhiza the growth rate is increased. Another intention of the project is the training of potential users of mycorrhiza and the propagation of the yielded knowledge of future reforestation projects in China especially Inner Mongolia.

P12.13 - AM Fungal diversity of the plant communities on two mountains of South China

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South China lies on the transitional area between the subtropical and tropical region. Nankunshan and Tonggufeng are the two major mountains in South China. They are covered by large patches of well developed secondary forest. The AM fungal richness and diversity of the plant communities along the altitude between 300 m and 1500 m on Nankunshan and Tonggufeng were studied. A total of 54 AMF spore types were isolated from the twenty three soil samples. Thirty four types were identified to species, including 16 of *Glomus*, 11 of *Acaulospora*, 3 of *Scutellospora*, 2 of *Diversipora*, 1 of *Entrophospora* and 1 of *Gigaspora*. The rest 20 spore types were only identified to the genus. The result showed that the AM fungal communities on the two mountains were both dominated by *Glomus* and *Acaulospora* with small-sized spores, but the dominant species were different. Those on Nankunshan were dominated by *Acaulospora bireticulata* and *Glomus multicaule*, while Those on Tonggufeng were dominated by *Acaulospora bireticulata* and *A. scrobiculata*. In addition, *Acaulospora foveata* was only isolated from the soil samples of Nankunshan while *Acaulospora* sp.1 was only isolated from the soil samples on Tonggufeng. In general, Nankunshan had low AMF spore richness, high spore density and diversity. However, the spore diversity and evenness were greatly varied with different communities on the same mountain. They varied with altitude, season, and soil depth.

P12.14 - Arbuscular mycorrhizal mediation of biomass-density relationship of *Medicago sativa* L. under two water conditions

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Biomass-density relationship was generally explained by competitive utilization of resources. Arbuscular mycorrhizal fungi (AMF) have been showed to be able to influence plant interaction through mediating resource utilization, but whether this mycorrhizally mediated interaction will shift biomass-density relationship is less documented. We hypothesized that AMF may shift biomass-density relationship through affecting intraspecific competition. Four populations (with density 10, 100, 1000 and 10000 seedlings per square meter respectively) of *Medicago sativa* L. were designed. Annual precipitations (1435 mm and 327.7 mm) simulating wet and arid areas

respectively were applied in the experiment. With or without fungicide benomyl application was used to create lowly or highly AM fungal treatment. Our results showed that AM fungal treatment affected biomass-density relationship. Highly AM fungi enhanced decrease of individual biomass with increasing density (biomass-density line sharper) under both water levels, and made log mass vs log density a linear shape. Low mycorrhiza treatment made bilogarithmic line a humped shape under drought stress. AM fungal treatment did not influence mortality rate of the populations but increased absolute competition intensity (ACI). Our results implied AM fungal status did not influence self-thinning process but shift biomass-density relationship through affecting intraspecific competition, and it was abiotically dependent. Key words: Biomass-density relationship, Arbuscular mycorrhizal fungi, self-thinning, intraspecific competition

P12.15 - Ectomycorrhizal fungal diversity: updating the wheat and the chaff

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Thousands of ectomycorrhizal (ECM) fungal species exist, but estimates of global species richness of ECM fungi differ widely. Many genera have been proposed as being ECM, but in a number of studies evidence for the hypothesized ECM habit is lacking. Progress in estimating ECM species richness is therefore slow. Recently, we have collected evidence for the ECM habit of fungal species and for the identification of the mycobiont(s) in specific ECM associations, using published and web-based mycorrhiza literature (Rinaldi et al, Fungal Diversity 33: 1-45, 2008). The identification methods considered were morpho-anatomical characterization of naturally occurring ECMs, pure culture synthesis, molecular identification, and isotopic evidence. In addition, phylogenetic information was also considered as a relevant criterion to assess ECM habit. Our published survey indicated that for some 343 fungal genera an ECM status has been alleged, and for about two thirds of these supportive evidence on ECM status exists or can be at least hypothesized. For the remaining taxa, currently no indication exists as for their ECM nutritional habit, besides field observations of associations with putative hosts. On the basis of our literature search we conservatively estimated ECM species richness around 7750 species. However, on the basis of estimates of knowns and unknowns in macromycete diversity, a final estimate of ECM species richness would likely be between 20000 and 25000. Here, we update our survey, by reporting the new evidence that become available after our study was released. We can confirm that current knowledge of ECM fungal diversity, as supported by experimental evidence, is only partly complete, and that inclusion of many fungal genera in this trophic and ecological category is not verified at this stage. Care must thus be used when compiling lists of ECM and saprotrophic fungi in community-level studies on the basis of published information only.

