icom 3

THE UNIVERSITY
OF ADELAIDE

ICOM 3

3rd International Conference on Mycorrhizas

"Diversity and Integration in Mycorrhizas"

8 - 13 July, 2001

ADELAIDE CONVENTION CENTRE
SOUTH AUSTRALIA

PROGRAM AND ABSTRACTS

The financial support of the following companies and institutions is gratefully acknowledged:

MAJOR SPONSORS

Australian Society of Plant Physiologists

Canadian Journal of Botany

Centre for Plant Root Symbioses

Forest & Wood Products Research and Development Council

Grains Research and Development Corporation

Kluwer Academic Publishers/Plant and Soil

New Phytologist

Samuel Roberts Noble Foundation

ADDITIONAL SPONSORS

Adelaide University Vice-Chancellor's Fund
Adelaide University Faculty of Science
Adelaide University Department of Soil and Water
AusAID
Biorize
CRC for Molecular Plant Breeding
Premiertech
Springer-Verlag GmbH & Co. KG
The Australian Academy of Science
Australian Institute of Biology Inc.

WELCOME FROM SALLY SMITH

Dear Friends and Colleagues

On behalf of the Organising Committee of ICOM3, I have great pleasure in welcoming you to the Conference and to Adelaide, Australia. It is a real honour for Adelaide to host ICOM3 in the Southern Hemisphere. Because all previous multinational conferences were held in USA or Europe the location is particularly significant – it emphasises that the mycorrhiza research community is truly international. At the time of going to press, ICOM3 has over 400 registrants from 48 countries.

The local Organsing Committee wishes to extend heartfelt thanks to many people, without whose help ICOM3 would not have happened:

The Australasian Committee, for help and advice.
Adelaide University for supporting the bid and much subsequent in-kind support.
Organisers of the Symposia, Concurrent Sessions and Workshops.
All our sponsors, who are named on the front inside cover of the program.
The Conference Organisers, Sally Jay, and her co-organiser, Jenny Blanchard, for all they have done to bring the Conference into being.
John Davey, who provided essential financial support and advice.
Alla Baklan, who maintained the web page.
Tracey Parish for secretarial help.
Mark Stevens for help and advice on data projection.
Bub Augé who maintained the Director of Mycorrhizologists.
Roger Finlay, who passed on "do's" and "don'ts" for ICOM organisers.
The members of the Mycorrhiza Group in the Department of Soil and Water who have helped in a huge number of ways.

I invite you all to make the most of the excellent scientific and social programs, the beautiful City of Adelaide and the opportunity to make friends and find new collaborators from all over our region. Enjoy yourselves.

Sally Smith
Chair, ICOM3

CONTENTS

ORGANISING COMMITTEE

Sally Smith (Convenor)
Andrew Smith (Treasurer)
Timothy Cavagnaro
Sandy Dickson
Debbie Miller
Yongguan Zhu
Alla Baklan (Web Master)
John Davey (Financial)

AUSTRALASIAN ADVISORY COMMITTEE

John Cairney (University of Western Sydney, Nepean)
Ian Hall (New Zealand Institute for Crop and Food Research)
David Jasper (University of Western Australia)
Andrew Smith (Adelaide University)
Sally Smith (Adelaide University)

Symposium Organisers

Dr B. Bago
Associate Professor J.W.G. Cairney,
Dr B. Dell
Dr V. Gaininazzi-Pearson
Dr P. Pfeffer
Dr I. Sanders
Dr A. Schüßler
Professor S. Smith
Dr M. van der Heijden

Concurrent Session Organisers

Associate Professor L.K. Abbott
Associate Professor M. Adams
Dr J.B.Baon
Mr T. Cavagnaro
Dr S. Dickson
Professor D. Janos
Dr D. Jasper
Ms R. S. Kasiamdari
Mr P. O'Connor
Professor R.L. Peterson,
Dr H. Rassmussen
Dr M. Ryan
Dr S. Timonen
Dr Y. Zhu

Workshop Organisers

Dr J. Baar
Dr B. Bago
Dr E. Dumas-Gaudot
Dr J. Garbaye
Dr P. Högberg
Dr P.A. McGee
Dr K. Nielsen
Dr P. Pfeffer
Dr S. Rosendahl
Dr G. Rosewarne
Dr I. Sanders
Dr P. Schweiger
Dr A. Taylor
Dr S. Timonen
Dr L. Watrud
Dr D. Wipf

Conference Secretariat

Sally Jay Conferences

GENERAL INFORMATION

Venues

The main scientific sessions will take place in Halls B and C at the Adelaide Convention Centre. Posters will be on display in Halls A and D. A floor plan of the Adelaide Convention Centre is on the inside back cover.

The Workshops on Tuesday and Wednesday evenings will be held in the Napier and Lower Napier Buildings at Adelaide University - see map on back cover.

Refreshments

Morning and afternoon refreshments will be served in Hall E, adjacent to the poster area.

Lunches

Lunches and light snacks can be purchased in Hall E of the Adelaide Convention Centre (next to the poster area) at the designated lunch breaks. A range of sandwiches, hot foods, snacks, coffee, tea and soft drinks will be available.
Prices start from $2.50 for an individual gourmet salad through to $8.50 for a substantial hot lunch.

Smoking

The Adelaide Convention Centre and Adelaide University are non-smoking venues. Smoking is prohibited by law in all eating establishments in South Australia.

Mobile Phones

Please switch all mobile phones to a silent mode during sessions.

Name badges and tickets

The name badge is the official pass and must be worn at all times to gain entry to scientific sessions, the mixer, the workshops, Wines of the World and the Student Resource Centre, Union Building, Adelaide University (for email facilities). Tickets must be shown at all social functions.

Registration Desk

The ICOM3 Registration Desk will be staffed at the following times:

Sunday 8 July	12:00-2:00 pm and 5:00 to 8:00 pm
Monday 9 July	7:30 am to 5:30 pm
Tuesday 10 July	8:00 am to 5.30 pm
Wednesday 11 July	8:00 am to 5:30 pm
Thursday 12 July	8:00 am to 4:40 pm

The telephone number for the ICOM3 Registration Desk is 8210 6801.

Notices and Messages

Program updates and personal messages will be displayed on the noticeboard near the ICOM3 Registration Desk. Any other housekeeping announcements will be made at Keynote Sessions in Hall B.

E-mail

The Adelaide Convention Centre does not provide internet access for conference delegates. Some of the hotels and motels provide this facility, but the University Colleges do not. Arrangements have been made for ICOM3 delegates to have free access to the computers in the Resource Centre run by the Adelaide University (Students') Union. It is in Level 3 of the Union Building on the University Campus, about 10 minutes' walk along North Terrace from the Convention Centre. There are detailed maps at the entrances to the campus. The Resource Centre will be open only from 10am to 4pm on weekdays. (This is the University vacation.) ICOM3 delegates must wear name badges so that they can be identified. Use of computers should be limited to 15 minutes per session. In addition, there are internet cafes close to the Adelaide Convention Centre. Details will be available at the ICOM3 Registration Desk.

Telephones

Public telephones are available on the ground floor of the Adelaide Convention Centre – coins are available at the Convention Centre Reception desk (not at ICOM3 Registration Desk).

Hotel/College Accounts

All accommodation deposits have been transferred to hotels and colleges. Please check your account and advise Sally Jay on the ICOM3 Registration Desk as soon as possible if the correct amount has not been transferred. The balance of monies owing must be paid direct to the hotel or college before you leave.

Parking

Car parking is available at the Exhibition Car Park underneath "Exhibition Hall" which is next to the Adelaide Convention Centre. An "early bird" rate of $5.50 per day applies to those who are in before 9.30am and out before 6.30pm (Monday to Friday).

Storage of Luggage

Check out at hotels/colleges is usually around 10.00am. Facilities will be available at the Adelaide Convention Centre to store your luggage if you are departing on Thursday 12th July.

Speaker Support Centre

The Speaker Support Centre is Room 6 in the Adelaide Convention Centre, which is located one level above the ICOM3 Registration Desk. This room can be reached by the stairs or lift behind the main Convention Centre Reception. A technician will be on duty from 8:00 am to 5:00 pm each day.

Please make sure that you check your slides or data projection with the Speaker Support Centre, Room 6. All speakers are encouraged to hand in their presentations at time of registration (Sunday, July 8 or Monday, July 9). Please test your presentation at least one day prior to your talk.

The technician in the Hall where you will make your presentation will provide assistance on the operation of the microphone and lectern controls.

The chairpersons will be keeping a strict eye on time, so please be sure to keep to the printed time allocation.

Small Meetings

A room for small meetings of up to 15 people can be arranged if required. Please contact the ICOM3 Registration Desk to make bookings.

Poster Displays

Posters will be on display in Halls A and D. There are two groups of posters: P1, displayed on Monday and Tuesday and P2, displayed on Wednesday and Thursday. Access to the hall is available from 7:30 am and posters should be mounted by 12:30 on the first day and taken down by 4:30 pm on the second day of your session. Velcro strips are provided to attach posters to the display boards - please do not use pins. Presenters should stand by their posters at the following times:

Poster Session 1 –
Monday 9 July and Tuesday 10 July

Poster P1 01-50 and P1 51-100
Attendance 1:30 to 2:30 pm Monday 9 July

Poster P1 101-150 and P1 151-170
Attendance 1:30 to 2:30 pm Tuesday 10 July

Poster Session 2 –
Wednesday 11 July and Thursday 12 July

Poster P2 1-50 and P2 51-100
Attendance 1:30 to 2:30 pm Wednesday 11 July

Poster P2 101-160 and P2 161-175
Attendance 1:30 to 2:30 pm Thursday 12 July

Student Competitions
(posters and oral presentations)

Judging for the competition will take place during the conference and Awards will be announced at the Closing Session at 4:10 pm on Thursday 12 July.

Insurance

The conference organisers are unable to accept responsibility for personal accidents and/or damage to private property, or damage to other person's property by any participant, during the conference or at the social events.

SOCIAL EVENTS

Tickets must be shown at all social events. If places are still available, tickets for accompanying guests can be purchased at the ICOM3 Registration Desk.

Sunday 8 July

Welcome Mixer 6:00 to 7:30 pm
Hall E Adelaide Convention Centre
Cost of Ticket: free of charge to registrants. $30 for accompanying guests

Monday 9 July

"Wines of the World" 7:15 pm to 10:00 pm
Bonython Hall, Adelaide University
Don't forget to bring a bottle of wine for competition tasting - prizes for the highest-scoring wines. Light finger food and soft drinks will be provided.
Cost of Ticket: $15

Thursday 12 July

Conference Dinner 7:00 - 11:30 pm
Hardy's Reynella Winery
Coaches will leave from North Terrace outside the Grosvenor Hotel at 6:30 pm and will return to the city from 10:45 pm onwards from Hardy's Winery. Entertainment by "Old Gum Tree-O" - a traditional Australiana band.
Cost of Ticket: $75

GENERAL INFORMATION (contin.)

TOURS AND EXCURSIONS

Coaches for all tours and excursions will leave from North Terrace outside the Adelaide Convention Centre. Tickets are still available for some of the Friday tours - enquire at the ICOM3 Registration Desk.

Sunday 8 July

Cleland Wildlife Reserve	1:30 to 5:00 pm

Friday 13 July

1. Barossa Valley	9:30 to 5:30 pm
2. Cleland Wildlife Reserve	1:30 to 5:00 pm
3. **St Kilda Mangroves/Winery Tour	9:00 am - 5:00 pm

***It is important that you register for coaches on this tour – please see the noticeboard near the ICOM3 Registration Desk*

ENJOY ADELAIDE . . .

Wining and Dining

Adelaide has many excellent cafes and restaurants. A list of eating places close to the Adelaide Convention Centre, Adelaide University and the Colleges is in the conference satchel. Some restaurants allow you to take your own wine (Bring Your Own - BYO) but there will be a corkage charge on each bottle.

Shopping

The main shopping district in the city is Rundle Mall, located only a few minutes walk from the Adelaide Convention Centre. Shops are open from 9:00 am to 5:30 pm Monday to Thursday and until 9:00 pm on Friday. Opening times vary on the weekends.

Entertainment

The Adelaide Casino is located in the Adelaide Railway Station on North Terrace. Cinemas and other places of entertainment are in Hindley Street one block south from North Terrace and (for more cinemas) in Regent Arcade, off Rundle Mall.

City-Airport Transfers

An airport shuttle bus operates between the domestic air terminal and major hotels.

Transport around Adelaide

A free bus service around the city centre operates on weekdays leaving from outside the Railway Station on North Terrace. Details are on the maps which are available at the ICOM3 Registration Desk.

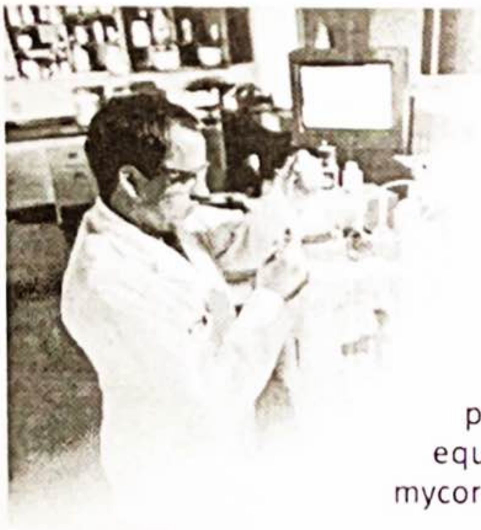
Currency Exchange and Banking

Currency exchange is available in banks and exchange bureaux and at the Adelaide Casino. Banking hours are 9:30 am to 4:00 pm Monday to Thursday and 9:30 am to 5:00 pm on Friday. There are several 24-hour Automatic Teller Machines close to the Adelaide Convention Centre - for directions ask at the Convention Centre Reception.

Major credit cards - Mastercard,Visa, AMEX and Diners Club are widely accepted at hotels, restaurants and shops.

Tipping

Tipping is not widespread in Australia, but is gratefully accepted as a reward for good service. A Goods and Service Tax (GST) applies to most products (except uncooked food) and is included in the cost of items displayed and purchased.



The Premier Line of 100% Natural Mycorrhizae-Based Products.

Since 1983, Premier Tech Biotechnologies has been working on important research and development studies and has made a significant technological breakthrough in mycorrhizal fungi production. The use of the most sophisticated techniques and equipment allows Premier Tech Biotechnologies to manufacture mycorrhizal-based products of unrivaled quality on an industrial scale.

This innovative process is revolutionizing the horticultural industry with three different product line of 100% natural growth stimulants.

Consumer

MYKE

- Used by amateur gardeners on fruits, vegetables, perennials, annuals, trees and shrubs
- Available in two formats: 1.5 and 4 L; and three formulations: MYKE™ Garden, MYKE™ Flowers and MYKE™ Tree and Shrub

Professional

MYCORISE PRO

- Specially adapted for greenhouses, nurseries, reforestation sites or hydroseeding
- Available in two formats: 18 and 30 L; and three formulations: Mycorise Pro® Endo, Mycorise Pro® Ecto and Mycorise Pro® Reclaim

Research

MYCORISE® ASP

- Pure and contaminant-free suspension of *Glomus intraradices spores*
- Available in three concentrates, 100 000, 500 000, 1 000 000 spores

---

Premier Tech
Biotechnologies

1-800-606-6926
ptb@premiertech.com
www.premiertech.com

1, avenue Premier
Rivière-du-Loup, QC
G5R 6C1 CANADA

	Sunday 8 July	Monday 9 July		Tuesday 10 July		Wednesday 11 July		Thursday 12 July	
8.45 am		**Welcome** Professor Sally Smith **Official Opening** Professor Mary O'Kane Vice-Chancellor Adelaide University		**Keynote address 3** Dr M Chalot Transport in ectomycorrhizas		**Keynote 4** Dr S Barker Molecular approaches to understanding mycorrhizal symbioses		**Keynote 5** Professor G Stewart Ecophysiology of mycorrhizal plants	
9.30		(9.15) **Keynote address 1** Professor B Soderstrom Challenges for mycorrhizal research into the new millennium		**Symposium 2** Metabolism and transport in arbuscular mycorrhizas		**Symposium 3** Fungus-plant interactions: from genes to structure and function		**Symposium 4** Mycorrhizal ecology	
10.30		(10.00) Refreshment break		Refreshment break		Refreshment break		Refreshment break	
11.00		(10.30) **Keynote address 2** Dr J Prosser Molecular and functional diversity in soil fungi (11.15) **Symposium 1** Molecular diversity, systematics and detection of mycorrhizal fungi		**Symposium 2** continued		**Symposium 3** continued		**Symposium 4** continued	
12.30 pm		(12.45) Lunch break		Lunch break		Lunch break		Lunch break	
1.30		Poster Session P1. 01- 085		Poster Session P1.101-179		Poster Session P2. 01-096		Poster Session P2.102-172	
2.30		**Concurrent 1** Molecular diversity, systematics and detection of mycorrhizal fungi	**Concurrent 2** Problem soils and extreme environments	**Concurrent 4** Functional Diversity in Mycorrhizas	**Concurrent 5** Mycorrhizal Ecology	**Concurrent 6** Mycorrhizas in agriculture and horticulture	**Concurrent 7** Plant fungus interactions	**Concurrent 8** Mycorrhizosphere	**Concurrent 9** Mycorrhizas in vegetation restoration
3.50		Refreshment Break							
4.20		**Concurrent 1** (continued)	**Concurrent 3** Mycorrhizas in the tropics	**Concurrent 4** (continued)	**Concurrent 5** (continued)	**Concurrent 6** (continued)	**Concurrent 7** (continued)	(4.10) **Overview:** Professor DJ Read (4.40) **Business meeting** Professor M. Allen	
Evening	6.00 – 7.30 pm **Social: Mixer**	7.15 – 10.00 pm **Social: Wines of the World**		7.30-9.30 pm **Workshops A,D,H,I,J**		7.30 – 9.30 pm **Workshops B,E,F,G**		Buses Depart 6.30 pm **Conference Dinner**	

ICOM3 PROGRAM AT A GLANCE

MONDAY, 9 JULY 2001

SCHEDULE OF EVENTS

WELCOME AND OFFICIAL OPENING

Location: Hall B

8:45 **WELCOME**
Professor S.E. Smith,
Chair, ICOM3

OFFICIAL OPENING
Professor M.J. O'Kane,
Vice-Chancellor, Adelaide University

KEYNOTE SESSION 1

Chair: Professor S.E. Smith (Australia)
Location: Hall B

9:15 **K 1**
Challenges for mycorrhizal research into the new millennium
Professor B. Söderström, Lund University, Sweden

10:00 Morning Tea
Location: Hall A

KEYNOTE SESSION 2

Chair: Professor T.D. Bruns (USA)
Location: Hall B

10:30 **K 2**
Molecular and functional diversity in soil fungi
Professor J.L. Prosser, The University of Aberdeen, Scotland

MONDAY, 9 JULY 2001

Note: The first author is the presenting author in most cases.
Underlining indicates presenting author if not first listed author

SYMPOSIUM 1
MOLECULAR DIVERSITY, SYSTEMATICS AND DETECTION OF MYCORRHIZAL FUNGI

Chair: Associate Professor J.W.G. Cairney (Australia) and Dr. A. Schüßler (Germany)
Location: Hall B

11:15	**S1 01** Molecular detection, community structure and phylogeny of ericoid mycorrhizal fungi Berch, S.M., Allen, T.R. and Berbee, M.L. (Canada)
11:45	**S1 02** Molecular approaches to ectomycorrhizal diversity studies Horton, T.R. (USA)
12:15	**S1 03** Molecular identification and phylogeny of arbuscular mycorrhizal fungi Redecker, D. (Switzerland)
12:45	LUNCH BREAK
1:30	**POSTER SESSION 1**
Location:	Hall A

Mycorrhizas in Agriculture and Horticulture

P1 01	Direct assessment of the relationship between mycorrhizal responsiveness and external hyphae-mediated P uptake by four barley cultivars Zhu, Y-G., Smith, S.E. and Smith, F.A. (Australia)
P1 02	Distribution of ^{32}P between roots and tops of white clover: effect of mycorrhizal fungi and placement distance from the roots Mehravaran, H., Mozafar, A. and Frossard, E. (Iran and Switzerland)
P1 03	The effect of vesivular-arbuscular mycorrhizal association on growth of cotton (*Gossypium hirsutum* L.) Kianmehr, H. (Iran)
P1 04	Maize growth curve in response to arbuscular mycorrhizal association Gholami, A. (Iran)
P1 05	Predicting potential phosphorus and zinc fertiliser savings by arbuscular mycorrhizas using a multiple Mitscherlich approach Seymour, N.P., Thompson, J.P. and Kelly A.M. (Australia)
P1 06	Are mycorrhizas more effective at P uptake in organic than conventional farmland? A test of mycorrhizal functioning in response to management in the UK Muckle, G., Leake, J. and Leake, A. (UK)

MONDAY, 9 JULY 2001

P1 07 Influence of different types of fertilizer on the colonisation of rye (*Secale cereale*) by arbuscular mycorrhizal fungi
Gollner, M.J. and Freyer, B. (Austria)

P1 08 Poor growth of field crops following canola (*Brassica napus*) due to insufficient mycorrhizas
Thompson, J.P., Owen, K.J., Clewett, T.G. and Reen, R. (Australia)

P1 09 Effects of preceding crops and soil temperature on growth and arbuscular mycorrhizal colonization of maize plants
Karasawa, T., Kasahara, Y. and Takebe M. (Japan)

P1 010 Distribution and density of arbuscular mycorrhizae and *R. leguminosarum* associated with faba bean grown in Egyptian soil
Attia, M., Bader El-Din, S.M.S. and Ishac, Y. (Egypt)

P1 011 Abundance of rhizobia and vesicular-arbuscular mycorrhizal fungi on groundnut (*Arachis hypogaea*) in semi-arid Zimbabwe
Besmer, Y.L. and Koide, R.T. (USA)

P1 012 Mycorrhizal communities of *Pinus sylvestris* seedlings from bare-root nurseries in Poland
Rudawska, M.L. and Leski T. (Poland)

P1 013 Characterisation of ectomycorrhizal communities of willows (*Salix viminalis, S. dasyclados*) in a short rotation forestry plantation
Püttsepp, Ü. and Taylor, A.F.S. (Sweden)

P1 014 Growth of wild and cultivated lettuce inoculated with *Glomus intraradices* : response to variations in nitrogen and phosphorus addition
Miller, D.J., Jackson, L.E. and Smith, S.E. (Australia)

P1 015 Arbuscular fungi and mycorrhizae at asian pear orchards in Korea
Choi, J.W., Yun, S.K. and Kim, H.L. (Korea)

P1 016 Mycorrhization and plant growth of highbush blueberry (*Vaccinium corymbosum* L.) on farmland in Germany
Goldack, J., Schubert, P., Schwärzel, H., Lentzsch, P. and Münzenberger B. (Germany)

P1 017 Dispersal and effectiveness of mycorrhizal fungi in a non-sterile strawberry field
Muamba, F.D., Hamel, C. and Dalpé (Canada)

P1 018 Mycorrhizal colonisation and its effect on phenolic content, P uptake and growth in olives (*Olea europaea L.*)
Ganz, T.R., Abbott, L.K. and Kailis, S.G. (Australia)

P1 019 Construction of sustainable fruit growing by vesicular-arbuscular mycorrhizal fungi: introduction of new soil management
Ishii, T., Kirino, S. and Kadoya, K. (Japan)

P1 020 Effect of AM fungi on growth and nutrient uptake in some endemic forest tree species of the Western Ghat forests of Karnataka, India
Rama Bhat, P. and Kaveriappa, K.M. (India)

P1 021 Effect of three different fertilization levels on the development of arbuscular mycorrhizal *Phoenix canariensis*
Dreyer, B., Morte, A. and Honrubia M. (Spain)

MONDAY, 9 JULY 2001

P1 022 Growth and nutrient uptake of *Calliandra calothyrus* as affected by arbuscular mycorrhizal inoculation and fertilization with two different P fertilizer
Simanungkalit, R.D.M. and Lukiwati, D.R. (Indonesia)

P1 023 Occurrence, activity and diversity of AM fungi in grapevines from the Pacific Northwest United States
Schreiner, R.P. (USA)

P1 024 Recent studies on AM symbiotic association with mangosteen in Malaysia
Masri, M. (Malaysia)

P1 025 New *in vitro* cultures @ Premier Tech
Béland, M. and Moutoglis, P. (Canada)

P1 026 PTB's Research Report
Moutoglis, P. and Béland, M. (Canada)

P1 027 Using alginate as a sticking agent for production of AM seedlings of *Acacia mangium* (Willd.) in aeroponic culture
Weber, J., Crassard, O., Lee, S.K., Tham, F.Y., Prin, Y., Durand, P. and Ducousso, M. (Singapore and France)

P1 028 Sustained root development *in vitro* obtained in *Pinus pinea* with the help of ectomycorrhiza-derived fungi
Oliveira, P., Barriga, J., Cavaleiro, C., Peixe, A. and Potes, A. (Portugal)

P1 029 Effect of seven indigenous mixtures of AMF in coffee plants under field conditions
Trejo, D., Ferrera-Cerrato, R., Garcia, R., Escalona, M. and Lara-Capistran, L. (Mexico)

P1 030 Integrated use of indigenous arbuscular mycorrhizal fungi and farm yard manure saved fertilizer input, improves grain yield and nutrient uptake by wheat (*Triticum aestivum*) grown in an alfisol
Sharma, M.P. and Adholeya, A. (India)

P1 031 Effect of conventional tillage versus no-till on indigenous arbuscular mycorrhizal fungi of triticale and wheat under field conditions
Brito, I., Antunes, P. and Carvalho, M. (Portugal)

P1 032 Functional diversity of AMF co-existing in agricultural soils subjected to different tillage
Mozafar, A., Jansa, J., Ruh, R., Anken, T., Sanders, I. and Frossard, E. (Switzerland)

P1 033 Effects of soil disturbance on the efficacy of indigenous mycorrhizal fungi in enhancing nitrogen fixation by soybeans
Goss, M.J. and de Varennes, A. (Canada and Portugal)

P1 034 Relationship between cultural practices and arbuscular-mycorrhizal activity in different orchards
Rutto, K.L., Mizutani, F., Moon, D-G and Kadoya K. (Japan)

P1 035 Agronomic significance of arbuscular mycorrhizal fungi activity in soil P dynamics in ridge-tilled corn production
Landry, C.P., Simard, R.R., Hamel, C. and Vanasse, A. (Canada)

P1 036 Inoculation of efficient arbuscular mycorrhizae saved phosphatic fertilizer input on the *post-vitro* micropropagated strawberry plant in an alfisol
Adholeya, A. and Sharma, M.P. (India)

MONDAY, 9 JULY 2001

P1 037 Arbuscular-mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter
Gaur, A. and Adholeya, A. (India)

P1 038 New cultivation methods for *Anoectochilus formosanus* Hayata
Chang, D.C.N., Chou, L.C. and Lee, K.C. (Taiwan, R.O.C.)

P1 039 Morphological and molecular characterization of tropical orchid mycorrhizas
Tham, F.Y., Zhang, X.S. and Lim-Ho, C.L. (Singapore)

P1 040 Fungi isolated from ericoid mycorrhizal roots of the Australian family *Epacridaceae* improve rooting of shoot cuttings
Lawrie, A.C., McDonald, N. and McLean C.B. (Australia)

P1 041 Stimulation of adventitious rooting on cuttings from woody perennial plants by exposure to inoculum of ericoid and arbuscular mycorrhizal fungi
Scagel, C.F. (USA)

P1 042 Alternation of nuclear phase in the filamentous basidiomycete, *Helicobasidium mompa*
Aimi, T., Yotsutani, Y. and Morinaga, T. (Japan)

P1 043 Molecular identification of truffle species in processed food products
Amicucci, A., Guidi, C., Zambonelli, A., Potenza, L. and Stocchi, V. (Italy)

P1 044 Ergosterol in the mycorrhiza formed by desert truffles
Gutiérrez, A., Morte, A. and Honrubia, M. (Spain)

P1 045 Scandinavian black truffles: distribution and habits
Wedén, C. and Danell, E. (Sweden)

P1 046 Potential for the cultivation of *Lactarius deliciosus* (L.:Fr.) S.F. Gray in New Zealand
Wang, Y., Hall, I.R., Dixon, C and Stephen, M. (New Zealand)

P1 047 *Amanita ponderosa*, an edible species rooted at the Sierra of Huelva, Spain
Daza, A., Santamaria, C., Romero, L., Camacho, M., Aguilar, A., Bernedo, M., Pérez, M., Diez, J., Moreno, G. and Manjón, J.L. (Spain)

P1 048 *In vitro* mycorrhizal synthesis of *Tricholoma matsutake* with *Pinus* and *Picea*
Yamada, A. and Murata, H. (Japan)

P1 049 Nutritional value of edible ectomycorrhiza from a Northern Thailand forest
Lumyong, S., Sanmee, R., Lumyong, P., Rerkkasem, B. and Dell B. (Thailand and Australia)

P1 050 Effect of industrial processing on desert truffles: *Terfezia claveryi* Chatin and *Picea juniperi* Vittadini, proximate composition and histochemical location of lipids
Honrubia, M., Murcia, M.A., Martinez-Tomé, M., Vera, A., Jioménez, A.M., Morte, A. and Gutiérrez, A. (Spain)

MONDAY, 9 JULY 2001

Problem Soils and Extreme Environments

P1 051	Influence of restoration practices on mycorrhiza of selected plants from calamine spoil mounds Turnau, K., Bartyzel, E., Zubek, Sz., Jurkiewicz, A. and Szarek-Lukaszewska, G. (Poland)
P1 052	Occurrence of ectomycorrhizas, and endophytic fungi associated with *Betula, Alnus* and *Salix* seedlings naturally regenerated at a disturbed site Hashimoto, Y. (Japan)
P1 053	Rehabilitation of Malaysian highway landscape plants using commercial arbuscular mycorrhizal fungi Raja, P., Herdhawati, J., Nazam, C.A. and Zubaidah, I. (Malaysia)
P1 054	The vertical distribution of *Quercus agrifolia* associated arbuscular mycorrhizae (AM) and ectomycorrhizae (EM) in weathered bedrock Bornyasz, M., Querjeta, I., Graham, R. and Allen, M. (USA)
P1 055	Mycorrhiza-related protein profiles for sensoring toxicity due to agricultural amendments Bestel-Corre, G., Dumas-Gaudot, E. and Gianinazzi, S. (France)
P1 056	Genetics and heavy metal (Ni) tolerance in *Cenococcum geophilum* Gonçalves, S.C., Portugal, A., Gonçalves, M.T., Vieira, R., Freitas, H. and Martins-Loução, M.A. (Portugal)
P1 057	Adaptive heavy metal tolerance in *Suillus* spp Adriaensen, K., Lambaerts, M., Muller L.A. and Colpaert, J.V. (Belgium)
P1 058	Ecology of ectomycorrhizal fungi isolated from *Pinus sylvestris* found at the Great Devon Consols, an old mine site contaminated with arsenic and copper Preston, S., Anderson, I.C., Poskitt, J., Hartley-Whitaker, J., Alexander, I.J. and Meharg, A.A. (UK)
P1 059	Chemical characterisation of extraradical fungal walls of AMF and their metal binding capacity Briones, R., Mustin, C., Joner, E., Belgy, G. and Leyval, C. (France)
P1 060	Interaction of arbuscular mycorrhizal fungi (AMF) with heavy metals: fungal isolates indigenous and non-indigenous in contaminated soils Vosátka, M., Malcová, R., Ilová, M and Rydlová, J. (Czech Republic)
P1 061	Mycorrhizal colonization of indigenous plants in polluted sites in Northern Portugal Oliveira, R.S., Dodd, J.C. and Castro, P.M.L. (Portugal and UK)
P1 062	Mechanism(s) of heavy metal metabolism: the role of arbuscular mycorrhizas Boddington, C.L. and Kuyper, T.W. (The Netherlands)
P1 063	*In planta* molecular monitoring of arbuscular mycorrhizal fungal diversity in field soils amended with sewage sludges Jacquot-Plumey, E., van Tuinen, D., Chatagnier, O., Gianinazzi-Pearson, V. and Gianinazzi S. (France)

MONDAY, 9 JULY 2001

P1 064	Effect of fire on the ectomycorrhizae in a *Quercus ilex* L. stand in NE Spain De Román, M., De Miguel A.M. and Etayo, M.L. (Spain)
P1 065	Drought resistance of Norway spruce seedlings with different mycorrhization, induced by boron supply Lehto, T., Mottonen, M. and Aphalo, P.J. (Finland)
P1 066	Mycorrhizal development in waterlogged soils Baar, J., Roelofs, J.G.M., Visser, E.J.W. and Vousenek, L.A.C.J. (The Netherlands)
P1 067	Effect of arbuscular-mycorrhizal colonisation on water relations and growth of *Zea mays* in compacted soils Sharples, J.M., Farquhar, G.D. and Masle, J. (Australia)
P1 068	A method for analyzing active internal colonization of dark septate fungal endophytes in grasses and shrubs native to arid southwestern USA rangelands Aaltonen, R.E. and Barrow, J.R. (USA)
P1 069	Unique active internal morphology of dark septate fungal endophytes associated with roots of native grasses and shrubs in an arid ecosystem Barrow, J.R. and Aaltonen, R.E. (USA)
P1 070	Establishment and infective capacity of *Gigaspora margarita* Becker & Hall and *Glomus clarum* Nicol. & Gerd. in eroded soil Dos Santos, A.L., de Souza, F.A., Berbara, R.L.L. and Guerra, J.M. (Brazil)
P1 071	Soil compaction and water stress effects on arbuscular mycorrhizal fungi and Norway maple trees Pezzente, M., Hamel, C., St-Arnaud, M. and Dalpé, Y. (Canada)
P1 072	Mycorrhizal relations of mangrove plant community of the Ganges River estuary in India Chaudhuri, S. and Sengupta, A. (India)
P1 073	Impact of flooding on a Glomalean fungal population in agricultural soil Watson, D.M.H., and Rufty, T.W. Jnr. (USA)
P1 074	The effect of summer flood of the river Oder in 1997 on the population of arbuscular mycorrhizal fungi Tauschke, M.,Lentzsch, P., Höflich, G. and Schalitz, G. (Germany)
P1 075	Arbuscular mycorrhiza in the aquatic plant *Littorella uniflora* (L.) Ascherson Nielsen, K.B., Andersen, F.Ø. and Thomsen, D.R. (Denmark)
P1 076	Ectomycorrhizae, soil substrates, and post-fire recovery in a permafrost-dominated watershed in subarctic Alaska Helm, D.J. (USA)
P1 077	VA mycorrhiza in a chenopod halophyte in highly saline soil Weber, D.J., Jefferies, J. and Fisk, H. (USA)
P1 078	Role of vesicular-arbuscular mycorrhiza (VAM) on growth of banana in acid soil containing high aluminium Sukarno, N., Hadisunarso and Shanti (Indonesia)
P1 079	The effect of two arbuscular mycorrhizal (AM) fungi on cowpea growth under low pH conditions Rohyadi, A., Smith, S.E., Smith, F.A. and Murray, R.S. (Australia)

P1 080	The responses of ectomycorrhizal biomass and production to elevated CO_2 Langley, J.A., Hungate, B.A. and Drake, B.G. (USA)
P1 082	The effects of low level ozone stress on the below- and above-ground compartments in the silver birch seedlings with three different mycorrhizal status Kasurinen, A., Mannoinen, A.M., Holopainen, T. and Holopainen J.K. (Finland)
P1 083	Effects of long-term CO_2 and O_3 exposure on silver birch rhizosphere Holopainen, T., Kasurinen, A. and Vapaavuori E (Finland)
P1 084	Effect of mycorrhizal fungus inoculations on *Castanea sativa* Mill. germination, growth and weaning Martins, A., Caldas, S., Lino, C., Alves, J., Sousa, M.J. and Pais, M.S. (Portugal)
P1 085	Occurrence of vesicular arbuscular mycorrhizae (VAM) in coastal habitats of Bahrain Gul, J. and Mandeel, Q. (Bahrain)

MONDAY, 9 JULY 2001

CONCURRENT SESSION 1
MOLECULAR DIVERSITY, SYSTEMATICS AND DETECTION OF MYCORRHIZAL FUNGI

Chairs: Dr. S.M. Chambers (Australia) and Dr. A. Schüßler (Germany)
Location: Hall B

2:30 **C1 01**
Resupinate thelephoroid fungi are world-wide and common plant root mycobionts - merging classical taxonomy, molecular phylogeny and bioinformatics
Kõljalg, U. and Dunstan, B. (Estonia and Australia)

2:50 **C1 02**
Are arbuscular mycorrhizal fungi homo- or heterokaryotic?
Pawlowska, T.E. and Taylor, J.W. (USA)

3:10 **C1 03**
A natural taxonomy of AM fungi (Glomales) based on the SSU rRNA-gene
Schwarzott, C., Walker, C. and Schüßler, A. (Germany and UK)

3:30 **C1 04**
The ectomycorrhizal fungal community in soil as identified from soil DNA extracts
Landeweert, R., Smith, E. and Kuyper, T.W. (The Netherlands)

3:50 Afternoon Tea
Location: Hall A

4:20 **C1 05**
Application of PCR-denaturing gradient gel electrophoresis (DGGE) in taxonomy, rDNA characterization and community analysis of Glomales (Zygomycetes)
de Souza, F.A., Leeflang, P., Smit, E., van Elsas J.D. and Kowalchuk, G.A. (Brazil and The Netherlands)

4:40 **C1 06**
Changes in community structure of AM fungi due to reduced tillage
Jansa, J., Mozafar, A., Ruh, R., Anken, T., Kuhn, G., Sanders, I. and Frossard, E. (Switzerland)

5:00 **C1 07**
Molecular diversity, phylogenetic affinities and mycorrhizal synthesis of root-associated ascomycetes of Helotiales in northern boreal plant communities
Vrålstad, T., Myhre, E., Taylor A.F.S. and Schumacher, T. (Norway and Sweden)

5:20 **C1 08**
Diversity and distribution of fungal endophyte genotypes in the root system of *Woollsia pungens* (Ericaceae)
Midgley, D.J., Chambers, S.M. and Cairney, J.W.G. (Australia)

MONDAY, 9 JULY 2001

CONCURRENT SESSION 2
PROBLEM SOILS AND EXTREME ENVIRONMENTS

Chair:	Dr. Y. Zhu (Australia)
Location:	Hall C

2:30 **C2 01**
Mycorrhiza in semi-aquatic environments: studies with *Lythrum salicaria*
Stevens, K.J., Spender, S., Peterson, R.L. and Reader, R.J. (Canada)

2:50 **C2 02**
The use of mycorrhizal associations for an *in situ* phytoremediation of contaminated soils
Heyser, W., Dobner, I. and Seebeck, A. (Germany)

3:10 **C2 03**
Ecophysiology of ectomycorrhizal fungi associated with *Pinus* spp. in low rainfall areas of Western Australia
Bell, T.L. and Adams, M.A. (Australia)

3:30 **C2 04**
Surviving the cold: ectomycorrhizal fungi and life at low temperature
Tibbett, M. and Cairney, J.W.G. (UK and Australia)

3:50 Afternoon Tea
Location: Hall A

CONCURRENT SESSION 3
MYCORRHIZAS IN THE TROPICS

Chair:	Dr. J.B. Baon (Indonesia) and Professor D. Janos (USA)
Location:	Hall C

4:20 **C3 01**
Phosphorus cycling by ectomycorrhizal trees in an African rain forest
Chuyong, G.B., Njampa, LO., McNewbery, D. and Alexander, I.J. (Switzerland and Scotland)

4:40 **C3 02**
Vertebrate exclusion reduces mycorrhizal inoculum potential of a rainforest soil
Gehring, C.A. and Theimer, T.C. (USA)

5:00 **C3 03**
Arbuscular mycorrhizal fungi preclude Eucalypt seedling establishment in a northern Australian monsoon rainforest soil
Janos, D.P., Scott, J. and Bowman, D.M.J.S. (USA and Australia)

5:30 **C3 04**
Differential benefit of forest and pasture mycorrhizas to seedling growth in a tropical tree
Aldrich-Wolfe, L. (USA)

7:15 **WINES OF THE WORLD**
Location: Bonython Hall, Adelaide University, North Terrace

TUESDAY, 10 JULY 2001

Note: The first author is the presenting author in most cases.
Underlining indicates presenting author if not first listed author

KEYNOTE SESSION 3

Chair: Professor L. Peterson
Location: Hall B

8:45 **K 3**
Transport in ectomycorrhizas
Dr. M. Chalot, Université Henri Poincaré, France

SYMPOSIUM 2
METABOLISM AND TRANSPORT IN ARBUSCULAR MYCORRHIZAS

Chairs: Dr. B. Bago (Spain) and Dr. P. Pfeffer (USA)
Location: Hall B

9:30 **S2 01**
Imaging transport along arbuscular mycorrhizal hyphae
Bago, B., Pfeffer, P.E., Lammers, P., Zipfel, W. and Shachar-Hill, Y. (Spain and USA)

10:00 **S2 02**
Regulation of plant nutrient transporters in arbuscular mycorrhizas
Burleigh, S.H. (Denmark)

10:30 Morning Tea
Location: Hall A

11:00 **S2 03**
Mechanisms of nutrient transport across interfaces in arbuscular mycorrhizas
Ferrol, N., Barea, J.M. and Azcón-Aguilar C. (Spain)

11:30 **S2 04**
P metabolism and transport
Ezawa, T., Smith, S.E. and Smith, F.A. (Japan and Australia)

12:00 **S2 05**
Carbon metabolism and transport in the arbuscular mycorrhizal symbiosis
Shachar-Hill, Y., Lammers, P.J., Jun, J., Abubaker, J., Allen, J.W., Bago, B., Douds, D.D. and Pfeffer, P.E. (USA and Spain)

12:30 LUNCH BREAK

TUESDAY, 10 JULY 2001

1:30 POSTER SESSION 1 (CONTINUED)

Location: Hall A

Plant/Fungus Interactions, including mycorrhiza/pathogen interactions

P1 101	Interaction effects of laser irradiated *Glomus mosseae* and *Bacillus pumilus* on *Fusarium oxysporum* f.sp. *lycopersici* in tomato plants Ouf, S.A. (Egypt)
P1 102	Interactions between two arbuscular mycorrhizal fungi and fungal feeding nematodes and control of the nematode with phenamiphos Bakhtiar, Y., Miller, D., Cavagnaro, T. and Smith, S.E. (Indonesia and Australia)
P1 103	Effect of mycorrhizal inoculation on tolerance of *Castanea sativa* Mill. plants to *Phytophtora cinnamomi* infection Martins, A., Gouveia, E., Coelho, V., Estevinho, I. and Pais, M.S. (Portugal)
P1 104	Influence of soil phosphorus on the interactions between the mycorrhizal fungus *Glomus coronatum* and binucleate *Rhizoctonia* or *Rhizoctonia solani* on mung bean Kasiamdari, R.S., Smith, S.E., Smith, F.A. and Scott, E.S. (Australia)
P1 105	Ultrastructural effects of a fungal endophyte in the roots of eggplant and their potential role in disease suppression Narisawa, K., Currah, R.S., Chen, M. and Tsuneda, A. (Canada)
P1 106	Fertilizer and inoculation of AM fungi effect on the development of a foliar disease (*Phoma costarricencis* Echandi) on coffee seedlings Escalona, M., Trejo, D. and Abato, M. (Mexico)
P1 107	Effect of arbuscular mycorrhiza fungi (AMF) on *Meloidogyne hapla* in pyrethrum in Kenya Waceke, J.W., Waudo, S.W. and Sikora, R. (Kenya and Germany)
P1 108	Testing of AMF propagation in tubular rhizoboxes with different plant pot culture and MHB co-inoculation Ksiezniak, A., Paszkowski, W.L., Wróblewska, B. and Doroszewska, T. (Poland)
P1 109	High cell density promotes the outgrowth of intraradical hyphae of *Gigaspora* from carrot root explants; a step towards axenic culture? Williams, P.G. (Australia)
P1 110	Agarose as a suitable substrate for growing arbuscular mycorrhizal fungi *in vitro* Karandashov, V. and Kuzovkina, I. (Russia)
P1 111	Effect of P-deficient root exudates on hyphal elongation and branch of arbuscular mycorrhizal fungi Tawaraya, K., Suda, S., Murasawa, M., Naeki, Y., Murayama, T. and Wagatsum, T (Japan)
P1 112	Red radiation stimulates hyphal growth of vesicular-arbuscular mycorrhizal fungi *in vitro* Yachi, Y., Ishii, T. and Horii, S. (Japan)
P1 113	Blue light and chemical signals from a host root exudate synergistically stimulate hyphal branching of the AM fungus *Gigaspora gigantea* Nagahashi, G. and Douds, D. Jr (USA)

TUESDAY, 10 JULY 2001

P1 114	Hypaphorine, an auxin antagonist delivered to the root by the ectomycorrhizal fungus *Pisolithus tinctorius* Lapeyrie, F., Ditengou, F.A., Kawano, T., Langrange, H., Jambois, A. and Legué, V. (France)
P1 115	Analysis of indole-3-butyric acid function in arbuscular mycorrhiza using the fluorinated IBA analog TFIBA Kaldorf, M., Fitze, D. and Ludwig-Müller, J. (Germany)
P1 118	Partner recognition in the *Geosiphon pyriforme* symbiosis - a model system for AM research Wolf, E. and Schüßler, A. (Germany)
P1 119	Secondary metabolites acumulation in monoaxenic culture *Prosopis laevigata-Gigaspora rosea* Rojas, R., Hernández, J., Frias, J.T., Dendooven, L., Cerda, C., Bécard, G., Ramos-Valdivia, A.C and Olalde-Portugal, V. (Mexico and France)
P1 120	Allene oxide cyclase in *Medicago truncatula* - implications for a role of jasmonates in the establishment of mycorrhiza Hause, B., Stenzel, I., Miersch, O., Kramell, R. and Strack, D. (Germany)
P1 121	A phosphate deficiency-induced flavonoid glucoside stimulates vesicular-arbuscular mycorrhiza formation in melon roots under low and high phosphate conditions Akiyama, K., Matsuoka, H. and Hayashi, H. (Japan)
P1 122	Study of nutrient and water availability of mycorrhizal *Daucus carota* hairy roots on *Glomus intraradices* AM fungus development Jolicoeur M. and Perrier, M. (Canada)
P1 123	Isolation and characterization of monakaryotic cultures of *Pisolithus* sp. Kasuya, M.C.M., Costa, M.D., Pereira, G.M.D. and Borges, A.C. (Brazil)
P1 124	Effect of AM symbiosis and phosphate on root ploidy Lingua, G., Fusconi, A., Trotta, A. and Berta, G. (Italy)
P1 125	Ectomycorrhiza development and cytoskeleton Raudaskoski, M., Tarkka, M., Lahdensalo, J., Hanif, M. and Laitiainen, E. (Finland)
P1 126	Cytoskeltal reorganization in *Medicago truncatula* root cells during development of an arbuscular mycorrhizal symbiosis Zhao, L., Blancaflor, E.B. and Harrison, M.J. (USA)
P1 127	The role of the motile tubular vacuole system in mycorrhizal fungi Allaway, W.G. and Ashford, A.E. (Australia)
P1 128	Microtubule arrangement of fungal hyphae and root cells in the *Entoloma - Rosa* mycorrhiza Kobayashi, H. and Hatano, K. (Japan)
P1 129	Microtubules and tubulin dynamics of *Glomus intraradices* and tomato in arbuscular mycorrhizal symbiosis Timonen, S., Smith, F.A., and Smith, S.E. (Finland and Australia)
P1 130	Proteomics of *in vitro* grown Ri T-DNA transformed carrot roots (*Daucus carota* L.) associated with the arbuscular mycorrhizal fungus *Glomus intraradices* Saravanan, R.S., Bestel-Corre, G., Fontaine, B., Gianinazi, S., St-Arnaud, M. and Dumas-Gaudot, E. (France and Canada)

TUESDAY, 10 JULY 2001

P1 131 Molecular cloning and expression analysis of an ammonium transporter and other nitrogen-regulated genes in the ectomycorrhizal fungus *Tuber borchii*
Montanini, B., Soragni, E., Moretto, N., Bolchi, A., Percudani, R. and Ottonello, S. (Italy)

P1 132 Proteomics as a tool to study plant microbe interactions
Mathesius, U., Keijzers, G., Natera, S., Weinman, J.J., Djordjevic, M.A. and Rolfe, B.G. (Australia)

P1 133 Differential expression of defence-related genes in wild-type and a mycorrhiza-defective mutant tomato
Gao, L-L., Smith, S.E., Knogge, W., Delp, G. and Smith. F.A. (Australia)

P1 134 Mapping the *rmc* locus: towards characterisation of the mutation
Larkan, N.J., Smith S.E. and Barker S.J. (Australia)

P1 135 The role of plasma membrane H^+-ATPases in arbuscular mycorrhizal roots
Rosewarne, G.M., Smith, S.E., Smith, F.A. and Schachtman, D.P. (Australia)

P1 136 Expression of two phosphate transporters in barley plants colonised by mycorrhizal fungi
Glassop, D., Schünmann, P.H.D., Smith, F.W. and Smith, S.E. (Australia)

P1 137 Investigations of phosphate transporter expression in the soybean AM symbiosis
Bougoure, D.S. and Dearnaley, J.D.W. (Australia)

P1 138 Functional characterization of *Mt4*-like genes, a common component of the phosphate-starvation signaling pathways in higher plants
Shin, H. and Harrison, M.J. (USA)

P1 139 Molecular cloning of an LTR-retroelement, *marY1*, from the ectomycorrhizal homobasidiomycete *Tricholoma matsutake*
Murata, H., Miyazaki, Y., Babasaki, K. and Yamada, A. (Japan)

P1 140 Molecular characterization of amino acid transporters in *Hebeloma cylindrosporum/Pinus pinaster* ectomycorrhizae
Wipf, D., Benjdia, M., Tegeder, M. and Frommer, W.B. (Germany)

P1 141 Increased arbuscule colonization in roots of *Lotus japonicus* hypernodulating mutants
Senoo, K., Solaiman, M.Z., Tanaka, S., Kawaguchi, M., Imaizumi-Anraku, H., Akao, S., Tanaka, A. and Obata, H. (Japan and Australia)

P1 142 An approach to elucidate molecular events in the recognition of phase of ectomycorrhiza formation
Herrmann, S., Krüger, A., Peskan, T., Oelmueller, R. and Buscott, F. (Germany)

P1 143 The influence of different arbuscular mycorrhizal fungi and phosphorus on gene expression of invertase and sucrose synthase in roots of maize
Ravnskov, S., Wu., Y. and Graham, J.H. (Denmark and USA)

P1 144 Cloning of β-1, 3-glucanase genes from mycorrhizal olive plantlets
Azcón-Aguilar, C., Barea, J.M., Ferrol, N. and Calvente, R. (Spain)

P1 145 Antagonistic interactions between symbiotic and saprophytic microbes in birch rhizosphere - evidence from manipulating carbon availability
Stark, S. and Kytäviita, M-M. (Finland)

P1 146	Comparison of the activites of isocitrate dehydrogenase and isocitrate lyase involved in isocitrate metabolism in *Laccaria amethystea* during symbiosis with *Pinus densiflora* Hattori, T., Itaya, M., Ohta, A. and Shimada, M. (Japan)
P1 147	Anatomy of carbohydrate utilization, metabolism, and transfer in an AM fungus Pfeffer, P.E., Bago, B., Douds, D.D., Jun, J., Abubaker, J., Allen, J.W., Lammers, P.J. and Shachar-Hill, Y. (USA & Spain)
P1 148	Cloning of *Suillus bovinus* GS/GOGAT genes and localisation of expression in the Scots pine mycorrhizosphere Juuti, J.T., Jokela, S., Timonen, S., Hess, M. and Sen, R. (Finland)
P1 149	Regulation of NO_3 uptake in the symbiotic association *Pinus pinaster - Rhizopogon roseolus* Plassard C. and Gobert A.M. (France)
P1 150	Nitrogen (N) allocation patterns of mycorrhizal fungi and N supply rate control N isotope patterns in *Pinus sylvestris* Hobbie, E.A. and Colpaert, J.V. (Germany and Belgium)

Mycorrhizas in vegetation restoration and phytoremediation

P1 151	Effect of ectomycorrhiza of Scots pine on dissolved nitrogen compounds in peat and nitrogen uptake and polyamines of host plant Sarjala, T. (Finland)
P1 152	The impact of peaty substrate on mycorrhization of Scots pine (*Pinus sylvestris* L.) seedlings and containerised stock Gaitnieks, T. (Latvia)
P1 153	Diversity of ectomycorrhizal fungi and ectomycorrhizae in forests of *Pinus culminicola* Andersen & Beaman in the state of Nuevo León, México Garza Ocañas, F. and Arias Mota, R. (Mexico)
P1 154	The role of mycorrhization in the reafforestation of agricultural soils with *Pinus halepensis* Diaz, G., Gutiérrez, A. and Honrubia M. (Spain)
P1 155	The potential for woody understory plants to provide refuge for ectomycorrhizal inoculum at an interior British Columbia Douglas-fir forest after clear-cut logging Durall, D.M., Hagerman, S.M. and Sakakibara, S.M. (Canada)
P1 156	The effects of disturbance (post-logging) on sequestrate fungal diversity Lebel T. (Australia)
P1 157	Pruning effects on root length density, root biomass, and arbuscular mycorrhizal colonization in two shrubs in a simulated xeric landscaped yard Whitcomb, S.A. and Stutz, J.C. (USA)
P1 158	Inoculation of Australian native seedlings with arbuscular mycorrhizal fungi improved growth in the field Pattinson, G.S, Hammill, K.A., Sutton B.C. and McGee, P.A. (Australia)

TUESDAY, 10 JULY 2001

P1 159 Effect of metallic trace elements on AM fungi diversity within roots of *Trifolium subterraneum* colonised with AM spores isolated from a polluted soil: application to soil remediation
Tonin, C., Vandenkoornhuyse, P., Joner, E.J., Straczek, J. and Leyval C. (France)

P1 160 Effect of native mycorrhizas on the growth and uptake of heavy metals and nutrients by native plant species growing on lateritic nickel soil
Boulet, F.M. and Jasper, D.A. (Australia)

P1 161 Molecular responses of ericoid mycorrhizal fungi to heavy metal
Perotto, S., Martino, E., Vallino, M., Lanfranco, L. and Bonfante, P. (Italy)

P1 162 Proteomics for studying cadmium-induced modifications in mycorrhizal pea roots
Repetto, O., Bestel-Corre, G., Gianinazzi, S., Gianinazzi-Pearson, V. Dumas-Gaudot, E. and Berta, G. (Italy and France)

P1 163 Molecular diversity of mycorrhizal fungi on the roots of trees grown with contaminated soil inoculum
Ridgway, K.P., Marland, L.A., Young, J.P.W. and Fitter, A.H. (UK)

P1 164 Cadmium tolerance and mechanisms of detoxification in mycorrhizal pea genotypes
Rivera-Becerril, F., van Tuinen, D., Turnau, K., Gianinazzi, S. and Gianinazzi-Pearson, V. (France and Mexico)

P1 165 Arbuscular mycorrhizae and restoration of endangered plants in subtopical Florida
Fisher, J.B. and Jayachandran, K. (USA)

Mycorrhizosphere

P1 171 Ectomycorrhizal and microfungal diversity on Norway spruce stands with different degrees of decline in the Krkonose Mountains (CZ)
Egli, S., Gonthier, P., Nicolotti, G., Peter, M., Varese, G.C. and Voyron, S. (Switzerland and Italy)

P1 172 Soil microbial population and activity affected by mycorrhizal and non-mycorrhizal winter cover crops in Southern Brazil
Lovato, P.E. and Kunze, A. (Brazil)

P1 173 Arbuscular mycorrhizal infection changes the bacterial 16S rDNA community composition in the rhizosphere of maize
Marschner, P., Crowley, D.. and Lieberei, R., (Germany and USA)

P1 174 Detection of Archaea in Scots pine mycorrhizospheres and boreal forest humus
Bomberg, M., Jurgens, G., Saano, A., Sen, R. and Timonen, S. (Finland)

P1 175 Biological activity in the mycorrhizosphere of *Trachypogun plumosus* Ness, dominant species in Venezuelan acid savannas
Toro, M., López-Gutiérrez, J.C., Hernández-Valencia, I and López-Hernández, D. (Venezuela)

P1 176 Mycorrhiza-regulated plant genes common to beneficial rhizobacteria interactions
Weidmann, S., Brechenmacher, L., Batoux, M., van Tuinen, D., Lemenceau, P. and Gianinazzi-Pearson, V. (France)

P1 177 To which extent observed changes on plant $\delta\ ^{15}N$ values are due to the symbiosis with arbuscular mycorrhiza fungi?
Fonseca, H.M.A.C. and Berbara, R.L.L. (Portugal and Brazil)

P1 178	Effects of hardened wood ash on microbial activity, plant growth and nutrient uptake by ectomycorrhizal spruce seedlings Mahmood, S., Finlay, R.D., Fransson, A-M. and Wallander, H. (Sweden)
P1 179	Bacteria associated with *Glomus clarum* spores influence mycorrhizal activity Germida, J.J. and Xavier, L.J.C. (Canada)

TUESDAY, 10 JULY 2001

CONCURRENT SESSION 4
FUNCTIONAL DIVERSITY IN MYCORRHIZAS

Chairs: Associate Professor L.K. Abbott (Australia) and Professor R. L. Peterson (Canada)
Location: Hall B

2:30 **C4 01**
Linking into CMNs: the implications of position and host preference for ecto- and arbuscular mycorrhizal colonization of oak seedlings
Lindahl, A.E., Allen, M.F., Egerton-Warburton, L.M., Lansing, J.L. and Tennant, T. (USA)

2:50 **C4 02**
Significance of the apoplastic pathway for nutrient acquisition and nutrient exchange in ectomycorrhizal root systems
Bücking, H., Kuhn, A.J., Hans, R. and Heyser, W. (Germany)

3:10 **C4 03**
Identification of genes for lignin peroxidases and manganese peroxidases in ectomycorrhizal fungi using PCR
Chen, D.M., Taylor, A.F.S., Burke, R.M. and Cairney, J.W.G. (Australia, Sweden and U.K.)

3:30 **C4 04**
Uptake of ^{15}N-labeled inorganic and organic N by *Picea engelmannii* seedlings growing in a high elevation clear-cut in association with different ectomycorrhizal fungi
Jones, M.D., Peat, H.C.L. and Philip, L.J. (Canada)

3:50 Afternoon Tea
Location: Hall A

4:20 **C4 05**
First record of VAM in the mycoheterotrophic *Arachnitis uniflora* (Corsiaceae): morphological development of the association
Dominguez, L.S. and Sérsic, A. (Argentina)

4:40 **C4 06**
Are mycorrhizal allocation patterns related to mutualistic function?
Johnson, N.C., Rowland. D.L., Corkidi, L. and Allen, E.B. (USA)

5:00 **C4 07**
Intraspecific variation in P uptake potential among isolates of *G. mosseae, G. clariodeum* and *G. geosporum/G.caledonium* in relation to their genetic variation
Knudsen, L.M, Vestberg, M., Rosendahl, S and Jakobsen I. (Denmark)

5:20 **C4 08**
Mycorrhiza and root evolution and diversity in ancient and living plant lineages.
Brundrett, M. (Australia)

TUESDAY, 10 JULY 2001

CONCURRENT SESSION 5
MYCORRHIZAL ECOLOGY

Chairs: Associate Professor M. Adams and Mr. P. O'Connor (Australia)
Location: Hall C

2:30 **C5 01**
Mycorrhizal ecology studied directly in the field of boreal forests
Högberg, P. and Högberg, M.N. (Sweden)

2:50 **C5 02**
Field responses of ectomycorrhizal community structure to elevated CO_2
Fransson, P.M.A., Taylor, A.F.S. and Finlay, R.D. (Sweden)

3:10 **C5 03**
Resource availability determines the outcome of competitive interactions between ectomycorrhizal and saprotrophic fungi
Lindahl, B. and Finlay, R.D. (Sweden)

3:30 **C5 04**
Mechanistic plant competition revisited: the intermediate role of mycorrhizal networks
Booth, M.G. (USA)

3:50 Afternoon Tea
Location: Hall A

4:20 **C5 05**
Consideration of ectomycorrhizal fungi in sustainable forestry: scaling in ecosystems from root tips to flying squirrels
Luoma, D.L. and Eberhart, J.L. (USA)

4:40 **C5 06**
Arbuscular mycorrhizas influence diversity and structure in a semi-arid plant community
O'Connor, P.J., Smith, S.E. and Smith F.A. (Australia)

5:00 **C5 07**
Patterns of blue oak (*Quercus douglasii*) fine root production and ectomycorrhizal colonization in a California oak woodland: Response to season and fertilizer
Bledsoe, C.S. and Cheng, X. (USA)

5:20 **C5 08**
Mycorrhizas and global climate change: a summary of experimental evidence for the effects of increasing temperature
Gavito, M.E., Schweiger, P. and Jakobsen, I. (Sweden, Australia and Denmark)

TUESDAY, 10 JULY 2001

7:30 **WORKSHOPS**

Location: Napier and Lower Napier Theatres, Adelaide University, North Terrace (Adjacent to Bonython Hall)

A: Mycorrhizal fungi in semi aquatic environments : functional or relict
Organisers: Dr. J. Baar (The Netherlands) and Dr. Nielsen (Denmark)

D: Transport and metabolism in mycorrhizas
Organisers: Dr P. Pfeffer (USA), Dr. B. Bago (Spain) and Dr. D. Wipf (Germany)

H: Potential effects of transgenic plants on mycorrhizal establishment and function
Organiser: Dr. L. Watrud (USA)

I: The "right controls" for gene expression studies of mycorrhizas
Organiser: Dr G. Rosewarne (Australia)

J: Mycorrhizas in agriculture and horticulture
Organisers: Dr. P. McGee (Australia) and Dr. P. Schweiger (Austria)

WEDNESDAY, 11 JULY 2001

Note: The first author is the presenting author in most cases.
Underlining indicates presenting author if not first listed author

KEYNOTE SESSION 4

Chair:	Associate Professor J.W.G. Cairney (Australia)
Location:	Hall B

8:45 **K 4**
Molecular approaches to understanding mycorrhizal symbioses
Dr S.J. Barker, The University of Western Australia

SYMPOSIUM 3
FUNGUS-PLANT INTERACTIONS: FROM GENES TO STRUCTURE AND FUNCTION

Chairs:	Dr. V. Gianinazzi-Pearson (France) and Professor S.E. Smith (Australia)
Location:	Hall B

9:30 **S3 01**
Developmental regulation of fungal genes in the arbuscular mycorrhizal symbiosis
Requena, N., Breuninger, M., Ocón-Garrido, A., Cappellari, S., Franken, P. and Hampp, R (Germany)

10:00 **S3 02**
Genetic and molecular analyses of ectomycorrhizal symbiosis
Tagu, D. and Martin, F. (France)

10:30 Morning Tea
Location: Hall A

11:00 **S3 03**
The role of the plant genome in modulating arbuscular mycorrhizal formation
van Tuinen, D., Brechenmacher, L., Weidmann, S., Chatagnier, O., Gianinazzi, S., Franken, P. and Gianinazzi-Pearson, V. (France and Germany)

11:30 **S3 04**
Plant and arbuscular mycorrhizal phosphate transporters
Versaw, W.K. and Harrison, M.J. (USA)

12:00 **S3 05**
Dissecting the plant's genetic program for endosymbioses using mutants of *Lotus japonicus*
Kistner, C., Coomber, S., Mulder, L., Pitzschke, A., Stracke, S., Tuck, E., Winzer, T. and Parniske, M. (UK)

12.30 LUNCH BREAK

WEDNESDAY, 11 JULY 2001

POSTER SESSION 2
Location: **Hall A**

Mycorrhizal Ecology

P2 01	Do plants selectively associate with species of ectomycorrhizal fungi that support highest plant growth? Kummel, M. (USA)
P2 02	Influence of plant community composition and diversity on ectomycorrhizal diversity in boreal mixed-woods Kernaghan, G., Légaré, S., Widden, P and Bergeron, Y. (Canada)
P2 03	Influence of habitat on diversity of putative mycorrhizal endophytes of *Epacris microphylla* (Ericaceae) Williams, A.F., Chambers, S.M. and Cairney, J.W.G. (Australia)
P2 04	Spatial relations of ectomycorrhizae and their relations to soil heterogeneities in natural soil of a Norway spruce stand as studied by the new method "Micromapping" (McMp) Agerer, R., Raidl, S., Grothe, R. and Göttlein, A. (Germany)
P2 05	The effects of long-term field fertilization on mycorrhizal fungal communities in a temperate oak savanna Avis, P.G., McLaughlin, D., Reich, P. and Charvat, I.D. (USA)
P2 06	Conservation of ectomycorrhizal fungi: Green-tree retention preserves species diversity Eberhart, J.L.. and Luoma, D.L. (USA)
P2 07	Preliminary data about how the ectomycorrhizal community changes in space and time in an evergreen oak forest Sarrionandia, E., Gartzia M. and Salcedo I. (Spain)
P2 08	Below ground community structure of ectomycorrhizas; roots and external mycelia Erland, S., Mahmood, S. and Rahm, J. (Sweden)
P2 09	Mycorrhiza fungal diversity as impacted by the loss of endemic, rainforest plants in Madagascar Fernandes, E.C.M., Styger, E. and Rakotondramasy, H.M. (USA & Madagascar)
P2 010	An investigation of arbuscular mycorrhizas in a South African coastal sand dune system Dames, J.F. and Haller, A.H.A. (South Africa)
P2 011	Below-ground ecology of Scottish heathlands: the role of mycorrhizas Genney, D.R., Alexander I.J. and Hartley, S.E. (UK)
P2 012	Established vegetation negates mycorrhizal benefit in seedlings - a case study of subarctic perennial herbs Kytöviita, M.-M., Vestberg, M. and Tuomi, J. (Finland)
P2 013	Diversity and host preference of ectomycorrhizal fungi in arctic heath communities Moyersoen, B., Knox, O., Shand, I. and Alexander, I.J. (Scotland)
P2 014	Ecology and mycorrhizae of *Craterellus tubaeformis* in the Pacific Northwestern United States Trappe, M.J. (USA)

WEDNESDAY, 11 JULY 2001

P2 015	Is man indirectly responsible in the dissemination of AMF spores? Lakshman, H.C. (India)
P2 017	Moisture retention properties of a mycorrhizal soil Augé, R.M., Stodola, A.J.W., Tims, J.E. and Saxton, A.M. (USA)
P2 018	Mycorrhizal diversity and niche requirements in relation to N-deposition and dryness Wöllecke, J., Münzenberger, B. and Hüttl, R.F. (Germany)
P2 019	Uptake of organic nitrogen in Dutch heathland ecosystems Zijlstra, J.D. and Berendse, F. (The Netherlands)
P2 020	Phylogenetic and environmental influences on nitrogen stable isotope ratios in sporocarps of ectomycorrhizal fungi Trudell, S.A., Rygiewicz, P.T. and Edmonds, R.L. (USA)
P2 021	Nitrogen fertilization promotes homogeneity in Glomalean communities Egerton-Warburton, L.M., Johnson, N.C. and Allen, E.G. (USA)
P2 022	N fertilization results in reduced growth of ectomycorrhizal (EM) mycelium in the field Nilsson, L.O., Wallander, H. and Bååth, E. (Sweden)
P2 023	Seasonal and host specific responses of AM fungi to elevated carbon dioxide and nitrogen Wolf, J., Johnson, N.C. and Rowland D.L. (USA)
P2 024	Determination of the carbon and nitrogen sources for arbuscular mycorrhizal fungi using stable isotopes Nakano, A., Takahashi, K., Koide, R.T. and Kimura, M. (Canada and Japan)
P2 025	*In situ* dynamics of carbon transfer through AM mycelium in an upland grassland Johnson, D., Leake, J.R., Ostle, N., Ineson, P. and Read, D.J. (UK)
P2 026	Element transport from minerals by the ectomycorrhizal mycelium in forest of different nutrient status Hagerberg, D. and Wallander, H. (Sweden)
P2 029	Distribution and persistence of Australian *Pisolithus* spp. genets at native sclerophyll forest field sites Anderson, I.C., Chambers, S.M. and Cairney, J.W.G. (Australia)
P2 030	Hypogeous fungal diversity on Rottnest Island, Western Australia Thomas, S.R., Dell, B., Malajczuk, N and Trappe, J.M. (Australia)
P2 031	*Tomentella* and related genera: ectomycorrhiza-forming resupinate basidiomycetes occurring in forests and woodlands of south-western Australia Dunstan, B., Kõljalg U and Dell, B (Australia and Estonia)
P2 032	Size and distribution of genets of five *Amanita* spp. native to Australia Sawyer, N.A., Chambers, S.M. and Cairney, J.W.G. (Australia)
P2 034	Impact of elevated CO_2 and nitrogen fertilization on fungal biomass of ectomycorrhizae of *Piloderma croceum* and *Tomentellopsis submollis* Raidl, S. and Agerer, R. (Germany)
P2 035	Genet size analysis of selected late-stage and multi-stage fungi Redecker, D., Szaro, T.M., Bowman, R., Tan, J. and Bruns, T.D. (Switzerland and USA)

WEDNESDAY, 11 JULY 2001

P2 036	Tracking the role of arbuscular mycorrhizal fungi in vegetation dynamics by molecular community analysis Kowalchuk, G.A., de Souza, F.A., van der Kaaij, R.M. and van der Putten, W.H. (The Netherlands)
P2 037	Establishment of mycorrhizas on *rolC*-transgenic Aspen in a field trial Buscot, F., Kaldorf, M., Fladung, M. and Muhs, H.-J. (Germany)
P2 038	Effect of fermentation of organic matter on changes in growth stimulatory and inhibitory substances for mycorrhizal fungi Ishii,T., Kitabayashi, H., Yachi, Y., Okuda, J., Yang, Y., Kirino, S. and Kadoya, K. (Japan)
P2 039	Ectomycorrhizal weathering of soil minerals Hoffland, E., Jongmans, T., Giesler, R. and van Breemen, N. (The Netherlands and Sweden)
P2 040	PIXE analysis to estimate the elemental composition of ectomycorrhizal rhizomorphs grown in contact with different minerals in forest soil Wallander, H., Johansson L. and Pallon, J. (Sweden)
P2 041	Importance of mycorrhizal symbiosis for tolerance to mowing in the grasses *Brachypodium pinnatum* and *Bromus erectus* Kahlert, B.R., Ryser, P. and Edwards, P. J. (Switzerland)
P2 042	Plant responses to earthworms and arbuscular mycorrhizas Scullion, J., Tuffen, F. and Eason W.R. (UK)
P2 043	Interactions of mycorrhizal symbiosis and herbivory in tallgrass prairie Wilson, G.W.T., Hartnett, D.C. and Todd, T.C. (USA)
P2 044	Chinese truffles (*Tuber*): Diversity and their geographical distribution Chen, Y.L., Dell, B. and Le Tacon, F. (China, Australia and France)
P2 045	Characteristics of hypogeous fungal colony of *Sarcodon aspratus* Kim, H.-E., Koo, C.-D., Park, J.-I., Kim, J.-S., Shin, W.-S. and Shin, C.-S. (South Korea)
P2 046	*Tricholoma matsutake*: a highly coveted ectomycorrhizal mushroom Guerin-Laguette, A., Vaario, L.M., Gill, W.M., Matsushita, N., Lapeyrie, F. and Suzuki, K. (France and Japan)
P2 047	A sampling technique for counting VAM fungal spores extracted from soil Wangiyana, W. and Cornish, P.S. (Australia)
P2 048	Effects of greenhouse environment on assessment of arbuscular mycorrhizal fungal diversity in trap cultures Stutz, J.C., and Morton, J.B. (USA)
P2 049	Ecology of the AM fungi in a star of Africa (*Cynodon pletostachyus*) preiri with different resting cycles Lara-Capistrán, L., Tablada-Aguilar, Y. and Trejo, D. (Mexico)
P2 050	What are the attributes of ectomycorrhizal fungi to be managed for future health of energy and nutrient flows in remnants of Australia's temperate tall tree woodlands? Tommerup, I.C. and Bougher, N.L. (Australia)

WEDNESDAY, 11 JULY 2001

Molecular diversity, systematics and detection of mycorrhizal fungi

P2 051	Diversity and species distribution in orchid-fungal symbioses McCormick, M.K., Whigham, D.F. and O'Neill, J.P. (USA)
P2 052	Characterization of ascomycetous fungi colonizing the liverwort *Cephaloziella exiliflora* in the Antarctic using direct PCR and cloning Jumpponen, A., Newsham, K.K. and Neises, D.J. (USA and UK)
P2 053	Diversity of fungi forming ericoid mycorrhiza in the Australian family Epacridaceae McLean, C.B., Collins, R.A., McDonald, N., Ramsperger, M. and Lawrie, A.C. (Australia)
P2 054	Mycorrhizal endophyte of medicinal plant *Homanotica aromatica* (*roxbo*) Schott (*Araceae*) Cuong, N.N., Long, H.H., Kieu, L.N., Ha, N.H., Nhung, V.T. and Thanh, L.T.M. (Vietnam)
P2 056	GINCO : Glomales *in vitro* collection Dalpé, Y. and Declerck S. (Canada and Belgium)
P2 057	Infrequent recombination in the supposedly asexual AM fungi Kuhn, G. and Sanders, I.R. (Switzerland)
P2 058	*Scutellospora castanea* spores as microbial communities Hijri, M., Redecker, D. and Sanders, I.R. (Switzerland)
P2 059	Community structure of AM fungi colonizing roots of rare and common *Pulsatilla* spp. in boreal forest and meadow habitats Öpik, M., Moora, M., Zobel, M. and Sen, R. (Estonia and Finland)
P2 060	Defoliation effects on the community structure of arbuscular mycorrhizal fungi based on 18S rDNA sequences Saito, K., Sato, S. and Sugawara, K. (Japan)
P2 061	Diversity of deeply branched arbuscular mycorrhizal fungi, *Archaeospora* spp., and their detection with PCR Sawaki, H., Kojima, T., Shinozaki, N. and Saito, M. (Japan)
P2 062	Molecular identification of arbuscular mycorrhizal fungi (Glomales) in roots from natural and agricultural settings Alie, I., Oehl, F., Ineichen, K., Mäder, P., Sieverding, E., Wiemken, A. and Redecker, D. (Switzerland and Germany)
P2 063	Detection and quantification of the arbuscular mycorrhizal (AM) fungi *Glomus mosseae* and *Gigaspora margarita* using species - specific PCR primers Antoniolli, Z.I., Schachtman, D.P., Ophel-Keller, K., Herdina and Smith, S.E. (Brazil and Australia)
P2 064	Polypeptides comprising a mycorrhizal specific esterase band on maize roots inoculated with four different AMF Sancayaningsih, R.P., Setiadi, Y., Artama, W.T., Hartiko, H., Moeljopawiro and Soedarsono, J. (Indonesia)
P2 065	Preliminary study on the arbuscular mycorrhizae of Namibia Uhlmann, E., Goerke, C., Strohbach, B and Oberwinkler F. (Germany and Namibia)

WEDNESDAY, 11 JULY 2001

P2 066	Fatty acids of total lipids from AM fungal spores and host roots can detect, identify and differentiate AM species Madan, R., Smith, S.E., Pankhurst, C.E. and Hawke, B. (Australia)
P2 067	*Glomus spinosum* sp. nov. in the Glomaceae from Taiwan and its mycorrhizal associations Hu, H.T. (Taiwan R.O.C.)
P2 068	Detection of introduced AM fungi in plant roots using nested PCR Zhao, B., Dong, X.L. and Zheng, S.X. (P.R. China)
P2 070	A novel method for extraction and purification of amplifiable DNA from ectomycorrhizas Koide, R.T. and Dickie, I.A. (USA)
P2 071	Characteristics of surface carbohydrates of hyphae in ectomycorrhizal fungi using fluorochrome labeled lectins and their diagnostic application to detect their ectomycorrhizas and external hyphae in soils Tateishi, T., Kohzu, A., Wada, E. and Marumoto, T. (Japan)
P2 074	Ectomycorrhizal species richness and composition in old growth mixed conifer and adjacent stands of Lodgepole pine (*Pinus contorta*) in Yellowstone National Park USA Parker, V.T., Douglas, R.B. and Cullings, K.W. (USA)
P2 075	Characterization of *Pinus* ectomycorrhizas from mixed conifer and pygmy forests using morphotyping and molecular methods Wurzburger, N., Bidartondo, M.I. and Bledsoe, C.S. (USA)
P2 076	Effect of soil moisture on mycorrhizal symbiosis in *Pinus sylvestris* L. nursery seedlings Hilszczaska, D. and Sierota, Z. (Poland)
P2 077	Molecular identification of ectomycorrhizae of *Hydnum albidum* Peck. on Austrian pine (*Pinus nigra* Arnold) Grebenc, T., Martin, M.P. and Kraigher H. (Slovenia and Spain)
P2 078	High species and intraspecific diversity in ectomycorrhizal fungi in *Eucalyptus* forest Glen, M., Bougher, N.L., Tommerup, I.C. and O' Brien, P.A. (Australia)
P2 079	Diversity of mycobionts of *Pisonia grandis* on coral cays in the Great Barrier Reef Chambers, S.M., Hitchcock, C.J. and Cairney, J.W.G. (Australia)
P2 080	Nitrogen addition changed macromycete sporocarp production and belowground ectomycorrhizal species composition in a Norway spruce stand Peter, M., Ayer, F. and Egli, S. (Switzerland)
P2 081	Community structure of ectomycorrhizal fungal sporocarps among successional forest age classes of *Pseudotsuga menziesii* Smith, J.E., Molina, R., Huso, M.M.P., Luoma, D.L., McKay, D., Castellano, M.A., Lebel, T. and Valachovic. Y. (USA and Australia)
P2 083	*Terfezia* species (desert truffles) : ITS and 25S rDNA analyses Kagan-Zur, V., Aviram, S., Ferdman, Y. and Roth-Bejerano, N. (Israel)
P2 084	Genet distribution of two related ectomycorrhizal fungal species: *Tricholoma scalpturatum* and *T. populinum* in a black poplar stand Gryta, H., Carré, F., Charcosset, J.Y. and Gardes, M. (France)

WEDNESDAY, 11 JULY 2001

P2 085	Population genetics of *Boletus edulis* in Western North America: genets and gene flow Camacho, F.J. and Bruns, T.D.(USA)
P2 086	A PCR-based method for identifying genets (=genetic individuals) of ectomycorrhizal fungi on ectomycorrhizal root tips Diéz, J. and le Tacon, F. (France)
P2 087	Spatial distribution of the subterranean mycelia and ectomycorrhizae of *Suillus grevillei* genets Zhou, Z., Miwa, M., Matsuda, Y. and Hogetsu, T. (Japan)
P2 088	*Pisolithus* : a worldwide ectomycorrhizal partner Ducousso, M., Prin, Y. and Thoen, D. (France and Belgium)
P2 089	Species and world wide evolutionary lineages within the ectomycorrhizal fungus *Pisolithus* Dell, B, Diez, J., Delaruelle, C. and Martin, F. (Australia and France)
P2 090	Genetic diversity in *Tuber uncinatum* in plantations and woodlands of Northeastern France Luis, P., Delaruelle, C., Dupré, C., Chevalier, G. and Martin F. (France)
P2 091	Morphological and molecular characterisation of the mycelium of different *Tuber* species in pure cultures Iotti, M., Amicucci, A., Stocchi, V. and Zambonelli, A. (Italy)
P2 092	Taxonomical re-evaluation of the mycorrhizal fungus "Matsutake-modoki" (*Tricholoma robustum* s. auct.) in Japan. Iwase, K. and Tanaka, C. (Japan)
P2 093	Molecular analyses of inter- and infrageneric relationships within the agaricoid Russulaceae (Basidiomycetes): correspondence with mycorrhizal anatomy Eberhardt, U. (Germany)
P2 094	Anatomical and molecular characterization of *Ramaria* ectomycorrhizae Nouhra, E, Horton, T., Cazares, E. and Castellano, M. (USA)
P2 095	Molecular phylogenetics of *Gomphus* and related genera inferred from nuclear large and mitochondrial small subunits ribosomal DNA sequences Giachini, A.J., Spatafora, J.W., Cazares, E. and Trappe, J.M. (USA)
P2 096	Identification of the ectomycorrhizae of *Helvella leucomelaena* on *Pinus sylvestris* confirmed by the analysis of the ITS sequence Urban, A. (Austria)

WEDNESDAY, 11 JULY 2001

CONCURRENT SESSION 6
MYCORRHIZAS IN AGRICULTURE AND HORTICULTURE

Chairs: Dr. M. Ryan and Mr. T. Cavagnaro (Australia)
Location: Hall B

2:30	**C6 01** Growers evaluate nursery and early field performance of 15 plant species inoculated with mycorrhizal and trichodermal fungi Hunter, M., Healy, C., Healy, B., Heaton, P., Hickey, M., Hockings, D., Kebblewhite, A., Macintyre, D. and Young, P. (Australia)
2:50	**C6 02** Response of Arabica and Robusta coffee to *Gigaspora margarita* and carbofuran application in parasitic nematode infested land Baon, J.B. and Wiryadiputra, S. (Indonesia)
3:10	**C6 03** Predicting edible mushroom productivity using forest carbon allocation modeling and immunoassays of ectomycorrhizae Pilz, D., Molina, R., Danell, E., Rose, C. and Waring, R. (Sweden and USA)
3:30	**C6 04** Legume trees in symbiosis with abuscular mycorrhizal fungi Gardezi, A.K., Cetina-Alcalá, V.M., Ferrera-Cerrato, R. and Larqué-Saavedra, M.U. (Mexico)
3:50	Afternoon Tea Location: Hall A
4:20	**C6 05** Mycorrhizal nutrient loading: a new cultural technique for forest planting stock production Quoreshi, A.M., Timmer, V.R. and Koike, T. (Japan and Canada)
4:40	**C6 06** Effect of rotary tillage versus no-till on the interaction between indigenous mycorrhizal fungi, *Bradyrhizobium japonicum* and soybean under field conditions Antunes, P. and Goss, M.J. (Canada)
5:00	**C6 07** The influence of different arbuscular mycorrhizal fungi on root proliferation in, and nitrogen capture from, organic patches in soil Hodge, A. (United Kingdom)
5:20	**C6 08** Role of VAM fungi in growth and nutrient uptake of wheat and field peas in the southern wheatbelt of Australia Ryan, M.H. and Angus, J.F. (Australia)

WEDNESDAY, 11 JULY 2001

CONCURRENT SESSION 7
PLANT FUNGUS INTERACTIONS

Chairs: Dr. S. Dickson and Ms. R. Kasiamdari (Australia)
Location: Hall C

2:30 **C7 01**
Evidence for increased tolerance to phytoplasma disease in arbuscular mycorrhizal tomato plants
Berta, G., Lingua, G., Massa, N., Antosiano M. and D'Agostino, G. (Italy)

2:50 **C7 02**
Nitrogen fixation and tuberculate ectomycorrhiza?
Paul, L., Chapman, B. and Chanway, C. (Canada)

3:10 **C7 03**
Reorganization of tobacco root plastids during establishment of the arbuscular mycorrhizal symbiosis
Fester, T., Strack, D and Hause, B (Germany)

3:30 **C7 04**
Tubular vacuoles observed in *Gigaspora margarita* hyphae using laser scanning confocal microscopy
Uetake, Y., Kojima, T., Ezawa, T. and Saito, M. (Japan)

3:50 Afternoon Tea
Location: Hall A

4:20 **C7 05**
Is the interfacial pH a value for the efficiency of nutrient transfer in ectomycorrhizas?
Nenninger, A., Griffith, G.W. and Heyser W. (Wales and Germany)

4:40 **C7 06**
Physiological markers target key genes in arbuscular mycorrhizal clover
Kelly, R., Teodosio, R., Eason, W., Hooker, J.E. and Webb, K.J. (UK)

5:00 **C7 07**
Transcript profiling during ectomycorrhiza development
Johannson, T., Ahrén, D., Le Quéré, A., Söderström, B., Erlandsson, R., Lundeberg, J., Uhlén, M. and Tunlid, A. (Sweden)

5:20 **C7 08**
The use of proteomics to elucidate cellular changes during development of an AM symbiosis between *Medicago truncatula* and *Glomus versiforme*
Mussa, H.J., Sumner, L.W. and Harrison, M.J. (USA)

WEDNESDAY, 11 JULY 2001

7:30 **WORKSHOPS**

Location: Napier and Lower Napier Theatres, Adelaide University, North Terrace (Adjacent to Bonython Hall)

B: Mycorrhiza-associated bacteria
Organisers: Dr J. Garbaye (France) and Dr S. Timonen (Finland)

E: Proteomics
Organiser: Dr E. Dumas-Gaudot (France)

F: Stable isotopes and fungal ecology
Organisers: Dr P. Högberg and Dr A. Taylor (Sweden)

G: Population and evolutionary genetics of AMF
Organisers: Professor S. Rosendahl (Denmark) and Professor I. Sanders (Switzerland)

THURSDAY, 12 JULY 2001

Note: The first author is the presenting author in most cases.
Underlining indicates presenting author if not first listed author

KEYNOTE SESSION 5

Chair: Professor A. Fitter (UK)
Location: Hall B

8:45 **K 5**
Ecophysiology of mycorrhizal plants
Professor G. Stewart, University of Western Australia

SYMPOSIUM 4
MYCORRHIZAL ECOLOGY

Chairs: Dr. M. van der Heijden (The Netherlands), Dr. B. Dell (Australia) and Dr. I. Sanders (Switzerland)
Location: Hall B

9:30 **S4 01**
Ecological dynamics within the plant-AM fungal mutualism
Bever, J.D. (USA)

10:00 **S4 02**
Ecological role of hypogeous ectomycorrhizal fungi in Australian vegetation
Claridge, A.W. (Australia)

10:30 Morning Tea
Location: Hall A

11:00 **S4 03**
Arbuscular mycorrhizal fungi influence soil structure
Miller, R.M. and Jastrow, J.D. (USA)

11:30 **S4 04**
Fungal diversity in ectomycorrhizal communities
Taylor, A.F.S. (Sweden)

12:00 **S4 05**
Mycorrhizal fungi as a determinant of biological diversity and ecosystem functioning
van der Heijden, M. (The Netherlands)

12.30 LUNCH BREAK

THURSDAY, 12 JULY 2001

POSTER SESSION 2 (CONTINUED)

Location: Hall A

Diversity in mycorrhizal structure and function

P2 102	The status of arbuscular mycorrhizal fungi in soil: a potential indicator of the sustainability of farming systems Abbott, L.K., Graham, J., Edwards, L., Djuuna, I. and Delaney, J. (Australia)
P2 104	Effects of mycorrhizal colonization on growth and phosphorus uptake by *Trifolium alexanderium* as affected by the size of soil aggregates Nadian, H. (Iran)
P2 105	Growth and function of AM external hyphae in sands of varying pore sizes Drew, E.A., Jakobsen, I., Murray, R.S. and Smith, S.E. (Australia and Denmark)
P2 106	The ectomycorrhizal fungi of four North American forest ecosystems: a cross-site comparison of EM diversity and community structure and the effects of nitrogen fertilization Allen, M.F. and Lansing, J.L. (USA)
P2 107	Models of nutrient partitioning by fungal-plant interactions in arbuscular mycorrhizal ontogeny Graves, D.W., Long, P.G., Macgregor, A.N., Outred, H.A. and Jameson, P.E. (New Zealand)
P2 109	Biodiversity of arbuscular mycorrhizal fungi from rhizosphere of *Macaranga denticulata* (Bl.) Mull. Arg. in Mae Hong Son province, Thailand Youpensuk, S., Lumyong, S., Rerkasem, B. and Dell, B. (Thailand and Australia)
P2 110	Biodiversity of mycorrhizae on *Quercus garryana* in Southern Oregon Southworth, D., Valentine, L.L., Fiedler, T.L., Haney, S.R. and Berninghausen, H.K. (USA)
P2 111	Structural diversity of arbuscular mycorrhizal fungi in a disturbed grassland Renker, C., Kaldorf, M. and Buscot, F. (Germany)
P2 112	High species richness and dominance of *Cortinarius* taxa in belowground communities of ectomycorrhizal fungi from the Canadian Arctic Dahlberg, A., Gardes, M. and Charcassot, J.Y. (Sweden and France)
P2 113	*Pterospora andromedea*: Morphological and molecular assessment of mycorrhizae from plants growing in a sub-boreal forest ecosystem in central British Columbia Catherall, J.M., Massicotte, H.B., Young, B.W., Tackaberry, L.E. and Egger, K.N. (Canada)
P2 114	Ectomycorrhizae between *Gyrodon monticola* Singer and *Cortinarius* sp. and *Alnus acuminata* H.B.K. (Argentina) characterized by anatomical and molecular tools Becerra, A.G., Daniele, G.M., Dominguez, L.S., Nouhra, E.R. and Horton, T.R. (Argentina and USA)

THURSDAY, 12 JULY 2001

P2 115	Characterization of growth, nutritional and arbuscular mycorrhizal colonization behaviours of *in vitro* tomato hairy roots clones Labour, K., St-Arnaud, M., Jolicoeur, M. (Canada)
P2 116	*Lactarius deliciosus* culture conditions and *in vitro* mycorrhization of *Pinus pinaster* plants raised from seeds Manjón, J.L., Bernedo, M., Pérez, M., Santamaria, C., Camacho, M., Aguilar, A., Romero, L. and Daza, A. (Spain)
P2 117	Influence of temperature and light on the functioning of arbuscular mycorrhizal (AM) and on the extraradical hyphae growth: implications of climate change Heinemeyer, A. and Fitter, A.H. (UK)
P2 118	Some ultrastructural aspects of the *Anadenanthera falcata* Speg. mycorrhizae Gross, E., Cordeiro L. and Caetano F.H. (Brazil)
P2 119	Formation of cross walls in trunk hyphae of AM fungal arbuscules and their relationship to metabolic activity Dickson, S. and Smith, S.E. (Australia)
P2 120	Tomato: a model plant to unravel the functional implications of AM structural diversity Cavagnaro, T.R. and Smith, F.A. (Australia)
P2 121	Do arbuscular mycorrhizal fungi determine the *Arum*- or the *Paris*-type morphologies seen in different host roots? Kubota, M., Hyakumachi, M. and McGonigle, T.P. (Japan)
P2 122	*Asphodelus fistulosus* and *Glomus coronatum*: physiological studies of a *Paris*-type AM Smith, F.A., Cavagnaro, T.R., Ayling, S.M. and Smith, S.E. (Australia)
P2 123	The interface between the arbuscular mycorrhizal fungus *Glomus intraradices* and root cells of ginseng: a *Paris*-type mycorrhizal association Peterson, R.L. and Armstrong, L. (Canada)
P2 124	Characterization of the ectomycorrhizal (*Paxillus involutus*) plant/fungus interface Rouhier, H. and Söderström, B. (Sweden)
P2 125	Visualisation of ectomycorrhizal rhizomorph structure using laser scanning confocal microscopy Schweiger, P., Rouhier, H. and Söderström, B. (Austria and Sweden)
P2 126	The morphology and anatomy of two mycoheterotrophic species in central British Columbia: Fungal specificity north of 50! Massicotte, H.B., Tackaberry, L.E., Melville, L.H. and Peterson, R.L. (Canada)
P2 127	Mycorrhizas of the Ladyslipper Orchids (*Cypripedium*), Orchidaceae Melville, L., Zelmer, C.D. and Peterson, R.L. (Canada)
P2 128	Morphological classifcation of Monotoropoid mycorrhizas on *Monotropastrum globosum* Matsuda, Y. and Yamada, A. (Japan)
P2 129	A mycorrhiza-defective mutant tomato (*Lycopersicon esculentum Mill.*) forms functional mycorrhizas with *Glomus versiforme* Poulsen, K.H., Gao, L.-L., Smith, S.E. and Smith, F.A. (Denmark and Australia)