P12.16 - AM fungi can alter flowering time in a desert ecosystem: implications in a changing environment

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Ecological consequences of global climate change may include earlier flowering (Fitter and Fitter 2002). Climatic change, including rainfall is known to affect phenology, but the indirect effects of global change, such as changes in soil biota, are poorly understood. The aim of this study was to examine how and to what extent the indigenous AM fungi would change the timing of flowering in a changing desert ecosystem in central Asia. A 2-year-experiment was conducted in glasshouse and field conditions to test the growth response of spring ephemerals to indigenous AM fungi. The fungicide benomyl was added in the equivalent of 40 mm of water to suppress the activity of indigenous AMF; controls included added water (40mm) and no additions. Both mycorrhizal colonization and spore abundance decreased in the benomyl treatment. Time of flowering was not affected by addition of 40 mm water comparing with no addition, but it was delayed by application of benomyl by, respectively, 3 or 4 d in the field and 4 or 5d in the glasshouse in *Trigonella arcuata* and *Erodium oxyrrhynchum*, relative to the controls that received added water. Both plants are heavily colonized by mycorrhizal fungi. It appears that AM fungi can strongly affect the flowering phenology of plants in the desert. Such an effect should be taken into consideration in assessing the biological consequences of global climate change.

P12.17 - Evidence for high specialization in mycorrhizal association of *Encyclia dichroma* and *E. ghillanyi*, closely related tropical orchid species

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The specialization level in mycorrhizal association for two closely related tropical photosynthetic orchid species was investigated for the first time. These species occur in two distinct environments: *Encyclia dichroma* in Restinga (strand vegetation) fragments near to the coast in the Bahia State, Brazil, and *E. ghillanyi* in rocky fields in land in

the same State. In order to assess specialization level and if the specialization is influenced by habitat and genotype, we sampled leaves and roots of 135 individuals from 10 populations (four populations of *E. dichroma* and six of *E. ghillanyi*). The relationships between conspecific and non-conspecific orchid populations were inferred using dominant molecular markers of Inter Single Sequence Repeats (ISSR). The fungal phylogeny was obtained from parsimony and Bayesian analysis of sequence data from Internal Transcribed Spacer (ITS – nrDNA). Based on genetic markers, *E. dichroma* and *E. ghillanyi* are genetically distinct species. With regard to the level of specialization, *E. dichroma* presented a more specialized mycorrhizal association than *E. ghillanyi*. We found evidence for a high specialization in mycorrhizal association in *E. dichroma* and *E. ghillanyi* possibly related to habitat distinction.

P12.18 - Tropical succession in three Brazilian biomes regulates soil nutrient content, fine root morphology and arbuscular mycorrhizal fungi abundance

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Fine root traits, AM fungi and soil fertility were assessed in grassland sites, secondary forests and mature forests chosen in Atlantic, Araucaria and Pantanal biomes in Brazil. Soil cores were collected at the 0-10 cm and 10-20 cm depths in three quadrants in each site and biomes. Among biomes and during succession advance, the soil fertility, fine root mass, fine root diameter and fine root tissue density increased, while fine root length, specific root length, root-hair length, root-hair incidence and AM fungi root colonization and AM fungi spore density in soil decreased. Many correlations within fine root traits as well in fine root traits and AM fungi variables were verified. Fine root morphological traits as: root length, specific root length, root diameter, root-hair length and root-hair incidence were the most important determinants related with AM fungi root colonization and sporulation. The results suggest that plant species of the early stages of tropical succession invest in fine root morphology and AM fungi association toward increase the soil resource exploration and fine root uptake capacity. Plant species belonging to late stages of succession display low investment in fine root morphology and AM fungi for nutrient acquisition. The fine root morphology and AM fungi mycotrophy employed as strategy for plant nutrient acquisition display narrow relations with plant ecological groups of the different succession phases among three Brazilian tropical biomes.

P12.19 - Observations of antagonism in Arbuscular Mycorrhizal fungi

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Abstract The majority of land plants are colonised by arbuscular mycorrhizal fungi, however, members of few families notably Cruciferae, Chenopodiaceae, Caryophyllaceae and Polygonaceae, so called non-host are not colonised by these fungi. Previous studies have shown that the growth and development of non-host species were severely inhibited when grown in the presence of active AMF mycelia. There is therefore a need to understand the mechanistic bases of adverse effects of AMF mycelia upon seedlings of non-host species. In this experiment young roots of non-hosts *Arenaria serpyllifolia* and *Cardamine hirsuta*, and host *Centaurium erythraea* were exposed to mycorrhizal and non-mycorrhizal extracts. The results of this experiment showed that mycorrhizal extracts significantly reduced the growth of radicles and root hairs development of non-host species whereas had no effect on radicles and root hairs development of host species. These results indicate that toxicity effects rather the nutritional factors are the drivers of the negative responses of non-host species to the presence of the AMF.

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