P2 131	Macro (N, P, K, Ca and Mg) and micronutrient (Fe, Mn, Cu and Zn) content of mycorrhizal and nonmycorrhizal micropropagated *Castanea sativa* Mill. plants along three months of *in vitro* growth Martins, A., Baptista, P., Sousa, M.J. and Pais, M.S. (Portugal)
P2 132	Nitrogen transfer between Australian native *Eucalyptus* and *Casuarina*: ^{15}N labelling experiments He, X., Stewart, G., Ng, H. and Critchley, C. (Australia)
P2 134	Nitrogen assimilation in a mycorrhizal *in vitro* culture system Toussaint, J-P., St-Arnaud, M. and Charest, C, (Canada)
P2 135	Genetic variation in growth and nutrient uptake of mycorrhizal and non-mycorrhizal *Picea abies* seedlings Mari, S., Jonsson, A., Eriksson, G. and Finlay, R. (Sweden)
P2 136	Nitrate reductase regulation in the ectomycorrhizal fungus *Tuber borchii* Vittad. cultured on different nitrogen sources Guescini, M., Vallorani, L., Pierleoni, R., Sacconi, C., Zeppa, S., Palma, F., Amicucci, A. and Stocchi, V. (Italy)
P2 137	Carbon uptake during the symbiotic, asymbiotic and presymbiotic stages of the AM fungus *Glomus intraradices* Séjalon-Delmas, N., Rogister, I., Jauneau, A., Roux, C. and Bécard, G. (France)
P2 138	A holistic view of chanterelle carbon transfer Rangel-Castro, J.I. and Danell, E. (Sweden)
P2 139	Respiratory metabolism of rhizosphere-dissolved inorganic carbon in arbuscular mycorrhizal roots under P starvation Valentine, A.J. and Cramer, M.D. (South Africa)
P2 140	A laboratory study of ectomycorrhizal fungi and below ground carbon transfer: problems and constraints Philip, L.J. and Jones, M. (Canada)
P2 141	Growth depression of mycorrhizal *Citrus* seedlings grown at high P supply is mitigated by elevated CO_2 Graham, J.H., Jifon, J.L., Drouillard, D.L. and Syvertsen, J.P. (USA)
P2 142	Phosphorus uptake by a community of arbuscular mycorrhizal fungi from the jarrah forest Solaiman, M.Z., Abbott, L.K., Jakobsen, I. and Lambers, H. (Australia and Denmark)
P2 143	AM-P Baylis, G.T.S. (New Zealand)
P2 144	Possible involvement of hyphal phosphatase in phosphate efflux from intraradical hyphae isolated from mycorrhizal roots colonized by *Gigaspora margarita* Kojima, T and Saito, M. (Japan)
P2 145	Phosphor imaging as a tool for non-invasive, real time studies of nutrient transport in arbuscular mycorrhizas Jakobsen, I., Nielsen, J.S., Joner, E.J. and Declerck, S. (Denmark)
P2 146	Phosphate pools and metabolism in arbuscular mycorrhizal fungi studied by ^{31}P NMR spectroscopy Rasmussen, N., Hansen, P.E. and Jakobsen, I. (Denmark)

P2 147	Influence of early stages of arbuscular mycorrhiza on uptake of zinc and phosphorus by red clover from a low-phosphorus soil amended with zinc and phosphorus Bi, Y. and Christie, P (P. R. China and UK)
P2 148	Studing Mg-transport across and via mycorrhiza of Norway spruce roots Kuhn, A.J., Jentschke, G., Stettien, A., Brandes, B., Godbold, D.G. and Schröder, W.H. (Germany and UK)
P2 149	Circadian cycles of water transfer between host plant and mycorrhizal extraradical mycelium Querejeta, I., Egerton-Warburton, L. and Allen, M.F. (USA)
P2 150	Effect of two species of arbuscular mycorrhizal fungi on growth, assimilation and leaf water relations in maize (*Zea mays* L.) Amerian, M.R., Stewart, W.S. and Griffiths, H. (UK)
P2 151	Characterization and histochemical localization of *Terfezia claveryi* tyrosinase Morte, A., Pérez-Gilabert, M., Honrubia, M., García-Carmona, F. (Spain)
P2 152	Monophenolase activity of *Tefezia claveryi* tyrosinase Pérez-Gilabert, M., Morte, A., Honrubia, M. and García-Carmona, F. (Spain)
P2 153	Electrophysiology of vesicular arbuscular mycorrhizal fungi Ayling, S.M., Edmonds, T., Smith, S.E. and Smith F.A. (Australia)

Mycorrhizas in tropical environments

P2 161	Effects of arbuscular mycorrhizal fungi on damage by *Striga hermonthica* on sorghum, *Sorghum bicolor* Kuyper, T.W. and Lendzemo, V.W. (The Netherlands and Cameroon)
P2 162	Changes in rhizopheric biological dynamics of *Trachypogon plumosus* Ness affected by phosphate fertilization in three acid savanna soils López-Gutiérrez, J.C., Toro, M. and López-Hernández, D. (Venezuela)
P2 163	Correlation of VA colonization with *Cercospora coffeicola* infection in leaves of coffee seedlings Lebrón, L., Lodge, D.J. and Rodríguez, R.P. (USA)
P2 164	Interactions of arbuscular-mycorrhizal fungi and wetland plant species in the everglades ecosystem Jayachandran, K., Kemp, S. and Jones, R.D. (USA)
P2 165	Host and environment controls on arbuscular mycorrhizal communities in a moist tropical forest Lovelock, C.E and Morton, J. (USA)
P2 166	Arbuscular micorrizal fungi in rain forest fragments in Sergipe, Northeast of Brazil Landim, M.F. and Heyser, W. (Germany and Brazil)
P2 167	Do native trees restore mycorrhizal communities to eroded tropical pasture? Carpenter, F.L., Palacios, S. and Gonzalez, E. (USA and Mexico)
P2 168	Ectomycorrhizal fungi associated with eucalypts in Africa and Madagascar Thoen, D., Abourouh, M. and Ducousso, M. (Belgium, Morocco and France)
P2 169	Ectomycorrhizal mushrooms from the Guatemala highlands Flores, R., Bran, M.C. and Honrubia, M. (Spain)

P2 170	Is incorporation into an ectomycorrhizal hyphal network important for the growth of dipterocarp seedlings? Brearley, F.Q., Press, M.C. and Scholes, J.D. (UK)
P2 171	Competition for nutrients between *Acacia mangium* and *Hopea odorata* Strömmer, R., Jokinen, H. and Lee, S.A. (Finland)
P2 172	Arbuscular mycorrhizal fungi under teak clones seed orchard Corry, Maryadi, and Irmawati, (Indonesia)

THURSDAY, 12 JULY 2001

CONCURRENT SESSION 8
MYCORRHIZOSPHERE

Chair: Dr. S. Timonen (Finland)
Location: Hall B

2:30 **C8 01**
AM fungal colonisation of sand compartments adjusted to different pH values
van Aarle, I.M., Olsson, P.A. and Söderström, B. (Sweden)

2:50 **C8 02**
Mycelial carbon allocation and weathering of minerals by ectomycorrhizal fungi
Rosling, A. and Finlay, R. (Sweden)

3:10 **C8 03**
Characterisation of bacterial communities associated with the hyphosphere and hyphoplane of *Glomus intraradices*
Mansfeld, K., Bødker, L. and Larsen, J. (Denmark)

3:30 **C8 04**
Comparison of antagonistic potential of rhizobacteria from mycorrhizosphere and rhizosphere soils against soilborne fungal pathogens
Linderman, R.G., Marlow, J.L. and Davis, E.A. (USA)

CONCURRENT SESSION 9
MYCORRHIZAS IN VEGETATION RESTORATION

Chair: Dr. D. Jasper (Australia)
Location: Hall C

2:30 **C9 01**
AM infectivity and spore status along chemico-physico and rehabilitation gradients in slimes dams of deep level gold mines in South Africa
Straker, C.J., Freeman, A.J., Witkowski, E.T.F. and Weiersbye, I.M. (South Africa)

2:50 **C9 02**
Role of arbuscular mycorrhiza in alleviation of zinc toxicity and mineral nutrition of host plants on zinc-contaminated soils .
Li, X.L., Christie, P. and Chen, B.D. (China and UK)

3:10 **C9 03**
Ectomycorrhizal protection of *Pinus sylvestris* L. against Cu toxicity
Colpaert, J.V., Van Tichelen, K.K. and Vangronsveld, J. (Belgium)

3:30 **C9 04**
Contribution of arbuscular mycorrhizal fungi to phytoremediation of polluted soils
Leyval, C., Binet, P. and Joner, E. (France)

3.50 Afternoon Tea
Location: Hall A

THURSDAY, 12 JULY 2001

CLOSING SESSION

4:10 **SCIENTIFIC OVERVIEW**

Chair: Professor F.A. Smith (Australia)
Location: Hall B

Professor D.J. Read (UK)

4:40 **BUSINESS SESSION**

Chair: Professor M. Allen (USA)

5:30 CLOSE

6:30 **COACHES DEPART FOR DINNER**

ABSTRACTS

■

Monday 9 July

K1

Challenges for mycorrhizal research into the new millennium

B. Söderström, Department of Microbial Ecology, Lund University, Sweden

A brief look 100 yrs backwards clearly demonstrates that the general development in our understanding of the mycorrhizal systems has been immense. Readiness for developing and applying new techniques is identified as a major driving force in the advancement of biological sciences. Comparisons in this respect with other biological research fields will be followed by a discussion of possible strengths and weaknesses of mycorrhizal research today. It is suggested that mycorrhizal researcher will adopt more techniques from other related research areas. For the future development of mycorrhizal research, the Kyoto Protocol to the UN's Framework Convention on Climate Change could be an important basis, since mycorrhizas sit as switching regulators between fixation and release of atmospheric carbon dioxide. Thus, by adopting a more holistic view, mycorrhizal researches may establish a stronger position in the research related to questions about sustainability. On the other hand, the inevitable very strong influence on mycorrhizal research of the so called new biology, may generate a more reductionistic approach. It will, however, be essential for many more laboratories than today to take full advantage of the molecular techniques available and the enormous mass of information, easily and openly accessible via the web, which has been produced within genome sequencing programs as well as all data available as indirect effects of these programs. A challenge will be to formulate the proper and relevant questions, which answers' will still have a strong impact on the understanding on the functioning of the mycorrhizal symbioses in natural environments.

K2

Molecular and functional diversity in soil fungi

J.I. Prosser The University of Aberdeen, Scotland

Traditional approaches to the study of microbial diversity have relied on laboratory cultivation of isolates from natural environments and identification by classical techniques, including analysis of morphology, physiological characteristics and biochemical properties. These approaches provide information on fine-scale diversity but suffer bias resulting from the media and cultivation conditions employed and from the inability to grow and isolate significant proportions of natural communities in the laboratory. An alternative approach is the amplification of ribosomal RNA and functional genes from nucleic acid extracted directly from environmental samples, with subsequent analysis by 'fingerprinting' methods or by sequencing and phylogenetic analysis. This approach avoids the need for laboratory cultivation and has already provided major insights into species and functional diversity of soil bacterial and archaeal populations. For example, considerable diversity in total populations and in specific groups has been characterised and quantified, the influence of environmental factors has been assessed and novel groups, with no known cultured representatives, have been detected at high relative abundance. The application of such molecular techniques to the analysis of species and functional diversity of fungal populations is relatively new and the potential benefits and pitfalls will be considered.

S1 01

Molecular detection, community structure and phylogeny of ericoid mycorrhizal fungi

S. M. Berch[1], T.R. Allen[2], and M. L. Berbee[2]. [1]British Columbia Ministry of Forests Research Branch, Canada [2]University of British Columbia, Canada

To understand the community structure of ericoid mycorrhizal fungi in the roots of the ericaceous plant salal (*Gaultheria shallon*), we have cultured hundreds of fungal isolates from thousands of mycorrhizal root pieces. Almost all were ascomycetes. More than half of the fungi in culture form spores, including *Scytalidium vaccinii* and *Oidiodendron maius*. However, many isolates do not sporulate and we characterized these by RFLP and sequence analysis. Of these, many are discomycetes related to the ericoid mycorrhizal fungus *Hymenoscyphus ericae*. We mapped the fine-scale distribution of the fungi cultured from roots and found that in most cases the same fungus did not grow out of adjacent 2mm root segments. However, not all ericoid root endophytic fungi are culturable. We showed that culturing fungi from roots and cloning PCR amplified DNA from the same roots revealed two different communities of fungi. We extracted total DNA from mycorrhizal roots and used PCR amplification, cloning and sequencing to identify all the fungal species that had contributed their DNA to the mixture. Most of the fungal DNAs from the mycorrhizae were from basidiomycetes. The basidiomycetes that predominated in the DNA had never been detected among the hundreds of fungal cultures from mycorrhizae. We have also accessed the 200+ sequences in GenBank of ericoid and epacrid mycorrhizal fungi and root endophytes from a number of research groups and carried out phylogenetic analyses that will be discussed.

S1 02

Molecular approaches to ectomycorrhizal diversity studies

T.R. Horton SUNY College of Environmental Science and Forestry.

In many ectomycorrhizal (EM) community studies, genetic data from multiple samples of an EM morphotype collected from various locations are compared to genetic data from a single sporocarp of each species for identification purposes. The mismatch between above and below ground species occurrence commonly observed may be influenced by these different sampling efforts. To address this, I revisit the question of intraspecific variation in the ITS region first investigated in Karén et al. (1997), but at a spatial scale in which intraspecific variation is expected to be low. Sporocarps were collected across a 7 km region of the Oregon Dunes National Recreation Area in western North America. ITS-RFLP data are presented for 3-18 sporocarps from each of 44 EM species in 18 genera. Consistent RFLP patterns were observed in 84% of the species. An additional 9% yielded two RFLP types with one rare type observed among common ones. On the other hand, multiple frequent RFLP types were observed in *Lactarius deliciosus* and *Tricholoma flavovirens*, and sequence analysis of the ITS region suggests the presence of cryptic species. A phylogenetic analysis of ITS sequence data revealed 2 well supported clades among collections of *Tricholoma flavovirens*. Although a low degree of intraspecific variation and apparent cryptic species were evident in a few cases, these results suggest that RFLP data from a single sporocarp were adequate for identifying most of the species at this scale. However, to identify the family or species group of unknown RFLP types, additional analyses of sequence data (ITS or other loci) is highly recommended.

S1 03

Molecular identification and phylogeny of arbuscular mycorrhizal fungi

D. Redecker
Institute of Botany, University of Basel, Switzerland

The fossil record and molecular data show that the evolutionary history of arbuscular mycorrhizal fungi (Glomales) goes back at least to the Ordovician (460 million years ago), coinciding with the colonization of the terrestrial environment by the first land plants. At that time the land flora only consisted of plants on the bryophytic level.

Ribosomal DNA sequences also indicate that the diversity within the Glomales on the family and genus level is much higher than previously expected from morphology-based taxonomy. Two deeply divergent lineages were found and described in two new genera, *Archaeospora* and *Paraglomus*, each in its own family.

Based on the fast-growing number of available DNA sequences, several systems for molecular identification of the Glomales within roots have been designed and tested in the past few years. These detection methods have opened up entirely new perspectives for studying the ecology of arbuscular mycorrhiza.

C1 03

A natural taxonomy of AM fungi (Glomales) based on the SSU rRNA-gene

D. Schwarzott[1], C. Walker[2], A. Schüßler[1]
[1]Darmstadt University of Technology, Germany [2] Bournemouth University, United Kingdom

One of the most widely distributed, ecologically and economically most important fungal groups is represented by the order Glomales, currently placed in the polyphyletic phylum Zygomycota. To obtain an clearer understanding of the phylogeny of the arbuscular mycorrhiza (AM) fungi, we sequenced the SSU rRNA-gene of 70 glomalean isolates belonging to 51 described and some undescribed species. For phylogenetic analyses, we included about 100 zygomycotan as well as a set of basidiomycotan and ascomycotan sequences available from the databases.

The results show that the Glomales do not belong to the Zygomycota and can be unequivocally separated from all other major fungal taxa. They form a monophyletic clade, which probably diverged from the same common ancestor as the Ascomycota and Basidiomycota. To give these fungi their proper status, we propose a new phylum, the Symbiomycota.

Besides the position of the Glomales in the Kingdom of Fungi, new conclusions about the taxonomic grouping concerning the classic order and family concept can be drawn from our study. For example, the *Glomaceae* as presently defined are polyphyletic with genetic distances between the three main clades as large as those between the families *Acaulosporaceae* and *Gigasporaceae*.

We propose a new order and family concept as a basis for a new and natural systematic taxonomy of AM fungi.

C1 04

- ergosterol d'ici meilleure dispo[illegible]
le p [illegible]

The ectomycorrhizal fungal community in soil as identified from soil DNA extracts

Renske Landeweert[1], Eric Smit[2] and Thomas W. Kuyper[1] *[1]Wageningen University, The Netherlands [2]RIVM, The Netherlands*

Many effects of ectomycorrhizal fungi on plants depend on the ability of the hyphae to colonize the soil effectively. The occurrence, quantity and activity of ectomycorrhizal mycelium in soil may be valuable indicators of the ecological relevance of ectomycorrhizal fungal species. It is therefore essential to identify and quantify extraradical mycelium of individual fungal species in soil. For this purpose novel molecular techniques are being developed which involve direct DNA extraction from soil. Subsequently, amplified basidiomycete DNA is analysed on a DGGE gel, visualizing the ectomycorrhizal fungal community diversity. Bands representing certain fungal species are sequenced and identified by comparing them to a database. The ectomycorrhizal fungal diversity in a Swedish podzol profile was studied by this new molecular approach and the results were compared to diversity data obtained from morphotyped ectomycorrhizal root tips collected from the same sites (in collaboration with Anna Rosling, SLU, Sweden). In order to compare molecular quantification with other techniques used for quantification of mycelium in soil, a pot experiment was performed. *Pinus sylvestris* seedlings were inoculated with one or two ectomycorrhizal fungi. After 30, 60 and 90 days of growth the quantity of ectomycorrhizal mycelium in the soil was analysed using novel molecular techniques, hyphal staining and counting and PLFA and ergosterol analyses (in collaboration with Hannu Fritze, Vantaa Research Centre, Finland and Håkan Wallander, Lund University, Sweden). Results will be presented that put these novel molecular techniques into perspective with conventional identification and quantification techniques.

C1 05

Application of PCR-Denaturing Gradient Gel Electrophoresis (DGGE) in taxonomy, rDNA characterization and community analysis of Glomales (Zygomycetes)*

F.A. de Souza[1, 2], P. Leeflang[3], E. Smit[3], J.D van Elsas[4] and G.A. Kowalchuk[2]. [1]Embrapa Agrobiologia (CNPAB), Brazil. [2]CTO-NIOO, [3]RIVM, [4]PRI, The Netherlands.

The characterization of AMF species and its detection in field conditions are essential steps to understand the AMF symbiosis. The use of molecular based techniques such as PCR-DGGE offers great possibilities to detect, identify and characterize these symbiotrophic fungi. The aim of this study was to develop and test different PCR-DGGE approaches for identification and characterization of AMF species and to study AMF community structure in field conditions. Applying this technique, discrimination among closely related *Gigaspora* species and other AMF was possible. A PCR-DGGE system to detect fungal contamination in AMF spores was developed and tested using spores of *Scutellospora castanea* and *Gigaspora margarita*. In both cases, distinction between uncontaminated and contaminated spores was clear. An approach to assess the rDNA heterogeneity in Glomales was tested using geographically distinct isolates of *Glomus clarum*. Amplicons generated using the primers NS1 x ITS4 were cloned and screened by applying PCR-DGGE in two variable regions of the 18S rDNA. Selected clones were sequenced and phylogenetically analyzed. This novel approach provided a robust means to characterize rDNA heterogeneity in Glomales. Finally its application to community analysis was performed using samples collected in a Dutch farm, where AMF diversity in an arable land was compared to the adjacent species-rich grassland. The diversity obtained by direct PCR-DGGE after sequencing excised bands was compared with those obtained by trap culture assays. Both techniques detected lower diversity in the arable land than in the grassland. However the PCR-DGGE provided a faster way to assess changes in AMF community structure.

*This research has being supported by CNPq (Research council of Brazil).

C1 06

Changes in community structure of AM fungi due to reduced tillage

J. Jansa[1], A. Mozafar[1], R. Ruh[1], T. Anken[2], G. Kuhn[3], I. Sanders[3] E. Frossard[1]. [1] Federal Institute of Technology Zürich, Switzerland, [2] Federal Research Station for Agricultural Economics and Engineering, Tänikon, Switzerland, [3]University of Lausanne, Switzerland.

Arbuscular mycorrhizal fungal (AMF) communities from a long-term tillage experiment were studied in order to see if this management treatment had an effect on AMF community structure. AMF were grown in trap cultures with different plant species (soybean, sunflower, leeks, maize, *Plantago*) and monosporic cultures were subsequently established. Identification of fungal spores was carried out by microscopy combined with isoenzyme and ITS-rDNA sequencing approaches. Non-*Glomus* fungi (e.g. *Gigaspora*, *Scutellospora* and *Entrophospora*) were more prevalent in reduced tillage treatments. We developed specific DNA markers (targeted to 25S rDNA) to identify AM fungi in the plant roots. A study of AMF colonizing field-grown maize confirmed previous findings of presence of *Scutellospora* and *Gigaspora* in reduced tillage treatments, while *Glomus* spp. was more prevalent in intensively tilled soils. High incidence of non-*Glomus* fungi is probably related to importance of undisturbed mycelium net for survival of these fungi. Data showing presence of *Gigaspora margarita* at the field sites is the first confirmed evidence of this genus in European soils. Sequencing of DNA fragments obtained by PCR amplification using AMF species-specific primers revealed specificity of our markers for closely related groups of fungi (specific primers for *G. caledonium* amplified for example also *G. mosseae* and *G. fragilistratum*). Finer identification was possible using SSCP (single strand conformation polymorhism) separation of DNA fragments.

C1 07

Molecular diversity, phylogenetic affinities and mycorrhizal synthesis of root-associated ascomycetes of Helotiales in northern boreal plant communities.

T. Vrålstad[1], E. Myhre[1], A. F. S. Taylor[2] and T. Schumacher[1]. [1]University of Oslo, Dept. of Biology, P.O. Box 1045, Blindern, N-0316 Oslo, Norway. [2]Dept. of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, S-750 07 Uppsala, Sweden.

Nuclear ITS1-5.8S-ITS2 (ITS) rDNA sequence diversity and phylogenetic affinities of root-associated ectomycorrhizal (ECM), ericoid mycorrhizal (ERM) and non-mycorrhizal ascomycetes were studied. Fungi obtained from the *Piceirhiza bicolorata* ECM-morphotype of conifers and hardwoods, and from fine-roots of ericoid plants and grasses, were sequenced. A total of 132 root-derived ITS-sequences showed affinity to families of Helotiales, i.e. Helotiaceae, Dermataceae and Hyaloscyphaceae, and 75% of these grouped within the *Hymenoscyphus ericae* aggregate (Helotiaceae). This aggregate, which include *H. ericae* and *Phialophora finlandia,* is not con-generic with *Hymenoscyphus fructigenus*, the genus type species. An inferred phylogeny divided the *H. ericae* aggregate into four major clades (clade 1-4), three of which comprised sequences of both ECM- and ERM-root origin. The majority of ECM-root sequences grouped with *P. finlandia* (clade 4), but 9 ECM-root sequences shared 97.8-99.1% sequence identity with *H. ericae* of clade 3. ECM- and ERM synthesis experiments performed on a limited sample of isolates showed that five ECM-derived strains of clade 2 and 4 formed ECM, three ERM-derived strains of clade 3 formed ERM, and four ECM-derived strains of clade 1 and 2 did not associate with any host roots. The confirmed ECM-strains formed the black-and-hyaline mantles of the *P. bicolorata* morphotype. This morphology represented an intermediate stage, where mantle-colour changed from light brown to charcoal black.

S vaccinii – Ecto ?

Eric → H ericae

Dermataceae – Helotiaceae
Hyaloscyphaceae

Polyphyletic

aggreg H. ericae – monophyletic

not in Hymenoscyph

C1 08

Diversity and distribution of fungal endophyte genotypes in the root system of *Woollsia pungens* (Ericaceae).

D. J. Midgley, S. M. Chambers and J. W. G. Cairney. University of Western Sydney, Australia.

We are investigating the diversity and distrubution of ericoid mycorrhizal fungal endophytes in root systems of *Woollsia pungens* Cav. (Muell) (Ericaceae). Using a novel mapping technique, fungal endophytes were isolated from hair roots and mapped on a digital image of the root system, allowing the spatial distribution of each isolate to be determined. One hundred and eight isolates were obtained from the root system of one *W. pungens* seedling. These comprised five ITS-RFLP types, however one ITS-RFLP type (Type I) was most abundant, representing ~75% of all isolates. Type I is ~89% similar to *Hymenoscyphus ericae* (Leotiales) by ITS rDNA sequence comparison and groups with other *Hymenoscyphus*-like taxa in phylogenetic analyses. ISSR-PCR using the primers $GACA_4$ and GTG_5 indicated that the type I isolates comprised six genotypes. Mapping the distribution of each genotype in the root syestem indicates that one genotype is spatially dominant. Physiological variation within the type I endophyte population, in terms of nitrogen utilisation, is being investigated and will be discussed in the context of functional endophyte diversity in *W. pungens* root systems.

Chatosphaeria chloroconia
[illegible] contaminatin

Thielama hyrcaniai

C2 01

Mycorrhiza in semi-aquatic environments: studies with *Lythrum salicaria*.
K.J. Stevens, S. Spender, R.L. Peterson, and R.J. Reader. The University of Guelph, Guelph, Ontario, Canada. *Lythrum salicaria* (Lythraceae) is an amphibious species thriving in water levels characteristic of upland environments, as well as under flooded conditions. We have conducted several studies to ascertain the mycorrhizal status of *L. salicaria*, and to understand the dynamics of the mycorrhizal association under wetland conditions. *L. salicaria* is a facultatively arbuscular mycorrhizal (AM) species forming associations with species of *Glomus* and *Gigaspora*. Under field conditions, arbuscular, vesicular and hyphal colonization levels were highest in dry and intermediate sites, while plant heights were greatest in flooded and intermediate sites. These results suggest the AM association may play a greater role under dry and intermediate rather than flooded conditions. In a greenhouse study, AM inoculated plants exhibited significantly lower shoot heights, shoot, root and total dry weights compared to non-inoculated plants under flooded conditions, suggesting a substantial cost to maintaining the AM association. More recently we have been examining the effects of phosphorus availability and AM fungi on plant performance when grown under saturated conditions. Specifically we are trying to determine if there is an advantage to maintaining the AM association under saturated conditions and if this is related to P availability. Given the ongoing concerns regarding P influx into aquatic systems, the increased awareness of the potential for AM fungi to influence community structure, and, based on terrestrial systems, the potential for the uncoupling of the AM association at high levels of P, the affect of P availability on the AM association in wetland areas deserves attention.

C2 02

The use of mycorrhizal associations for an *in situ* phytoremediation of contaminated soils

W. Heyser, I. Dobner, A. Seebeck. The University of Bremen, Germany
The use of plants and their associated microorganisms for the *in situ* phytoremediation of contaminated soils is an emerging technology with potential for the effective and inexpensive clean-up of environmental pollutants. A predominant role in phytoremediation with respect to the biodegradation and uptake of contaminants can be expected for plant-associated mycorrhizal fungi, who explore large soil volumes by their extramatrical mycelium. Similar to white rot fungi some mycorrhizal species are able to degrade organic xenobiotics due to non-specific ligninolytic enzymes (e.g. Mn-peroxidase). However, it is still unknown whether mycorrhizal fungi produce these oxidizing enzymes also in symbiotic association with their host plants. We tested the potential of different *Populus* species for the biodegradation of petroleum hydrocarbons in contaminated soils, and found that distinct species differed significantly in their degradation capacity. In order to demonstrate the degradation capability of mycorrhizal associations for highly persistent pollutants we carried out experiments with mycorrhizal poplar trees on trinitrotoluene (TNT) contaminated soils. It was shown that the removal of TNT from the soil can be enhanced by mycorrhizal systems, obviously due to the following pathways: (1) reductive transformation of TNT at least to aminodinitrotoluene (ADNT) as primary reduction product, (2) uptake of TNT and metabolites by the host plants resulting in an accumulation within the root system and (3) partially also to a ligninolytic degradation by oxidizing enzymes.

C2 03

Ecophysiology of ectomycorrhizal fungi associated with *Pinus* spp. in low rainfall areas of Western Australia

T.L. Bell[1] and M.A. Adams[2] [1]The University of Western Australia, Australia, [2]Forest Science Center, Victoria, Australia

Deep-rooted trees are being re-established in the low rainfall (< 500 mm year^{-1}) wheat growing region of Western Australia as a means of ameliorating rising water tables and associated increases in salinity. As a means of monitoring successful tree establishment, aspects of the ecophysiology of ectomycorrhizal fungi associated with roots of plantations of *Pinus pinaster* and *P. radiata* growing in the wheatbelt of WA were examined. A single species of mycorrhizal fungi, *Rhizopogon rubescens*, was found to predominate, with sporocarps and mycorrhizal-infected root tips appearing with the onset of winter rains in May, increasing in number and total biomass to peak in September and decreasing to negligible levels at the beginning of summer drought in December. Greatest numbers of active root tips were consistently located at depths of 10 - 20 cm despite the bulk of the soil organic matter being in the 0 - 10 cm layer. This suggests the overriding importance of soil moisture rather than nutrient status of the soil in determining root and fungal distribution throughout the profile. Results from a wide range of assays determining enzyme activity of soil (protease, phosphomonoesterase, cellulase, *L*-asparaginase, *L*-glutaminase and β-glucosidase) surrounding mycorrhizal roots indicated seasonal patterns of enzyme activity to be similar to those described for presence of mycorrhizal fungi. Information relating to factors responsible for patterns of seasonal activity and distribution of active ectomycorrhizal roots is essential for managing soils in order to maximise tree growth and form while effectively restoring soil water balance.

C2 04

Surviving the cold: ectomycorrhizal fungi and life at low temperature

M. Tibbett[1] and J.W.G. Cairney[2] [1]School of Conservation Sciences, Bournemouth University, Poole, Dorset, BH12 5BB, UK. [2]Mycorrhiza Research Group, School of Science, University of Western Sydney, (Nepean) PO Box 10, Kingswood, NSW 2747, Australia.

Edaphic temperatures in tundra, taiga and alpine regions are predominantly close to or below freezing. In these biomes ectomycorrhizal fungi are apparently common and appear to be an important component on the soil microbiota, yet little is know about how they survive and function in the cold. Evidence will be presented that ectomycorrhizal fungi (*Hebeloma* spp.) have evolved physiological adaptations to cold that allow them to survive and function at low temperature.

Ectomycorrhizal fungi are able to 'harden' when subjected to cold, enhancing the ability of mycelia to survive subsequent freezing. This seems to be associated with changes in levels of soluble carbohydrates in the mycelia, which may play a role in freeze tolerance. The mycelia also excrete specialised low temperature active enzymes, thought to be involved in nutrient mobilisation, that are induced only at low temperature. This has ecological significance and is also important for laboratory based studies that take place outside of the ambient temperature range of the fungus.

C3 01

Phosphorus cycling by ectomycorrhizal trees in an African rain forest.

G.B.Chuyong[1],L.Njampa[2] D.Mc.Newbery[1] & I.J.Alexander[2]. [1]University of Berne,
Switzerland & [2]University of Aberdeen, Scotland

Do ectomycorrhizal (EcM) trees enhance their fitness by altering their edaphic environment? On sandy, acid, phosphorus(P)-poor soils in Korup National Park ,Camerooon, a few EcM legumes contribute a disproportionately high proportion (up to 70%) of stand basal area in discrete groves in a matrix of otherwise species-rich, arbuscular mycorrhizal (AM) forest. We know that organic matter and P cycles within the groves are subtly different from those in the forest matrix because of the ability of the EcM trees to access P and concentrate it in soil surface horizons from where P is presumed to be efficiently recycled. Here we examine mechanisms which might contribute to efficient P recyling and minimise losses of P by leaching or fixation.

Retranslocation of P prior to leaf abscission was lower in EcM than AM species. Litter decomposition rates were high (k=3.3) but litter of EcM species decayed at a significantly slower rate than that of AM species, although the former had higher N & P concentrations at the start. Some EcM species had very high levels of condensed tannins in their leaves, but there was no simple relationship between tannins and decomposition rate. In the strongly seasonal climate P release from accumulated litter at the start of rainy season is a critical phase of the P cycle. We found that the turnover of P in light fraction organic matter in the mineral soil was also slower in EcM forest at this time. We conclude that leaf chemistry combined with the mat of ectomycorrhizas at the soil surface regulate decomposition and mineralisation processes and ensure efficient recycling of P in the EcM groves.

C3 02

Vertebrate exclusion reduces mycorrhizal inoculum potential of a rainforest soil

C.A. Gehring, and T.C. Theimer. Northern Arizona University, Flagstaff, Arizona, USA

By consuming and defecating mycorrhizal spores in viable condition, mammals can be important in dispersing spores to areas devoid of mycorrhizal inoculum. However, whether vertebrate spore dispersal limits colonization of plants in undisturbed habitats remains unclear. We experimentally tested the importance of terrestrial vertebrates to the arbuscular mycorrhizal inoculum potential of an Australian primary rainforest soil by comparing field plots that were fenced to exclude ground-dwelling vertebrates for three years to unfenced control plots. We tested whether: 1) the mycorrhizal inoculum potential of soil from vertebrate exclosure plots was reduced relative to that of control plots, and 2) whether higher levels of mycorrhizal inoculum potential in soils where vertebrates had unrestricted access resulted in enhanced seedling growth. We assayed mycorrhizal inoculum potential by growing seedlings of corn (*Zea mays*) and a rainforest tree (*Flindersia brayleana*) in replicate intact soil cores collected from vertebrate exclosure and control plots. Corn seedlings were grown at greenhouse light conditions (25% PAR) and *F. brayleana* seedlings were grown at natural rainforest understory light intensities (3% PAR). In both plant species, seedlings grown in cores from control plots had significantly higher mycorrhizal colonization than seedlings grown in cores from vertebrate exclusion plots. Corn plants grown in cores from control plots were significantly larger as well, but there were no significant size differences in the *F. brayleana* seedlings. This study provides experimental evidence that the exclusion of ground-dwelling vertebrates can limit the amount of mycorrhizal inoculum present in a tropical rainforest soil and suggests that loss of vertebrates from fragmented forests could potentially change rates of mycorrhizal colonization and associated plant and soil processes.

C3 03

Arbuscular mycorrhizal fungi preclude Eucalypt seedling establishment in a northern Australian monsoon rainforest soil

D.P. Janos[1], J. Scott[2] and D.M.J.S. Bowman[2] [1]University of Miami, U.S.A., [2]Parks and Wildlife Commission of the Northern Territory, Australia

Eucalyptus tetrodonta, an ectomycorrhizal tree species, dominates the tropical savannas of northern Australia. Its seedlings do not invade monsoon rain forest adjacent to savannas except after fire. Facilitation of seedling invasion by fire is a consequence of partial soil sterilization, suggesting "microbial suppression" of *E. tetrodonta*. We investigated whether or not arbuscular mycorrhizal (AM) fungi contribute to seedling suppression. We constructed containers with three compartments separated by double nylon cloth (ca. 43 µm pores) dividers. One end compartment could be lifted slightly every 2-3 d to retard hypha spread from the middle compartment which was filled with rain forest soil into which we transplanted field-collected AM *Litsea glutinosa*. We filled end compartments with either non-treated or methyl-bromide fumigated rain forest soil. After 4.5 mo growth of *L. glutinosa* AM fungus "donor" plants, we planted the end compartments to either *E. tetrodonta* or *Ceiba pentandra*, an AM species used as a check for the presence of AM fungi. Examination of *C. pentandra* roots indicated that fumigation but not lifting reduced the spread of mycorrhizal fungi. *E. tetrodonta* seedlings grew well, however, only in "lifted" compartments containing fumigated soil. In other compartments, the addition of chelated iron overcame *E. tetrodonta* seedling suppression. These results suggest that arbuscular mycorrhizal fungi are an agent of suppression of *E. tetrodonta* seedling establishment in monsoon rain forests.

C3 04

Differential benefit of forest and pasture mycorrhizas to seedling growth in a tropical tree

L. Aldrich-Wolfe, Ecology & Evolutionary Biology, Cornell University, Ithaca, New York, USA

During previous work in Costa Rica, I determined that the AMF (arbuscular mycorrhizal fungi) community differs in species composition between pasture and forest. This difference could be important to pasture recolonization by tropical forest trees, if pasture AMF form fewer or less effective mycorrhizas than forest AMF with tropical forest tree seedlings. I conducted a growth chamber experiment to assess whether the common AMF species found in forest and pasture are equally effective as mycorrhizal associates of the neotropical forest tree species *Terminalia amazonia* (Combretaceae). *T. amazonia* seedlings were inoculated with either a) a forest AMF species mix, b) a pasture AMF species mix, or c) no AMF; grown in individual pots and harvested after six months. On average, seedlings inoculated with forest AMF were three times larger than either uninoculated seedlings or seedlings inoculated with pasture AMF. Root colonization was high in seedlings inoculated with forest AMF and low in seedlings inoculated with pasture AMF. Shoot phosphorus concentration of seedlings inoculated with forest AMF was twice that of seedlings inoculated with pasture AMF, and almost three times higher than the phosphorus concentration observed in uninoculated seedlings. Shoot phosphorus concentration was positively correlated with percent root colonization. These results suggest that pasture AMF are less effective than forest AMF at forming mycorrhizas with *T. amazonia* seedlings. A field-test of these findings is needed, to determine whether an absence of common forest AMF species from pastures limits seedling establishment in pastures by tropical forest trees.

ABSTRACTS

·

Tuesday 10 July

K3

Transport in ectomycorrhizas

M. Chalot[1], A. Javelle[1], BR Rodriguez-Pastrana[1], R. Lambilliote[2], R. Cooke[3], H. Sentenac[2], A. Brun[1], F. Martin[1], B. Botton[1]

[1] UMR 1136 INRA/UHP IaM, Nancy, France. [2] BPMP, UMR 5004 CNRS / ENSA-M - / INRA / UM2, Montpellier, France. [3] LPBMP, UMR 5545 CNRS, Université de Perpignan, Perpignan, France.

Nutrient transport, namely absorption from the soil solution as well as nutrient transfer from fungus to plant and carbon movement from plant to fungus are key features of mycorrhizal symbiosis. This review will summarize our current understanding of nutrient transport processes in ectomycorrhizal fungi and ectomycorrhizas, as well as transport regulation in response to intracellular needs and to changing external environment. With the ongoing functional analysis of nutrient transporters, identified during sequencing of fungal and tree genomes, a picture of individual transport systems should be soon available, with their molecular functions assessed by functional characterization in, *e.g.*, yeast mutant strains or *Xenopus* oocytes. Beyond the molecular function, systematic searches for knockout mutants will allow to obtain a full understanding of the role of the individual transporter genes in the physiology of the symbionts. Based on this information, the mechanisms by which fungal cells obtain, process and integrate information regarding nutrient levels in the external environment and the plant demand will be analysed.

S2 01

Imaging transport along arbuscular mycorrhizal hyphae

B. Bago[1], P.E. Pfeffer[2], P. Lammers[3], W. Zipfel[4], Y. Shachar-Hill[3]
[1]Estación Experimental del Zaidín (CSIC), Granada, Spain [2]ERRC (USDA) Wyndmoor PA, USA; [3]Department of Chemistry and Biochemistry, NMSU, Las Cruces NM, USA [4]Applied and Engineering Physics, Cornell University, Ithaca NY, USA

The arbuscular mycorrhizal (AM) symbiosis is responsible for huge fluxes of mineral nutrients from the soil to the roots of most of the land plants, and of photosynthetically fixed carbon from plants to the soil. Such transport should be carried out from extraradical to intraradical phases of the fungal colony and vice-versa, along long, coenocytic hyphae. In the case of transport of carbon, previous isotopic labeling experiments showed that the fungus exports storage lipid from the intraradical to the extraradical mycelium, however the mechanisms through which such transport occurs remain unknown. We have used *in vivo* multiphoton microscopy to observe the movement of lipid bodies through the fungal colony and to determine their sizes, distribution and velocities. The distribution of lipid bodies along fungal hyphae suggests that they are progressively consumed as they move toward growing tips. Time-lapse image sequences show lipid bodies moving in both directions along hyphae and NMR analysis of labeling patterns after supplying ^{13}C labeled glycerol to either extraradical mycelium or colonized roots shows that there is indeed significant bi-directional translocation between intraradical and extraradical mycelium. We conclude that large amounts of lipid are translocated within the AM fungal colony, and that while net movement is from the intraradical to the extraradical mycelium, there is also substantial recirculation throughout the fungus. We also report the isolation of an AM fungal EST for a putative acyl-CoA dehydrogenase whose deduced amino acid sequence suggests that it may function in the anabolic flux of carbon from lipid to carbohydrate.

S2 02

Regulation of plant nutrient transporters in arbuscular mycorrhizas

Stephen H. Burleigh
Risø National Laboratory, Roskilde, Denmark

One likely mechanism for the regulation of plant nutrient transporters in roots is through a feed-back mechanism, whereby these transporters are down-regulated as a result of nutrient uptake by the plant and a corresponding improvement of plant nutrition. Their down-regulation in mycorrhizal roots, therefore, would also be predicted as a result of symbiotic function. We have recently showed that a variety of AMF species can down-regulate high-affinity Pi transporters from two diverse dicots. This down-regulation was correlated with shoot P concentration, which indicated that P supply from the fungus was likely responsible for the observed alterations in plant gene expression. A similar mechanism of regulation was observed for a zinc transporter from *Medicago truncatula*. However, there is some evidence that these plant nutrient transporters are also down-regulated within mycorrhizal roots by a mechanism not involving nutrient supply by the fungus. The nature of this duel mechanism of regulation will be discussed.

- Relation lumière - Phospho
si lumière ↗ P part ↘ - [illegible]

Rapport Fresh weight / Dry weight → relié à expression d'un stress

S2 03

Mechanisms of nutrient transport across interfaces in arbuscular mycorrhizas

N. Ferrol, J.M. Barea and C. Azcón-Aguilar
Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Granada, Spain

The mutualistic nature of arbuscular mycorrhizal symbiosis relies on the ability of the fungal mycelium to take up mineral nutrients from the soil solution and to transfer them to the symbiotic roots in exchange for carbohydrates. This bidirectional nutrient exchange between the plant and the fungus takes place across the symbiotic interfaces which are bordered by plant and fungal plasma membranes. Nutrient movement between partners must be controlled by the membrane proteins that mediate controlled exchange of solutes into and out of the symbiont cells. Membrane transport proteins responsible for movement of phosphate and carbon compounds across the individual plant and fungal membranes have not so far been identified. However, increasing information about plasma membrane H^+-ATPases, a family of enzymes that drive active secondary transport systems, is being obtained from the use of molecular biology techniques. Current knowledge on the expression of genes encoding plant and fungal plasma membrane H^+-ATPases will be presented and discussed in relation to the implication of the different plant and fungal plasma membrane H^+-ATPase isozymes in transport mechanisms operating at the membranes of both symbionts in arbuscular mycorrhiza. Expression analysis of a gene encoding a novel tomato sugar transporter will be also presented.

S2 04

P metabolism and transport

T. Ezawa[1], S.E. Smith[2], and F.A. Smith[2].
[1]Nagoya University, Japan, [2]The University of Adelaide, Australia.

P (phosphate) is the most important 'currency' in the arbuscular mycorrhizal symbiosis, in which the fungi obtain a carbon source from the host plant and the plant receives P from the fungi. After taking up P from soil, the fungi first incorporate it into the cytosolic P pool which is utilized for maintenance of various cell functions, and the excess P is transferred to the vacuoles. One essential function of the vacuolar P pool is probably to buffer cytosolic P concentration. It is considered that the vacuolar P pool also plays a central role in P supply to the host plant. The main forms of inorganic P in vacuoles are orthophosphate and polyphosphate (a linear polymer of P, linked by high-energy bond). The vacuolar P pool consists of not only inorganic but also organic P, including various intermediate metabolites such as sugar phosphates and glycerophosphate. The vacuolar components are probably transported by protoplasmic streaming or the motile tubular vacuole-like system to the site (maybe arbuscules) at which P is released into the interfacial apoplast and thence to the plant. Biochemical and histochemical studies suggest that the fungi have several phosphatase-type enzymes which have different substrate specificities which may be responsible for liberation of orthophosphate from the vacuolar P pool. A scheme of P-flow and possible regulatory factors are proposed with respect to recent advances in the study of P metabolism in arbuscular mycorrhizal fungi and other eukaryotic microorganisms.

Carbon metabolism and transport in the arbuscular mycorrhizal symbiosis

Y. Shachar-Hill[1], P.J. Lammers[1], J. Jun[1], J. Abubaker[1], J.W. Allen[1], B. Bago[2], D.D. Douds[3], P.E. Pfeffer[3]
1 Dept Chem & Biochem, New Mexico State University, Las Cruces NM, USA. 2 [2] Departmento Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, CSIC, Spain.
3 US Department of Agriculture, Wyndmoor Pennsylvania USA

Experiments using isotopic labeling and NMR analysis have identified the forms of carbon transferred to the fungus and point to key pathways by which this carbon is transformed, translocated and utilized by the fungal partner. Two essential tools for further progress have recently emerged: testable working models of carbon flow in the symbiosis, and molecular biological methods for studying fungal gene expression. This presentation will highlight our recent work on testing and extending current models of the mechanisms of carbon movement. This is being done by a combination of functional analysis of pathways and fluxes (NMR, in vivo microscopy, mass spectrometry) with analysis of metabolic gene expression (gene identification, quantitative real-time PCR). Also to be presented are hypotheses about the regulation of metabolism and transport in response to developmental, metabolic and environmental signals. The results have implications for central questions about C handling in the AM symbiosis, such as the nature and extent of C transfer from fungus to host, and increase our fundamental understanding of the biochemistry of a unique, important but poorly understood symbiosis.

C4 01

Linking into CMNs: The implications of position and host preference for ecto- and arbuscular mycorrhizal colonization of oak seedlings

A. E. Lindahl, M. F. Allen, L. M. Egerton-Warburton, J. L. Lansing, T. Tennant
University of California, Riverside USA

Common mycorrhizal networks (CMNs) are thought to link plants, via their mycorrhizal fungi, in meaningful ways. Some oak species are capable of dually hosting both ecto- and arbuscular mycorrhizal (AM) fungi, and may link into CMNs of varying form and function over the course their maturation. The mycorrhizal status of seedlings may also be largely determined by the mycorrhizal status of their immediate plant neighbors. For example, oak seedlings that colonize AM grassland ecosystems usually become highly colonized with AM fungi. As seedlings are planted closer to established, mature oaks, they are more likely to share the ectomycorrhizae of these fungal hosts. This trend was observed in three oak species (*Querus agrifolia*, *Q. garryana*, and *Q. douglasii*) transplanted into a southern Californian oak (*Quercus agrifolia*) ecosystem. Although approximately 15 ectomycorrhizal morphotypes were shared by both seedlings and the mature, host trees, seedling mycorrhizae typically corresponded with those fungi found in the immediate vicinity on the mature oak. RFLP data collected from seedling and mature oak root tips showed similar trends. The diversity and identity of the CMNs found with transplanted seedlings appears to be largely regulated by the position of planting rather than by seedling preference.

C4 02

Significance of the apoplastic pathway for nutrient acquisition and nutrient exchange in ectomycorrhizal root systems

H. Bücking', A. J. Kuhn", R. Hans', W. Heyser' 'The University of Bremen, Germany, "Research Center Jülich, Germany

The contribution of the apoplastic pathway to the nutrient acquisition of ectomycorrhizal roots is controversely discussed. Based on fluorescence microscopical investigations, it was assumed that the only pathway available for a nutrient transport across the sheath is via the fungal symplast and therefore under physiological control of the fungal symbiotic partner (Ashford et al. 1989). The apoplastic movement of a fluorescent dye, however, might differ significantly from the movement of ions across the fungal sheath. We have investigated the entry of different nutrients into the cortex of mycorrhizal roots by laser microprobe mass analysis, microautoradiographic techniques and EDXS. The results show, that the fungal sheath does not seal the root cortex and the interfacial apoplast completely from the external conditions. However, entry and radial movement depends on (1) the ion, (2) the fungal species and (3) the growth conditions. In contrast to Mg and Ca, which can move via the apoplast, P is mainly symplastically translocated across the fungal sheath to the host plant. In further experiments, we examined conditions affecting P uptake and P efflux across the fungal plasma membrane and found that the P efflux into the interfacial apoplast is affected e.g. by (1) the intracellular P concentration, (2) the external cation concentration and (3) the phosphate demand of the host plant. The results are summarized in a model system showing the main factors influencing the apoplastic movement and/or apoplastic exchange of nutrients in ectomycorrhizal systems.

C4 03

Identification of genes for lignin peroxidases and manganese peroxidases in ectomycorrhizal fungi using PCR

D. M. Chen[*], A. F. S. Taylor[§], R. M. Burke[^], and J. W. G Cairney[*]
[*]Mycorrhiza Research Group, School of Science, Food & Horticulture, University of Western Sydney, Australia; [§]Department of Forest Mycology & Pathology, Swedish University of Agricultural Sciences, Sweden; [^]Department of Biomolecular Sciences, UMIST, UK.

ECM basidiomycetes were screened for genes for lignin peroxidases (LiP) and manganese peroxidases (MnP) by PCR using isozyme-specific primers. PCR fragments indicative of LiP genes were obtained from a broad taxonomic range of ECM basidiomycetes. DNA sequences of the amplified fragments showed high percentage similarity to sequences for LiP isozymes from white-rot fungi, confirming that LiP genes are widespread in ECM basidiomycetes. In contrast, MnP genes were detected in only a limited number of the screened basidiomycete taxa. The presence of these genes supports putative evolutionary relationships between ECM and saprotrophic fungi. Expression of the ligninolytic genes may facilitate ECM fungal access to nutrients associated with dead plant material in soil and potentially a supplementary carbon supply. The data suggest that functional boundaries between ECM and decomposer fungi may be less clear-cut than previously thought, and will be discussed in relation to roles of ECM fungi in nutrient cycling processes.

C4 04

Uptake of ^{15}N-labeled inorganic and organic N by *Picea engelmannii* seedlings growing in a high elevation clear-cut in association with different ectomycorrhizal fungi

M.D. Jones, H.C.L. Peat, and L.J. Philip Okanagan University College, Kelowna, Canada

The area of high elevation *Picea engelmannii/Abies lasiocarpa* forest being logged in British Columbia is increasing, as more easily-accessible forests are no longer available for harvest. Laboratory studies on nonmycorrhizal *P. engelmannii* seedlings, the species typically selected for replanting in the sites, show that this species prefers ammonium over nitrate as a N source. This is interesting given the increase in soil nitrate concentration that frequently occurs following clearcut logging. As part of a major study on N transformations in high elevation spruce/fir forests following logging, we compared uptake of ^{15}N-labeled nitrate, ^{15}N-labeled ammonium or ^{15}N/^{13}C-labeled aspartate by 15 month-old *P. engelmannii* seedlings that had become naturally colonized by ectomycorrhizal fungi after growing on the site for 12 months. ^{15}N from all N sources was detected in roots after 24 hours, and in shoots after 72 hours. Differences in uptake by seedlings mycorrhizal with different fungi (those formed by dark septate fungi and several unidentified basidiomycetes, and Estrain mycorrhizae) and nonmycorrhizal seedlings will be discussed.

C4 05

First record of VAM in the mycoheterotrophic *Arachnitis uniflora* (Corsiaceae): morphological development of the association.

L. S. Domínguez & A. Sérsic - Univ. Nac. Cba. - IMBIV – C.C. 495. 5000 – Córdoba – Argentina - laura@imbiv.unc.edu.ar – asersic@com.uncor.edu

The achlorophyllous *Arachnitis uniflora* is native to southern South America (Bolivia, Chile and Argentina). This study describes the morphological development of vesicular-arbuscular mycorrhizal fungi (Glomales) within the tuberous roots of this plant regarding the modifications of plant tissues. Individual plants, at different developmental stages, were excavated under *Nothofagus pumilio* and *Austrocedrus chilensis*. (Chubut- Argentina) forest soil. Different stains were proved for the fungal simbiont and lugol solution for detecting plant starch. Three very different stages of development were recognized: Young stage (short and turgent root system, shoot and flower restricted to a small bud), mycorrhizal colonization of the roots occurred between epidermal cells. In the outer most layers (epidermis and peridermis) inter- and intracellular hyphal development was detected. Young arbuscules and vesicles developed inside the parenchymatic cells of the outer cortical layers of the root intermingled with starch storage cells. Intermediate stage: (elongated and turgent roots, partial shoot and flower development). Prolific development of hyphae, relatively sparse senescent arbuscules and vesicles bundles were seen between degenerating parenchymatic plant cells. Starch grains had been completely consumed. Senescent stage: (collapsed and hollowed roots, withering flowering shoot). Epidermal and peridermal layers remained intact, while a massive development of a loosely arranged mycelium remains within the completely degenerated parenchymatic cortical cells. Innermost parenchymatic cortical layers and the vascular tissues were no longer present.

C4 06

Are mycorrhizal allocation patterns related to mutualistic function?

+ de consumo estructuras [illegible] Glomus [illegible] esporas? [illegible] vesículas, no [illegible] células para mantenimiento

N.C. Johnson, D.L. Rowland, L. Corkidi and E.B. Allen. Northern Arizona University, Flagstaff, AZ, USDA/ARS National Peanut Research Laboratory, Dawson GA, Tree of Life Nursery, San Juan Capistrano, CA, University of California, Riverside, CA, USA.

Total percent root length colonized by arbuscular mycorrhizal (AM) fungi is often unrelated to plant performance, but it has been suggested that the formation of arbuscules, coils, and extraradical hyphae may better predict the mutualistic function of AM associations. Arbuscules, coils, and extraradical hyphal networks are directly involved in supplying nutrients and water to host plants so we reasoned that allocation to these "supplier structures," relative to total AM colonization, should relate to plant performance. We examined the ratio of supplier structures to total colonization in over 1,200 root samples from a three-year field study of mycorrhizal responses to nitrogen (N) enrichment at five grasslands. Our results show that, at sites with sufficient soil phosphorus, N enrichment consistently reduces relative allocation to AM supplier structures, but not total colonization. We then used greenhouse trials to examine the mutualistic function of the same communities of AM fungi with their indigenous grass species. In seven out of ten trials, the biomass of grasses inoculated with communities of AM fungi from N enriched plots was significantly lower than that of grasses inoculated with AM fungi from unfertilized plots. These findings correspond with N-induced changes in the species composition of AM spore communities (see abstract by Egerton-Warburton et al.) and suggest that, at the community scale, mutualistic function of mycorrhizae may be related to relative allocation to supplier structures. Studies are underway to assess allocation plasticity of individual AM fungal species to N enrichment.

Consumen estructuras [illegible] [illegible] 5 [illegible] con la reservas.

[illegible] [illegible] más dependientes de AM [illegible] [illegible]

C4 07

Intraspecific variation in P uptake potential among isolates of *G. mosseae*, *G. clariodeum* & *G. geosporum/G. caledonium* in relation to their genetic variation

L.M. Knudsen[1], M. Vestberg[2], S. Rosendahl[3] & I. Jakobsen[1]. [1]Risø National Laboratory, Roskilde, Denmark. [2]Laukaa Research and Elite Plant Station, Vihtavuori, Finland. [3]Botanical Institute, University of Copenhagen, Denmark.

Recent studies have revealed a substantial genetic variation among AMF, not only between, but also within isolates (Clapp *et al.* New Phytol. 149:539-54, 2001). It is unknown whether this variation is expressed at the functional level, e.g. in terms of fungal uptake of P from soil to plant. Earlier studies showed that P uptake per unit length of hyphae clearly differed between *G. clariodeum* (16 isolates) and *G. mosseae* (4 isolates), but that the intraspecific variation was relatively small. New data will be presented on the variation in P uptake of *G. mosseae* (13 isolates), *G. clariodeum* (5 isolates) and *G. geosporum/G. caledonium* (6 isolates) of different geographical origin. The fungi were grown in symbioses with cucumber in cross tubes equipped with a hyphae-only compartment containing ^{33}P-labelled soil. Fungal P uptake was quantified by measuring ^{33}P content in the plant and results were compared to the genetic variation in the large subunit rRNA gene.

C4 08

Mycorrhiza and root evolution and diversity in ancient and living plant lineages

Mark Brundrett, Soil Science and Plant Nutrition, The University of Western Australia, Nedlands WA 6097 and Kings Park and Botanic Garden, West Perth WA 6005

Early Paleozoic fossil rhizomes and spores, DNA data and their ubiquitous associations with plants show the Glomales are ancient fungi. VAM probably evolved from endophytes in early bryophyte-like land plants. Most mycorrhizas are balanced with dependant, specialised root-soil fungi and plants adapted to balance association costs with nutrient requirements. Further evolution can result in achlorophyllous myco-heterotrophic plants that exploit fungi for energy and nutrition, but are uncommon due to habitat specificity. Mycorrhizal fungi can also be endophytes, necrotrophs and antagonists. Most land plants have mycorrhizas and endophytes. Some bryophytes have VAM. Most ferns, horsetails and club mosses have VAM, but some have facultative VAM or orchid-like associations. Ectomycorrhizal (ECM) and nonmycorrhizal (NM) roots arose with flowering plants. Efficient nutrient capture is a major driving force in plant evolution. New root types evolved with plant lineages and are consistent in most families. Roots evolved from rhizomes with VAM to supply water and nutrients to support leaves and complex branching. Root structures control solute transport and guide and confine mycorrhizal fungi, resulting in two morphological categories of VAM. NM roots are thinner, more branched, active, with more hairs and typically have more advanced chemical defences than mycorrhizal roots. ECM plants have dimorphic root systems with slow-growing short laterals to allow fungal colonisation. ECM have epidermal or cortical Hartig nets due to root structure. Definitions of mycorrhizal association types and categories defined by host-controlled features are proposed. Arbuscules alone do not define VAM. Ectendomycorrhizal, arbutoid and monotropoid associations are subcategories of ECM. Orchid mycorrhizas differ greatly from other types. The success of plants with different types of roots are considered.

C5 01

Mycorrhizal ecology studied directly in the field in boreal forests

P. Högberg and M.N.Högberg, Dept. of Forest Ecology, SLU, Umeå, Sweden.

We and our collaborators have conducted *in situ* studies in boreal forests of the importance of mycorrhizal symbiosis. For example, double (C-13, N-15) labeled amino acid (glycine) was injected into the soil, and traced into roots of ectomycorrhizal (ECM) trees, ericoid mycorrhizal (EM) shrubs and arbuscular mycorrhizal (AM) grass. Between 40 and 90% of the N-15 tracer in roots came from amino acid taken up in intact form; there was no major difference between types of mycorrhizal symbiosis in this respect (Näsholm et al. 1998 Nature). In another experiment, in which >700 trees were girdled, soil respiration fell rapidly on girdled plots, demonstrating the role of current photosynthates and that ECM roots accounted for more than 50% of soil respiration (Högberg et al. 2001 Nature). Moreover, studies of the natural abundance of C-13 in trees and their ECM fungi revealed that dominant trees in mixed forests provided most of the C to the community of ECM fungi (Högberg et al.1999 PNAS). Laboratory micro- and mesocosm studies during the last decades have preceded the above findings, but field demonstrations, like those reported here, may be necessary to convince a wider audience of biogeochemists and ecologists about the importance of mycorrhizal symbiosis.

C5 02

Fieldresponses of ectomycorrhizal community structure to elevated CO_2

Petra M. A. Fransson, Andrew F. S. Taylor and Roger D. Finlay. Department of Forest Mycology and Pathology, SLU, Uppsala, Sweden.

Ectomycorrhizal (ECM) fungi play an important role in mediating carbon sequestration into forest soil. They have a direct influence on both sequestration and emission of carbon, and changed ECM community structure may thus contribute to changes in the carbon balance of boreal forest ecosystems. The effects of elevated CO_2 on ECM community structure and species abundance were monitored for three years in a 37 year old Norway spruce forest in N Sweden. Twelve *Picea abies* trees in whole tree chambers (6 in a control plot and 6 in a liquid fertilisation plot) were treated with ambient or elevated (700 ppm) CO_2-levels. Soil cores were sampled from whole tree chambers and reference trees. ECM root tips were extracted and classified morphologically, and possible treatment effects evaluated statistically. Significant effects on ECM community structure were found in response to elevated CO_2 under both nutrient regimes. Neither elevated CO_2 or fertilisation affected species richness. There was considerable variation among samples which may have masked treatment effects on individual species. After three years, the effects of elevated CO_2 were of the same magnitude as those seen after 15 years of fertilisation treatment. The results indicate that elevated CO_2 can have a major impact on the community structure of ECM fungi in mature forests.

C5 03

Resource availability determines the outcome of competitive interactions between ectomycorrhizal and saprotrophic fungi

B. Lindahl and R.D. Finlay, Dept. Forest Mycology and Pathology, SLU, Uppsala, Sweden

Mycelia of ectomycorrhizal and saprotrophic fungi both occupy the same organic substrates in the forest floor, and there is a clear potential for combative interactions between fungi from these two groups. The outcome of such interactions should influence the partitioning of nutrients between decomposer organisms and plants. Interactions between the ectomycorrhizal fungus *Suillus variegatus* and the wood decomposing fungus *Hypholoma fasciculare* were studied in soil microcosms. The mycorrhizal fungus grew in association with *Pinus sylvestris* seedlings and the saprotrophic fungus extended into the soil from either small or large wood blocks. When the saprotrophic fungus was supplied with large wood blocks, it overgrew the mycorrhizal fungus with dense mycelium, but when supplied with small wood blocks, it was often overgrown by the mycorrhizal fungus, which formed dense mycelial patches over the saprotrophic mycelium. The interacting mycelia were labelled with ^{32}P and transfer of the tracer isotope between the fungi was measured non-destructively using electronic autoradiography. When the saprotrophic fungus extended from large wood blocks it captured significantly more ^{32}P from labelled mycorrhizal mycelium than when growing from small wood blocks. The mycorrhizal fungus captured significantly more ^{32}P from labelled saprotrophic mycelium growing from small wood blocks than from saprotrophic mycelium growing from large wood blocks. These results suggest that there is a combative balance between ectomycorrhizal and saprotrophic fungi that is strongly influenced by resource availability. This balance affects the partitioning of nutrients between the fungi.

C5 04

Mechanistic plant competition revisited: the intermediate role of mycorrhizal networks

Michael G. Booth Yale University, School of Forestry & Environmental Studies, New Haven, CT, USA

In plant communities dominated by ecto- or ericoid mycorrhizal species, individuals are frequently connected to each other below ground by mycelial networks of host-generalist mycorrhizal fungi. Several authors have examined the capacity of such networks to transport carbon and nutrients between connected plants, and many reviewers have speculated about the importance of such transfers relative to plant competition and biodiversity. Here, I develop a graphical model, adapted from Tilman's resource competition model, of an alternative mechanism by which the presence of interspecific mycorrhizal networks may influence the structure and dynamics of plant communities. The model shows that mycorrhizal fungi are important intermediaries between plants and their nutrient environments. Proportional to plants' dependence on mycorrhizal fungi for nutrient capture in a community, the fungi – not the plants - are the principle competitors for plant limiting nutrients. Further, the model predicts that the growth responses of competing plant species to environmental nutrient supplies grow alike proportionally to the importance of common mycelial networks to plant species' nutrition. This similitude diminishes the effects of interspecific competition for one or more limiting nutrients and increases the time to competitive exclusion in an equilibrial system. I consider the importance of these effects in the context of non-equilibrial systems and relate the features of this model to other mechanistic and phenomenological models of plant competition.

C5 05

Consideration of ectomycorrhizal fungi in sustainable forestry: scaling in ecosystems from root tips to flying squirrels.

Luoma, D.L. and Eberhart, J.L.
Department of Forest Science, Oregon State University, Corvallis, Oregon, USA.

At the last ICOM we were challenged to think holistically and across multiple scales. Here, we intend to meet that challenge. Our research is part of a large, interdisciplinary study designed to test the biological and silvicultural effects of green-tree retention in Douglas-fir forests: The Demonstration of Ecosystem Management Options program. Six treatments were replicated on six blocks in the northwestern USA: no harvest, 75% aggregated, 40% (dispersed and aggregated), and 15% (dispersed and aggregated) green tree retention. We have quantified treatment effects on several measurable parameters that pertain to ectomycorrhizal (EM) fungi. These include assessment of hypogeous and epigeous sporocarp production, determination of the relative importance of sporocarps in the diets of small mammals, and quantification of EM morphotypes. We found a reduction of EM sporocarp production in clearcut and heavily thinned treatments. However, individual retention trees serve as important refugia for EM and support limited sporocarp production. Ectomycorrhiza type richness was found to be significantly and positively correlated with fine-root tip density. This biological legacy may be important for recolonization of EM onto seedlings. We also observed statistically significant changes in the fungi consumed by all small mammals studied.

C5 06

Arbuscular mycorrhizas influence diversity and structure in a semi-arid plant community

P. J. O'Connor, S. E. Smith and F.A. Smith
Department of Soil and Water and The Centre for Plant Root Symbioses, University of Adelaide, Australia

We determined the contribution of arbuscular mycorrhizas to community structure and diversity in a semi-arid herbland, after reducing mycorrhizal colonisation in field plots by applying the fungicide benomyl as a soil drench. We also assessed mycorrhiza-responsiveness of the six most abundant species from the field in two glasshouse experiments. The herbland was situated in semi-arid southern Australia and dominated by the introduced species, *Medicago minima*, *Carrichtera annua* and *Salvia verbenaca*. These three plant species showed different growth responses to colonisation by AM fungi, with *M. minima* being highly mycorrhiza-responsive, *S. verbenaca* becoming colonised but exhibiting no growth response when colonised, and *C. annua* remaining uncolonised. However, the highly mycorrhiza-responsive species *Vittadina gracilis* and *Velleia arguta* were equally abundant and productive in control and mycorrhiza-suppressed plots, suggesting that competition from the mycorrhiza-responsive dominant *M. minima* offset the benefits of mycorrhizal association for other highly mycorrhiza-responsive species.

Floristic diversity increased in mycorrhiza-suppressed field plots due to a decrease in biomass of *M. minima* and competitive release of the non-positively responsive species *S. verbenaca* and *C. annua*. There was no change in plant species richness after fungicide treatment; all the increase in diversity was attributable to an increase in plant species evenness within the community.

C5 07

Patterns of blue oak (*Quercus douglasii*) fine root production and ectomycorrhizal colonization in a California oak woodland: Response to season and fertilizer.

Caroline S. Bledsoe and Xiaomei Cheng, University of California, Davis CA USA.

Northern California oak woodlands have cool wet winters (Jan-Mar) and hot, dry summers (Jul-Sep). Using root in-growth cores, we studied seasonal patterns of fine (<2mm diam.) root production (FRP) for annual grasses and blue oaks at 3 sites (River, Upland, Hilltop). Oak FRP decreased little with soil depth except in summer (max. growth, 20-40 cm). Grass FRP was greatest in fall and winter (26 and 18 $g\ m^{-2}\ mo^{-1}$ (*gmm*), less in spring (3.3 *gmm*), negligible in summer. In contrast, oak FRP was greatest in spring and summer (4.8 and 2.9 *gmm*) and lowest during fall+winter (1.5 *gmm* combined). Oak ectomycorrhizal roots were found primarily in spring and fall at all soil depths. The 3 sites differed in oak FRP, soil available P (P), and ectomycorrhizal colonization (EC). EC was high at the Upland site (24 tips/m) where FRP was also high (63 g/m2/y) and soil P was low (21 mg/kg). EC was low at River and Hilltop sites (0-6 tips/m) where FRP was low (9-10 g/m2/y), but P was high (40-60 mg/kg). Fertilization (NPK) increased grass FRP, decreased oak FRP and doubled EC (21 to 40 tips/m). We do not understand why oak FRP and EC both increased without fertilization, but did not both decrease with fertilization -- oak FRP decreased while EC increased. Oaks may respond to fertilization and increased grass FRP by increasing mycorrhizal colonization, suggesting that mycorrhizas play a role in temporal and spatial competition for soil resources between grasses and oaks.

C5 08

Mycorrhizas and global climate change: a summary of experimental evidence for the effects of increasing temperature.

M. E. Gavito[1], P. Schweiger[2] and I. Jakobsen[3]. [1]University of Lund, Sweden, [2]University of Vienna, Austria, [3]Risø National Laboratory, Roskilde, Denmark

Low temperature is at present a common but poorly understood growth-limiting factor for many organisms in temperate zones. Temperature has been predicted to increase in the future. We conducted a series of experiments with different temperate crops and arbuscular mycorrhizal fungi to look at the direct or indirect effects of increasing soil temperature on the formation of mycorrhizas, their capacity to take up P and their influence on plant dry matter production and allocation. We also looked at potential interactions of temperature with other climate change factors such as increasing atmospheric CO_2. Increasing soil temperature by 5°C duplicated mycorrhiza development and plant P uptake in the 5°-15°C range. We observed intraradical colonisation at soil temperatures as low as 5°C but, although intraradical colonization reached 50% of the root length at 10°C, extraradical mycelium developed poorly below 15°C. Therefore, below 15°C soil temperature, mycorrhizas affected plant dry matter production and allocation through their use of plant C but could not contribute much to plant nutrition. Atmospheric CO_2 and soil temperature effects were in most cases independent. We conclude that low soil temperature reduced mycorrhiza development directly since plant C and root spaces to colonise were always available. The fact that colonisation progressed inside roots only until soil temperature reached 15°C may be due to limited capacity for C translocation within the mycelium below 15°C.

ABSTRACTS

▪

Wednesday 11 July

K4

Molecular approaches to understanding mycorrhizal symbioses

S.J. Barker and N. Larkan. The University of Western Australia, Crawley Campus, Australia.

Molecular analyses of plant-microbe interactions have become common place in the last two decades. Although there are philosophical considerations about the application of a reductionist approach to some areas of research, the collaborative interface (eg molecular ecology) can provide specialised insight to the generalist, whilst adding broader relevance to the research of the specialist. However, the expense of this discipline has tended to restrict research to work on model host-microbe interactions of perceived economic importance to research funding bodies. Molecular techniques were embraced early on by a few pioneers from the field of mycorrhizal research but despite some high profile research, the number of molecular mycorrhizal research programs around the world remains comparatively limited. More recent efforts to establish a few model plant molecular species as hosts for mycorrhizal research, along with broadening recognition of the fundamental importance of mutualistic symbioses to life on earth, should begin to redress this issue. In this paper we will explore the diversity of questions that can be addressed using molecular techniques and the diversity of techniques that can be utilised. Where possible we will highlight results that have been obtained by molecular researchers in the field, or illustrate possibilities based on results from other research fields. The presentation will focus on clear illustration of these approaches with a minimum of gene jockey jargon, to foster potential collaboration and uptake of this technology across a broader range of mycorrhizal research projects.

S3 01

Developmental regulation of fungal genes in the arbuscular mycorrhizal symbiosis

N. Requena, M. Breuninger, A. Ocón-Garrido, S. Cappellari, P. Franken and R. Hampp
Botanical Institute, University of Tübingen, Germany. Natalia.Requena@uni-tuebingen.de

The developmental programme in the arbuscular mycorrhizal is approached in this study, where regulated fungal genes from *Glomus mosseae* (BEG12) are investigated by means of the Selective Subtractive Hybridization technique (SSH). Pre-symbiotic fungal material was compared either to extraradical mycelium or to pre-symbiotic material exposed to contact with a host plant. Two cDNA libraries containing clones regulated during development were obtained and several of the genes further analysed. Among those, a cDNA fragment with homology to H^+ATPases was found. The cDNA was putatively up-regulated during pre-symbiosis and showed homologies to other P-types H^+ATPases from Archaea and fungi. The full cDNA clone was obtained by RACE technique and the corresponding genomic clone isolated from a genomic library. Surprisingly, although five other partial genomic clones coding for different isoforms of H^+ATPases from *Glomus mosseae* (ha1 to ha5) were already known (Ferrol et al., 2000), the cDNA clone isolated by suppressive subtractive hybridization appeared to be a new isoform with higher homology to the isoform ha5. Differential expression for the two isoforms was confirmed, both spatially and temporally by RT-PCR experiments. Thus, the new isoform, GmPMA1, appears preferentially expressed during pre-symbiotic development and in intraradical hyphae, in contrast to isoform ha5 highly expressed in extraradical hyphae. Complementation experiments in yeast confirmed that indeed the genes are functional H^+ATPases and could play a differential role in nutrient uptake at different developmental stages of the fungal life cycle. These results show that developmental gene expression analyses can be a powerful tool to investigate structure and function at the fungal site of the arbuscular mycorrhizal symbiosis.

S3 02

Genetic and molecular analyses of ectomycorrhizal symbiosis

D. Tagu and F. Martin.

UMR INRA-UHP, Interactions Arbres Microorganismes (IaM), I.N.R.A.-Nancy, France.

Living in symbiosis sounds being in harmony with each partner. However, constant communication in a couple is essential for a stable and enriching relationship. It seems to be the case for the mutualistic ectomycorrhizal symbiosis, a well described association which allow trees to adapt and support adverse conditions. The key of this equilibrium probably consists in a finely tuned balance between genetic determinism of the ability to form a symbiosis, and environmental conditions which may enhance or repress the establishment of the relationship. That is what our recent genetic analyses proved on poplar trees. The demonstration that the ability of the plant to form symbiosis is heritable opens the way to the localization on genetic maps of Quantitative Trait Loci (QTL) linked to ectomycorrhiza formation. The genes responsible for these QTL have not yet been identified, but protein and gene characterization by transcriptome and proteome analyses of the symbiotic tissues pointed out putative cellular functions regulated in the interaction. Among them, membrane trafficking, cell wall and cytoskeletal organization are probably needed for the structuration of new cellular compartments involved in cell-to-cell communication. Polypeptides belonging to signal tranduction pathways might in turn link environmental and extracellular changes perceived at the cell surfaces with modifications of the cellular response through transcriptionnal and post-traductionnal regulations. Data from different worldwide research groups will be discussed.

S3 03

The role of the plant genome in modulating arbuscular mycorrhizal formation*

van Tuinen, D. Brechenmacher, L., Weidmann, S., Chatagnier, O., Gianinazzi, S. Franken, P. and V. Gianinazzi-Pearson. UMR INRA-Université de Bourgogne, BBCE-IPM, CMSE-INRA Dijon, France. [1] Max-Planck-Institut für terrestrische Mikrobiologie, Karl-von-Frisch-Strasse, 35043 Marburg, Germany.

The establishment of a functional arbuscular mycorrhiza requires a permanent dialogue between the plant and the fungal symbionts. The complexity of the physiological and structural modifications occurring during mycorrhiza development implies modifications in the expression pattern of the genomes of both partners. Several experimental approaches have been adopted to decode this molecular dialogue. A limited number of genes with modulated expression in the symbiosis have been identified by targeted approaches, and these include those expressed during plant defense or nodulation and those encoding proteins with metabolic or membrane transport functions. Modulated expression of novel plant genes or plant genes with unknown functions has also been detected using untargeted approaches like DDRT or differential screening. Most of these plant genes are related to processes active in the late stages of symbiotic interactions and only a few to early stages. More extensive searches for plant gene expression profiles have recently been undertaken in *Medicago truncatula* by large scale sequencing of cDNA libraries from symbiotic and non-symbiotic root tissues, and by suppressive subtractive cDNA analysis of mycorrhizal and non-mycorrhizal roots at different stages of the mycorrhizal development. These strategies will give a more global picture of plant genes involved in the molecular process modulated by/or during the development of arbuscular mycorrhiza.

*Research is part of an EU QLRT project (QLRT2-CT-2000-30676)

S3 04

Plant and Arbuscular Mycorrhizal Phosphate Transporters

W.K. Versaw and M.J. Harrison
The Samuel Roberts Noble Foundation, Plant Biology Division, Ardmore, Oklahoma, USA

Phosphate movement throughout the plant requires transporters to mediate uptake from the soil and to transport phosphate across membranes separating multiple cell types and tissues. Most vascular plants acquire phosphate from their environment either directly, via the roots, or indirectly, via a symbiotic interaction with arbuscular mycorrhizal (AM) fungi.

In our investigation of the phosphate translocation process, we have identified phosphate transporter genes from an AM fungus, *Glomus versiforme* (*GvPT*) and from the roots of a host plant, *Medicago truncatula* (*MtPT1, MtPT2*). Expression analyses and localization studies indicate that each of these transporters has a role in phosphate uptake from the soil. *GvPT* is expressed primarily in external hyphae and *MtPT1* transcript and protein both localize to the epidermal cells and root hairs of *M. truncatula* roots. *MtPT1* transcript and protein levels increase in roots in response to phosphate starvation and decrease during AM fungal colonization, which suggests a role in uptake under conditions of limited phosphate availability, but not in symbiotic phosphate transport.

We have also isolated a shoot-specific, phosphate transporter gene (*PHT2;1*) from *Arabidopsis thaliana*, a non-mycorrhizal host. Although *PHT2;1* expression is unaffected by external phosphate concentrations, a null mutant does display altered expression patterns for root-specific transporters and is at least partially defective in the retranslocation of shoot phosphate.

S3 05

Dissecting the plant's genetic program for endosymbioses using mutants of *Lotus japonicus*

Catherine Kistner[1], Scott Coomber[1], Lonneke Mulder[1], Andrea Pitzschke[1], Silke Stracke[1], Elaine Tuck[2], Thilo Winzer[1] and Martin Parniske[1]
[1]The Sainsbury Laboratory; Norwich, UK; [2]IGER, Aberystwyth, UK

Three classes of symbiotic mutants have been isolated from *Lotus japonicus*, these are affected in either arbuscular mycorrhiza (AM) or the formation of nitrogen-fixing root nodules or both symbioses. The identification of mutants impaired in both root symbioses led to the formulation of the hypothesis that plant functions required for the evolutionarily older AM have been recruited to allow rhizobial infection to occur. We are using mutants belonging to this class to study early symbiotic signal transduction events. Sixteen of these mutants, belonging to at least 6 complementation groups, have been identified. The affected genes are required for the successful infection of the root epidermis, but are dispensable for arbuscule development in cortical cells. The cell-type specific function of these genes suggests regulatory roles during symbiotic development. We are cloning the affected genes using map-based strategies. Gene expression analysis by cDNA-AFLP has revealed that at least 400 plant genes are transcriptionally upregulated during AM development. A subset of these is also induced upon inoculation with Rhizobium; supporting the idea of a common plant accommodation program for endosymbiotic microorganisms. The majority of genes are activated in inoculated roots of the wild-type but not in roots of symbiotic mutants, consistent with a role for the mutated genes in symbiotic signal transduction pathways. Hierarchies between these genes are revealed by differential expression of marker genes between symbiotic mutants.

Wegel 1998 MPMI 9:933-6; Parniske 2000 Cur. Opin. Plant Biol. 3:320-8.

C6 01

Growers evaluate nursery and early field performance of 15 plant species inoculated with mycorrhizal and trichodermal fungi.

M.Hunter*, C.Healy, B.Healy, P.Heaton, M. Hickey, D. Hockings, A. Kebblewhite, D. Macintyre, P.Young, Nursery~Garden Industry Queensland, Australia. *(ANOVA Solutions)

Beneficial fungi are generally not utilised in the Australian nursery industry despite evidence from elsewhere of their value in field growth promotion and disease resilience. Eight nursery operators from south east Queensland each conducted statistically valid experiments in a nursery phase followed by a P deficient field phase with the same plants. 16 factorial treatments included media inoculation with mycorrhizal (*Glomus invermaium*, WUM 10) and trichodermal (*T.koningii* and *T.harzianum*) fungi at the start of the nursery phase. Mycorrhizal inoculation significantly depressed nursery plants at one site and interacted with fertiliser and *Trichoderma* at one site, with fertiliser at two sites and with fertiliser and species at two sites. *Trichoderma* inoculum depressed growth at one site, promoted growth at another, interacted with fertiliser at two sites, with species at one site and with species and fertiliser at one site. Five media (ex nine) were contaminated with mycorrhizal fungi from elsewhere. Very high (80%) mycorrhizal colonisation levels were recorded *Ceratopetalum, Platysace, Stenthenum, Cassinia, Westringia* and *Zantedeschia,* with intermediate levels (20-60%) in *Zephranthes*, *Gamelepsis* and *Carica.* Traces of colonisation were observed in *Rumohra, Pentas* and *Syzygium* and none in *Phelobodium, Cyphomandra* or *Murraya*. Despite contamination, a 21% field growth response in *Westringia* to mycorrhizal inoculum initially applied in the nursery was significant (P=0.02). Most rapid growth in the field occurred in plants most colonised with mycorrhiza. High densities of arbuscules were observed in 182 day old *Westringia* roots.

C6 02

Response of Arabica and Robusta coffee to *Gigaspora margarita* and carbofuran application in parasitic nematode infested land

John Bako Baon and Sukadar Wiryadiputra
Indonesian Coffee and Cocoa Research Institute, Jember, Indonesia

Nematode is one of the most destructive pests in coffee plantations, which may reduce the growth, development and yield of coffee. To overcome this problem in biological means, it has been reported that arbuscular mycorrhizal (AM) fungi have the capacity to increase the tolerance of coffee plants to the infestation of parasitic nematodes. The objective of this work held in Sumber Asin Experiemntal Station is to study the role of AM fungus, *Gigaspora margarita* compared to nematicide carbofuran in increasing the tolerance and resistance of Arabica and Robusta coffees grown in land endemic to parasitic nematodes. Mycorrhizal inoculation of *G. margarita* was carried out only once when the coffee seedlings were transplanted into polybags in nursery, while carbofuran was applied when the plants were planted in the field and the application was repeated once every three months. Results of this study indicated that optimum plant growth in 36 months after planting was obtained in combination of *G. margarita* and carbofuran. Application of carbofuran on Arabica resulted in better growth and yield potence than that of *G. margarita*, but both were not different in Robusta coffee. Nevertheless, both applications were better than control. G. margarita and carbofuran reduced the population of *Pratylenchus coffeae* but not effective to *Rotylenchulus reniformis*, although population of *R. reniformis* and *P. coffeae* was reduced in treatment combination of carbofuran and *G. margarita.* Robusta coffee was more resistance to P. coffeae than Arabica coffee.

C6 03

Predicting edible mushroom productivity using forest carbon allocation modeling and immunoassays of ectomycorrhizae

D. Pilz[1], R. Molina[1], E. Danell[2], C. Rose[3] and R.Waring[3]. [1]PNW Research Station, USDA Forest Service, Corvallis, OR. [2]Swedish University of Agricultural Sciences, Uppsala, Sweden. [3]Oregon State University, Corvallis, Oregon.

Foresters could better evaluate habitat conditions for edible ectomycorrhizal mushroom production across a wide range of forest types if they had a mechanistic model that predicted how stand conditions and alternate silvicultural choices affect mushroom productivity. We hypothesize that the average potential of a site to produce the sporocarps of chanterelles and matsutake can be reasonably well predicted by (1) the food resources available to ectomycorrhizal fungi in a stand and (2) the occupancy of a site by these particular fungi (as measured by the percentage of ectomycorrhizal root tips they colonize). A recently refined and widely applicable forest carbon allocation model allows prediction of how much net primary productivity in a forest stand is allocated to fine roots and mycorrhizae. The model will be modified to calculate the fraction of net primary productivity produced by ectomycorrhizal host tree species that are symbionts with chanterelles or matsutake. Immunoassay tests will provide quick and inexpensive evaluation of ectomycorrhizal root tips extracted from sample soil cores to determine the percentage colonized by the fungi of interest. Field trials will test the model's predictions across a range of climate, stand age, and edaphic factors believed to influence the quantity of net primary productivity allocated below-ground.

C6 04

Legume Trees in Symbiosis with Arbuscular Mycorrhizal Fungi

A.K. Gardezi, V.M. Cetina-Alcalá, R.Ferrera-Cerrato and M.U. Larqué-Saavedra.
IRENAT, Especialidad Forestal, Colegio de Postgraduados. 56230 Montecillo, México.
Kabdul@colpos.colpos.mx, vicmac@ colpos.colpos.mx

This paper presents advances obtained in seedlings of four leguminous trees: Acacia saligna (Acacia), Acacia farnesiana (Acacia), Pithecellobium dulce (guamuchil), and Caesalpinia cacalaco (tehuixtle). Arbuscular endomycorrhizal fungi represent a symbiosis between higher plants and fungi in the family Endogonaceae. These fungi improve the capacity of host plants to take up and translocate phosphates and other nutrients, especially in soils with low available phosphorus.Under these conditions mycorrhizal plants can reach growth rates similar to those obtained with high levels of fertilization. Experiments were established under greenhouse conditions, using soil from three localities with different properties. There were nine treatments, in each soil type. Six strains of mycorrhizal fungus Glomus sp. (locally identified as Zac-1, Zac-2, Zac-6, Zac-10, Zac-15 and Zac-19) and three levels of ordinary superphosphate (0, 50 and 100 ppm of $P_2 O_5$ were evaluated. Mycorrhizal symbiosis increased plant height as follows: Acacia saligna, 814% Acacia farnesiana 187.5% Pithecellobium dulce 414%, and Caesalpinia cacalaco, 89%. The mycorrhizal inoculation also induced more leaf area, greater dry weight of shoots, greater stem diameter, more dry weight of the root, greater root volume and greater phosphorus and nitrogen contents, in the aerial part. The total percentage of colonization varied from 7.3 to 100%. These results demonstrate that mycorrhizal fungi increase the productivity of plants either by uptake of phosphorus as in andosol or acid soils, or in soils of low fertility. It is important to mention that these legumes have been contemplated for rehabilitation of eroded soils, which exist in extensive regions of Mexico.

C6 05

Mycorrhizal nutrient loading: a new cultural technique for forest planting stock production

A.M. Quoreshi[1], V.R. Timmer[2] and T. Koike[1]
[1]*Hokkaido University Forest, Sapporo, Japan,* [2]*Faculty of Forestry, University of Toronto, Toronto, Canada*

Conventional fertilization schedule that practices intensive fertilization in the nursery may inhibit the development of adequate mycorrhizas on seedlings in forest nurseries. The aim of this research was to demonstrate a new cultural approach for forest planting stock, which integrates exponential fertilization with ectomycorrhizal inoculation without impeding symbiotic association, seedling growth, and nutrition. Exponential fertilization induced steady-state nutrition that corresponds with sustained symbiosis. The improved ectomycorrhizal formation, even at high loading rates, was attributed to low initial nutrient additions and progressive increase in nutrient supply according to the exponential growth and nutrient demand of plants. The mode of nutrient delivery rather than the amount of fertilizer was crucial for the production of nutrient loaded ectomycorrhizal seedling. The traditional view that poor nutritional condition is the prerequisite for development of mycorrhiza in pants was found untenable based on present experimental findings. Mycorrhizal nutrient loading significantly increased nutrient uptake of seedlings, demonstrating advantage of combining nutrient loading and fungal inoculation for more efficient builds up of internal nutrient reserves. When transplanted, mycorrhizal nutrient loaded seedlings outperformed conventional seedlings by enhancing growth and nutrient assimilation. The findings of this study were integrated into simplified conceptual model to illustrate the key mechanisms involved in mycorrhizal nutrient loading both in nursery, and when outplanted.

C6 06

Effect of rotary tillage versus no-till on the interaction between indigenous mycorrhizal fungi, *Bradyrhizobium japonicum* and soybean under field conditions

P. Antunes and M.J. Goss, University of Guelph, Ontario, Canada

A soil disturbance effect produced by rotary tillage versus no-till was used to investigate the interaction between indigenous mycorrhizal fungi, *Bradyrhizobium japonicum* and soybean (*Glycine max* L. Merr. cv. Evans) under field conditions in Ontario over 2 seasons. The seed of soybean was sown with a peat moss inoculum of *Bradyrhizobium japonicum*, strain 532C a very effective strain for Ontario conditions. Two levels of AMF inoculum potential were established between seasons. A site fallow from the previous summer was chosen for use in the first season. For the second season the chosen site was planted with a cover crop after barley to support the extra-radicle hyphae mycelium over winter. A non-nodulated line of soybeans was used as the non-fixing crop, so that N-fixation capacity could be measured accurately, and treatment effects related to the effect of soil disturbance on the relationship between soybean and AMF in the absence of *B. japonicum* could be examined. Two different levels of P were set. The growth and activity of plants from rotary tilled and no-tilled soil were compared. The study focused on the early stage of colonization with both groups of microbes, specifically the consequences on the formation and growth of root nodules for different AMF inoculum potentials. The impact on the later growth and development of plants was also evaluated, and symbiotic N_2-fixation in the soybean crop was determined. Both nodule function as well as the level of AMF colonization was significantly greater in the plants under no-till than in the plants under rotary till. The results were therefore consistent with previous laboratory experiments. A tillage effect and positive interaction between the two microbial symbionts was observed in the field.

C6 07

The influence of different arbuscular mycorrhizal fungi on root proliferation in, and nitrogen capture from, organic patches in soil.

A. Hodge Department of Biology, University of York, UK

Nutrients in soil are distributed in a patchy manner. Plants can proliferate roots in these nutrient-rich patches however, most studies have ignored the possible role of mycorrhizal fungi despite their key role in nutrient acquisition. Proliferation of AM hyphae instead might be expected because of the lower carbon costs associated with hyphal construction. Arbuscular mycorrhizal (AM) fungi can enhance nutrient capture, especially phosphate, for their associated host plant from patches, however their ability to capture nitrogen from organic N sources remains a matter of debate. I have measured the ability of different AM fungi to exploit N-rich simple (glycine) to more complex (*Lolium perenne* shoot material) organic residues in soil when both roots and fungi were present in these nutrient-rich zones. The patches were dual-labelled with ^{15}N and ^{13}C to follow capture of these nutrients in the plant tissues. In addition, the effect of AM colonisation on root proliferation within the patch zone was followed by *in situ* minirhizotron techniques. Root proliferation was altered in different ways depending on the AM fungus present. Nitrogen capture however was not enhanced by the presence of AM fungi and hyphae did not proliferate within the patch. However, when roots were excluded from a N-rich complex organic patch by use of a nylon mesh which allowed hyphal but not root penetration, N-capture was greater than when both hyphae and roots were excluded. The consequences for AM colonised roots exploiting the heterogeneous distributed resources in soil will be discussed.

C6 08

Role of VAM fungi in growth and nutrient uptake of wheat and field peas in the southern wheatbelt of Australia

M.H.Ryan and J.F.Angus, CSIRO Plant Industry, Canberra, ACT, Australia

A field trial examining the contribution of VAM fungi to crop growth was conducted on a red earth at Junee in southern NSW. In year one the level of inoculum was manipulated through five treatments; legume-based pasture, linola (*Linum usitatissimum*), canola (*Brassica napus*), fallow maintained with chemicals and fallow maintained with tillage. In year two, canola, wheat (*Triticum aestivum*) and field peas (*Pisum sativum*) were sown with either no P fertiliser or 20 kg/ha of P as triple superphosphate. Crop growth and P and Zn uptake were measured four times during the season. A comprehensive investigation of the functioning of VAM fungi was possible due to: 1) use of several treatments to manipulate inoculum levels; 2) inclusion of the non-VAM control in year two (canola); 3) monitoring of potentially confounding factors such as crop emergence and levels of root disease; and 4) repeated measuring of VAM colonisation, crop biomass and crop nutrient status. In year two, P fertiliser greatly increased crop growth and decreased VAM colonisation. VAM colonisation was high after pasture and linola and low in the remaining treatments. The responses to high VAM colonisation were similar for wheat and peas with no effects on crop biomass or crop P uptake at any stage during the season. Harvest index, yield, and grain P, Cu and Mn concentrations were also unaffected. However, zinc uptake after anthesis was greatly increased by higher VAM colonisation in both the unfertilised and fertilised treatments. As a result, grain Zn concentrations were elevated and this may have implications for the nutritional value of the grain.

C7 01

Evidence for increased tolerance to phytoplasma disease in arbuscular mycorrhizal tomato plants

G. Berta[1], G. Lingua[1], N. Massa[1], M. Antosiano[1] and G. D'Agostino[2]
[1]DiSTA, Università del Piemonte Orientale "A. Avogadro", Alessandria - ITALY
[2]IFA, CNR Torino - ITALY

Phytoplasmas are prokaryotes responsible for severe diseases in wild and cultivated plants. Analyses, particularly of roots, by morphometry, light and electron microscopy, and flow cytometry showed that tomato (*Lycopersicon esculentum*) plants infected with the phytoplasmas (Phy) of the Stolbur group showed milder symptoms when they were colonized by the arbuscular mycorrhizal (AM) fungus *Glomus mosseae*. In AM plants, the phytoplasmas were often degenerated and morphometric parameters differed significantly from those of Phy plants, and were nearer to those of the uninfected controls. At each ploidy level, Phy caused a reduction of the DNA content, but in AM+Phy plants the results were identical to the controls and significantly higher than those of Phy plants. In AM+Phy plants the percentage of diploid nuclei, whose increase is a marker of pathogen-induced stress, was also intermediate between those of AM and Phy plants, although the difference was no significant.

C7 02

Nitrogen fixation and tuberculate ectomycorrhiza?

Leslie Paul[1], Bill Chapman[2] & Chris Chanway[1]. [1]University of British Columbia, Vancouver, B.C., Canada. [2]B.C. Ministry of Forests, Williams Lake, B.C., Canada

In lodgepole pine forests of British Columbia, Canada, woody debris (CWD) is believed to be a major source of nutrients for stand productivity. Tuberculate ectomycorrhiza (TEM) are commonly found within woody debris in these stands and may be linked to stand productivity. This study focused on the possibility that TEM on lodgepole pine (*Pinus contorta*) roots within CWD contribute to the host's nutrient status by supplying fixed nitrogen from diazotrophic bacteria found to reside within tubercles. The acetylene reduction assay was used to quantify *in situ* N_2 fixation associated with TEM on lodgepole pine. Values of N_2 fixation ranged from 0 – 4.27 $g/m^3/yr$ (wet basaltic soil), 0 – 0.78 $g/m^3/yr$ (dry basaltic soil), and 0.00 to 1.23 $g/m^3/yr$ (granitic soil). Fixation rates determined according to stand age were 0 – 0.46 $g/m^3/yr$ (old stands, >140 years) and 0 – 2.63 $g/m^3/yr$ (young stands, < 40 years). Enumeration of the diazotrophic bacteria was conducted by extraction and dilution plating on to N free media using tissue extracted from the inner matrix of tubercles. Tubercle tissue consisted of fungal hyphae and host root tips. Three bacterial species were isolated and identified using GC Fame and BIOLOG: *Bacillus sp.*, *Micrococcus luteus* and *Methylobacterium extorquens*. The isolates were evaluated for nitrogenase activity *in vitro* using the acetylene reduction assay. *Bacillus sp.* and *M. luteus* both showed positive nitrogenase activity (> 1.5 nmoles N/ml) whereas *M. extorquens* did not. This study therefore shows that nitrogen fixation does occur in association with TEM on lodgepole pine and that two identifiable nitrogen fixing bacteria may be responsible for the activity observed. Although the amounts of N_2 fixation appear to be small, there appears to a larger contribution in younger stands. It may well be that the contributions of N from TEM may be specific to seedlings growing in CWD since both are commonly found in this substrate.

C7 03

Reorganization of tobacco root plastids during establishment of the arbuscular mycorrhizal symbiosis

T. Fester, D. Strack and B. Hause, Institute of Plant Biochemistry, Halle, Germany

Root plastids are responsible for a large variety of biosynthetic pathways, e.g. fatty acid biosynthesis, nitrite reduction and assimilation or starch metabolism. Some of these pathways may be essential for the establishment of the arbuscular mycorrhizal symbiosis. In addition, biosynthesis of some plastid derived secondary metabolites (apocarotenoids) has been described to be induced by arbuscular mycorrhiza. Using epifluorescence microscopy and confocal laser scanning microscopy we analyzed transgenic tobacco plants expressing the green fluorescent protein (GFP) targeted to the plastid compartment. We observed a dramatic reorganization of plastids in root cortex cells containing arbuscules. Arbuscules were covered by large network-like compartments formed by these plastids. Plastid volume and surface were strongly increased, pointing to profound changes in metabolic activities. Upon disintegration of fungal arbuscules we observed a concomittant disintegration of the plastid networks covering these structures. Another characteristic of mycorrhizal roots – apart from the networks described – is the existence of „octopus“- or „millipede“-like plastid structures surrounding some root cortex cell nuclei, a phenomenon which had only been observed in cells from tobacco suspension cultures. The cytological changes during arbuscular mycorrhizal symbiosis described so far in literature were mainly observed by means of electron microscopy. Examination of cell organelles labeled with GFP, however, allows a deeper insight into the extent and dynamics of the actual changes.

C7 04

Tubular vacuoles observed in *Gigaspora margarita* hyphae using laser scanning confocal microscopy

Y. Uetake[1], T. Kojima[2], T. Ezawa[3], M. Saito[2] [1]BRAIN, Natl. Grassland Res. Inst., Japan, [2]Natl. Grassland Res. Inst., Japan, [3] Nagoya University, Japan.

Hyphae of *Gigaspora margarita* were stained with Oregon Green® 488 carboxy-DFFDA that is known to accumulate in lumen of fungal vacuole, and were observed by laser scanning confocal microscopy. Germ tube was filled with vacuoles observed as one of follows: A, a longitudinally oriented bundle of long tubules (about 0.3 μm diam.); B, both tubular and various sizes of spherical vacuoles in various proportions; C, a masses of spherical vacuoles. The germ tube where the cytoplasmic streaming was observed and vacuoles were stained showed always A type of vacuole, but never showed C type. Tubular vacuoles were extremely fragile against laser irradiation which broke down the tube into many small spherical form. These suggested that the vacuole system in active germ tube was tubular bundle. Extra and intra-radical hyphae of *G. margarita* were obtained by co-culture with onion seedlings. The bundle of tubular vacuoles was also observed in extraradical hyphae. Intercellular hyphae and trunks of arbuscules were often occupied with lipid bodies centrally and peripherally with vacuoles. The tubular vacuoles were occasionally observed in intercellular hyphae, and fine branches of arbuscules were rarely stained, in hand-sectioned roots. Function of the highly developed tubular vacuole system within germ tubes and extra- and intra-radical hyphae will be discussed in relation to high potential of long distance translocation of nutrients through the hyphae in the arbuscular mycorrhiza.

C7 05

Is the interfacial pH a value for the efficiency of nutrient transfer in ectomycorrhizas?

A. Nenninger[1], G.W. Griffith[1] and W. Heyser[2]
[1] Institute of Biological Sciences, University of Wales, Aberystwyth, Wales
[2] Centre of Environmental Research and Technology, University of Bremen, Germany

Interfacial pH-conditions in *Pinus nigra* ectomycorrhizas were determined using the pH-sensitive ratio-dye Cl-NERF and a confocal laser scanning microscope (CLSM). A pseudo-ratiometric method is commonly used for determining apoplastic pH-values. In the present study a non-invasive ratiometric method relying on a single fluorescence dye was devised and tested.

Results show homogeneous pH-values near 3 in non-mycorrhizal roots from different plants and inhomogeneous interfacial pH-conditions between 3.1 and 4 in various pine ectomycorrhizas. In mycorrhizas reduced sucrose and phosphate leads to decreasing interfacial pH-conditions, whereas external applied glucose induced an increase of the interhyphal pH-values in the mantle. Based on the results we developed a model for the interfacial transfer processes in ectomycorrhizas.

Transfer processes on the ectomycorrhizal interface are not pH-regulated. The processes generate an interfacial pH-value to maintain the symbiotic balance. In balance you find optimal pH-conditions for the nutrient transfer. The lack of only one of the transfer goods induces a decrease of the interfacial pH-conditions. A decreasing pH-value in the interface of ectomycorrhizae leads to reduce transfer processes and the mutualistic balance in the symbiosis can be maintained.

C7 06

Physiological markers target key genes in Arbuscular Mycorrhizal clover

Kelly, Rhys[1, 2]; Teodosio, Rita[1]; Eason, William[1]; Hooker, John, E.[2] and Webb K. Judith[1], [1] Institute of Grassland and Environmental Research, Aberystwyth, UK. [2] School of Applied Sciences, University of Glamorgan, Pontypridd, UK.

Uniquely available near-isogenic lines (NILs) of *Trifolium repens* (white clover), are being used in studies of the molecular genetic interactions of the plant-arbuscular mycorrhizal symbiosis. These NILs have exhibited contrasting growth responses to inoculation with arbuscular mycorrhizal (AM) fungi, indicating a strong plant genetic control over the functioning or effectivity of the symbiosis. NILs colonised with AM fungi were analysed to determine gene expression at the level of transcription, using differential display and compared with uncolonised controls. Preliminary results indicate differences in gene expression in leaf tissue between control plants and plants colonised with AM fungi. One differentially expressed gene fragment has been cloned and sequenced. Future studies will 1) include the screening of EST and genomic databases to determine the gene's identity or homology with other genes (to resolve its function), and 2) relate phenotypic differences in AM plants to gene expression. We have developed a phosphate limiting nutrient-flow culture system in which all environmental variables (and so phenotype response) can be controlled and related to gene expression. The system permits the *in situ* study of plants and offers a way of linking the study of differentially expressed genes with AM facilitated phosphate uptake. This system, combined with the use of NILs will provide a powerful, targeted method for the study and identification of new AM-regulated genes.

C7 07

Transcript profiling during ectomycorrhiza development

Tomas Johansson*, Dag Ahrén*, Antoine Le Quéré*, Bengt Söderström*, Rikard Erlandsson§, Joakim Lundeberg§, Mathias Uhlén§, and Anders Tunlid*, §Department of Biotechnology, Royal Institute of Technology (KTH), Stockholm, Sweden; *Microbial Ecology, Lund University, Lund

Mycorrhiza, a symbiotic interaction between plant and fungi, is found on a vast majority of all plant species including many important forest trees and crop plants. The symbiotic interaction, which involves the development of specific infection structures in the plant rhizosphere, improve the growth of the host plant by facilitating nutrient uptake from the soil, while the fungus gains carbohydrates from the plant. The developmental process is anticipated to depend on a well-tuned regulation at both temporal and spatial levels, and the objective of this research is to isolate and characterize symbiosis-related (SR) genes by analyses of gene expression during the ectomycorrhizal development between the fungus *Paxillus involutus* and the plant *Betula pendula* (birch).

Several cDNA libraries have been constructed from symbiotic ectomycorrhizal tissue, from free-living hyphae of *P. involutus* and from non-infected roots of *B. pendula*, respectively, and a total of approximately 11,000 EST clones have been analyzed by DNA sequencing. After assemblage of data into functional categories based on sequence homology, we observe major differences between libraries in frequencies and content, such as a significant shift within subgroups of metabolism, i. e. phosphate and nucleotide metabolism, C-compound and carbohydrate metabolism, and lipid, fatty-acid and isoprenoid metabolism.

C7 08

The use of proteomics to elucidate cellular changes during development of an AM symbiosis between *Medicago truncatula* and *Glomus versiforme*

H.J. Mussa, L.W. Sumner and M.J. Harrison. Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, Oklahoma, USA

The colonization of *Medicago truncatula* roots by *Glomus versiforme* results in the formation of an arbuscular mycorrhiza. The colonization process is accompanied by a myriad of changes in gene expression and protein content. Our laboratory is involved in transcriptome and proteome analysis to investigate the cellular changes in *Medicago truncatula* roots during development of a mycorrhizal association. Changes in transcript levels do not necessarily result in concommitant changes in the cognate protein levels due to post-transcriptional and post-translational control mechanisms such as mRNA stability, nonsense mediated decay and ubiquination. Recently, the use of proteomics to study cellular changes in *Medicago truncatula* roots has been made possible due to the advances in mass spectometric analysis, two-dimensional gel electrophoresis technology as well as bioinformatics. By the use of expression proteomics we hope to discover and characterize additional genes that are regulated during development of the mycorrhizal symbiosis. So far, we have generated 2D protein maps from cytosolic, cell wall and microsomal fractions of colonized and uncolonized *Medicago truncatula* roots. In addition, we were able to identify some microsomal proteins that were generated from 2D gels by MALDI-TOF-MS (matrix-assisted laser desorption ionization time-of-flight mass spectometry). However, some proteins with good tryptic digest maps could not be identified by MALDI-TOF-MS due to the scarcity of plant proteins in the non-redundunt databases. Progress in these areas will be discussed.

ABSTRACTS

■

Thursday 12 July

Ecophysiology of Mycorrhizal Plants

G.R. Stewart[1], and S. Schmidt[2] [1]University of Western Australia, [2]University of Queensland, Australia

Mycorrhization is widespread, around 90% of extant plant species are presumed to be mycorrhizal. Of course only a few per cent of plants have been directly investigated. The general occurrence of these associations has prompted much investigation into their physiological and ecological significance. Traditionally mycorrhizas have been considered as nutrient-absorbing organs although a wider set of functions has been proposed in recent years. Included in these, are effects on plant water relations, resistance to toxins, pathogens and predators and impacts on elemental cycling. This review will consider the extent to which mycorrhization, qualitatively and quantitatively, alters the ecophysiology of a plant. Our own work has focussed on the impact of mycorrhization on the nitrogen nutrition of Australian native species and we will assess our findings in relation to the development of a model for the ecophysiology of mycrrorhization.

Root types vs Mycorrhiza types
CR - ?? crustulenoid, chez Empodisma plants
Proteoid

Source d'azote - leur usage par plante
NH4 - majeur la + élevé
Glycine = toutes plantes peuvent métaboliser la glycine - ce qui ne veut pas dire qu'elles l'utilisent comme source d'azote
Nitrate - ↘↘ très bas -

S4 03

Arbuscular mycorrhizal fungi influence soil structure.

R.M. Miller and J.D. Jastrow Argonne National Laboratory, Argonne, IL, USA.

A series of closely related processes will be discussed, by which arbuscular mycorrhizal fungi (AMF) contribute to the formation of relatively stable aggregate structures. Fibrous roots and AMF hyphae can be viewed as a "sticky-string bag" that contributes to the entanglement and enmeshment of soil particles to form macroaggregates, a basic structural component of soil structure. Furthermore, these fungi produce a glycoprotein, glomalin, which appears to be deposited on their outer hyphal walls and on adjacent soil particles. Glomalin appears to be a rather stable hydrophobic glue that might reduce macroaggregate disruption during wetting and drying events by retarding water movement into the pores within the aggregate structure. Examples from various soil types are used to demonstrate the contributions of AM fungi to soil aggregation and its subsequent stabilization. Over a wide range of soil types, these contributions depend largely on broad textural characteristics and whether the soil's structure is hierarchical in construction, among other factors.

S4 04

Fungal diversity in ectomycorrhizal communities

Andy F.S. Taylor, Department of Forest Mycology and Pathology, SLU, Uppsala, Sweden.

Recent years have seen a considerable increase in the number of publications examining ectomycorrhizal fungal diversity below ground. The great majority of these have focused, understandably, upon anthropogenic factors that influence species richness and community structure, with regrettably few studies dealing with diversity *per se* and the 'natural' factors that contribute to the observed complexity. Consequently, we still know little concerning the underlying ecological processes that govern ECM diversity. This contribution will examine a number of interconnected issues relating to root dynamics, spatial distribution, species attributes, and interactions with other soil organisms that all potentially influence ECM diversity.

Root production and longevity are likely to be major factors affecting the competitive interactions among and within ECM species. The importance of the colonisation strategies (primary, secondary, direct colonisation of emerging tips) adopted by ECM species may change under varying root dynamics and some potential consequences of this will be considered here. The general view that ECM communities are highly diverse will be examined in relation to that recorded for other soil dwelling organisms. Special emphasis will be given to those groups of soil animals that, through their action or trophic interactions, are likely to directly influence ECM fungi. Many studies have recorded both high species densities and spatial variation in ECM communities. In light of these findings, the potential influence of current sampling techniques upon how we perceive ECM diversity will also be examined.

S4 05

Mycorrhizal fungi as a determinant of biological diversity and ecosystem functioning

Marcel van der Heijden, Department of Systems Ecology, Vrije Universiteit Amsterdam, The Netherlands.

Arbuscular mycorrhizal fungi (AMF) are abundant and widespread. Descriptive studies suggest that AMF play a key role in many natural ecosystems. However, experimental evidence is scarce and relatively few studies have addressed the ecological function of these mutualistic soil fungi. We have simulated experimental grasslands and manipulated the presence and diversity of AMF to assess the importance of AMF and of AMF diversity for plant diversity and ecosystem functioning. During this talk I will summarize the findings we obtained in these experimental grasslands. We observed that AMF enhance plant diversity. They do this by promoting seedling establishment and growth of subordinate plant species. Moreover, different AMF species had different effects on plants. This led to a change in plant species coexistence and plant community structure when different AMF species were present. Furthermore, the phosphorus content was 30 percent higher in communities with AMF, despite of a 40 percent reduction in root length in the mycorrhizal grasslands compared to the non-mycorrhizal grassland communities. It appears thus, that mycorrhizal hyphae, and not plant roots, are the primary factor that contribute to phosphorus nutrition in these grasslands. Finally, AMF not only affected plants, they also altered soil structure (by enhancing the number of soil aggregates) and AMF influenced the growth and survival of herbivores that feed on the plants occurring in these grasslands. In conclusion, our results show that AMF contribute to biological diversity and to the functioning of grassland ecosystems.

C8 01

AM fungal colonisation of sand compartments adjusted to different pH values

I.M. van Aarle, P.A. Olsson and B. Söderström, Dept. of Microbial Ecology, Lund University, Sweden

In nature the extraradical mycelium (ERM) of arbuscular mycorrhizal (AM) fungi can grow into soil not explored by roots, where e.g. P can be absorbed and translocated to roots. Local pH changes in soil might influence the availability of nutrients. The ERM can possibly respond on these changes by altered growth or activity and in that way influence the AM colonisation. To test the response of AM fungi on a pH change, the ERM of *Scutellospora calospora* and *Glomus intraradices* were exposed to two different pH values, whereas the pH of the root compartment was unaffected. Seedlings of *Plantago lanceolata* colonised by either of the fungal symbionts, and non-mycorrhizal controls, were grown in meshbags placed in pots containing pH buffered sand. The systems were harvested 5 times between 20 and 80 days of growth. Colonisation by *S. calospora* was slower than by *G. intraradices*. No differences were observed early in the experiment. However, later a high pH reduced the arbuscular and vesicular formation of *G. intraradices*. Total root colonisation was not influenced by the pH. Both fungi formed more ERM at high pH and almost no ERM could be detected at low pH for *G. intraradices*. Acid and alkaline phosphatase activity of the ERM was higher for *S. calospora* than for *G. intraradices*. Further, at a high pH the ERM of *S. calospora* had lower phosphatase activities than at low pH. No significant phosphatase activities were found in the sand. These results show that the pH did influence not only the mycorrhizal ERM but also root colonisation. AM fungi respond on pH changes, though the response seems partly dependent on the fungal symbionts.

C8 02

Mycelial carbon allocation and weathering of minerals by ectomycorrhizal fungi

A. Rosling and R. Finlay, Dept. Forest Mycology and Pathology, SLU, Uppsala, Sweden

Ectomycorrhizal fungi colonise coniferous trees and benefit tree growth mainly through improved soil colonization but also have qualitative effects on the availability of different organic and inorganic substrates. Selective, spatial allocation of carbon has been shown in association with colonisation and exploitation of organic resources by ectomycorrhizal fungi but interactions of ectomycorrhizal hyphae with different mineral substrates are less well studied. The aim of this study was to investigate patterns of carbon allocation within ectomycorrhizal mycelia colonising different mineral resources and to investigate the role of fungal associated bacteria in mineral weathering processes. Our preliminary results suggest higher allocation of carbon to more easily weatherable feldspar substrates, compared with quartz. This result is consistent with the idea that photosynthetically derived carbon allocated to the mycorrhizal mycelium may be involved in weathering interactions. Exudation of oxalic acid by mycorrhizal hyphae has been frequently demonstrated but in order to establish the possible significance of the increased carbon allocation in relation to weathering, complementary studies of element mobilisation from the different mineral substrates must also be performed. These are currently in progress in our laboratory.

C8 03

Characterisation of bacterial communities associated with the hyphosphere and hyphoplane of *Glomus intraradices*

K. Mansfeld, L. Bødker and J. Larsen, Danish Institute of Agricultural Science, Department of Crop Protection, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark.

The external phase of AM fungi play a key role in the functioning of AM symbioses transporting phosphorus from the soil to the host plant, forming soil aggregates and perhaps suppressing root pathogens. Bacteria most likely influence all these functions of external mycelium of AM fungi. In order to better understand the role of mycorrhizal fungi in plant growth and health, it is therefore important to know more about interactions between AM fungi and bacteria. The objective of the present work was to characterise the culturable aerobic-heterotrophic bacterial communities in the hyphosphere and the hyphoplane of *Glomus intraradices*. Non-mycorrhizal and mycorrhizal cucumber plants were grown in pots with two root-free compartments. 52 days after sowing, sample fractions representing rhizosphere, hyphosphere and hyphoplane were collected. Total population of bacteria was determined on TSBA medium. 1400 bacterial colonies from the four fractions were isolated and identified by fatty acid methyl ester analysis using the Sherlock System (MIDI Inc.). 87 species within 48 genera were identified with a Similarity Index > 0.30. *Pseudomonas*, *Arthrobacter* and *Burkholderia* were the most frequent genera encountered. Large differences in bacterial community structure were observed between rhizosphere and hyphosphere fractions whereas significant differences between mycorrhizal and non-mycorrhizal treatments were observed for a few species only. However, isolates identified as *Paenibacillus* spp. were more frequently found in the mycorrhizal treatment and in the hyphoplane fraction indicating that bacteria from this genus may live in close associations with mycelium of *G. intraradices*.

C8 04

Comparison of antagonistic potential of rhizobacteria from mycorrhizosphere and rhizosphere soils against soilborne fungal pathogens

R.G.Linderman, J.L.Marlow and E.A.Davis USDA-ARS Horticultural Crops Research Laboratory, Corvallis, Oregon, USA

Specific functional groups of rhizobacteria in rhizosphere soil can influence plant growth and health, and formation of mycorrhizae can alter their populations in the mycorrhizosphere. We isolated rhizobacteria from the soil around roots of onion plants inoculated (mycorrhizosphere – MR) or not (rhizosphere – R) with the VA mycorrhizal fungus *Glomus intraradices* and analyzed them for their in vitro antagonistic potential against several soilborne fungal pathogens. The antagonistic potential index (API) was calculated as the sum of all the zones of inhibition (in mm) from rhizobacteria from a treatment for a specific pathogen. The API at 10 weeks was higher in MR than R soil for all the fungal pathogens tested (*Phytophthora cinnamomi*, *Pythium irregulare*, *Rhizoctonia solani*, *Thielaviopsis basicola*, *Cylindrocladium scoparium*, and *Sclerotium cepivorum*. The API against *P. cinnamomi* at 6 weeks was equally low for MR and R soil, but increased significantly at 8 and 10 weeks in MR but not R soil. In another experiment, three antagonistic isolates of *Bacillus* from onion MR soil were added at sowing to onion plants with (MR) or without (R) mycorrhizae. The APIs at 6 weeks for R treatments, with (+BC) or without (-BC) *Bacillus*, were low, whereas the APIs for MR treatments, with or without *Bacillus*, were significantly higher. The percentages of isolated bacteria that were antagonistic were 24, 13, 63, and 73% for R-BC, R+BC, MR-BC, and MR+BC treatments, respectively. These results indicate that mycorrhizae stimulate antagonistic rhizobacterial populations that could suppress fungal pathogens and thereby the diseases they cause.

C9 01

AM infectivity and spore status along chemico-physico and rehabilitation gradients in slimes dams of deep level gold mines in South Africa

C.J. Straker, A.J. Freeman, E.T.F. Witkowski and I.M. Weiersbye, Restoration and Conservation Biology Research Group, University of the Witwatersrand, Johannesburg, South Africa

Slimes dams consist of pulverized, high pyrite waste from the mining of gold and uranium and are a significant environmental hazard. The dams are inhospitable physical and chemical environments for plant growth and current revegetation methods have proven ecologically and economically unsustainable. An infectivity study was undertaken to determine mycorrhizal status along a physico-chemico gradient from midway (neutral pH) to bottom (acidic pH) of the dam and the adjacent natural vegetation ('veld'). Infectivity was also assessed for dams based on their age since rehabilitation (recently-revegetated; old-revegetated and non-revegetated). Infectivity was determined using a bioassay with *Eragrostis curvula* as the host plant, and spores were extracted, counted and determined for viability by tetrazolium chloride reduction. AM infectivity, total spore numbers and spore viability increased down the slope, being greatest in the veld. This pattern was associated with increasing aerial vegetation cover and increasing soil organic matter. Substrata with low pH and high soil conductivity corresponded with low vegetation cover and low spore numbers. Recently-revegetated dams had higher infectivity and spore status than old- and non-revegetated dams. AM infectivity was weakly correlated with spore number, and infectivity and spore numbers were weakly correlated with soil organic matter, pH and soil conductivity. Trends in infectivity and spore status reflected those found in a previous survey of root colonization levels and rhizosphere spore status of plants growing in slimes. A high proportion of naturally-colonizing and introduced plants showed AM mycotrophy including the salt bush, *Atriplex semibaccata*, a member of the Chenopodiaceae. Results are discussed in terms of the desirability of including AMF inoculum in revegetation programmes.

C9 02

Role of arbuscular mycorrhiza in alleviation of zinc toxicity and mineral nutrition of host plants on zinc-contaminated soils

X.L. Li[1], P. Christie[1,2] and B.D. Chen[1]
[1]China Agricultural University,Beijing,China; [2] Queen's University of Belfast, UK

A series of experiments investigated the mechanism of alleviation of Zn phytotoxicity. We grew red clover (*Trifolium pratense* L.) in three-compartment rhizoboxes, pasture soil containing 50 mg·kg^{-1} 'total' (*aqua regia-*) Zn was amended in the outer compartments with four application rates of Zn (0-1000 mg·kg^{-1}). Mycorrhizal infection rate was little affected by Zn addition, and plant Zn concentrations and uptakes were lower in mycorrhizal plants than non-mycorrhizal controls, this being more pronounced with increasing Zn addition. Using two soil moisture samplers in each container, we found that soil solution Zn was lower and pH was higher in mycorrhizal treatments. Thus the protective effect of mycorrhiza against plant Zn uptake may have been associated with changes in Zn solubility mediated by changes in soil solution pH, or by immobilization of Zn in the extramatrical mycelium. In further studies using a modified glass bead compartment cultivation system and *G. versiforme* as the fungal symbiont, up to 30 mg of fungal dry matter was recovered from the hyphal compartment of mycorrhizal maize and about 6 mg from red clover. The Zn concentration of the fungal biomass was about 10 fold higher than in the roots of the host plants, and very much higher than in the shoots. The high affinity of the mycelium for Zn was very striking.

C9 03

Ectomycorrhizal protection of *Pinus sylvestris* L. against Cu toxicity.

J.V. Colpaert, K.K. Van Tichelen and J. Vangronsveld
Limburgs Universitair Centrum, Diepenbeek, Belgium

The effect of the ectomycorrhizal fungi, *Suillus bovinus* and *Thelephora terrestris*, on the copper sensitivity of *Pinus sylvestris* seedlings was studied. Plants were grown under P limitation and were exposed to 0.3 (control), 16, 32 or 47 μM Cu for 22 or 36 days. Plant and fungal development, P nutrition, sorption of Cu on roots and external mycelia as well as transfer of Cu to shoots were assessed. Root growth of nonmycorrhizal (NM) pines was severely reduced at elevated Cu in contrast to root growth of mycorrhizal plants. Both ectomycorrhizal fungi protected their host plant against Cu toxicity and this benefit was not due to a metal dilution effect. Growth of mycorrhizal root systems and fungi was hardly affected in the presence of elevated Cu and mycorrhizal plants could maintain their P status. The *T. terrestris* isolate was less sensitive to Cu stress than the *S. bovinus* strain, both *in vitro* and in symbiosis. Exposed to elevated Cu, NM plants had shoot Cu concentrations 1.4 to 4.5 times higher than shoots from corresponding mycorrhizal plants. The extraradical mycelium of *S. bovinus* retained large amounts of Cu and Cu sequestered in its mycorrhizas could not be desorbed by an excess of Pb^{2+} or H^+ (pH 1.0). In contrast, these desorption solutions could efficiently remove Cu from *T. terrestris* mycorrhizas and NM roots. Binding of Cu in fungal tissues is not a prerequisite for low Cu sensitivity since mycorrhizas and extraradical mycelia of the highly Cu resistant *T. terrestris* isolate adsorbed considerably less Cu than *S. bovinus*. The results suggest that the mechanisms of mycorrhizal amelioration of Cu toxicity are probably diverse and species-dependent.

C9 04

Contribution of arbuscular mycorrhizal fungi to phytoremediation of polluted soils

C. Leyval, P. Binet, E. Joner. Centre de Pédologie Biologique, CNRS, BP5, 54501 Vandoeuvre-les-Nancy, France

Phytoremediation is the use of plants for remediation of polluted soils. Heavy metals cannot be degraded and can only be extracted from the soil or immobilized in a non toxic form. AM fungi may contribute to plant establishment and survival in heavy metal polluted sites and could be used as a complement to immobilization strategies. Organic pollutants such as polycylic aromatic hydrocarbons (PAH) can be transformed or degraded in soil and this is enhanced in the rhizosphere through improved microbial activity. We showed that AM fungi may also improve the establishment of a plant cover on PAH-polluted soil. Pot and column experiments with industrially contaminated or PAH-spiked soil were performed with a mixture of clover and ryegrass, inoculated or uninoculated with *G.mosseae* (BEG 69).The dissipation of 4-5 ring PAH was enhanced in mycorrhizal treatments compared to non-mycorrhizal treatments after prolonged periods (>100 days) of phytoremediation. Mycorrhizal effects on PAH dissipation was accompanied by a modification in biomass partitioning between two co-occurring plant species (clover and ryegrass) as well as changes in soil microbial community structure estimated by phospholipid fatty acid analyses.

Although these results are promising,there is still a need to improve our understanding of the mechanisms involved in the contribution of AM fungi to organic pollutant availability and degradation, as well as in the transfer and immobilisation of heavy metals, to improve the chances of successful application under practical conditions.

POSTER
SESSION
ONE

■

Monday & Tuesday
9 & 10 July

P1 01

Direct assessment of the relationship between mycorrhizal responsiveness and external hyphae-mediated P uptake by four barley cultivars

Y-G. Zhu[1,2], S. E. Smith[1,2] and F. A. Smith[2]
[1]CRC for Molecular Plant Breeding, [2]Centre for Plant Root Symbioses and Department of Soil and Water, Adelaide University, Australia

A 'cross-pot' experiment was carried out in a greenhouse to investigate phosphorus (P) efficiencies of four barley cultivars (Forrest, Skiff, Clipper and Sahara) and P acquisition mediated by external hyphae of arbuscular mycorrhizal fungi (AMF). Plants were grown in compartmented cross-pots filled with grey calcareous soil collected from Minnipa, South Australia. The grey calcareous soil has $CaCO_3$ content of nearly 40% and pH of 8.7. Therefore the P availability is extremely low in this soil. The side arm of each cross-pot was filled with the same soil but labelled with ^{32}P. Mycorrhizal colonisation significantly reduced plant shoot biomass in all cultivars, and root biomass in some cultivars. Mycorrhizal colonisation significantly increased tissue P concentrations and specific P uptake (SPU). Mycorrhizal colonisation did not affect the total P uptake. There were significant differences in activity concentrations, specific activities and total activities of ^{32}P between different barley cultivars. Increase in SPU due to mycorrhizal colonisation was positively correlated with total activities and specific activities of ^{32}P in shoots. Mycorrhizal P responsiveness (MPR) was positively correlated with shoot specific activities of ^{32}P. The results indicated that it might be possible to assess the relationship between mycorrhizal responsiveness and external hyphae-mediated P acquisition by a range of different cultivars.

P1 02

Distribution of 32P between roots and tops of white clover: effect of mycorrhizal fungi and placement distance from the roots

H .Mehravaran', A. Mozafar ' and E. Frossard' 'Urmia University 'Urmia ,Iran 'Swiss Federal Institute of Technology, Switzerland

In a greenhouse experiment we compared the effects of *Glomus intraradices* and indigenous mycorrhizal isolates from soils under plow and no-till treatments on the uptake of32 P placed at 1,2.5, and 4 cm from the roots of white clover (*Trifolium repence*) at 23, 37, or 46 days after planting. The colonization of the roots with regard to hyphae, vesicle or arbuscles was significantly different between fungi. Uptake of 32P from different distances from the roots was strongly dependent on the mycorrhizal isolate used and the plant symbiont age. Indigenous fungi were more effective in transporting 32P from short distances compared with *G. intraradices*. At the age of 46 days, for example, fungi from no-till and plowed plots transported 8-1o times more 32P to the plants as compared with *G.intraradices*, when 32P was placed at 1-cm distance from the roots. These differences, however disappeared when 32P was placed at 2.5 and 4 cm from the roots. As the plant-symbiont grew older, relatively more 32P was transported to the tops and the differences between fungi with this respect became more pronounced. In 21-, 37-, and 46-day-old plants, for example, and, when 32P was placed 1 cm from the root surface, 18, 21, and 56%, respectively, of the absorbed 32P was transported to the tops in plants inoculated with fungi from plowed fields. Progressively lesser amounts of total absorbed 32P were transported to the tops as 32P was placed farther away from the roots. For example 46 days after planting 51, 30, and 11% of the total absorbed 32P was transported to the tops when 32P was placed at 1, 2.5, and 4 cm from the roots, respectively, in white clovers inoculated with fungi from no-till soil. It is concluded that different mycorrhizal fungi may have different effects on the partitioning of absorbed phosphorus within the white clover.

P1 03

The effect of vesicular-arbuscular mycorrhizal association on growth of cotton (Gossypium hirsutum L.)

KIANMEHR, H.
Ferdowsi Univ. Mashhad, IRAN

Abstract:

In this investigation influence of VAM association and the effects of inoculum level on growth of cotton was studied. Cotton plants were grown under three treatments, sterile field soil (treatment III), natural field soil without inoculation (treatment II) and natural field soil with inoculation (treatment I). Inoculum consisted of a suspension of mycorrhizal roots from infected field cottons. Plants were harvested 3 months after planting,the amount of root colonization, spore population, available phosphorus concentration in each treatment were determined. Analyses were made , on stem diameter, plant height, leaf number, leaf area, P concentration, plant dry and fresh weight , and shoot to root ratio. using the SAS software, analysis of variance (ANOVA) was performed to examine differences in plants growth between various treatments. Inoculated mycorrhizal plants showed increase in growth and high concentration of phosphorus as compared with the other two treatments. The phosphorus content of inoculated soil were lowest, which indicates increased phosphorus absorption by inoculated plants. High colonization of roots of inoculated plants showed that amount of root colonization in cotton directly influences plant growth.

P1 04

Maize growth curve in response to arbuscular mycorrhizal association

A.Gholami . Shahrood university , Iran.

Arbuscular mycorrhizal fungi have symbiotic association with many crops and therefore increased crop yields. In this study potential benefits of arbuscular mycorrhizal fungi (AMF) were evaluated in two years farm experiments. The fungal treatments were:*Glomus mosseae*,*Glomus caledonium*,*Glomus intraradices* and sterile (control) plots. Also growing degree days (GDD) values calculated and used for curve fitting and estimation of growth equations. The results showed that application of fungal inoculum had significant effects on maize dry weight(DW) at both years. *G. caledonium, G. mosseae* , *G. intraradices* and sterile plots had the highest and lowest effects on total Dw, respectively.Results showed the maize growth curve along growing season follows logistic equations and regression coefficient of total DW on *G. mosseae* ,G . *caledonium* ,*G. intraradices* and sterile plots were 0.97, 0.96 , 0.98 and 0.98 respectively. Also comparison of growth curves showed that the trend of increasing total DW at the end of growing season were stopped at the lower GDD values in sterile plots and *G. intraradices* treatments and therefore resulted the lower maize DW in these treatments.In this research , crop growth rate (CGR) increased during growing season then decreased at the end of season. But the rate of crop growth (curve slope) and time of maximum CGR were different in fungal treatments.The highest CGR obtained from application of G . *caledonium* . In this treatment CGR maximized at 1540 GDD and then lightly decreasead. The lowest CGR obtained from sterile plots.

P1 05

Predicting potential phosphorus and zinc fertiliser savings by arbuscular mycorrhizas using a multiple Mitscherlich approach.

N.P. Seymour, J.P Thompson and A.M. Kelly
Queensland Department of Primary Industries, Leslie Research Centre, Toowoomba.

In Queensland vertisols, crops following a long fallow or non-mycorrhizal host may show deficiencies of phosphorus and zinc, requiring high fertiliser inputs to compensate for the decline in AM inoculum. Significant savings in P and Zn fertiliser can be made by the presence of high levels of AM colonisation in the crop roots. Glasshouse experiments determined the response of linseed to jointly varying rates of P (0 to 800 mg kg^{-1} of soil), Zn (0 to 60 mg kg^{-1}) and mycorrhizal inoculum (0 to 75 *Glomus mosseae* spores g^{-1}) added to a pasteurised vertisol.

The results confirmed the very high mycorrhizal dependency of linseed. Without mycorrhiza, linseed required extremely high, uneconomic additions of P and Zn to achieve full growth potential (400 mg P and 15 mg Zn kg^{-1}). With AM, good growth was achieved with no added P and just 3.75 mg Zn kg^{-1}, and maximum growth with 200 mg P and 7.5 mg Zn kg^{-1}. Eight spores g^{-1} of soil gave good colonisation levels causing no plant growth or yield penalties compared to higher levels of VAM inoculum. High levels of spores in the soil (up to 75 spores g^{-1}) led to better P and Zn nutrition and faster growth of the linseed plant due to faster colonisation of the roots (shorter colonisation lag phase). A multiple Mitscherlich model was fitted to dry biomass for extractable-levels of P and Zn, and AM inoculum level. Solving the equation given P or Zn status, AM levels, or yield allows prediction of potential savings in P or Zn.

P1 06

Are mycorrhizas more effective at P uptake in organic than conventional farmland? A test of mycorrhizal functioning in response to management in the U.K.

Gemma Muckle[1], Jonathan Leake[1] & Alastair Leake[2] [1]University of Sheffield, U.K.
[2]CWS Agriculture, Stoughton, Leicestershire, U.K.

Sustained yields in low intensity, organic management, where soluble phosphatic fertilisers are not permitted, are dependent on greater efficiency of nutrient use than in conventional agriculture. Arbuscular mycorrhizal fungi (AMF) are thought to play a significant role in these processes - but little is known about the responses of AMF functioning to different management intensities in the U.K. It was hypothesised that under organic management the lower P status and reduced frequency of ploughing would enhance mycorrhizal functioning and plant P uptake efficiency in comparison to integrated and conventional management.

This hypothesis was tested using intact turfs taken from arable (wheat) and grassland fields under organic, integrated and conventional management in Leicestershire, U.K. The turfs were placed in the outer compartments of pots containing a central root-free hyphal compartment (containing a sand-soil mixture) separated by a 35μm pore sized nylon mesh barrier. Anion-exchange resin membrane, loaded with ^{33}P, was inserted into the hyphal compartment, and the uptake of ^{33}P from the membrane by AMF was traced into plants. Controls had hyphae severed by regular cutting along the dividing mesh to prevent ^{33}P movement out of the central compartment by the AMF hyphal pathway. The results are discussed in relation to the possible long-term effects of management intensity on mycorrhizal functioning in U.K. farming.

P1 07

Influence of different types of fertilizer on the colonisation of rye (*Secale cereale*) by arbuscular mycorrhizal fungi

M.J. Gollner and B. Freyer, Institute of Organic Farming, University of Agricultural Sciences, Vienna, Austria

In our experiments the hypothesis was tested that farmyard manure fertilization increases the degree of colonisation of mycotrophic arable crops with AMF. In two field trials in different climate regions of Austria the effect of different fertilization treatments on the colonisation of *Secale cereale* with AMF was examined. One trial is located in the pannonic region (average temperature: 9.8 °C, average rainfall: 554 mm a^{-1}) with the following fertilization treatments: no fertilizer, mineral fertilizer (N/P/K in kg ha^{-1}: 122/135/208), farmyard manure (equiv. N/P/K in kg ha^{-1}: 110/33/96). The second trial is located in the alpine region (average temperature: 6.8 °C, average rainfall: 1010 mm a^{-1}) and three different organic fertilizers were used (slurry (equiv. N/P/K in kg ha^{-1}: 91/16/105), rotted farmyard manure (equiv. N/P/K in kg ha^{-1}: 81/27/83) and farmyard manure compost (equiv. N/P/K in kg ha^{-1}: 71/27/75)). The field trial in the pannonic region was designed as a large plot without replication. This field trial was used for our research because of the long continuous management duration (since the year 1906 *Secale cereale* is grown in monoculture and in rotation with *Hordeum vulgare* and complete fallow with constant fertilization). On both sites it could be shown that the fertilization with farmyard manure has positive effects on the colonisation of *Secale cereale* with AMF compared with mineral fertilizers or semi-liquid manure. At the "rye long-term experiment" a higher colonisation rate could be determined with *Secale cereale* in monoculture than in rotation with *Hordeum vulgare* for all fertilizer treatments.

P1 08

Poor growth of field crops following canola (*Brassica napus*) due to insufficient mycorrhizas

J.P. Thompson, K.J. Owen, T.G. Clewett and R. Reen
Queensland Department of Primary Industries, Leslie Research Centre, Toowoomba

Arbuscular mycorrhizas are important contributors to sustainable field crop production in the northern grain region of Australia where various cereals, grain legumes and oilseed crops are grown in both winter and summer seasons. The most important limiting nutrients in the alkaline clay soils are nitrogen, phosphorus and zinc. Rainfall is quite variable resulting in enforced fallows and flexible 'opportunity cropping'. Our previous research has shown that decline in inoculum of arbuscular mycorrhizal (AM) fungi during clean fallowing can result in increased severity of P and/or Zn deficiencies in following crops.

Canola has been used to reduce the incidence of soil-borne diseases in other cropping systems and we are researching its value for managing root-lesion nematodes (*Pratylenchus thornei*) in wheat and chickpea rotations. However, canola is a non-host of mycorrhiza. We have found poorer growth of a number of crop species (linseed, cotton, chickpea and wheat) after canola than after wheat in both farmers fields and research experiments. Sampling roots has shown much lower proportions of mycorrhiza in crops after canola than after other crops that are AM hosts. In one experiment involving 15 pre-crops including five canola varieties, wheat produced nearly twice as much biomass after mycorrhizal pre-crops than after canola. There was a highly significant correlation between biomass or head density of the wheat crop and level of mycorrhizal colonisation of the wheat roots. Similar experimental results were obtained with poor chickpea following canola compared with mycorrhizal host crops.

P1 09

Effects of preceding crops and soil temperature on growth and arbuscular mycorrhizal colonization of maize plants

T. Karasawa', Y. Kasahara' and M. Takebe' 'National Agricultural Research Center for Hokkaido Region, Japan
Preceding crops have been reported to affect the growth and P uptake of succeeding crops because of their distinct effects on AM colonization. It has also been reported that low soil temperature particularly reduces plant P uptake. Therefore, the effects of preceding crops on the growth, P uptake and AM colonization of succeeding maize plants were examined at three soil temperatures in order to clarify the influences of low soil temperature on the growth responses of maize plants to preceding crops. Maize plants were grown in pots in which sunflower (AM host) or mustard (nonhost) crops had been cultivated as preceding crops. Soil temperature was adjusted to 15, 20 or 25 °C during cultivation of maize plants. Air temperature was adjusted to 25 °C (day) and 20 °C (night) in each treatment. The growth and AM colonization of maize plants declined as the soil temperature fell. However, the growth of maize plants after cultivation of sunflower crops was superior to that after mustard cropping at each soil temperature. Shoot dry weight of maize after sunflower cropping was 5.1-times (at 15 °C), 6.5-times (at 20 °C) and 5.0-times (at 25 °C) higher than that after mustard cropping. The AM colonization of maize roots after sunflower cropping was much higher than that after mustard cropping at each soil temperature. The results therefore suggested that the AM colonization of maize roots was affected by the preceding crops at each soil temperature and that cultivation of sunflower (AM host) crops improved the AM colonization of succeeding maize, which accelerated the P uptake and growth of maize even at a low soil temperature.

P1 10

Distribution and density of arbuscular mycorrhizae *and R. leguminosarum* associated with faba bean Grown in Egyptian soi

M. Attia*, S.M.S. Bader El-Din* and Y. Ishac****Agriculture Microbiology Dep. National Research Centre Dokki, Cairo, Egypt.***.**Agriculture Microbiology, Faculty of Agricultural, Ain Shams Univ., Cairo, Egypt. Arbuscular mycorrhizal infection and nodulation of faba bean plants; growing in different location in Egypt; were studied in relation to physicochemical properties. The population densities of *R. leguminosarum* bv. *viceae* in Egyptian soils ranged between 3.6x10 to 40x104 cells g-1 soil and nodule numbers were between 37 to 200 nodules plant-1 weighing 0.4 to 1.6 plant-1. The rhizobial counts and nodule mass correlated significantly with organic matter, total N, total P, clay; silt and sand. However, no significant correlation were recorded between either rhizobial counts or nodule mass and any of available phosphorus, CaC3 and salinity.The numbers of AM spores associated with faba bean plants grown in different soils ranged between 3x102 to 1.2x104 spores kg-1 soil and the levels of root infection ranged between 5 to 68%. The spores' numbers in soils and the levels of root infection correlated significantly with organic matter, total N, total P, salinity, clay, silt and sand. However, no significant correlation was found between spore numbers or mycorrhizal root infection and CaCO3. Significant correlation was found between numbers of AM spores in soils and levels of mycorrhizal root infection.The physicochemical conditions of soils that enhanced the development of *Rhizobium* were generally found to increase also the fungal colonization and spore production. The numbers of *R. leguminosarum* in soils were significantly correlated with the AM spore numbers. The nodule and mass significantly correlated with percentages of mycorrhizal infection.

P1 11

Abundance of rhizobia and vesicular-arbuscular mycorrhizal fungi on groundnut *(Arachis hypogaea)* in semi-arid Zimbabwe

Y.L. BESMER and R.T. KOIDE. Intercollege Graduate Degree Program in Ecology, Penn State University, University Park, PA USA.

Rotations with N-fixing legumes have been suggested as a means to increase cereal production in low-input agriculture in Zimbabwe. However, N-fixation in our sites is P limited. Vesicular-arbuscular mycorrhizal fungi can increase plant P uptake. The objective of this initial study was to determine the inoculum potential of rhizobia and VAM fungi on groundnut in eight soils collected from semi-arid Zimbabwe. The soils ranged widely in pH and texture. Both nodulation and VAM colonization varied significantly between the soils. Results show that 68 % of the variation in nodulation could be explained by the soil available P (Olsen). Contrary to expectations, VAM colonization was not a good predictor of nodulation, probably due to the sensitivity of the fungi to the low pH and high available P in some of the soils. P applications and VAM inoculations in one sandy soil which was low in available P, slightly acidic and with moderate nodulation and VAM colonization, increased nodulation and significantly increased the N content in the plant. Based on this, optimizing the tripartite interaction between the legume, bacteria and fungi holds the promise of increasing legume growth and N-fixation in subsistence agriculture without applying costly fertilizers.

P1 12

Mycorrhizal communities of *Pinus sylvestris* seedlings from bare-root nurseries in Poland

M.L Rudawska, T. Leski 'Institute of Dendrology Polish Academy of Sciences', Kornik, Poland

Mycorrhizas on 1-0 and 2-0 *Pinus sylvestris* seedlings produced in bare-root nurseries situated in north-west part of Poland were estimated. Mycorrhizal status these seedlings ranged from mycorrhizal-free to complete mycorrhization. Most roots were spontaneously colonized by indigenous ecto- and ectendomycorrhizal symbionts. The rate of ecto- to ectendomycorrhizal colonization was significantly correlated with nitrogen fertilization as measured by nitrogen concentration in the needles. Fungal communities associated with the fine feeder roots of these seedlings were assessed by a combination of morphological and molecular techniques. Morphological typing of mycorrhizas based on the structure and colors of their external hyphae and fungal mantle distinguished several ecto- and few ectendomycorrhizal morphotypes. To identify these mycorrhizas fungal rDNA was amplified using polymerase chain reaction and digested with endonucleases (*Hinf* I, *Mbo* I, *Taq* I). *Thelephora terrestris* followed by *Hebeloma*, *Laccaria* and *Suillus* species were the most ubiquitous among basidiomycetes found on tested seedlings. Morphologicaly variable complex of ectendomycorrhizal morphotypes was dominated by ascomycetes giving identical RFLP pattern.

P1 13

Characterisation of ectomycorrhizal communities of willows (*Salix viminalis, S. dasyclados*) in a short rotation forestry plantation

Ü. Püttsepp[1] and A. F. S. Taylor[2], [1]Department of Ecology and Environmental Research, [2]Department of Forest Mycology and Pathology; the Swedish University of Agricultural Sciences, Uppsala, Sweden.

The aim of the study was to analyse the diversity of ectomycorrhizal communities associated with two different clones of *Salix* (*S. viminalis* and *S. dasyclados*) commonly used in short rotation forestry plantations. Based on morphological characterisation, nine groups of ectomycorrhizal morphotypes were distinguished. Root-tips of the morphologically similar groups were examined using molecular techniques (PCR and sequencing) for taxonomical identification. Morphological groups with well-defined features (*Tomentella* spp.) showed limited taxonomical variation, while groups with ill-defined features contained wider taxonomic variation, including unrelated species (eg. *Laccaria* spp., and *Hebeloma* spp.). Colonisation was not restricted to root tips, but occurred on any unsuberised root surface. This colonisation was estimated using the magnified intersections method. The most common ectomycorrhizal structure was the combination of the Hartig net and the mantle, which occupied nearly 20% and 32% of the root length of *S. viminalis* and *S. dasyclados* respectively. The preliminary results indicate little difference between the ectomycorrhizal communities in the two studied *Salix* species.

P1 14

Growth of wild and cultivated lettuce inoculated with *Glomus intraradices* : response to variations in nitrogen and phosphorus addition

D.K. Miller,[1] L.E. Jackson[2] and S.E. Smith[1]. [1]Adelaide University, Australia, [2]University of California, Davis, USA.

Horticultural crops have been bred for increased yield over many years with little or no concern for possible effects on mycorrhizal symbioses. In some crops breeding is thought to have reduced the capacity of the plants to benefit from the symbiosis. At present *Lactuca serriola* (wild lettuce) is being used in breeding experiments to increase rooting depth of *Lactuca sativa* (cultivated lettuce). *L sativa* is a high input crop with a high water and nutrient demand whilst *L .serriola* is tolerant to low water and nutrient conditions. With such broad environmental requirements, the mycorrhizal response along with corresponding growth, colonization, phosphorus and nitrogen tissue concentrations may differ with mycorrhizal inoculation.

Wild and cultivated lettuce were inoculated with *Glomus intraradices* under varying N and P conditions to compare rates of colonisation and to assess whether use of *L. serriola* in breeding programs is likely to have a significant influence on potential mycorrhizal benefits. Results will be presented and their importance/implications discussed.

P1 15

Arbuscular fungi and mycorrhizae at asian pear orchards in Korea

J. W. Choi[1], S. K. Yun[1] and H. L. Kim[2]
[1]Naju Pear Research Institute, National Horticultural Research Institute, Korea [2]Horticultural Soil Management Lab., National Horticultural Research Institute, Korea

Arbuscular mycorrhizal fungi (AMF) of asian pear (*Pyrus pyrifolia*) trees growing in the Naju region of southern Korea was surveyed from 1999 to 2000. Thirty-nine soils of pear rhizosphere from three cultivation types, weed control with chemical or tillage, sod cover and 60ℓcontainer were examined. All soil samples contained spores of arbuscular fungi, container cultured soils had more numerous than field soils. AMF spores were generally abundant in the conditions of pH 5.5~6.5, total nitrogen content under 4.0 g/kg, available phosphate under 1,000 kg/kg and negatively correlated with soil P levels. Pot cultures made with sample soil were placed in a growth room and spore populations were assessed. Six *Glomus* and three *Gigaspora* were found in the identified fungi on the basis of spore extraction.

Colonization was obrerved in feeder roots of two year old seedlings of four pear species after growth in a nursery with silty lome soil in July 2000. The major features of endomycorrhizae, hyphae, arbuscules, vesicles were developed. Proportion and intensity of root colonization were varied, but the means were respectively 76.3%, 43.1%. 'Sakwabae' seedling with thicker feeder roots than others was most heavily colonized.

P1 16

Mycorrhization and plant growth of highbush blueberry (*Vaccinium corymbosum* L.) on farmland in Germany

J. Golldack[1], P. Schubert[2], H. Schwärzel[2], P. Lentzsch[1], B. Münzenberger[1], [1]Center of Agricultural Landscape and Land Use Research (ZALF), [2]Institute for Horticulture, Müncheberg, Germany

A highbush blueberry production system with a surface mulch amendment of Scots pine wood chips was established on sandy soils in the north-eastern lowlands of Germany. In this study, mycorrhizal infection and growth of three different varieties of *Vaccinium corymbosum* L. influenced by different amounts of inorganic and organic fertilizers were investigated. According to long-term plot studies, the highest yield was correlated with lowest and highest amount of inorganic fertilizer using variety ‚Duke‘ and ‚Reka‘, respectively. Mycorrhizal infection rates of the varieties ‚Duke‘ and ‚Reka‘ differed by fertilizer regimes. It could be shown that ‚Duke‘ had a high mycorrhizal infection rate at all fertilizer regimes. The mycorrhizal infection rate of ‚Reka‘ decreased with increasing amounts of inorganic fertilizer. These facts suggest a different sensitivity of the mycorrizal symbiosis. The mycorrhizal infection rates of young plants were increased by Agro-Biosol®, a fertilizer derived from fungal mycelium. However, these young plants showed signs of nutrient deficiency after application of Agro-Biosol®. The influence of decomposition on microbial activities was tested in a climatic chamber in pots with mulch amendment and soil from the above described field trial. In the mulch layer, microbial activity (DMSO reduction), alkaline phosphatase, nitrification and nitrate reductase activities were significantly increased by Agro-Biosol®. Nutrient supply by Agro-Biosol® decomposition seems to be used by microbial biomass and not primarily by plants. Thus, a change in management of Agro-Biosol® application in new plantations could be necessary and will be tested.

P1 17

Dispersal and Effectiveness of Mycorrhizal Fungi in a Non-sterile Strawberry Field.

F. D. Muamba[1], C. Hamel[1] and Y. Dalpé[2]. [1]McGill Univ., Ste-Anne-de-Bellevue, Canada; [2]Agriculture and Agri-Food Canada, Ottawa, Canada. There is very little information on the dispersal of introduced arbuscular mycorrhizal fungi (AMF) and on their competitive ability to persist in agricultural field soils from one season to the next, in the presence of resident fungal species. The objectives of this study were to measure the ability of mycorrhizal fungi to spread out from their initial points of introduction and to estimate the impact of mycorrhizal inoculation on strawberry growth and yield. Strawberry plants, non-inoculated or inoculated with *Glomus mosseae, Glomus macrocarpum*, or *Glomus versiforme*; or inoculated with both *G. mosseae* + *G. macrocarpum* were field-grown for two years. All of the introduced species became established in the field, against a background of indigenous AM species and were detected up to 24 cm away from the mother plants. Dispersal rates of fungal species were variable: 0.78 cm per week for *G. versiforme*, 0.94 cm and 0.90 cm for *G. mosseae* and *G. macrocarpum* respectively; and 1.02 cm/week for dually inoculated plants. Mixed inoculation with *G. mosseae* + *G. macrocarpum* improved mycorrhizal effectiveness, as measured by combined fungal spores and strawberry yield.

P1 18

Mycorrhizal colonisation and its effect on phenolic content, P uptake and growth in olives (*Olea europaea* L.)

T.R. Ganz, L.K. Abbott and S.G. Kailis Faculty of Agriculture, The University of Western Australia, Australia

The extent of arbuscular mycorrhizal (AM) colonisation in olives, phosphorus uptake, growth and tissue phenolic content was assessed after five months growth of olive rootlings in a glasshouse. The olive cultivar Mission was grown under five levels of applied soluble phosphate, with or without inoculation with a mixture of three AM fungi (*Glomus invermaium*, *Acaulospora laevis* and *Scutellospora calospora*). Colonisation was high across all phosphate treatments ranging from 58-77% of root length mycorrhizal. Without inoculation, maximum growth of olives occurred at the fourth highest level of applied phosphate. With inoculation, maximum growth occurred at the first level of application of phosphate. Total leaf and root phenolics in inoculated plants was the same with increasing applied phosphate but both decreased markedly with increasing phosphate supply for non-inoculated plants. The reduction in the non-inoculated plants was due to a reduction in two phenolic fractions (phenolic acids + aldehydes and phenolic glycosides + tannins). Although the total phenolic content was not affected by phosphate supply for the inoculated plants, there was a change in its composition. The phenolic acid + aldehyde fraction decreased and the cell wall conjugated phenol fraction increased.

P1 19

Construction of sustainable fruit growing by vesicular-arbuscular mycorrhizal fungi: Introduction of new soil management

T. Ishii[1], S. Kirino[2] and K. Kadoya[3] [1] Faculty of Agriculture, Kyoto Prefectural University, Kyoto, Japan, [2] Ehime Chuo Agricultural Cooperative Association, Ehime, Japan, [3] Faculty of Agriculture, Ehime University, Ehime, Japan

We propose that the introduction of a sod culture system using Naginatagaya (*Vulpia myuros* (L.) C. C. Gmel.) and bahiagrass (*Paspalum notatum* Flügge.) that enhances the activity of vesicular-arbuscular mycorrhizal (VAM) fungi has a good effect on constructing the low input and sustainable fruit growing. By introducing the sod culture system, we can reduce the amounts of agrochemicals and chemical fertilizers. Particularly, no herbicides will be necessary for weed control. In Japan, the *V. myuros* plants die in summer season, cover the surface of soil like mulching of rice straw, and inhibit the growth of weeds in this season. Since a great network system between fruit trees and these grasses by VAM hyphae is formed in the soil of the orchards, the competition for absorption of nutrients and water is scarcely observed. Besides, erosion of both top soil and minerals is prevented by these grasses, and minerals will circulate in the plan-soil system without being lost. Use of liquid fertilizers containing some VAM stimulants through an overhead sprinkler system is also very effective not only in enhancing the activity of indigenous VAM fungi in soils and in increasing the percentage of VAM infection in roots, but also in reducing the fruit production costs.

P1 20

Effect of AM fungi on growth and nutrient uptake in some endemic forest tree species of the Western Ghat forests of Karnataka, India

P.Rama Bhat and K. M. Kaveriappa
Dept. of Applied Botany, Mangalore University, Mangalagangothri – 574 199, Karnataka, India

Inoculation of the seedlings of three tree species viz., *Gymnacranthera farquhariana, Knema attenuata* and *Myristica fatua* var. *magnifica* with five species of AM fungi namely, *Acaulospora laevis, Gigaspora gigantea, Glomus fasciculatum, G. geosporum* and *G. macrocarpum,* individually and in combinations such as *A. laevis* and *G. gigantea, A. laevis* and *G. macrocarpum, G. gigantea* and *G. macrocarpum* showed significant growth increment and increase in NPK content of the inoculated plants after twelve months. The effects of different types of inocula on the parameters such as height, root length, number of leaves, dry weights of leaves, stem and root and plant dry biomass of each plant species varied. The effect of combined inocula of all the five AM species was maximum on the biomass of *G. farquhariana*, and *M. fatua* var. *magnifica*, while the effect of combined inocula of *Gigaspora gigantea* and *Glomus macrocarpum* was maximum on the biomass of *K. attenuata*. The phosphorus content was maximum in *G. farquhariana* with the combined inocula of all the five AM species was. In *K. attenuata* the phosphorus content was maximum with the combined inocula of *G. gigantea* and *G. macrocarpum* and in *M. fatua* var. *magnifica* it was maximum with combined inocula of *A. laevis* and *G. gigantea*. Maximum spore density was obtained in the rhizosphere of all the three plant species inoculated with combined inocula of all the five AM species. The mycorrhizal dependency was maximum in *G. farquhariana* and *M. fatua* var. *magnifica* with combined inocula of all the five AM species, while in *K. attenuata* it was maximum with *G. geosporum*.

P1 21

Effect of three different fertilization levels on the development of arbuscular mycorrhizal *Phoenix canariensis*

B. Dreyer, A. Morte and M. Honrubia
Dpto. Biología Vegetal, Universidad de Murcia, Spain

Palms are known to be mycorrhizal. The potential of mycorrhizae to enhance plant growth would be of great interest for the management of cultivated palms, due to their slow rate of growth. Inoculation of canary palms (*Phoenix canariensis* Hort. *et* Chabaud) with *Glomus mosseae*, *G. intraradices* and *G. deserticola* increased plant growth compared with non-mycorrhizal plants. Three different fertilization levels were tested in combination with inoculation and *G. mosseae* was the most efficient arbuscular mycorrhizal (AM) fungus in all conditions. The results also showed that a reduction in the amount of fertilizer used accompanied by inoculation with *G. mosseae* resulted in greater plant growth than normal fertilization treatment without inoculation.

Mycorrhizal colonization was low for all treatments and the proportion of arbuscules, vesicles and hyphae varied between the different AM fungi assayed.

There were no great differences between the nutrient content of mycorrhizal and non-mycorrhizal plants, suggesting that the growth enhancement observed involved a non-nutritional mechanism.

The use of AM fungi in palm production systems would allow a significant reduction in fertilizer levels and accelerate growth, thus reducing the cost of the palm culture.

P1 22

GROWTH AND NUTRIENT UPTAKE OF *Calliandra calothyrsus* AS AFFECTED BY ARBUSCULAR MYCORRHIZAL INOCULATION AND FERTILIZATION WITH TWO DIFFERENT P FERTILIZER

R.D.M. Simanungkalit[1] and D.R. Lukiwati[2]
[1]Research Institute for Food Crops Biotechnology, Bogor, Indonesia
[2]University of Diponegoro, Semarang, Indonesia

The effect of arbuscular mycorrhizal fungi inoculation and application of two different P fertilizer on growth and nutrient uptake of *Calliandra calothyrsus* was studied in a glass house experiment using an acid latosolic soil as a growth medium. The treatments were mycorrhizal inoculation (control and inoculation with *Glomus fasciculatum*) and P fertilizer forms (control, Christmas Island rock phospate and triple superphosphate). Mycorrhizal inoculation could improve significantly the growth and nutrient uptake of calliandra. The effect of inoculation on plant height was shown as early as 8 weeks after planting with an average increase of 22%. Inoculation increased dry matter weight by 649-2000%, and the uptake of N, P, S, and Zn by 989%, 1039%, 831% and 1067% respectively. The application of triple superphosphate in combination with *Glomus fasciculatum* showed much better effect on all variables measured than that of Christmas Island rock phosphate.

P1 23

Occurrence, Activity, and Diversity of AM fungi in Grapevines from the Pacific Northwest United States

R.P. Schreiner. USDA-ARS-Horticultural Crops Research Lab, Corvallis, OR, USA

The importance of arbuscular mycorrhizal (AM) symbiosis in perennial cropping systems is largely unknown. Grapevines (*Vitis spp.*) may be particularly dependent on mycorrhizal fungi, owing to their low root densities in soil. An examination of 70 commercial vineyards in Oregon and Washington has shown grape roots to be extensively colonized by AM fungi. Mean colonization of fine roots by AM fungi at veraison was 69% and 73% in Oregon and Washington vineyards, respectively. Arbuscular colonization was significantly greater in Oregon vineyards, averaging 48% at veraison in 1999, versus 19% in Washington vineyards at veraison in 2000. Arbuscular colonization of roots in Oregon vineyards was correlated most strongly to soil pH. The diversity of AM fungi in grape roots from 7 Oregon vineyards was assessed using amplified and cloned 18s ribosomal gene sequences. We have identified 15 potential AM-fungus species from grape roots with an average richness of 8 species per vineyard. However, 4 *Glomus* species dominate our root samples, representing 85% of the cloned products. Two of the four dominant species are *G. intraradices* and *G. mosseae*. A study of the spatial and temporal dynamics of mycorrhizae in a mature *V. vinifera* vineyard showed that arbuscular colonization of fine roots increased prior to bud break, continued to rise until veraison, dipped slightly, and increased again after harvest. Arbuscular colonization was related to known carbon allocation patterns within vines and to the timing of nutrient uptake from soil. AM fungi play a major role in real-world, grapevine production systems.

P1 24

Recent studies on AM symbiotic association with mangosteen in Malaysia

M. Masri, Malaysian Agricultural Research and Development Intitute, Malaysia

Mangosteen (*Garcinia mangostana* L.) is a delicious tropical fruit but has not been commercially grown in Malaysia mainly due its long juvenile period resulting from the extremely slow growth of the seedlings. We have been investigating the potential of inoculating arbuscular mycorrhizas (AM) to promote growth. Two-months old seedlings were inoculated with *Glomus mosseae* (GM), a mixture of *G. mosseae* and *Scuttelospora calospora* (MS) and uninoculated seedlings (-M) were regarded as controls. Growth of these seedlings in polyethylene bags was monitored and they were subjected to water stress conditions after 18 months.

Results show that the introduced fungi infected more than 60% of the total root length. Infection increased plant height by 25-34%, total dry biomass by 40-64% and number of leaves by 23-43% as compared to the controls. Improvement in growth was primarily due to increased P-uptake and positive alterations of the root systems. P-uptake efficiency was 22-28% higher while P-utilization efficiency was 9-11% higher in mycorrhizal plants. AM inoculation was also found to increased root length density by 58-60%, root branching density by 20-30%, number of laterals by 15-26% and number of root tips by 22-25%. Leaf water potential of inoculated seedlings were maintained relatively higher and dropped gradually during the stress period. Similarly, stomatal conductance of inoculated seedlings also dropped gradually with slower rate as compared to uninoculated controls. These investigations clearly indicate that AM association with mangosteen seedlings could promote growth and improved the overall quality of the planting materials.

P1 25

New *In vitro* cultures @ Premier Tech

Beland, Marc and Moutoglis Peter, Premier Tech, Riviere-du-Loup, Canada

To create future products that will meet customer needs and respect the environment, new, more efficient species characterized as growth stimulators or pathogen deterrants are being cultured via conventional and *in vitro* means at Premier Tech Biotechnologies.

Several species from different genera were grown with conventional greenhouse methods using soil-less substrates (mineral-organic) and traditional plant hosts (leek, sorghum, etc). Plants were harvested, the roots and substrate were analyzed for internal colonization, total propagule numbers and for the presence of plant pathogens. The new species produced in the greenhouse were introduced into aseptic culture using a modified spore disinfection procedure. Surface sterilized spores were placed on minimal (M) media and incubated at 27°C with or without a CO_2 enriched environment depending on the species. Treatments of buffered versus non-buffered media, varying pH, as well as different host plants were compared. Germination of spores occurred within 2 weeks after being placed in CO_2 incubators. The presence of CO_2 and biological buffers increased spore germination of certain species. Media with a pH below 7 (non-buffered) however, seemed to inhibit germination. Non-colonized roots of both carrot and clover were placed near the germinating hyphae to initiate colonization. After eight weeks incubation at 27°C, a dense hyphal network was established upon which new spores were produced indicating colonization had occurred. At 12 weeks, new spores were transferred to fresh media with new roots to maintain the symbiosis. Root samples were stained and the colonization morphology was compared to conventionally cultured mycorrhizae.

P1 26

PTB's Research Report

Moutoglis, Peter and Beland, Marc, Premier Tech Biotechnologies, Riviere-du-Loup, Canada

Since 1983, Premier Tech Biotechnologies has been working on important research and development studies in order to come up with leading edge technology in mycorrhizal fungi production. The use of the most sophisticated techniques and equipment allows Premier Tech Biotechnologies to manufacture mycorrhizal-based products of unrivaled and superior quality on an industrial scale.

This along with a long history of collaborative university partnerships geared to test and validate product performance and efficiency have resulted in a compilation of a published and non-published mass of knowledge and information some of which will be shared for the first time. Results of increases in biomass, yield, and plant stress tolerance using Premier Tech's MYKE™ and MYCORISE® PRO products, will be presented from studies in the following sectors: nursery, fruit & vegetable production, turf and land re-vegetation.

This innovative process is revolutionizing the horticultural industry by bringing a solution to the reduction and in some cases, the loss of a most versatile and effective organism: the mycorrhizal fungus. Premier Tech Biotechnologies is developing a unique manufacturing expertise for products containing components which are essential to optimal plant growth. Due to its concern with environmental protection and public health, Premier Tech Biotechnologies creates environmentally-friendly products which supply natural elements critical to healthy plantations.

P1 27

Using alginate as a sticking agent for production of AM seedlings of *Acacia mangium* (Willd.) in aeroponic culture.

J. Weber[1,3], O. Crassard[2], S.K. Lee[1], F.Y. Tham[1], Y. Prin[2], P. Durand[2], M. Ducousso[2].

[1]Nanyang Technological University/ Nanyang Institute of Education, Biology Division, 1 Nanyang Walk, 637616 Singapore

[2]CIRAD-Forêt. Laboratoire des Symbioses Tropicales et Méditerranéennes, 34398 Montpellier Cedex 5, France

[3]UMR INRA-UHP Nancy "Interactions Arbres/Micro-organismes" Faculté des sciences BP 239 F. 54506 Vandoeuvre lès Nancy, Cedex, France

Sodium alginate was used as a sticking agent for direct inoculation of AM spores, as well as crushed root inoculum, of *Glomus* sp. on aeroponically grown *Acacia mangium*. The root systems of one-month old saplings were cut to 6cm length prior to inoculation with AM. Six weeks after inoculation, for both treatments, up to 18 cm of the well-developed root systems in aeroponics had AM with typical arbuscules and vesicles. No spores were observed even after another month in aeroponic culture. A single inoculated sapling in aeroponics generated enough crushed root inoculum to inoculate 50 new trees. Aeroponic culture appears to be an easy way to produce in one step, both mycorrhizal saplings of *Acacia mangium* and large quantities of AM inoculum.

P1 28

Sustained root development in vitro obtained in *Pinus pinea* with the help of ectomycorrhiza-derived fungi

Oliveira, P.[1], Barriga, J.[1], Cavaleiro, C.[2], Peixe, A.[2], Potes, A.[2]

[1]Centre for Applied Ecology and [2]Institute for Mediterranean Agriculture Sciences, University of Évora, Portugal oliveira@uevora.pt

Stone pine (*Pinus pinea* L.) is an important fruit crop in mediterranean climates and has been the target for selection efforts through micropropagation. Previous attempts reached incipient rooting after induction with a combination of auxin and hypertonic shock, but their development *in vitro* was not sustained. At this stage, coculturing the stalled plantlets with fungi isolated from ectomycorrhiza succeeded in overcoming this barrier and the satisfactory development of small trees in vermiculite and later in soil. Different fungi were used, isolated from ectomycorrhiza collected in mature pine stands and also from eucalyptus and oak. Pine plantlets were obtained through micropropagation *via* organogenesis from mature seeds cotyledons. About half of the fungal isolates tested have produced the desired effect, but diffusible signals, rather than a direct contact of the mycelia with the roots, seemed to be involved. Although control plants (in the absence of fungi) were found to develop roots on the acclimation stages in vermiculite, their growth was by comparison quite modest. The root systems of some inoculated plants apparently had ectomycorrhiza, presumably carried over when the plants were transferred from the co-cultures to vermiculite.

Supported in part by PAMAF 2090 project

P1 29

EFFECT OF SEVEN INDIGENOUS MIXTURES OF AMF IN COOFFEE PLANTS UNDER FIELD CONDITIONS.

D. Trejo., R. Ferrera-Cerrato., R. García., M. Escalona., and L. Lara-Capistrán. Agriculture Science School, Universidad Veracruzana ,Mexico

The purpose of this work was to compare the effect of seven indigenous mixtures of AMF in coffee plants in one orchard with a great damage of "corchosis" (*Meloydogine incognita + Fusarium* sp.). Four months old coffee plants were planted around a coffee plant in productive stage. The experiment design was a randomized complete (7 indigenous mixtures AMF inoculo) with eight replicates.Thirty days after the they were planted measurement were carried out of the following; hight ,diameter, leaves number and survival. It was found statistical differences among inoculum and the best results were obtained from soil with low fertility and strong dry season. The survival percentage of inoculated plants was 90 percent while the copntrol was 45 percent. The inoculum efficiency was different in nursery as compare with field.

P1 30

Integrated use of indigenous Arbuscular mycorrhizal fungi and farm yard manure saved fertilizer input, improves grain yield and nutrient uptake by wheat (*Triticum aestivum*) grown in an alfisol

Mahaveer P Sharma and Alok Adholeya
Centre for mycorrhizal research, Tata Energy Research Institute, Habitat Place Lodhi Road, New Delhi -110003, INDIA

Abstract

The effect of inoculating wheat with the indigenous mycorrhizal fungi with and without the combinations of fertilizer and farmyard manure (FYM) application in a nutrient-deficient soil was studied in a field experiment using split-plot; randomized block design. Four levels of fertility combinations viz., I, N: P: K+FYM 100, 50, 40 kg/ha and 20 ton/ha; II, N: P: K+FYM 100, 25, 40 kg/ha and 20 ton/ha; III, N: P: K+FYM 100, 50, 40 kg/ha and 40 ton/ha; IV, N: P: K+FYM 200, 100, 80 kg/ha and 20 ton/ha ± AM inoculation constituting 8 treatments. The mycorrhizal inoculated plots irrespective of any fertility level showed significantly higher removal of macro- and microelements over their respective uninoculated counterparts. Significantly higher soil dehydrogenases activity was observed in the inoculated plots at all the fertility levels with maximum in the plots where FYM was applied @ 40 ton/ha. Plant P and N uptake analysed at harvest increased significantly following inoculation with AM fungi with FYM applied @40 ton/ha. At harvest, grain yield, straw yield and number of tillers was significantly higher in the inoculated plots with maximum at 40 ton/ha FYM over their respective uninoculated counterparts. Inoculation with indigenous mycorrhizal fungi also significantly improved root colonization and number of infectious propagules in soil determined at harvest

P1 31

Effect of conventional tillage versus no-till on indigenous arbuscular mycorrhizal fungi of triticale and wheat under field conditions.

I. Brito, P. Antunes and M. Carvalho, University of Évora, Évora , Portugal,

Winter cereals are the most important crops in rotation in the southern region of Portugal. Traditionally wheat is the main crop, however the area cultivated with triticale is increasing. Although it is a promising crop there are only few studies on triticale as host plant for AMF.

The present study examined the influence of soil disturbance on the mycorrhizal colonisation of wheat and triticale included in a particular rotation (wheat - sunflower - triticale). Experimental plots have been managed under no-tillage and conventional tillage for 6 years. In the 6th year, during the vegetation period, roots were sampled every 15 days and AMF colonisation was assessed.

A significant tillage effect was observed on both triticale and wheat roots. When the maximum colonisation was reached, no tillage resulted in 20 % increase for triticale and 18 % for wheat. AMF colonisation levels rose gradually at different rates between the two species until late spring, dropping thereafter. At the end of the vegetation period this increase was less evident, 2 % for triticale and 6% for wheat.

P1 32

Functional diversity of AMF co-existing in agricultural soils subjected to different tillage

A. Mozafar[1], J. Jansa[1], R. Ruh[1], T. Anken[2], I. Sanders[3], E. Frossard[1]. [1] Federal Institute of Technology Zürich, Switzerland, [2] Federal Research Station for Agricultural Economics and Engineering, Tänikon, Switzerland,[3]University of Lausanne, Switzerland.

Monosporic isolates of four *Glomus* spp. obtained from both conventional and no-tillage treatments of a long-term tillage experiment were studied. Physiological properties of the fungi growing in symbiosis with maize (*Zea mays*) were assessed in terms of P transport over different distances from the roots, employing ^{33}P labeling and two types of compartmented system. (1) A system containing a large contact screen area (180cm^2), which assured linear growth of mycorrhizal mycelium through a root-free segment, and (2) a system where plant and radioactively-labeled soil segments were connected by a narrow tube (contact area 3.5cm^2), where rebranching capabilities of mycelium were assessed. Two physiological groups were identified among our fungal isolates. (a) *G. mosseae* and *G. intraradices* were able to transport P from a distance of 10 and 14 cm from the roots, resp. In the first system (1), the amount of P transported by AMF to the plants decreased almost linearly with distance, while in the second system (2) the decrease was similar to exponential function of distance. We also showed that *G. mosseae* obtained P mainly by colonization of a new space, while *G. intraradices* could take up P more efficiently from already colonized substrates. (b) Less efficient group of AMF (*G. clarum* and *G. claroideum)* showed exponential decrease of P transport with distance at the first experimental design, with maximum transport distance of only 6 cm from plant roots. In the second system, these fungi did not transport any P from distant compartments.

P1 33

Effects of soil disturbance on the efficacy of indigenous mycorrhizal fungi in enhancing nitrogen fixation by soybeans

M.J. Goss[1] and A. de Varennes[2], [1]University of Guelph, Canada, [2]I.S.A. Lisbon, Portugal

We used soil disturbance to study the interaction between indigenous AM fungi, *Bradyhizobium japonicum*, and soybean. Two cycles of corn, each of three weeks, were grown in sieved or undisturbed soil before soybeans were sown. Corn plants from undisturbed soil were taller and produced twice the biomass than plants from sieved soil. Plants from undisturbed soil accumulated more P and N. This was consistent with results from other studies which indicated that improved growth was due to the presence of an intact, extraradical mycellium at the start of AM fungal colonization. Soybean was sown in the pots with peat-based inoculum *Bradyrhizobium japonicum*, strain 532C. Soybean plants grown in undisturbed soil developed faster than did plants from sieved soil. There was a positive interaction between the two microbial symbionts in undisturbed soil that resulted in both colonizing roots of soybean earlier than in sieved soil. Colonization of soybean roots by AM fungi was still greater at podfill in undisturbed soil. Plants from undisturbed soil took up P from 10 days after emergence, while plants in sieved soil only started accumulation thirteen days later. P accumulation was 55% greater in undisturbed soil, than in sieved soil by podfill The dry weight of plants was 42% greater in undisturbed soil. Although the nodule number was not different, nodule dry weight was also greater in undisturbed soil than in sieved soil and the larger nodules fixed 3.5 times more atmospheric N_2

P1 34

Relationship between cultural practices and arbuscular-mycorrhizal activity in different orchards

K. L. Rutto[1], F. Mizutani[1], D-G. Moon[1] and K. Kadoya[2]
[1]The Experimental Farm, Ehime University, Hojo, Japan
[2]College of Agriculture, Ehime University, Matsuyama, Japan

It has been demonstrated that cultural practices involving soil disturbance and the use of agrochemicals affect population dynamics and function of arbuscular-mycorrhizal (AM) fungi. In order to study the effect of different production practices on mycorrhizal activity in orchards, a survey of spore populations, soil and plant mineral content and mycorrhizal infection (%) was conducted monthly on satsuma mandarin (*Citrus unshiu* Marc.), Iyokan (*Citrus iyo* hort. ex Tanaka), apple (*Malus pumila*), peach (*Prunus persica* Batsch), kiwifruit (*Actinidia chinensis* Planch.), Japanese persimmon (*Diospyros kaki* Thunb.), Japanese plum (*Prunus salicina* Lindl.), Japanese chestnut (*Castanea crenata* Sieb. et Zucc.) and grape (*Vitis labrusca* L.) subject to various cultural practices and growing in the Ehime University Experimental Farm. The survey was conducted over one production season (May-October, 2000).

Soil nutrient levels in all orchards were high owing to the periodic application of inorganic fertilizers, and consequently, leaf content of major elements was within the recommended range for normal growth and function. However, there was a considerable variation in mycorrhizal factors, viz. spore population and diversity and root infection levels depending on the presence or absence of under-storey plants and on the particular cultural activities performed on the different orchards. It is noted that cultural practices involving the extensive use of pesticides coupled with weed control by soil disturbance have a more diverse effect both AM fungal populations and function than soil nutrient level, in particular soil P content.

P1 35

Agronomic Significance of Arbuscular Mycorrhizal Fungi Activity in Soil P Dynamics in Ridge-Tilled Corn Production.

C.P. Landry[1,2], R.R. Simard[2], C. Hamel[1] and A. Vanasse[2]. [1]McGill Univ., Ste-Anne-de-Bellevue, Canada; [2]Agric. Ag. Can., Ste-Foy, Canada. The activity of arbuscular mycorrhizal (AM) symbiosis is greatly influenced by cultural practices. Because ridge-tillage (RT) favours soil organic matter accumulation and creates little soil disturbance, it is conducive to a better soil AM hyphal network development. Higher soil AM potential and corn grain yields were reported under RT as compared to mouldboard plowing. The role of the AM symbiosis on soil P dynamics has been studied very little especially in field situations. An on-farm study was conducted to clarify the effects of the AM symbiosis on soil P fertility under RT. Three P fertilizer rates (0, 40, 80 kg ha^{-1}) applied to corn (*Zea mays* L.) were randomized into main plots. Subplots were either left untreated or received Quentozen™ (Qt) applied as a drench to suppress AM growth. Qt application had no significant impact on overall soil biological activity (fluorescein diacetate assay), but decreased corn AM root colonization (18-72%). Plants in untreated plots were more colonized, took up more P and produced higher biomass than those in Qt plots. In mid June, *in situ* soil solution inorganic P (Pi), rhizospheric labile Pi and organic P levels in untreated plots were 56%, 33% and 27% lower than those in Qt plots. These results suggest that the AM symbiosis has a significant influence on soil P dynamics. P fertilizer recommendations for RT corn should be revised to consider the contribution of AM symbiosis to soil P mobilization

---

P1 36

Inoculation of efficient arbuscular mycorrhizae saved phosphatic fertilizer input on the post-vitro micropropagated strawberry plant in an alfisol.

Alok Adholeya and Mahaveer P. Sharma
Centre for Mycorrhizal Research, Tata Energy Research Institute, Habitat Place,
Lodhi Road, New Delhi- 110 003, INDIA

Abstract

The benefit to fruit yield of micropropagated strawberry cv. Pajaro due to inoculation of indigenous arbuscular mycorrhizal (AM) fungi was evaluated in two field experiments. AM response was evaluated at two doses of phosphorus i.e., 25 and 50 kg P ha^{-1} during the first experiment and at four doses i.e., 50, 100, 150 and 200 kg P ha^{-1} in the second experiment in a P deficient alfisol. In both the trials, inoculation significantly increased mycorrhiza formation over that caused by the level of native AM fungi. Also during hardening period, AM inoculated plants survived 7-8.5% more and saved almost 11 days for total hardening period compared to uninoculated plants. During the first trial, the threshhold level of P application for beneficial AM symbiosis was not met since the external P requirement of the plants was not found to be achieved. In second experiment, the doses were increased based on the P fixation capacity (experimental soil showed 54% P fixation). At harvest, all inoculated plants except those at the highest level of P applied (200 kg ha^{-1}), showed higher values of fruit size, yield/plant, unit weight, no. of runners, shoot dry matter, shoot P contents. However, the magnitude of AM response in yield was found to be different at various levels of P. Inoculated plants tend to have significantly greater fruit yield in plants grown at 150 kg P ha^{-1} and yield was comparable with the uninoculated plants grown at 200 kg P ha^{-1}. AM inoculation could be at least 35-40 % (calculated on the basis of saving on P fertilisers, hardening time and survival) cost economic when compared to uninoculated plants.

P1 37

Arbuscular-mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter

A Gaur and Alok Adholeya , Centre for Mycorrhizal Research, Tata Energy Research Institute, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi - 110 003, INDIA

Five fodder crops, *Zea mays*, *Medicago sativa*, *Trifolium alexandrinum*, *Avena sativa*, and *Sorghum vulgare* were inoculated with a consortia of indigenous arbuscular mycorrhiza (AM) in non-sterile phosphate deficient sandy loam soil amended with organic matter under field condition. Shoot and root dry weights and total uptake of P and N of all the test plants were significantly increased by AM inoculation. Mycorrhizal inoculation with exceptions in *M. sativa* and *S. vulagre* increased green yield by 160% in *T. alexandrinum* followed by 35% in *A. sativa*, and 27% in *Z. mays*. A great variation in dependence on mycorrhiza was observed among the fodder crops. *T. alexandrinum* showed a maximum dependence of 72% in contrast to 5.7% dependency in *S. vulagre*. Plant species showed differences in percentage AM colonization with a high root infection recorded in *Z. mays* (76%). Spore production and infectious propagules (IP) were as high as 78 and 103 spore/IP g^{-1} in *S. vulgare*. This study clearly indicates the potential of using indigenous AM inoculations in fodder crops grown in marginal soils along with *insitu* production of large-scale AM inocula.

P1 38

New Cultivation Methods for *Anoectochilus formosanus* Hayata

D.C.N. Chang', L.C. Chou' and K.C. Lee' Department of Horticulture, National Taiwan University, Taipei, Taiwan, ROC

Anoectochilus formosanus Hayata is a native perennial and terrestrial orchid plant grown in the forests of Taiwan. It is one of the beautiful Jewel orchids with medical uses in treating many kinds of diseases, such as cancer, high blood pressure, diabetes, snake bite, even liver, heart and lung diseases. Now they are mostly grown by using micropropagated plantlets or seedlings. But this orchid plant is very susceptible to *Fusarium*, *pithium* and mites. So pesticides and insecticides are applied very often to ensure their growth. In order to solve this problem, many attempts were made, and results showed that using (1) large glass jars containing agar medium, or (2) pots contained commercial growth medium then covered with OPP plastic bags, as the protection shield was very effective to prevent the infection of insects. As to diseases, more cautions should be made. Moreover, this cultivation practice needed no more watering and fertilization for the orchid plant up to one year. If beneficial orchid mycorrhizal fungi (OMF) were inoculated into the growth medium during transplanting, it would result in significant growth enhancement of these plants *in vivo*. The cost of the OPP plastic bags was only 1/50 of the big glass jars. This orchid was identified as a CAM plant even under the protection shield. It is highly recommended to use plastic bags to cultivate this orchid free from pesticides and insecticides.

P1 39

Morphological and molecular characterization of tropical orchid mycorrhizas

F.Y.Tham[1], X.S.Zhang[1] and C.L. Lim-Ho[2]
[1]Nanyang Technological University, Singapore [2]Singapore Botanic Gardens, Singapore

Eighty-seven fungal isolates were established from pelotons and surface-sterilized root sections of twelve terrestrial orchid species in Singapore. Sixty-seven of these isolates were studied in detail and characterized. A number of these appeared to be *Rhizoctonia* or *Rhizoctonia*-like fungi. Four morphological groups could be distinguished. To further distinguish these mycorrhizal isolates, molecular data was generated using polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) and randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). Molecular identification corresponded with morphological groupings, and further discriminated the fungi into 13 RFLP groups and 14 RAPD-PCR groups. Molecular analysis distinguished among isolates in the same morphological group and revealed that a single orchid host may harbour more than one type of fungal endophyte. The symbiotic relationship between orchid seedlings of a terrestrial orchid hybrid, *Vanda* Tan Chay Yan and some of the mycorrhizal isolates in co-culture experiments indicated that *in vitro* orchid seedlings inoculated with fungi may be useful in establishment and survival of plantlets on transplantation to the field.

P1 40

Fungi isolated from ericoid mycorrhizal roots of the Australian family Epacridaceae improve rooting of shoot cuttings

A.C. Lawrie[1], N. McDonald[1] and C.B. McLean[2]
[1]RMIT University, Melbourne, [2]Burnley College, University of Melbourne, Australia

Fungi isolated from ericoid mycorrhizal roots of *Epacris impressa* plants in Victoria produced typical ericoid mycorrhiza in *E. impressa plants in vitro.* Two of these fungi (E4-1-2 and Ei1-1) were tested for their ability to improve rooting of shoot cuttings in sterilised potting mix *in vivo*. Shoot cuttings were treated with IBA and left in potting mix for several days before inoculating with small cubes of one of the cultures grown on potato dextrose agar for several weeks. Control cuttings were uninoculated. After 3 months, cuttings were dug up and roots, shoot height and flowering assessed. Both fungi improved rooting significantly and formed abundant hair roots, whereas control plants formed very few thick roots. There was no significant difference in shoot height or flowering between inoculated and control plants. Despite the abundant hair root formation, little or no ericoid mycorrhizal infection was present in inoculated plants. This may be because of the limited time of incubation with the fungi. This suggests that the rooting response is hormonal in the first instance and that these fungi could be used as inocula to improve the low success rate in rooting of cuttings in horticulture. Longer incubation (6-8 months) with habitat soil produced abundant ericoid mycorrhizal infection in inoculated plants of several genera. Differences in survival, rooting and mycorrhizal infection were attributed to season of soil collection.

P1 41

Stimulation of adventitious rooting on cuttings from woody perennial plants by exposure to inoculum of ericoid and arbuscular mycorrhizal fungi

C.F. Scagel USDA-ARS, Horticultural Crops Research Unit, Corvallis, OR, 97330.

Whole plant responses to colonization with mycorrhizal fungi have been well documented; however there has been little research on how inoculation can alter adventitious root production during cutting propagation. We assessed the influence of ericoid and vesicular-arbuscular mycorrhizal fungi (VAMF) on rooting of cuttings from different woody perennial plants, including several cultivars of *Rosa, Kalmia, Arctostaphylos, Leucothoe, Gaultheria, Vaccinium, and Rhododendron spp.* In general, adding mycorrhizal fungi into the rooting media increased the number of roots and root growth of cuttings from several cultivars, but the level of response depended on plant species, the cultivar within a plant species, and the type of fungi. For some species, the presence of mycorrhizal fungi in the rooting medium stimulated a rooting response that was equal to or better than the response obtained by using hormone alone. Increases in root initiation and root growth were not always associated with increased levels of colonization.Responses of cuttings to mycorrhizal fungi were detectable prior to root formation and colonization. Plants that do not normally form associations with VAMF showed increased root initiation when VAMF were mixed into the rooting medium. Differences in protein, amino acid, and carbohydrate composition between cuttings exposed to VAMF or not were detectable within two to seven days after cutting. Our results show that incorporation of mycorrhizal inoculum into rooting media during cutting propagation can increase the root initiation and quality of rooted cuttings for different cultivars of woody perennial plants.

P1 42

Alternation of nuclear phase in the filamentous basidiomycete, *Helicobasidium mompa*

Tadanori Aimi; Yoshiki Yotsutani and Tsutomu Morinaga

Department of Bioresource Development, Hiroshima Prefectural University, Japan

Variation in the number of nuclei and cellular ploidy were observed in eight strains of *Helicobasidium mompa*. The basidiospores, single-spore isolates and field-isolated strains were all dikaryons. The cellular ploidy, which was assessed by analyzing the fluorescence emitted by DAPI-stained nuclei, was unstable: monokaryotic strains derived from the original dikaryotic strains by successive subcultures were mainly tetraploid, although the original dikaryon was in most cases diploid. On the other hand, a dikaryotic strain derived by treatment with benomyl was haploid. These results suggest that diploid dikaryon is a normal nuclear phase of *H. mompa* in nature, and the alternation of ploidy may be due to a feature of the mating system of this fungus. This research was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences.

P1 43

Molecular identification of truffle species in processed food products

A. Amicucci[1], C. Guidi[1], A. Zambonelli[2], L. Potenza[1] and V. Stocchi[1]
[1]Istituto di Chimica Biologica "Giorgio Fornaini", University of Urbino, Urbino (PU), Italy
[2]Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna, Bologna, Italy

Truffles are known above all for the organoleptic properties of the fruitbodies of several species. These, and other edible fungi, are sold both fresh and as conserved cooked products (canned, frozen, dried, etc.).

In our research, molecular identification techniques were applied to analyse food products containing edible mushrooms and truffle species. The fungal DNA samples were processed by analyses of the ITS region. PCR procedures using truffle species-specific primers, multiplex PCR, RFLP analysis, sequencing of the ITS region and specific oligonucleotide probe hybridisation were used. The analyses not only confirmed the presence of the prized truffles in some food products, but also highlighted the fraudulent use of other, less-prized species. The results reported herein demonstrate that the molecular identification methods offer enormous advantages in the identification of food products containing truffles or other edible fungi, in that **1** - their high sensitivity allows a reliable identification even when starting from minimum amounts of sample or degraded DNA; **2** - the method can be successfully applied to analyse samples with morphological features that have been highly altered due to the drastic treatments utilised for food preparation or to the use of unripe fruitbodies (lacking spores); **3** - such strategies have important applications in the analysis of food product, in both the preparation and commercialisation phases, in attempt to avoid and reveal commercial frauds and detect the possible presence of toxic fungi or truffles.

P1 44

Ergosterol in the mycorrhiza formed by desert truffles.

A. Gutiérrez; A. Morte; and M.Honrubia
Dpto. Biología Vegetal, Universidad de Murcia, Spain

Two prominent sterols, ergosterol and brassicasterol, were detected in *Tuber melanosporum* and *Terfezia claveryi* carpophore tissues by GLC – Mass spectrometry. The mass spectrum of ergosterol contained a molecular ion at m/e 396, with prominent ion fragments at m/e 337, 262 and 211. The other sterol, brassicasterol, whose mass spectrum contained a molecular ion at m/e 398, showed prominent ion fragments at m/e 300, 271, 255 and 173.

GLC analysis showed that the most abundant sterol in *T. claveryi* is brassicasterol, while ergosterol is the most abundant in *T. melanosporum*. We used ergosterol analysis to estimate the active mycelium in the mycorrhiza formed by desert truffles. For this purpose, the ergosterol concentrations in *T. melanosporum*, *T. claveryi* and *Picoa juniperi* carpophore were estimated. Of these, *T. melanosporum* showed the highest amount of ergosterol, 5.659 µg/mg MF, followed by *T. claveryi* with 5.065 µg/mg MF, and finally, *P. juniperi* with 0.246 µg/mg MF.

Two *Helianthemum* species (*H. almeriense* and *H. viscarium*) were used as a host plant, and *T. claveryi* as mycorrhizal fungus. Mycorrhizal symbioses were carried out in *in vitro* conditions. The ergosterol content in the mycorrhiza formed was analysed 4, 8 and 12 weeeks after inoculation. The ergosterol present in the mycorrhizal roots of *H. viscarium* is less abundant than in the mycorrhizal roots in *H. almeriense*. In both *Helianthemum* species, the ergosterol concentrations 4 weeks after inoculation were 0.1566 and 0.0208 µg/mg MF for *H. almeriense* and *H. viscarium* respectively. These levels increased considerably at 8 weeks (0.214 and 0.163 µg/mg MF), and decreased markably at 12 weeks (0.1819 and 0.0237 µg/mg MF).

P1 45

Scandinavian black truffles: Distribution and habitats.

Christina Wedén & Eric Danell.

Dept. of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Only four specimens of *Tuber aestivum* (syn. *T. uncinatum*) were recorded in Sweden until 1997. In Denmark there are currently seven known *T. aestivum* sites while in Finland, Iceland and Norway there are no reported findings. Using trained truffle dogs in a valuable cooperation with Dr. Gérard Chevalier, we performed inventories in Sweden (Gotland) in 1999 and 2000. At present there are 22 known localities of *T. aestivum* in Sweden. Nine localities have also been found for *T. mesentericum*, a new species for Sweden. *T. mesentericum* was the only truffle found in seven sites, coexisting with *T. aestivum* in only two sites, thus indicating different habitat preferences. Ongoing RAPD analyses indicate that the Gotland population is composed of many heterogenic genotypes. One aim is to compare Swedish *T. aestivum* with Danish and Central European specimens in order to trace the origin of the Swedish *T. aestivum*. The next step is to understand how the hypogeous fungus *T. aestivum* is dispersed to the island of Gotland, which arose from the sea after the last ice age. Our hypothesis is that insects play a vital role in dispersal. The genetics of the genus *Tuber* is still poorly understood. The mating type system will therefore be the object of further studies.

P1 46

Potential for the cultivation of *Lactarius deliciosus* (L.: Fr.) S.F. Gray in New Zealand

Y. Wang', I.R. Hall, C. Dixon and M. Stephen
New Zealand Inst for Crop & Food Research Ltd, Invermay, Mosgiel, New Zealand

Lactarius deliciosus (L.: Fr.) S.F. Gray (saffron milk cap in English, chilpán in Spanish, lactare délicieux in French, lapacendro buono in Italian and meiwei rugu in Chinese) is a popular edible ectomycorrhizal mushroom in the Northern Hemisphere. It has also been accidentally introduced to Australia. It grows under coniferous trees, in particular pine and spruce species and fruits during autumn. It is widely eaten and sold in the markets in many countries. It is also very popular with chefs and gourmets because of its saffron-coloured cap and latex, and fruity odour. Isolates of *Lactarius deliciosus* were imported from Europe and a series of inoculation experiments carried out in the laboratory. Seedlings of *Pinus radiata*, *P. densiflora* and *Picea abies* formed good ectomycorrhizas with all the isolates. Growth responses in response to infection were also detected. The mycorrhizas were orange coloured when they were young and became dark green with age. Orange coloured branched lactifers in a mantle layer were visible under the microscope. Technology for producing infected *Pinus radiata* seedlings with *Lactarius deliciosus* was developed and two experimental plantations established in spring 2000, one in the North Island and the in the South. Further plantations are planned for spring 2001 with the aim of producing fruiting bodies of *Lactarius deliciosus* as a secondary crop in radiata pine forests.

P1 47

Amanita ponderosa, an edible species rooted at the Sierra of Huelva, Spain.

A.Daza[1], C. Santamaría[1], L. Romero[1], M. Camacho[1], A. Aguilar[1], M. Bernedo[2], M. Pérez[2], J. Diez[2], G. Moreno[2] and J.L. Manjón[2].
[1]CIFA Las Torres, Junta de Andalucía. Alcalá del Río (Sevilla), Spain
[2]Dpto. Biología Vegetal, Universidad de Alcalá, Alcalá de Henares (Madrid), Spain.

Amanita ponderosa is a poorly known species very abundant and gastronomically appreciated in the Southwest of Spain (commonly named as "gurumelo") and in the North of Marocco, where was described by the first time (Malençon and Heim, 1942). Their sporocarps are robust, white but toning to pink at the air contact and also at maturity, bag-shaped volva and ephemeral annulus. It shows a characteristical earthy smell that helps the harvesters to identify it in the field. Fruiting bodies of *Amanita ponderosa* ("gurumelos") appear only in Spring, showing a semihypogeum behaviour. Fruit harvesting of this species takes place before it appears on the surface and needs specific tools (named "cook") to remove the sporocarps as soon as swellings or small fissures appear in the soil.

The "gurumelos" grow in lightly acid, slaty, limited in organic matter soils. In natural conditions probably forms ectomycorrhizas with evergreen oak, rock-rose and cork-oak, but there are not studies on their preferences and symbiotic characteristics. We have been successful in growing the mycelium in synthetic conditions and we are studying their best laboratory growth conditions (pH, temperature, C and N resources, etc.). We are also analysing under field natural conditions the fruiting pattern and the effect of common forest treatments on sporocarp formation.

P1 48

In vitro mycorrhizal synthesis of *Tricholoma matsutake* with *Pinus* and *Picea*

Yamada A.[1] and Murata H.[2] [1]Shinshu University, Japan [2]Forestry and Forest Products Research Institute, Japan.

Tricholoma matsutake is one of the best-known edible ectomycorrhizal mushrooms in the world. The fungus has been recently suggested the distribution at least around the Eurasian continent based on the morphological and DNA analyses of the fruit body. Ectomycorrhizal status has been also confirmed both in situ and in vitro in association with *Pinus densiflora* that distributes East Asia (Japan, Korea, and northeastern China). However, the mycorrhizal status with other host plants has not been clarified. The present study aimed to test the mycorrhizal potential of a Japanese *T. matsutake* isolate with some *Pinus* and *Picea* in vitro. Targeted species were *Pin. thunbergii*, *Pin. sylvestris*, *Pic. jezoensis*, and *Pic. glehnii*, all of which have been suggested the potential of mycorrhizal association with *T. matsutake* in the natural field conditions. Axenically germinated seedlings were inoculated with liquid-cultured mycelium of *T. matsutake* and incubated 5 months under customary synthetic condition. Anatomical observations revealed that all *Pinus* species and *Pic. glehni* form Hartig net at the root cortex as same as *Pin. densiflora*. However, the Hartig net development was discontinuous in *Pin. thunbergii*. Colonization rate (mycorrhizal/total root length) was the highest in *Pin. densiflora* and lowest in *Pic. glehni*.

P1 49

Nutritional value of edible ectomycorrhiza from a Northern Thailand forest

S. Lumyong[1], R. Sanmee[1], P. Lumyong[2], B. Rerkkasem [3]and B. Dell[4]
[1] Department of Biology, Faculty of Science, Chiang Mai University, Thailand
[2] Deaprtment of Plant pathology, Facultuty of Agriculture, Chiang Mai University, Thailand.
[3] Agronomy Department , Faculty of Agriculture, Chiang Mai University, Thailand.
[4] School of Biological Science and Biotechnology, Murdoch University, Western Australia.

Ectomycorrhiza (ECM) play an important role in forest ecology. In Thailand the growth of forest trees such as Fagaceae family tend to associate with many species of ECM. Most of these ECM are edible. The price of some edible ECM are higher than cultivated mushroom. The local people in northern, north-east and southern part of Thailand eat wild ECM. Conservation and reforestation will conserve these edible ECM. Seven popular edible ECM were selected to analyze for nutritional value. The results show that all edible ECM tested have high protein range of 12,5-19%, fat 2-9%, fiber 8-15%, carbohydrate 38-60%, ash 7-25% and organic mater 65-84%. The main sugar components after extraction with hot water and analyzed by HPLC were manitol, fructose, glucose, ribulose and arabinose. *Russula* sp. had the highest manitol, at 308 .2 µmole/l. *Lactarius piperatus* had the highest fructose, at 122.6 µmole/l.

P1 50

Effect of industrial processing on Desert Truffles: *Terfezia claveryi* Chatin and *Picoa juniperi* Vittadini, Proximate Composition and Histochemical location of lipids

M. Honrubia [2], M A. Murcia [1], M. Martínez-Tomé [1], A. Vera [1], A. M Jiménez [1], A. Morte [2] and A. Gutiérrez [2].

Depto. Bromatología[1]. Depto. Biología Vegetal [2]. Universidad de Murcia.

The aim of the present study was to investigate the proximate composition of two desert truffles (*Terfezia claveryi* Chatin and *Picoa juniperi* Vittadini) and to determine the effects of canning and freezing on proximate composition. The histochemical location of lipids was also studied in order to ascertain the places where lipids concentrate in the ascocarps of these desert truffles.
Little loss of ashes, proteins and lipids was observed as a result of industrial processing. The fatty acids showed high quantities of linoleic acid 18:2 (45.4% in *T. claveryi* and 53.0% in *P. juniperi*).
The greatest lipid concentrations were found in the ascogenic hyphae of the gleba and in immature spores inside the asci, but not in the peridium.

P1 51

Influence of restoration practices on mycorrhiza of selected plants from calamine spoil mounds

K. Turnau', E. Bartyzel', Sz. Zubek', A. Jurkiewicz', G. Szarek-Łukaszewska" 'Jagiellonian University and "Polish Academy of Sciences, Krakow, Poland

Research on the influence of simple restoration practices on arbuscular mycorrhiza of *Biscutella laevigata* (Brassicaceae) and *Plantago lanceolata* (Plantaginaceae) was carried out on calamine spoil mounds in Bolesław (southern Poland). While *P. lanceolata* is a known mycorrhizal species, the symbiosis in the case of *B. laevigata* is surprising for two reasons: it is a member of a family generally accepted as nonmycorrhizal and a heavy metal hyperaccumulator. Careful analysis of the roots revealed not only coils, but also arbuscules. The calamine waste had two parts: the first was 100 years old and was spontaneously revegetated; on the second part trees were introduced about 30 years ago. Both parts were characterised by high total heavy metal content but low metal bioavailability. The main difference concerned the organic matter content. Heavy metal levels were measured in soil and in plant root and shoot samples. In *B. laevigata* only Cd levels were higher in roots than in shoots. *P. lanceolata* generally accumulated more heavy metals than *B. laevigata*, mostly in roots. Only slight differences in heavy metal content were found between plants from the restored and the non-restored part. Mycorrhiza frequency was usually high in *P. lanceolata* and low in *B. laevigata* what is probably due to different root systems. M% and A% values were usually much higher in *P. lanceolata* while m% and a% values were lower. M% and A% were increased in *P. lanceolata* from the restored part while almost no differences were found in *B. laevigata*. Restoration seems to have a negative effect on *B. laevigata,* which is a rare species in Europe, easily outcompeted and deserves protection.

P1 52

Occurrence of ectomycorrhizas, arbuscular mycorrhizas, and endophytic fungi associated with *Betula*, *Alnus* and *Salix* seedlings naturally regenerated at a disturbed site

Y. Hashimoto, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan

Mycorrhizal symbioses are thought to be important for newly establishing pioneer-plant seedlings at disturbed sites. Some pioneer plants are capable of forming different symbiotic association with ectomycorrhizal, arbuscular mycorrhizal, and endophytic fungi. In this study what types of association seen in the seedlings of *Betula platyphylla* var. *japonica*, *Alnus hirsuta* and *Salix* sp. naturally regenerated at a disturbed site were investigated. The samples of each plant seedling were taken monthly from a site where the surface soil layers had been removed by disturbances. The ectomycorrhizal formations in *Betula* seedlings (59-91%) were higher than that in *Aluns* (31-66%) and *Salix* (28-52%) seedlings. The frequency of colonization by endophytic fungi to *Betula*, *Alnus* and *Salix* were 9-60%, 17-85% and 16-68%, respectively. The colonization of arbuscular mycorrhizal fungi to *Betula, Alnus* and *Salix* in September was 0%, 9% and 7%, respectively. In addition, the dominant morphological types of ectomycorrhizas formed in each host species were investigated using PCR/RFLP analysis of the ITS region. Twelve types of ectomycorrhizas were identified among three host species. No types of ectomycorrhizas that colonized to three or two host species in common were observed. These results suggest that ectomycorrhizal and endophytic fungi might be the most dominant type associated with *Betula*, *Alnus* and *Salix* seedlings compared to arbuscular mycorrhizal fungi during the initial stage of re-vegetation following site disturbances.

P1 53

Rehabilitation of Malaysian highway landscape plants using commercial arbuscular mycorrhizal fungi

P. Raja[1], J. Herdhawati[1], C.A. Nazam[1] and I. Zubaidah[1] Malaysian Agri Hi-Tech, Block: Stroma, UKM-MTDC Smart Technology Centre, University Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

Malaysian government has promoted Malaysia to be a garden country. The importance of landscape is apparent in the recent and fast development of Malaysian highway projects. However, the development of these areas have often been characterised by poor establishment of plants due to the disturbed and compacted soils found along highways. In this study the application of commercially available AMF inoculum (MycoGold^{TM}) and organic matter (MycoOrganic^{TM}) in Malaysian high way landscape plants are UPM Interchange (PLUS) and Sepang Toll Plaza (ELITE) to improve root growth. In UPM Interchange the soil pH ranged from 4.6-6.5 and bulk density is 5.2g/ cm^{-3}. Total of 7 trees, one shrub and 7 cover crops were treated with MycoGold^{TM} and MycoOrganic^{TM}. All plant roots had colonized with AM fungi. The highest per cent infection (70 %) and spore count (227/10g soil) were recorded in *Acacia mangium* roots and rhizosphere soils. In Sepang Toll Plaza soil pH ranged between 5.2-6.8 and bulk density was about 4.8 g/ cm^{-3}. Mycorrhizal infection was noted on all treated plants. Out of 6 plants the highest percentage of infection (35%) and spore count (20/10g soil) were recorded in *Duranta repen 'gold'*. Twelve AM fungal spores were isolated from both rhizosphere soil samples. Out of 12, nine species from Commercial product and 3 from native spores. After application of commercial AMF inoculum and organic matter in landscape plants, we observed the fast recovery of cover crops and trees are as evidenced by the high production of new leaflets. These preliminary results are indicates that AMF inoculation can be profitably exploited for Malaysian high way landscape plant development.

P1 54

The vertical distribution of *Quercus agrifolia* associated arbuscular mycorrhizae (AM) and ectomycorrhizae (EM) in weathered bedrock.

M. Bornyasz, I. Querjeta, R. Graham and M. Allen. University of California, Riverside, Center for Conservation Biology and Soil and Water Program.

Quercus agrifolia most commonly occurs on deep coastal alluvial sediments, but can occur on slopes with rocky outcrops. In southwestern California, *Q. agrifolia* distribution closely matches regions of soils developed within granitic regolith. In these regions annual evapotranspiration demand is high, leading to a dependence on a deep rooting system with the ability to access plant available water from deep within the regolith. The establishment of an extensive root system, and hyphal network, can be essential for a plant's water and nutrient requirements. Recent investigations in deep alluvial sediments have confirmed that the subsurface architecture of *Q. agrifolia* is deep and dual in function, and found EM infected tips below 2 meters. Weathered granitic bedrock has a water-holding capacity similar to these coarse-textured soils, but plant roots are restricted to joint fractures. Most of the plant available water in weathered granitic rock is held within micropores, which are generally too small for roots to access. It is not clear how water held in the rock matrix reaches roots contained within the fractures. Hyphae are narrow and able to explore a greater soil and rock volume for resources. This study addresses the vertical composition of both AM and EM symbionts in weathered granitic rock in an effort to determine if mycorrhizae act as water channels, obtaining the water held within the rock matrix between joint fractures. We described the properties of the soil and weathered bedrock, measured water infiltration and retention characteristics, quantified plant available nutrients, and related these profile characteristics to mycorrhizal community composition and vertical distribution.

P1 55

Mycorrhiza-related protein profiles for sensoring toxicity due to agricultural amendments

G. Bestel-Corre, E. Dumas-Gaudot, S. Gianinazzi
INRA-Université de Bourgogne, Dijon, France

In recent years, proteome analysis has emerged as a powerful technique to get insight into protein expression and regulation within arbuscular mycorrhizal (AM) symbiosis [1]. Based on the combination of two dimensional electrophoresis and mass spectrometry, it allowed the mapping and identification, in *Medicago truncatula* roots inoculated with the AM fungus *Glomus mosseae*, of several symbiosis-related proteins [2]. Due to high sensitivity and throughput, this technique offers the possibility of detecting phenotypic changes at the cellular level, before any macroscopically visible trait. This feature comes out particularly interesting for evaluating the ecotoxicity of xenobiotics present in agricultural amendments. Indeed, recycling of sewage sludges and other wastes in agriculture, if interesting on a fertilizing point of view, however presents a potential risk, due to high content in heavy metals and organic pollutants. In particular, soil microorganism populations are exposed to such risk and among them, AM fungi were revealed utterly sensitive, leading to a depletion in plant mycorrhization [3]. In order to evaluate the early impact of sewage sludge pollution on AM symbiosis, changes in root protein profiles of *M. truncatula* plants inoculated with *G. mosseae* are now under investigation following treatment with sludges loaded with either heavy metals or organic pollutants.

[1] Dumas-Gaudot *et al*, 2001, Proteomics, a powerful approach towards understanding functional plant root interactions with arbuscular mycorrhizal fungi, in: Pandalai S (Ed.) *Recent developments in plant biology*, Research Signpost, in press.

[2] Bestel-Corre *et al*, 2001, Identification of symbiosis-related proteins from *M. truncatula* Gaertn. by two-dimensional electrophoresis and mass spectrometry, *Electrophoresis*, submitted.

[3] Jacquot *et al*, 2000, Monitoring species of AMF *in planta* and in soil by nested PCR: application to the study of the impact of sewage sludges, *Plant and Soil*, *226*, 179-188.

P1 56

Genetics and heavy metal (Ni) tolerance in *Cenococcum geophilum*

S.C. Gonçalves[1*], A. Portugal[1], M.T. Gonçalves[1], Vieira, R.[1], Freitas, H.[1] & Martins-Loução, M.A.[2] [1]Departamento de Botânica. Universidade de Coimbra. Coimbra, Portugal. [2]Departamento de Biologia Vegetal. FCL. Lisboa, Portugal [*]*scgoncal@ci.uc.pt*

A comparative study of the metal tolerance (Ni) and genetic diversity within two groups of the ectomycorrhizal fungus *Cenococcum geophilum* is being carried out. Several isolates were screened for axenic tolerance to Ni. The isolates came from two different origins: a serpentine soil and a nearby non-serpentine soil in north-east Portugal. Serpentine soils from north-east Portugal contain naturally elevated levels of Ni. Therefore, adaptive Ni tolerance can evolve. Molecular characterisation of all isolates was performed by means of amplification and subsequent restriction analysis of rDNA and microsatellite-primed PCR. The first results suggested that elevated Ni levels could be responsible for the evolution of adaptive Ni tolerance among serpentine isolates. Increased addition of Ni to the growth media induced differential responses of the isolates according to their origin. Namely, biomass production of the serpentine isolates was not negatively affected by Ni. *C. geophilum* isolates exhibited considerable intraspecific genetic variability. However, no correlation was found between Ni levels in the soil and genetic variation. Instead, genetic variation was correlated with morphotypes. This result does not support the former hypothesis. Research is in progress to further investigate the mechanisms of tolerance among serpentine isolates of *C. geophilum*.

P1 57

Adaptive heavy metal tolerance in *Suillus* spp.

K. Adriaensen, M. Lambaerts, L.A. Muller and J.V. Colpaert
Limburgs Universitair Centrum, Diepenbeek, Belgium

An extensive screening of different populations of the ectomycorrhizal genus *Suillus* showed that isolates from *Suillus luteus* and *Suillus bovinus* on metalliferous sites possessed increased levels of tolerance, to those metals that are enriched in the polluted soil. Growth of tolerant isolates was studied in dose-response experiments and was compared to growth of *Suillus* isolates from nearby control sites in uncontaminated areas. The observed metal tolerance was not phenotypically acquired and was genetically stable.
The aim of the present project is to investigate the mechanisms responsible for the adaptive Zn and Cd tolerance of the *Suillus* populations. Secondly the effect of the metal tolerance of *Suillus* on the development of a host plant, *Pinus sylvestris*, will be analysed in the presence of elevated metal concentrations. Experiments in polluted soil as well as in semi-hydroponic systems have been performed. We are interested to know whether ectomycorrhizal fungi can protect their host plant against metal toxicity in polluted soil and whether a metal tolerant isolate performs better in respect of a sensitive isolate.

P1 58

Ecology of ectomycorrhizal fungi isolated from *Pinus sylvestris* found at the Great Devon Consols, an old mine site contaminated with arsenic and copper

S. Preston[1], I.C. Anderson[1,2], J. Poskitt[3], J. Hartley-Whitaker[3], I.J. Alexander[1] & A.A. Meharg[1] [1]University of Aberdeen, UK [2]MLURI, Aberdeen, UK, [3]CEH Merlewood, UK

Ectomycorrhizal fungi and their hosts have adapted to survive on highly contaminated soils. An important function of the ectomycorrhizal fungi in the symbiosis is the acquisition of phosphate for their host. In arsenate-contaminated soils such a function poses a particular challenge to ectomycorrhizal fungi since arsenate is a phosphate analogue for fungal phosphate transporters. In this study, several ectomycorrhizal fungi were isolated from root tips of *Pinus sylvestris* seedlings and sporocarps found on arsenic and copper contaminated mine spoils at the disused Devon Great Consols Mine, Devon, south-west England. The ectomycorrhizal fungi were identified by extracting genomic DNA and sequencing the PCR-amplified ITS regions. Mycorrhizal synthesis experiments were also carried out on the isolates to confirm their ability to form ectomycorrhizas on *Pinus sylvestris* seedlings. Ectomycorrhizal fungi isolated from the mine site and from uncontaminated sites were then compared with regard to their sensitivity to arsenate, measured as inhibition of biomass production in solution culture. Isolates from the mine site demonstrated resistance to arsenate compared to isolates from uncontaminated sites. The results are discussed in terms of the ecological significance of adaptive arsenate resistance in ectomycorrhizal fungi.

P1 59

Chemical characterisation of extra radical fungal walls of AMF and their metal binding capacity.

R. Briones, C. Mustin, E. Joner, G. Belgy and C. Leyval. Centre de Pédologie Biologique, CNRS, Vandoeuvre-lès-Nancy, France.

Mycelium of four *Glomus* species, metal tolerant and not tolerant, produced individually in compartment pots with ryegrass as host plant, were characterised with respect to their chemical heterogeneity by high resolution acido - basic potentiometric titration (HRT-TPH) and infrared spectroscopy (FTIR). The wall surface of the four AMF was composed of different functional groups (carboxylic, amino acid, sulfonic, phosphate and phenolic) able to bind metal ions. The total acidity was divided into strong, weak and very weak. The pKa values ranged from 4 to 5.5 and from 8.5 to 9.6. The results showed that the total organic acidity of AMF mycelia are two times higher than that of a saprophytic fungus (*Rhizopus arrhizus*) and three times higher than that of an ectomycorrhizal fungus (*Laccaria laccata*). The sorption of copper and cadmium by metal-tolerant AM fungus (protonated and not protonated biomass) was evaluated using batch experiments with increasing ion concentrations. Metal sorption was accompanied by a release of Zn2+ and Ca2+ that had originally been adsorbed from the nutrient solution. The presence of strong acidity (which bound ions in a labile fashion and comprised ca. 60% of the total acidity) could explain the release of these cations into the solution phase. Adsorption of copper and cadmium was observed using TEM – EDAX and infrared spectroscopy and the latter verified that the adsorbed metal were held by chemical bonds on hyphal walls. Our results indicate that AMF play an important role in the mobility and availability of cations in the rhizosphere.

P1 60

Interaction of arbuscular mycorrhizal fungi (AMF) with heavy metals: Fungal isolates indigenous and non-indigenous in contaminated soils.

M. Vosátka, R. Malcová, M. ilová, J. Rydlová Institute of Botany, Academy of Sciences, Pruhonice, Czech Republic

A series of experiments was conducted to study the adaptation abilities of AMF to elevated concentrations of heavy metals (HM). Indigenous AMF isolates from soils contaminated with Mn or Pb were compared with the same species from unpolluted soils to determine their tolerance to HM. Results suggest differences in HM-tolerance between indigenous and non-indigenous AMF isolates. Nevertheless, some non-indigenous isolates were found to have a high resistance to HM pointing out to a high adaptability to HM. Two linia of each AMF isolate were maintained for 2-3 years either in inert media or in original soil. Process of losing the tolerance to HM when cultivated in inert media was compared to gaining HM-tolerance of non-indigenous strains when exposed to HM-stress. Indigenous isolates of *Glomus intraradices* and *G. fistulosum* from Mn-contaminated soil showed higher sporulation in original soil in comparison with non-indigenous isolates of the same species. Better development of indigenous *G. intraradices* compared with non-indigenous one was also found in hydroponic experiment where Mn was supplied. While indigenous isolate maintained in original soil survived elevated Mn concentration, the isolate maintained in inert substrate was suppressed. Similarly, indigenous *G. intraradices* showed better performance as compared to non-indigenous one in Pb-contaminated soil, but no difference was observed between the two linia. These linia represent unique material for ecophysiological comparison, however, the studies should be supported by use of biochemical and molecular techniques to find out principles of changed HM-tolerance.

Trans - remplies d'une solution métaux lourds
l'ajoute segment de racine

- Subculture of AM → perte de
tolérance
aux métaux lourds

P1 61

Mycorrhizal colonization of indigenous plants in polluted sites in Northern Portugal

R.S. Oliveira, J.C. Dodd[1] & P.M.L. Castro
Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal.
[1]PlantWorks Limited, 1-19 Innovation Building 1000, Sittingbourne Research Centre, Sittingbourne, Kent, United Kingdom. (www.plantworksuk.co.uk)

The use of plant-microbe-based systems (phytoremediation) for the treatment of contaminated soil has been increasing in recent years. *Phragmites australis* (common reed) grows in a wide range of habitats, but has been little studied with respect to its mycorrhizal status in polluted sites. Roots of *P. australis* and other plant species from three contaminated sites were sampled over a one-year cycle of plant growth and the degree of colonisation by arbuscular mycorrhizal fungi (AMF) was assessed. Root colonisation was low (<5% root length colonised) but arbuscule presence peaked in *P. australis* during the spring and autumn prior to flowering. A parallel greenhouse trial using *P. australis* seeds taken from one of the sites was established and the same trend in arbuscule presence was observed. Samples of roots of *Juncus effusus* and *Salix atrocinerea* were also taken to assess the presence of AMF through the year. Roots of *J. effusus* contained mainly low levels of vesicular colonisation but arbuscule activity peaked during the winter months (Dec-Mar). *S. atrocinerea* roots were found to be ectomycorrhizal throughout the year but the fine feeder roots were colonised by AMF. The analyses confirm that semi-aquatics, like *P. australis*, can become arbuscular mycorrhizal but that this status changes during the year depending on soil moisture content and plant phenology. The data obtained was used to plan and establish an on-going revegetation field trial on a sedimentation pond, including *S. atrocinerea* inoculated both with AMF and ectomycorrhizal fungi (ECMF) provided by PlantWorks Limited and indigenous ECMF. There is potential to establish a more diverse plant ecosystem during the phytorestoration of polluted sites using adapted plant and mycorrhizal ecotypes.

P1 62

Mechanism(s) of heavy metal metabolism: the role of arbuscular mycorrhizas

Claire L. Boddington and Thomas W. Kuyper; Wageningen University, The Netherlands.

Currently, phytoremediation is a popular approach to alleviate problems of heavy metal toxicity in polluted areas. Increasing evidence suggests that arbuscular mycorrhizal fungi (AMF) may also play a role in alleviating heavy metal toxicity. One of the questions that continues to remain largely unanswered, however, is how AMF might alleviate the effects of heavy metal toxicity. Determination of the mechanism(s) involved in the metabolism of heavy metals is an essential step to enable us to select those fungi with characteristics which increase their bio-remediation efficiency. Evidence suggests that at least two compounds found in AMF may play a role in the metabolism of heavy metals; polyphosphate and Glomalin. A detailed study of these two compounds and hyphal processes may indicate how heavy metals are taken up, translocated, and localised/stored by AMF. An isolate each of *Gigaspora* and *Glomus* (colonising *Plantago lanceolata*) were selected due to potential differences in both Glomalin (Wright et al., 1999) and polyphosphate (Boddington & Dodd, 2000) metabolism in these fungi. Two divalent heavy metals, Zn^{2+} and Cd^{2+}, will be studied. A combination of imaging/staining studies to locate the metals in fungal structures and the use of metabolic inhibitors to determine the nature of the uptake, translocation, and localisation/storage processes will be used. Suggestions relating to the mechanism(s) for the involvement of AMF in the metabolism of heavy metals will be discussed.

P1 63

In planta molecular monitoring of arbuscular mycorrhizal fungal diversity in field soils amended with sewage sludges

E. Jacquot-Plumey, D. Van Tuinen, O. Chatagnier, V. Gianinazzi-Pearson, and S. Gianinazzi.
UMR Université de Bourgogne/INRA, BBCE-IPM, CMSE-INRA, Dijon, France

Recycling of sewage wastes in agriculture is susceptible to affect the biological activity of soils through contamination of ecosystems by pathogens and metallic or organic micropollutants. We have developed a biological test to evaluate the toxicity of sewage sludges destined to be spread in agriculture and shown, by molecular monitoring using nested PCR with taxon-discriminating primers, that sewage sludges differentially affect AM fungal species in simulated communities in microcosm experiments (Jacquot *et al.*, 2000). Subsequent investigations were aimed at evaluating the usefulness of this molecular approach to monitor the effect of sewage sludge spreading on the natural diversity *in planta* of AM fungi in field plots. PCR primers were designed after sequence analysis of the large ribosomal subunit of five AM fungal morphotypes observed in field plots. *Medicago truncatula* was grown in soils from field plots amended or not with composted sludges, and the relative abundance of each fungus was estimated in trypan blue stained root fragments by nested PCR using the appropriate primers. Results show that input of composted sludges modified the relative diversity of AM fungal populations within host roots. Non amended and organic-spiked sludges had a generally positive effect on the relative abundance of the different species whilst no variation was observed after spreading of a sludge spiked with metallic pollutants.

P1 64

Effect of fire on the ectomycorrhizae in a *Quercus ilex* L. stand in NE Spain.

M. De Román, A.M. De Miguel, M.L. Etayo.
Departamento de Botánica, Universidad de Navarra, Pamplona, Spain.

This study aims to compare the mycorrhizal colonization at a burned and a non-disturbed sites in a *Quercus ilex* L. stand located in Navarra (NE Spain). The evolution of the percentages of mycorrhization at both study sites has been monitored for two years.

On the other hand, the diversity and abundance of the ectomycorrhizal morphotypes occurring at both sites have also been studied. The ectomycorrhizal morphotypes found have been described, and the fungal partner has been identified when possible. The ecology of the identified mycorrhizal fungi found at the burned and/or the non-disturbed sites is also discussed.

P1 65

Drought resistance of Norway spruce seedlings with different mycorrhization, induced by boron supply

Tarja Lehto, Marjoriitta Möttönen & Pedro J. Aphalo
University of Joensuu, Faculty of Forestry, Joensuu, Finland
tarja.lehto@joensuu.fi

Earlier studies indicate that improved boron (B) nutrition does not always affect growth of aboveground parts of plants as much as it increases formation of root tips and mycorrhizas. When the other environmental conditions are optimal, this does not necessarily affect uptake of other nutrients (Möttönen, Lehto & Aphalo, unpublished). However, when another environmental stress factor affects nutrient uptake, the decrease in root tips and mycorrhizas could be severe enough to reduce the fitness of plants. Water uptake under drought could also be affected by these changes in the root system.

The controlled environment experiment with Norway spruce seedlings included two B regimes, optimal and deficient. The drought treatments were applied after the seedlings had been growing under the contrasting B regimes for 117 days. The drought treatments were either one drying and rewatering cycle, or two drying and rewatering cycles. Photosynthesis and water relations, growth, mycorrhiza formation, and nutrient accumulation were recorded at the different phases.

P1 66

Mycorrhizal development in waterlogged soils

J. Baar[1], J.G.M. Roelofs[1], E.J.W. Visser[2] and L.A.C.J. Vousenek[3]
1. Aquatic Ecology and Environmental Biology; 2. Experimental Plant Ecology, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands. 3. Plant Ecophysiology, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands.

The aim of the study was to investigate mycorrhizal development in waterlogged soils. Evidence is accumulating that mycorrhizal fungi associate abundantly with plants in (semi) aquatic ecosystems. However, knowledge is limited about the mechanisms through which mycorrhizal fungi are able to survive in oxygen-poor waterlogged soils. Therefore, we studied the development of ectomycorrhizal fungi in wet Alder carr forests. In North-Western Europe, wet Alder carr forests are mostly found in peatlands and along streams forming an important component of wetland ecosystems. The soils are predominantly fed by groundwater and permanently waterlogged. The dominant tree species in wet Alder carr forests is *Alnus glutinosa* (Black alder). The present study showed that the majority of *A. glutinosa* roots in various wet Alder carr forests in The Netherlands were associated with ectomycorrhizal fungi, even in anoxic waterlogged soils. Ectomycorrhizal root tips and hyphae were observed up to two metres from the *A. glutinosa* trees in waterlogged soil. Ectomycorrhizal development under wet and oxygen limited conditions was related to radial oxygen loss from *A. glutinosa* roots. PCR-based techniques showed that the abundance and diversity of ectomycorrhizal fungi below ground was higher in wet Alder carr forests in a peatland than along a stream. In the wet Alder carr forests in the peatland, a relatively high redox potential of the waterlogged soil was measured together with radial oxygen loss from roots of the dense understory vegetation indicating that ectomycorrhizal development in waterlogged soils is stimulated by oxygen input of the surrounding vegetation.

P1 67

Effect of arbuscular-mycorrhizal colonisation on water relations and growth of *Zea Mays* in compacted soils.

JM Sharples*, GD Farquhar, J Masle
Environmental Biology Group, Research School of Biological Sciences,
The Australian National University, PO Box 475, Canberra, ACT 2601, Australia

The significance of arbuscular mycorrhizal infection on plant host water transport is relatively unclear, however their role in increasing nutrient absorption has been firmly established. Under compacted soil conditions, plant root functioning may be restricted by mechanical impedance, or by poor soil aeration which may in turn limit water and nutrient availability. Under such conditions, mycorrhizal association may be advantageous to the host plant by increasing their root surface area and in turn increasing their nutrient and water acquisition.

In an attempt to clarify the role of mycorrhizal associations in water relations in compacted soil, *Zea mays* was grown in soils with bulk densities of 1.1 g cm^3 and 1.4 g cm^3 in association with two different isolates of the arbuscular mycorrhizal fungus *Glomus pellucidum*. Non mycorrhizal *Z. mays* were also grown at the two different bulk soil densities. After 6 weeks, growth characteristics and water relations of all plants were analysed. The results are discussed in relation to mycorrhizal associations.

P1 68

A method for analyzing active internal colonization of dark septate fungal endophytes in grasses and shrubs native to arid southwestern USA rangelands

R.E. Aaltonen and J.R. Barrow USDA-ARS, Jornada Experimental Range, Las Cruces, NM, USA.

Dominant native grasses and shrubs in arid southwestern USA rangelands are more extensively colonized by dark septate fungal endophytes (DSE) than by conventional mycorrhizal fungi. These endophytes are widespread colonizers in most ecosystems, but their ecological function is currently unresolved. They are readily observed as stained or melanized septate hyphae and microsclerotia on the root surface and in the cortex using fungus specific stains that bind to chitin, a common wall component. Roots of grasses and shrubs, native to the northern Chihuahuan Desert, were stained with sudan IV, specific for lipids, and analyzed with high magnification differential interference microscopy. Inconspicuous internal hyphal extensions of stained or pigmented hyphae were observed that were not visible using fungus specific stains. These structures were observed in a much greater frequency than the commonly recognized stained or pigmented structures in physiologically active roots. Differential responses to staining were attributed to structural differences and levels of chitin incorporation in the fungal wall. Internal hyphae varied in diameter, wall thickness, and visibility and in many cases were detectable only because of the positive staining of lipids in fungal vacuoles. Internal hyphae grew both inter and intracellularly within the cortex and sieve elements of the vascular cylinder and at time contained substantial quantities of lipids. This method promises to be effective for studying the extent, nature, and possible function of DSE fungi in native ecosystems.

P1 69

Unique active internal morphology of dark septate fungal endophytes associated with roots of native grasses and shrubs in an arid ecosystem.

J.R. Barrow, and R.E. Aaltonen USDA-ARS, Jornada Experimental Range, Las Cruces, NM, USA.

Plants in arid ecosystems are exposed to severe nutrient and drought stress. Mycorrhizal fungi alleviate these stresses in natural ecosystems. Native grasses and shrubs in arid southwestern USA rangelands are more extensively colonized by dark septate fungal endophytes (DSE) than by conventional mycorrhizal fungi. The function of these endophytes is presently unresolved. Roots of grasses and shrubs, native to the northern Chihuahuan Desert, were stained with sudan IV, specific for lipids, and analyzed with high magnification differential interference microscopy. Unique, atypical extensions of DS fungi were observed that are not visible using fungus specific stains. Their prevalence in physiologically active roots suggested an active phase of DSE. Hyaline hyphae grew both inter- and intracellularly within the cortical cells and sieve elements and varied in visibility. Some hyphae had distinct hyaline walls, while others in the sieve elements walls were evident only by positive staining of lipids in their vacuoles. These vacuoles were arranged in a typical linear and branched hyphae manner or they were highly irregular in shape. Large 5-8 *u*m, lipid filled, hyphal vacuoles extended from the vascular cylinder through the cortex to the root surface which was coated with mucilage. The substantial lipid accumulation in hyphae extending from sieve elements to the root surface suggested that DSE fungi enhance rhizodeposition of carbon, the formation of a protective mucilage sheath that maintains hydraulic continuity and root function in dry soil.

P1 70

Establishment and infective capacity of *Gigaspora margarita* Becker & Hall and *Glomus clarum* Nicol. & Gerd. in eroded soil.

A.L. dos Santos[1], F.A. de Souza[2], R.L.L.Berbara[1] and J.M.Guerra[2]. [1] Universidade Federal Rural do Rio de Janeiro, Departamento de Solos, Seropédica, RJ, 23851-970; [2] Embrapa – CNPAB, Seropédica, RJ, Brazil.

The processes of recovering degraded lands may be benefited by the inoculation of selected arbuscular mycorrhizal fungi (AMF), for effectivety and competitiveness. Two greenhouse experiments were carried out with the aim of evaluating the establishment and infective capacity (IC) of introduced fungi in relation to community of autochthonous fungi (AF). In the first study, 10 plant species were cultivated (three grasses and seven legumes) with three inoculum treatments [control; *G. margarita* (CNPAB 001); *Gl. Clarum* (CNPAB 005)]. In the second study, the IC of the AMF was evaluated in soil after the first experiment in bioassays, with trap plants transplanted weekly to pots containing autoclaved soil. The establishment and IC of AMF were based on the presence of spores after harvest. The introduction of AMF and the plant species influenced the sporulation of AF (Acaulospora rugosa, Entrophospora colombiana, Gigaspora margarita and Glomus macrocarpum) in a different way. The inoculation made the establishment of the inoculated fungus in all tested plants possible. However, only *G. clarum* presented IC to compete with the AF. The IC of theses isolates showed no relationship to number of spores. The production of commercial inoculm using these fungi is discussed.

P1 71

Soil Compaction and Water Stress Effects on Arbuscular Mycorrhizal Fungi and Norway Maple Trees

Mauro Pezzente[1], Chantal Hamel[1], Marc St-Arnaud[2] and Yolande Dalpé[3]. [1]McGill Univ., Ste-Anne-de-Bellevue, Canada; [2]IRBV, Montreal, Canada; [3]Agric. Ag. Can., Ottawa, Canada

Certain AM fungi have been implicated in improving the health status of plants living under various environmental stresses. However, most experiments have been conducted with annual herbaceous plants and little is known about the effects of mycorrhiza on long-living plants such as trees. Our experiment involved 1-year old Norway maples, *Glomus intraradices*, 3 levels of compaction (1.6, 1.3 and 1.1 g/cm^3), and 2 water stresses (drought cycles or a final drought). Results indicate AM fungi increased shoot dry weight by 12% under the highest compaction and by 36% for the drought cycle treatment. There was a significant positive interactional effect at the highest stress levels by mycorrhiza. Mycorrhiza also increased fine root growth by 19% under the lowest compaction level and 4% under the highest level. Tree survival was also increased by mycorrhiza under all treatments. These results suggest AM fungi can improve health status of trees growing under high soil compaction and water stress. AM fungal inoculations should be considered when attempting to grow these trees under similar field conditions.

P1 72

Mycorrhizal relations of mangrove plant community of the Ganges River estuary in India

S. Chaudhuri and A. Sengupta
Department of Plant Pathology, BCKV (Agriculture University), West Bengal-741235, India.

Mangroves, the climax formation of hydrohalophytes, inhabit the strongly basic, inundated, estuarine salt marshes in the tropics and sub-tropics. Thirty-one species of mangroves and associates and fourteen species of transported flora, differentially distributed in four physiographic situations at the terminal part of the Ganges River estuary in India were examined for nature, extent and possible functions of the root endophytic fungus association(s).

Dominant members of the mangrove community were invariably colonized by (V)-A root endophytes, forming mostly '*Paris* type' structures, either alone or dually with dark septate mycelial endophyte(s). Members of non-mycotrophic plant families, except Cyperaceae, showed (V)-AM association with intracellular hyphae, coils and vesicles as the discernible structures. Intensity of (V)-AM colonization varied both with plant species and situations of their occurrence, being more intense and extensive in less saline ridge mangroves than in more saline formative or developed swamp mangroves. Introduced exotic trees on the ridges were less infected than the declining mangroves there. Seven common species of (V)-AM fungi isolated from mangrove roots colonized mycorrhiza responsive native trees and herbs in steamed locational silt and upstream alluvial soil giving significant dry matter yield and P-uptake response. Two dark septate mycelial root endophytes, isolated in axenic culture, colonized salinity tolerant herbs and trees in saline sand with evident improvement in their phosphorus nutrition and biomass yield. Significance of the findings for stress adaptation and community development of mangroves in semi-aquatic, nutrient limited saline environment is discussed.

P1 73

Impact of flooding on a Glomalean fungal population in an agricultural soil

D.M.H. Watson and T.W. Rufty, Jr. Department of Crop Science, North Carolina State University, USA

In the spring of 1999, using trap culture methodology, a survey of the Glomalean fungal population of an agricultural field was conducted to provide base-line data for a long-term systems experiment. Located at the Center for Environmental Farming Systems (CEFS) near Goldsboro, North Carolina, the field site covers approximately 200 acres and is situated at the confluence of two rivers. On September 16th, the field site was flooded by rain, as Hurricane Floyd swept over North Carolina. As much as three meters of water covered some of the field plots and soils were saturated for approximately three weeks. Glomalean fungi are aerobic organisms and are obligately dependent on living host plants to supply them with carbohydrate and a niche in which to live. While the vegetative stage of the fungus is susceptable to hypoxia, dormant resting spores can remain viable after extended periods in waterlogged soils. In the spring of 2000, using Global Positional Systems technology, post-flood soil samples were taken from permanently marked sampling sites and set up in trap cultures. Some Glomalean fungi had survived the flood. Of the eighteen identified Glomalean species recovered before the flood, twelve were recovered after the flood. The relative occurrence of one fungus, *Glomus clarum*, increased after the flood and the occurrence of the remaining eleven fungi decreased to varying degrees.

P1 74

The effect of summer flood of the river Oder in 1997 on the population of arbuscular mycorrhizal fungi

M. Tauschke, P. Lentzsch, G. Höflich, G. Schalitz, Centre for Agricultural Landscape and Landuse research (ZALF e.V.), Germany

Through the construction of summer dikes in river floodplains, such as in the "Lower Oder valley" large areas of flood polder may be used in the summer as grassland and meadow. Normally, these areas are transiently flooded in the winter season. As a result from violent rainstorms in the catchment area of the river occured a long time summer flood in July-September in 1997.

In the present study the influence of the 1997 summer flood on the genetic diversity of AM spores was analysed. It was part of a project concerning the effect of flooding incidences on the interaction of plants and microorganisms in the soil and rhizosphere. The analyses were carried out over three years at distinct locations of the Oder River flood polder in the National Park, "Lower Oder Valley".

In large areas around the river Oder, inundandet with water over 2-3 months, the vegetation was destroyed. This loss of host plants significantly reduced the AM population, which was genetically characterized by ITS - RFLP analyses of single spores. After comparison of all ITS-RFLP pattern of the predominant spore phenotype five genetically different groups were defined. At the end of the summer flood only three genotyps could be detected. The predominant genetic group (%) isolated in june 1997 was completely suppressed. In summer 1998 and 1999 this group had a portion of 5% and 7% resp. of the whole population. Therefore the summer flood 1997 caused in a change of the genetic structure of the population.

P1 75

Arbuscular mycorrhiza in the aquatic plant *Littorella uniflora* (L.) Ascherson

K.B. Nielsen, F.Ø. Andersen, D.R. Thomsen.
Institute of Biology, University of Southern Denmark, Odense, Denmark

The existence of arbuscular mycorrhizal (AM) symbiosis in the aquatic plant *Littorella uniflora* (shoreweed) has been known since 1977. *L. uniflora* often grows under very phosphorus limited conditions. The presence of arbuscular mycorrhiza is therefor thought to play an important role in the plants P acquisition. The objective of the present experiment was to study effects of mycorrhiza on *L. uniflora* and its root exudation of organic carbon. To do this we wanted to compare plants with and without active mycorrhiza under different phosphorus (P) concentrations. Sediment cores vegetated *with L. uniflora* were exposed to one of four treatments: no added fungicide (benomyl) or added fungicide, and low or high P-concentration in artificial porewater percolated through the sediment. Initial and terminal P-, carbohydrate- and low molecular organic acids (LOA) content of plants were measured as well as mycorrhizal colonization. Porewater concentrations of P, DOC, LOA and carbohydrates were followed during the four week experiment.

No LOA or carbohydrates were identified in porewater during the experiment. However, glucose was identified in porewater obtained by centrifugation of sediment before and after the experiment. After the experiment the highest concentration was found in cores not subject to benomyl treatment. LOA were only identified in a few cases. Mycorrhizal colonization was higher in plants treated with P and no benomyl than in plants before start and in the three other treatments.

P1 76

Ectomycorrhizae, soil substrates, and post-fire recovery in a permafrost-dominated watershed in subarctic Alaska

D.J. Helm. University of Alaska Fairbanks, USA

Distribution of ectomycorrhizae (EM) is related to plant species, their age, and soil conditions, which are functions of disturbance history. FrostFire was a prescribed burn in 1999 in a 700-ha, permafrost-dominated watershed, northeast of Fairbanks, Alaska, USA. Vegetation types included (1) mixed hardwood forest dominated by *Betula papyrifera* which also contained *Populus tremuloides* and *P. balsamifera* and (2) black spruce forest dominated by *Picea mariana* with ericaceous understory. The hypothesis is that since EM differ by vegetation type and soil substrate, some are more likely to survive a fire than others, either because of their depth or habitats where they occur. The survivors would be the most likely EM to colonize new plant seedlings or new roots from pre-existing plants. Pre- and post-fire samplings have used soil-litter cores to collect roots by horizon. The EM morphotypes present are being described and the EM community composition quantified by substrate depth and horizon in the burn and separate control areas. Black spruce sites burned more than the hardwood sites, but sphagnum patches frequently were not burned resulting in a mosaic of fire damage. Roots with mycorrhizae have been found in unburned moss and litter layers in pre-fire samplings. Ectomycorrhizae were found next to charcoal after the fire, supporting the concept that lethal temperatures do not extend very far into the soil. Where fire removed the insulating moss layer over permafrost, trees were uprooted rather than being consumed by fire. To date (August 2000), we have only sampled mature roots after the fire since seedlings have not yet colonized the site.

P1 77

VA Mycorrihza in a chenopod halophyte in highly saline soil

D.J. Weber, J. Jefferies, and H. Fisk. Brigham Young University. Provo, Utah

VA mycorrhizal fungi are normally not present in high saline soils, although there has been a couple of reports of VA mycorrhiza in saline environments. A negative correlation with sodium content of the soil and percent mycorrhizal colonization of roots of *Distichlis spicata* has been reported. Chenopods were normally considered to be non-mycorrhizal. *Allenrolfea occidentalis* (S. Wats.) Kuntze (iodine bush) is one of the most salt tolerant stem succulent shrub species (Chenopodiaceae) in the salt playas of Western United States. Investigations were made to determine if mycorrhizal spores were present in the soil and if the roots of *A. occidentalis* were colonized by mycorrhizal fungi. The pH of the soil over two summer seasons in the salt playa where *A. occidentalis* was the dominant plant was 7.3 to 8.3. The soil salinity varied over the two growing seasons from 29 to 146 dS m-1 and the soil moisture ranged from drought conditions (9.2% H_20) to wet (19% H_20). The salt concentration was significantly higher in the surface soil layers(upper 15 cm) than in the subsurface layers (15 to 30 cm). Soil and root samples were taken. The soil was processed by the wet sieve method to obtain potential mycorrhizal spore samples. The number of spores per gram in the saline soil was 3680 spore/g in young *Allenrolfea* and 1600 spores/g in old *Allenrolfea*. The roots were fixed in formaldehyde and stained with cotton blue and VA mycorrhiza observed. The estimated percent of infection of the roots was 24.3% in young *Allenrolfea* and 16.55% in old *Allenrolfea*.

P1 78

Role of vesicular-arbuscular mycorrhiza (VAM) on growth of banana in acid soil containing high aluminium

Nampiah Sukarno, Hadisunarso and Shanti. Bogor Agricultural University, Indonesia.

The main agricultural problem in acid soils containing high Al in Indonesia is the availability of phosphorus (P). This experiment aimed to study the role of VAM fungi in acid soil containing high Al on growth of Musa acuminata (banana) obtained from tissue culture. Three fungi, Glomus manihotis, G. fasciculatum, and Entrophosphora sp. were used to inoculate M. acuminata grown in pots containing 2 kg sterile soil and compared with non-inoculated controls. P application of 25.1 kg/ha to non-inoculated plants was also used to provide non-mycorrhizal, P supplemented, control plants. The three fungi colonised the plant roots and increased the growth of plants significantly, compared to the control plants. VAM fungi inoculation also improved the quality of plants in terms of plant health and survival. Inoculated plants did not severely show the toxicity symptoms due to low pH and high Al. Non-inoculated plants, however, suffered from Al toxicity. Glomus manihotis was the most effective mycorrhizal fungus and increased growth of M. acuminata to a greater extent than inoculation with either of the other fungi or application of P.

P1 79

The effect of two arbuscular mycorrhizal (AM) fungi on cowpea growth under low pH conditions

A. Rohyadi, S.E. Smith, F.A. Smith and R.S. Murray
The University of Adelaide, Australia

Arbuscular mycorrhizal fungi (AMF) may benefit various crop plants grown in acid soils, but their effectiveness highly depends on the fungi, plant and soil conditions. Many factors may influence AMF contributions under acidic soil conditions, such as excessive amounts of H^+ ions per se and/or increased aluminium (Al) toxicity at low pH. Management of the effects of low pH depends on understanding which mechanisms exert the strongest effect and in consequence it will be very valuable to distinguish the effects of pH itself from those of pH-related factors.

The effect of *Gigaspora margarita* and *Glomus etunicatum* on the growth of a cowpea cultivar 'Red Caloona' had been assessed by growing the plants, with and without inoculation, individually in pot containing a mixture of sand and soil (90:10 ratio) previously set up to have pH levels of 4.6, 4.9 and 5.2, and soluble-aluminium concentrations at sub-toxic levels of 1.18, 0.85 and 0.42 ppm respectively.

Non-mycorrhizal cowpea grew poorly particularly at pH 4.6. However, the growth was improved by inoculating with the two mycorrhizal fungi, regardless the pH. The improvement was highest at pH 4.6, and it decreased with increasing the pH. *Gi. margarita* was much more effective than *G. etunicatum*. The effectiveness may be related to the fungal ability to colonise the plant roots and grow actively in the medium. The results suggest that *Gi. margarita* preferred or tolerated low pH conditions, whilst *G. etunicatum* required a higher soil pH.

P1 80

The responses of ectomycorrhizal biomass and production to elevated CO_2

J.A. Langley[1], B.A. Hungate[1] and B.G. Drake[2] [1]Northern Arizona University, Flagstaff, Arizona, [2]Smithsonian Institute, Washington D.C., USA

In the context of global change, it is important to quantify the effects of CO_2 on soil inputs in intact ecosystems. Though ectomycorrhizal (EM) biomass can account for large portions of input into the soil, it is rarely considered in elevated CO_2 studies. In our study, portions of a regenerating scrub-oak system were enclosed in open-top-chambers and exposed to either ambient or elevated CO_2 concentrations. EM abundance was assessed in terms of colonization, but also in terms of biomass and production of EM roots. While standing EM and fine root mass (estimated from soil cores) showed no treatment effect after four years, EM and fine root production (estimated from ingrowth bags) increased sharply with elevated CO_2. The EM portion of total fine root mass was five times greater in ingrowth bags than in soil cores. This EM portion was not affected by CO_2 treatment in either ingrowth bags or soil cores. EM colonization frequency was 40% higher in ingrowth bags but also showed no CO_2 treatment effect. Innately slow belowground turnover in this system could mask CO_2 stimulation of fine root and EM growth in the standing crop. Because EM proportion of fine roots was much higher in newly produced roots than in the standing crop suggests that mycorrhizal roots turn over more rapidly than uncolonized ones. Decomposition of EM roots must be better addressed to accurately characterize the role of ectomycorrhizae in the carbon cycle.

P1 81

P1 82

The effects of low level ozone stress on the below- and above-ground compartments in the silver birch seedlings with three different mycorrhizal status

A.Kasurinen[1], A.M. Manninen[1], T.Holopainen[1] and J.K.Holopainen[1,2] 1) University of Kuopio, Finland 2) Agricultural Research Centre of Finland, Finland

Ozone is a phytotoxic and widespread air pollutant that is predicted to cause global forest decline in the future. It is also suggested, that tropospheric ozone increment is very likely to affect mycorrhizal root growth. In this study one-year-old potted silver birch (*Betula pendula* Roth.) seedlings were exposed to either ambient or 1.5 times the ambient ozone concentrations in the field during a three month period in 1996. Some of the seedlings had natural mycorrhizal status, whereas other seedlings were artificially inoculated with a *Paxillus involutus*-strain or treated with propiconazole fungicide to restrain mycorrhizal formation in their roots. The total mycorrhizal infection levels were highest in seedlings inoculated with *P. involutus* (40.69-44.23%) and lowest in fungicide (22.09-28.28%) treated birch seedlings. The lowest amount of ergosterol was observed in seedlings grown under ambient O_3 with *P. involutus*-inoculation (13.4 $\mu g^{-1}\ g^{-1}$). However, elevated ozone did not have any clear effect on the total mycorrhization. Shoot and foliage growth was not affected by elevated ozone level and there were no clear changes in root-to-shoot-ratios either. The observed total mycorrhizal infection levels did not have any significant effect on the shoot and foliage growth. In other words, high or low total mycorrhizal infection level of roots did not restrain or stimulate the above-ground growth of the seedlings in this study.

P1 83

Effects of long-term CO_2 and O_3 exposure on silver birch rhizosphere

T.Holopainen1), A. Kasurinen1) and E.Vapaavuori2) 1) University of Kuopio, Finland 2) Finnish Forest Research Institute, Finland

There is still limited information about the interactive effects of CO_2 and O_3 on the belowground compartments of forest trees. Moreover, most CO_2 and O_3 studies have been either short-term field experiments or experiments performed with seedlings grown under laboratory conditions. In this long-term (1999-2001) field experiment we are studying the effects of increased CO_2 and O_3 levels on fine root and mycorrhiza formation in ozone-sensitive and ozone-tolerant silver birch clones by root ingrowth cores. In addition to mycorrhizal root growth, we analyse total fungal biomass from roots and soil (ergosterol concentrations) plus measure rhizosphere soil CO_2 efflux. In 1999, before the experiment, the total mycorrhizal infection levels of trees varied between 79.55%-92.54%, whereas in 2000 the total mycorrhizal colonisation percentages were somewhat lower, ranging from 57.11% to 86.91%. The ergosterol concentrations of the fine roots were also normal for forest trees, values varying between 134-565 $\mu g^{-1}\ g^{-1}$. After two summers exposure (2000), elevated CO_2 and O_3 alone or in combination did not have any significant effect on the total mycorrhizal infection and root ergosterol level of the trees. Soil respiration rates were slightly higher in trees exposed to elevated ozone alone or in combination with elevated CO_2 during the first and second exposure season. Both root and soil analyses as well as soil respiration measurements will be continued during 2001.

P1 84

Effect of mycorrhizal fungus inoculation on *Castanea sativa* Mill germination, growth and weaning.

Anabela Martins*, Sandra Caldas*, Cidália Lino*, Joaquim Alves* M. J. Sousa* and M. S. Pais**. *Escola Superior Agrária de Bragança. Qta. de Sta. Apolónia. 5300 - 855 Bragança. Portugal. ** Laboratório de Biotecnologia Vegetal - ICAT, Campo Grande, 1749 - 016, Lisboa, Portugal.

Castanea sativa Mill seeds were surface disinfected and stratified in moistened sand under atmospheric temperature and humidity. After pre - sowing, seedlings were inoculated with the mycorrhizal fungus *Pisolithus tinctorius*. Weaning was performed under two different climatic conditions: 1) Controlled temperature and humidity (25°C, 70% RH), and 2) atmospheric temperature and humidity (5°C -25°C, 50 - 70% RH).

Several parameters were studied along germination, seedling development and plant growth: germination rate, plant growth rates (height and stem diameter, fresh and dry weights) and weaning rates.

From the results obtained we can consider that mycorrhizal inoculation does not influence neither seed germination nor plant growth in height or stem diameter when inoculation is made during sowing. If a second inoculation was performed, plant growth in terms of thickening, ratio stem diameter/height and FW/DW ratio are significantly higher in M plants. Mycorrhization can influence plant survival when weaning is performed under natural atmospheric conditions which accounts for the consideration that mycorrhiza can constitute na advantage for plants mainly when they are under environmental stressing conditions.

This work was supported by the Project PRAXIS MESOCASMIC.

P1 85

Occurrence of vesicular arbuscular mycorrhizae (VAM) in coastal habitats of Bahrain

Gul, J. and Mandeel, Q.
[1] Pakistan School/College, Bahrain. [2] University of Bahrain, Department of Biology

The occurrence of vesicular arbuscular mycorrhizae (VAM) in the rhizosphere soil and roots of wild and cultivated plants were investigated in coastal habitats in Bahrain. Soils were generally highly saline, with salt crusts on the top layer, low in soluble salts, slightly alkaline and poor in organic matter. The slide length method was used to quantitatively estimate VAM infection in roots and the wet-seiving and decanting method was used to isolate VAM spores from the rhizosphere soil. All the recorded isolates in this survey represent one species of *Glomus mosseae*, which is recovered for the first time from the hot, arid desert environment of Bahrain. The abundance of VAM in the soil and roots revealed an irregular distribution pattern and generally vesicular colonization and arbuscular infections were less frequent, when compared to similar desert systems. Spores were also present in low numbers in the cortex of some plants. When compared to wild flora, cultivated plants revealed a high VAM infection rate. The results indicate that salinity may have a detrimental effect upon mycorrhizal establishment, distribution and abundance in such habitats.

P1 101

Interaction effects of laser irradiated *Glomus mosseae* and *Bacillus pumilus* on *Fusarium oxysporum* f.sp. *lycopersici* in tomato plants. S. A. Ouf. Botany Department, Faculty of Science, Cairo University, Giza 12613, Egypt.

A pot experiment was conducted to study the interaction between vesicular arbuscular mycorrhizal (VAM) fungus, *Glomus mosseae* treated with different doses of He-Ne laser irradiation and bacterial antagonist, *Bacillus pumilus*, on soilborne pathogen *Fusarium oxysporum* f.sp. *lycopersici* in the rhizosphere of tomato. The percentage mycorrhizal root colonization and mycorrhizal spore count were higher in the rhizosphere soil of plants inoculated with laser irradiated mycorrhizal fungus than non-irradiated one. The best irradiation dose was 7.3 mW for one minute. The presence of the bacterial antagonist enhanced VAM colonization and increased spore count. The interaction effect of bacteria not only increased plant growth and yield but also improved P nutrition and plant resistance to the pathogen.

P1 102

Interactions between two arbuscular mycorrhizal fungi and fungal feeding nematodes and control of the nematode with phenamiphos

Yenni Bakhtiar[1,2], Debbie Miller[2], Tim Cavagnaro[2], and Sally Smith[2]
[1]Biotech Center - BPP Teknologi, Indonesia [2]The University of Adelaide, Australia

A pot experiment was conducted to investigate the effect of the fungal feeding nematode (*Aphelenchus avenae*) on two arbuscular mycorrhizal (AM) fungi, *Gigaspora margarita* and *Glomus coronatum* grown with clover (*Trifolium subterraneum* L.) and their control with the nematicide phenamiphos.

Colonisation and sporulation of the fungi were influenced by the nematodes. In mycorrhizal pots, the number of nematodes increased with a concomitant decrease in percent mycorrhizal colonisation. The nematode populations in non-mycorrhizal pots declined below the starting density. The number of fungal spores was little affected by the presence of nematodes, but the percentage of spores with contents decreased, particularly for *G. coronatum*. These results indicate that both species of AM fungi acted as food sources for nematodes. The greater susceptibility of *G. coronatum* is probably related to the thinner spore walls which could be more easily penetrated by the nematode stylet. There were non-linear mycorrhizal growth responses to the different nematode densities. Application of phenamiphos was effective in diminishing nematode populations and increasing the colonisation and sporulation by AM fungi. Treatment of pot cultures with phenamiphos is an effective control for contaminating nematodes.

P1 103

Effect of mycorrhizal inoculation on tolerance of *Castanea sativa* Mill plants to *Phytophtora cinnamomi* infection

Anabela Martins*, Eugénia Gouveia*, Valentim Coelho*, Isabel Estevinho* and M. S. Pais**.
*Escola Superior Agrária de Bragança. Qta. de Sta. Apolónia. 5300 - 855 Bragança. Portugal. **
** Laboratório de Biotecnologia Vegetal - ICAT, Campo Grande, 1749 - 016, Lisboa, Portugal.

Two different trials (1) and (2) were made in order to test mycorrhizal inoculation effect against *Phytophtora cinnamomi in vivo*. *Castanea sativa* Mill seedlings were inoculated with *Pisolithus tinctorius* at sowing (M1). Two month later, half of the M1 plants were reinoculated (M2) with *P. tinctorius*. 1) At the same time, M1, M2 and nonmycorrhizal (NM) plants were inoculated with 0,1%, 0,5% and 2% of *P. cinnamomi*; 2) One month after reinoculation, M2 and nonmycorrhizal (NM) plants were inoculated with 0,1% and 0,5% *P. cinnamomi*

The results obtained for the two trials were very different in terms of mycorrhizal protection against *P. cinnamomi*. Trial 1) shows that mycorrhizal inoculation simultaneous to reinoculation could not induce protection against *P. cinnamomi*. Results of trial 2) showed mycorrhizal protection against *P. cinnamomi* for both percentages of inoculation

From the results obtained we can consider that mycorrhizal protection against *P. cinnamomi* depends on the time of mycorrhizal association. Trial 2) shows that 3 month after mycorrhizal fungus inoculation and 1 month after reinoculation plants present some degree of protection against *P. cinnamomi*, suggesting that after an adequate period, mycorrhization induces plant protection against this pathogenic fungus.

This work was supported by the Project ECOPLUGMIC.

P1 104

Influence of soil phosphorus on the interactions between the mycorrhizal fungus *Glomus coronatum*, and binucleate *Rhizoctonia* or *Rhizoctonia solani* on mung bean

R.S. Kasiamdari, S.E. Smith, F.A. Smith and E.S. Scott
The University of Adelaide, Australia

Previous studies on the interactions between arbuscular mycorrhizal (AM) fungi and soil-borne pathogens have shown that increased disease resistance of plants might be related to enhanced phosphorus (P) nutrition. In order to investigate this possibility, the influence of P fertilization and inoculation of the AM fungus (*G. coronatum*) on the infection and disease severity of binucleate *Rhizoctonia* (BNR) and *R. solani* on mung bean were investigated. Two experiments were conducted in two levels of P addition (P0 and P1), in which BNR or *R. solani* were inoculated at the same time or 3 weeks after *G. coronatum* was established in the roots. After 6 weeks, *R. solani* reduced the growth of non-mycorrhizal plants in P0 soil, whereas BNR had no effect. Simultaneous inoculation with *G. coronatum* and BNR or *R. solani* in P0 soil resulted in improved growth, reduction in disease severity due to BNR or *R. solani*, and reduction in colonisation of roots by BNR, but not *R. solani*. When *G. coronatum* was established in the roots 3 weeks before BNR or *R. solani* was added to the potting mix, there was no significant effect of BNR or *R. solani* on growth of mung bean. Early colonisation by *G. coronatum* protected plants from disease caused by BNR or *R. solani*. In both experiments, addition of P stimulated plant growth, but had little effect on disease severity. The results showed that the reduction of the effect of BNR or *R. solani* on mung bean could not be explained by improved P nutrition, but could be attributed to the presence of *G. coronatum* within and among the roots.

P1 105

Ultrastructural effects of a fungal endophyte in the roots of eggplant and their potential role in disease suppression.

K. Narisawa, R. S. Currah, M. Chen and A. Tsuneda University of Alberta, Canada

In eggplant, roots that are first colonized by endophytic fungi, before being challenged with *Verticillium dahliae*, exhibit a high level of resistance to the wilt caused by this pathogen. The underlying mechanism for this phenomenon is not understood but it is possible that resistance develops because the host plant's defense response has been triggered by the endophyte, thus creating in advance a barrier to the ingress of the pathogen. To find support for this hypothesis the ultrastructural aspects of the interaction between an unidentified but effective fungal endophyte, Sterile White Mycelium (SWM), and the cells of eggplant roots was examined. Two weeks after inoculating plants with SWM, hyphae had grown over the root surface, formed appressoria at hyphal tips, and had penetrated host cell walls enzymatically via narrow infection hyphae. Opposite penetration points and along the inner wall surface, host cells formed electron dense papillae that elongated and lignified as penetration continued. Hyphae that extended through papillae subtended swollen globose infection vesicles. By four weeks post inoculation, closely packed masses of hyphae had formed within host cells in regions of the epidermis. By eight weeks, the fungus was common in cortical cells but was not in cells of the vascular tissues. Ultrastructural features did not indicate necrosis in the cells of either host or endophyte and the relationship between the two organisms may be a type of balanced antagonism. For the plant, these antagonistic effects of the endophyte may provide an early line of defense against subsequent challenges by a pathogen.

P1 106

FERTILIZER AND INOCULATION OF MA FUNGI EFFECT ON THE DEVELOPMENT OF A FOLIAR DISEASE(*Phoma costarricencis* Echandi)ON COFFEE SEEDLINGS.

M. Escalona., D. Trejo., and M. Abato. Agriculture Science School, Universidad Veracruzana , Mexico

There are few reports on the benefits of AM fungi on the biocontrol of foliage disease and these seem contradictory. When coffee seedlings are inoculated with AM fungi they are not affected by a foliage disease known as Brown eye spot ,this due either to the positive effect of a grater phosphorus assimilation or to the resistance factor attributed to the AM fungi. Ten treatments were carried out in this work:1)Sterile soil(SS)+filtrate(fil)+Phosphorus(P), 2) SS + P, 3) SS + MA fungi (native complex MTZ 1) + fil +P, 4) SS +MA fungi + P, 5)No sterile soil (NSS) + P, 6) SS + MA fungi, 7) SS + fil, 8) SS + MA fungi , 9) NSS y 10) SS; during six months there were measured: severity of disease, number of leaves, height, air and root biomass ,percentage of colonization and the number of spores .It was found that disease severity was less in treatments with Phosphorus and MA fungi and with MA fungi + filtrate, being not statistical significant differences between treatment with phosphorus and MA fungi , suggesting a lowering on disease severity as a positive effect of nutrition not mattering the source ;at the present time there is a work related with the measurement of Poliphenoloxidase activity.

P1 107

Effect of arbuscular mycorrhiza fungi (AMF) on *Meloidogyne hapla* in pyrethrum in Kenya

J.W. Waceke,[1] S.W.Waudo[1] and R.Sikora[2]
[1] Botany Department, Kenyatta University, P.O.Box 43844, Nairobi, Kenya
[2] Institute of Plant Diseases, University of Bonn, Nussallee 9, D- 53153, Germany

Two arbuscular mycorrhiza fungi (AMF) isolated from a pyrethrum growing region in Kenya were screened for efficacy against a nematode, *Meloidogyne hapla* in the greenhouse. The fungi were identified at INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi) as *Glomus etunicatum* (Isolate KS18) and *Glomus* sp. (Isolate KS14). Isolate KS14 (*Glomus* sp.) significantly suppressed nematode population, growth and development by up to 54%, egg production by up to 75% and disease severity by up to 71%. *Glomus etunicatum* (Isolate KS18) suppressed nematode growth and development by up to 50%, egg production by up to 75% and disease severity by up to 57%. In addition, *G. etunicatum and* Isolate KS14, significantly improved top dry biomass of pyrethrum by up to 33% and 47%, respectively. *Glomus etunicatum* and *M. hapla* were mutually inhibitory as root colonization by G. *etunicatum* was significantly reduced (up to 24%) by the presence of the nematode. The presence of the nematodes, on the other hand, did not significantly affect root colonization by Isolate KS14.

P1 108

Testing of AMF propagation in tubular rhizoboxes with different plant pot culture and MHB co-inoculation

A.Ksiezniak, W.L.Paszkowski, B.Wróblewska and T.Doroszewska
Institute of Soil Science and Plant Cultivation in Pulawy, Poland

Effectivity of AMF sporulation depends on many environmental and soil factors, which can affect both the host plant root growth and AM fungus activity. The plant roots growth conditions and their susceptibility to the mycorrhizal fungi may be modified in the arable soils by allelopathic interactions in the rhizosphere between different kind species plants i.e. cereals and weeds, and by plant roots-AMF-rhizobacteria interactions.

We have undertaken the research to exploit some dependences between root architecture, the plant root metabolites and selected rhizobacteria on AMF activity and spore propagation in the controlled pot culture conditions.

The tubular rhizoboxes can help us to assess the ability of selected plants - as cooperators for host plant - to stimulate or inhibit the host plant root mycorrhizal colonization and AMF sporulation. Simultaneous use the "sunbag" technique and tubular rhizoboxes with screen (50 µm) may help search for the effect of the co-inoculation of the cooperator plants with rhizobacteria which showed the root stimulation effect.

Tubular rhizoboxes technique should be also useful in the search for macronutrients translocations and other interactions in the conterminous mycorrhizosphere different plants.

Project supported by Polish Committee for Scientific Research (KBN), Grant No P06 A 00120

P1 109

High cell density promotes the outgrowth of intraradical hyphae of *Gigaspora* from carrot root explants; a step towards axenic culture?

P..G.Williams, University of New South Wales, Sydney, Australia. Plant and animal cells in culture require high cell densities for optimum growth. Likewise, the obligately biotrophic rust fungi leak amino acids in axenic culture so that hyphal growth is dependent on high cell numbers per unit volume of medium. The possibility that the growth of arbuscular mycorrhizal (AM) fungi is also sensitive to cell density was investigated using mycorrhizas of *Gigaspora* in transformed carrot roots. Single, isolated explants incubated for 4-6 days on M medium agar produced only the common, sparingly branched extraradical hyphae. But when two or more mycorrhizal explants were incubated closely side-by-side, not only extraradical hyphae grew into the agar but also intensely branched, swollen, monilioid hyphae interpreted as intraradical (assimilative) hyphae of *Gigaspora*. Hyphae of intermediate morphological complexity were also seen. It seemed that an interaction was occurring between mycelia in adjacent explants. To explore this possibility, single mycorrhizal explants were incubated on agar of different thickness and the occurrence of hyphae with complex morphology was recorded. The results showed that the frequency (%) of explants producing both intraradical and extraradical hyphae was inversely related ($p<0.05$) to the thickness of the agar. These observations may prompt new kinds of experiments in the search for a method for growing *Gigaspora* in axenic culture.

P1 110

Agarose as a suitable substrate for growing arbuscular mycorrhizal fungi *in vitro*

V. Karandashov, I. Kuzovkina. Timiryazev Institute of Plant Physiology, Moscow, Russia Monoxenic culture on pRi T-DNA transformed plant roots is a valuable tool for studying the biology of arbuscular mycorrhiza (AM). Agar or gellan gum usually used for solidifying nutrient medium have high level of impurities. In the present study, these substances were replaced with agarose which contain the lowest concentration of contaminant elements, foremost phosphorus. Such culture conditions allowed the establishment of well developed AM of eight *Glomus* isolates. All fungi, except *G. etunicatum*, formed extensive mycelium exploring the whole volume of nutrient medium. *Glomus etunicatum* hyphae grew within 1-2 cm from host roots. Sporulation started after 1-4 wk of growth and followed a classical sigmoid curve with most spores produced within 2-3 mo of dual culture. After 4-5 mo of growth on transformed carrot roots, *G. caledonium* formed up to 490 mature resting spores, *G. etunicatum* – 1180, and two isolates of *G. intraradices* – 37800 and 87200 spores per plate. Four unidentified AMF recovered from Russian soils produced from several hundreds to several thousands of spores. In plant hairy roots, AMF formed *Arum*-, *Paris*-, and intermediate type mycorrhizas. Percentage root colonization approximated 10-15% (max 25%) for different isolates. *Glomus etunicatum* colonized less than 5% of total root length. Intraradical hyphae of *G.* sp. MR1 sometimes grew inside carrot xylem cells. All isolates were successfully subcultured *in vitro* for several generations. Fungal development remained similar to that of initial cultures. However, for some isolates, increase of sporulation rates as well as decrease of spore size were observed during the successive subcultures.

P1 111

Effect of P-deficient root exudates on hyphal elongation and branch of arbuscular mycorrhizal fungi.

K. Tawaraya, S. Suda, M. Murasawa, Y. Naeki, T. Murayama and T. Wagatsuma
Yamagata University, Japan

Effect of root exudates on arbuscular mycorrhizal colonisation was investigated. P-deficient root exudates were collected from solution culture of onion (*Allium cepa* L.) and purified fractions were obtained by TLC. Purified fraction (0.7-3.1 mg) was separated from 1000 ml of root exudates. Spores of *Gigaspora margarita* were incubated with or without (control) root exudates and their purified fractions for 10 days. Hyphal elongation from spores of *G. margarita* was increased with P-deficient onion root exudates and the fractions. Primary hyphal branches were not affected by root exudates and the fractions but secondary hyphal branches were increased. These results suggest that both increased hyphal elongation and increased hyphal branch enhance opportunities for contacts between fungi and host plants and thereby mycorrhizal colonisation.

P1 112

Red radiation stimulates hyphal growth of vesicular-arbuscular mycorrhizal fungi *in vitro*

Y. Yachi, T. Ishii and S. Horii Faculty of Agriculture, Kyoto Prefectural University, Kyoto, Japan

We succeeded in the axenic culture of vesicular-arbuscular mycorrhizal (VAM) fungi, such as *Gigaspora margarita, Glomus caledonium, Glomus fasciculatum* and *Glomus* sp. R-10, in an agar medium amended with 25% MeOH eluates of bahiagrass root extracts obtained by using a flash chromatograph (Ishii et al, 1995; 1998). New spore formation was also observed in this culture. However, the successful re-growth of sub-cultured hyphae to form new hyphae under the axenic conditions was slow. Therefore, we examined the effect of an environmental factor such as radiation on the hyphal growth of VAM fungi *in vitro*. When the hyphae of *Gigaspora margarita* (Central Glass Co., Ltd.) in the petri dishes were incubated in the dark and under the conditions with fluorescent lamp and red lamp at 27°C, the hyphal length under the condition with red lamp was 4 to 9 times of that under the conditions with fluorescent lamp or in the dark. In this congress, we will minutely present the effects of red radiation on hyphal growth of VAM fungi *in vitro*.

P1 113

Blue light and chemical signals from a host root exudate synergistically stimulate hyphal branching of the AM fungus *Gigaspora gigantea*

G. Nagahashi and D. Douds, Jr U. S. Dept. Agriculture, Eastern Regional Research Center, Wyndmoor, PA, USA.

Recently, light-induced hyphal branching of arbuscular mycorrhizal (AM) fungi was reported (Nagahashi and Douds, 2000). It is also well documented that chemical components of the host root exudate also induce hyphal branching of AM fungi. Compounds that can induce the same morphogenetic or biochemical response as light are referred to as photomimetic agents. We report here the synergistic stimulation of hyphal branching of *G. gigantea* by blue light and photomimetic components semipurified from the exudate of a host root. With a short exposure to blue light and an application of diluted exudate components, the induced hyphal branching was greater than the sum of branches generated by blue light and chemical components tested separately. The synergistic stimulation was greatest at the higher levels or orders of branches

---

P1 114

Hypaphorine, an auxin antagonist delivered to the root by the ectomycorrhizal fungus *Pisolithus tinctorius*.

F. Lapeyrie, F.A. Ditengou, T. Kawano, H. Lagrange, A. Jambois, V. Legué - UMR INRA-UHP Interactions Arbres/Micro-organismes, Nancy, France

The role of fungal auxin in ectomycorrhiza has repetitively been suggested and questioned suggesting that if fungal auxin regulates some steps of colonised root development, its activity might be tightly controlled in time and in space, by plant and/or fungal regulatory mechanisms. Hypaphorine, the betaine of tryptophan, accumulation in *Pisolithus tinctorius* hyphae is controlled by root diffusible molecules, including zeatin. In return, fungal hypaphorine counteracts specifically the activity of IAA in host plant root and shoot. Recent results strongly suggest that IAA and hypaphorine interact during the very early steps of IAA perception or signal transduction pathway. In roots, hypaphorine and endogenous IAA interact to control root hair elongation, during ectomycorrhiza development, the absence of root hairs might be due in part to fungal release of root hair elongation inhibiting molecules, such as hypaphorine. While there are numerous examples where plant microbe interactions lead to increased auxin accumulation in plant tissues, due either to massive microbial auxin delivery, to host plant synthesis of auxin following microbe genome integration, or to auxin transport disruption, this might be the first reported case where the microbe is down regulating the auxin activity in the host plant.

P1 115

Analysis of indole-3-butyric acid function in arbuscular mycorrhiza using the fluorinated IBA analog TFIBA

M. Kaldorf[1], D. Fitze[2] and J. Ludwig-Müller[2]
[1]Institute of Ecology, University Jena, Germany
[2]Institute of Botany, Technical University, Dresden, Germany

The establishment of an arbuscular mycorrhizal (AM) symbiosis requires a continuous exchange of signals between host roots and fungus. Inoculation of maize with the AM fungus *Glomus intraradices* resulted in a distinct root phenotype with significant increase in the percentage of lateral roots during early stages of colonization. Auxins are known to play a role in different developmental processes including fine root formation and are therefore suitable candidates for the regulation of AM symbiosis. The increase in lateral root development coincided with an increase in the levels of the auxin indole-3-butyric acid (IBA). The phenotype of mycorrhizal maize roots could be mimicked by exogenously applying IBA to non-mycorrhizal roots. Addition of trifluoro-IBA (TFIBA), an inhibitor of IBA-induced root growth and lateral root induction, simultaneously with IBA resulted in a phenotype resembling that of untreated controls. In roots treated with TFIBA the inoculation with AM-fungi did not increase the formation of fine roots. The TFIBA-treatment also reduced endogenous free IBA and the percentage of colonization (intraradical hyphae and arbuscule formation) in mycorrhizal roots. The mechanism of TFIBA action is investigated by using a two-compartment system. A model for the possible role of IBA in the establishment of AM symbiosis will be presented.

P1 116

P1 117

P1 118

Partner recognition in the *Geosiphon pyriforme* symbiosis - a model system for AM research

E. Wolf, A. Schüßler
Darmstadt University of Technology, Germany

Geosiphon pyriforme (Kütz.) v. Wettstein is a unique endosymbiosis of a fungus and a cyanobacterium. The fungal partner was shown by sequence analysis to belong to the obligate symbiotic arbuscular mycorrhiza (AM) forming fungi. *Geosiphon* shows similarity to the AM fungi in morphology, the structure of the symbiotic interface and is also obligately symbiotic. In contrast to the plant root associated AM fungi, *Geosiphon* offers advantages for microscopic investigations: observation of the living organism can be performed with good optical properties even for several days. Thus, in our opinion *Geosiphon* represents an interesting model system for AM research.

A typical pear-shaped 1-2mm long *Geosiphon* "bladder" develops after the incorporation of a cyanobacterial filament (*Nostoc punctiforme* (Kütz.) Hariot) into the cytoplasm of a fungal hypha. Each individual bladder is the result of a new incorporation. As only a certain stage in the cyanobacterial life cycle is incorporated by the fungus, the recognition between the partners is to a large degree specific. By synchronisation of the *Nostoc* life cycle using alternating red and green light illumination we are able to induce and study the incorporation process. Time-lapse microscopy revealed that the recognisable *Nostoc* stage is an early primordium stage that exists only in a narrow time range. *Nostoc* filaments change the carbohydrate composition of their surface mucilage during their life cycle. The possible role of glycoconjugates as specific recognition signals in the *Geosiphon* symbiosis is investigated by saturation experiments and CLSM studies of surface carbohydrate changes, visualised by fluorescence-coupled lectins.

P1 119

Secondary metabolites acumulation in monoaxenic culture *Prosopis laevigata-Gigaspora rosea.*

R. Rojas [1], J. Hernández [1], J. T. Frías [2], L. Dendooven [1], C. Cerda [1], G. Bécard [3], A.C. Ramos-Valdivia [1] and Olalde-Portugal V [1].
[1]Centro de Investigación y Estudios Avanzados del IPN, Mexico; [2]Universidad de Guanajuato, Mexico; [3]Université Paul Sabatier, France.

The molecular mechanism involved in the bi-directional recognition between host plant and arbuscular mycorrhizal (AM) fungi in pre-symbiotic phase is poorly understood. Stimulation of hyphal elongation and extensive ramification *in vitro* has been observed in spores of genus *Gigaspora* when partially purified exudates of hairy roots of carrot and other host plants were added. This stimulatory effect has not been observed when the exudates were not from plant host indicating the lost of the inductor compound or its dilution. The active chemical compounds which produces those morphogenetic changes are synthesized by host plant and have not been identified yet. Mesquite or *Prosopis laevigata* is a leguminose shrub endemic of Mexico which grows in semiarid zones and establish arbuscular endomycorrhiza. We investigated possible mechanisms of recognition by inoculating spores of *Gigaspora rosea* in a monoaxenic culture of Mesquite. An elongation and hyphal ramification occurred after one week similar to those found when root exudates were added. Plants in contact or not with spores were analysed for secondary metabolites on HPLC. A maxplot showed accumulation of secondary metabolites which will be identified by NMR. Phenols, flavonoids and recently ciclohexanones derivates have been proposed like to be related with the mechanisms in the establishment of mycorrhizal symbiosis. Our system let us observed biochemical responds induced in the host plant at the presence of the spores while pre-simbiotic phase.

P1 120

Allene oxide cyclase in *Medicago truncatula* – implications for a role of jasmonates in the establishment of mycorrhiza.

Bettina Hause, Irene Stenzel, Otto Miersch, Robert Kramell, and D. Strack
Institute of Plant Biochemistry, Halle, Germany

Jasmonates (JA) and their precursors, the octadecanoids, are lipid-derived signaling compounds in plant defense reactions and plant development. In order to inspect possible role of JA in the interaction of arbuscular mycorrhizal fungi with plants we analysed mycorrhization of barley (*Hordeum vulgare* cv. Salome) roots with *Glomus intraradices*. Here, an increase in the endogenous levels of JA as well as of its amino acid conjugate JA-Ile correlates with the degree of mycorrhization. Data will be presented on temporal and spatial expression pattern of genes coding for JA-biosynthetic enzymes during different stages of mycorrhization.

The crucial step in JA-biosynthesis is the formation of its correct stereoisomeric precursor, *cis*(+)12-oxo-phytodienoic acid. This step is catalyzed by allene oxide cyclase (AOC) which has been cloned recently from tomato (1). In this report, cloning and enzymatic activity of recombinant AOC from *Medicago truncatula* will be presented. AOC mRNA accumulation during mycorrhization as well as upon environmental stress will be shown.

Based on data about AOC expression and formation of JA and its amino acid conjugates, a putative role of these compounds in establishment of mycorrhiza is discussed in terms of consequences for sink-source relationships and plant defense reactions.

(1) Ziegler, J., Stenzel, I., Hause, B., Maucher, H., Miersch, O., Hamberg, M., Grimm, M., Ganal, M. and Claus Wasternack, C. (2000) Molecular cloning of allene oxide cyclase: The enzyme establishing the stereochemistry of octadecanoids and jasmonates. *J. Biol. Chem.* **275**, 19132-19138

P1 121

A phosphate deficiency-induced flavonoid glucoside stimulates vesicular-arbuscular mycorrhiza formation in melon roots under low and high phosphate conditions

K. Akiyama, H. Matsuoka and H. Hayashi Osaka Prefecture University, Osaka, Japan

Melon (*Cucumis melo*) grown under phosphate-limiting conditions was analyzed for the levels of secondary metabolites in roots during colonization by the vesicular-arbuscular mycorrhizal fungus *Glomus caledonium* using HPLC. A compound in the methanol extracts from non-inoculated roots showed a significant increase 40 days after sowing. No accumulation was observed in *G. caledonium* colonized roots or in roots grown under high phosphate conditions. The compound was isolated by column chromatography and identified to be flavone *C*-glucoside, isovitexin 2"-*O*-β-glucoside by spectroscopic methods. The effect of the compound on root colonization of melon by *G. caledonium* was examined under low (0.05 mM) and high (2 mM) phosphate conditions. Forty-five days after inoculation, the percentage of colonization in control roots grown under low and high phosphate conditions were 22% and 8.8%, respectively. The compound applied to the soil at 20 μM increased root colonization to 36% under low phosphate conditions. Surprisingly, high degree of colonization (25%) was observed in roots grown under high phosphate conditions upon treatment by the compound at 20 μM. Isovitexin 2"-*O*-β-glucoside might be a endogenous stimulatory factor for mycorrhizal colonization in melon.

P1 122

Study of nutrient and water availability of mycorrhizal *Daucus carota* hairy roots on *Glomus intraradices* AM fungus development

M. Jolicoeur and M. Perrier École Polytechnique de Montréal

Among the multiple positive effects of AM fungi on host plants, the increase in draught resistance and nutritional stresses resistance are well known. This study presents the use of those parameters as a way to stimulate fungal development in *in vitro* cultures using carrot hairy roots as host. An aeroponic culture system was studied using a bed of perlite particles to support the mycorrhizal roots. Multiple culture strategies were assessed: 1) the culture process was separated in two steps: a first step using a modified White's culture medium which was continuously fed as a mist, followed by a step using a minimal medium which was also continuously fed as a mist; 2) a first step using a modified White's culture medium continuously fed as a mist, followed by a step using a static minimal medium placed at the bottom of the supporting bed; and 3) a three steps culture process with the use of a static liquid medium prior to the second culture process described. The amount of spores in the supporting bed was estimated and the amount of vesicles and their distribution density within the roots' tissues were measured. Results were compared with submerged air-lift bioreactor. The second culture process strategy resulted in an increase in spore level by a factor of 50 as compared to an air-lift bioreactor, from an average of 5 000 spores to 280 000 spores per bioreactor of 1 L in 120 d. However, the third culture process strategy led to an increase in the vesicles level from hundreds to 80 000 per bioreactor of 1 L in 120 d.

P1 123

Isolation and characterization of monokaryotic cultures of *Pisolithus* sp.

M.C.M. Kasuya, M.D. Costa, G.M.D. Pereira, A.C. Borges; Universidade Federal de Viçosa, Departamento de Microbiologia, Viçosa, MG, Brazil

The germination of spores of ectomycorrhizal fungi occurs at low rates, representing an obstacle for obtaining monokaryotic cultures. The objective of this work was to study the germination of *Pisolithus* sp. spores in the presence of two eucalypt species in media supplemented with yeast extract (YE) or tea extract (TE), and to characterize the monokaryotic strains obtained. The spores were distributed onto Petri dishes containing malt medium. After 7 d of incubation at 25 °C, spores free of contamination were spread onto modified Melin-Norkrans medium (MMN) containing activated charcoal and the supplements. One *E. grandis* or one *E. citriodora* seedling was placed in the Petri dishes and incubated at 25 °C. Fungal colonies were observed on the 20th day after spreading the spores onto the medium. No differences in spores germination were recorded for the media tested. However, germination rates varied due to the presence of different host plants. In the treatments with *E. citriodora* a total of 124 colonies were obtained, with *E. grandis* 14 colonies, and without any host plant 7 colonies. Monokaryotic cultures displayed wide variation in colony morphology, color and growth rates and are now being tested for mycorrhizal colonization using the Petri dish technique. The higher germination rates observed with *E. citriodora* may be related to specificity phenomena or to a higher production of stimulating factors of spore germination. Interspecific differences within *Eucalyptus* in stimulating spore germination may affect the efficiency of spore-based inoculants for the production of mycorrhizal seedlings.

P1 124

Effect of AM symbiosis and phosphate on root ploidy

G. Lingua[1], A. Fusconi[2], A. Trotta[2], and G. Berta[1]
[1]DISTA, Università degli Studi del Piemonte Orientale "A. Avogadro"
[2]DBV, Università degli Studi di Torino

Endopolyploidy is developmentally regulated according to the age, position and function of the tissue within the plant. It is also related to the metabolic activity of the cells and recent data suggest an involvement in the regulation of gene expression. It is a widespread phenomenon in plants with low DNA content such as tomato, in which colonisation by the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* induces an increase of the nucleus population with the highest DNA content (octoploid - 8C) in the cells where the arbuscule is formed.

In this work, we have investigated by flow cytometry the effect of colonisation by *G. mosseae* (BEG 12) on the DNA content of the root cells of leek (*Allium porrum* L.), a plant with high DNA content. Control and mycorrhizal plants were grown with two nutrient solutions, differing in phosphate concentration (3.2 and 96 µM), to test the nutritional effects on polyploidisation. Both fungus and high levels of phosphate lead to an increase of tetraploid (4C) nuclei. Accurate statistical analyses suggested that this event probably depends on the P concentration in the plant, which was measured with chemical techniques, as a significant correlation between phosphorus content and percentage of 4C nuclei was observed, independent of mycorrhization. Therefore, in leek the fungus may induce polyploidization by improving P nutrition. In contrast, AM colonisation is likely to affect the ploidy of tomato root cells in a way less directly dependent on P.

P1 125

Ectomycorrhiza development and cytoskeleton

M. Raudaskoski, M. Tarkka, J. Lahdensalo, M. Hanif and E. Laitiainen Plant Physiology,
Department of Biosciences, FIN-00014 University of Helsinki

Short roots are a special feature of *P. sylvestris* root system and only these roots are infected by the ectomycorrhizal fungus *S. bovinus*. The penetration of fungal hyphae in the short root takes place in a special region just behind the root meristem and causes an increase in short root meristematic activity. We are analysing in more detail the cell wall composition and cytoskeletal organization of the cortical root cells in the infection zone. The change in wall composition could also lead to production of signal molecules that indirectly or directly affect the cell cycle regulation in the root meristem. Several cyclin fragments have been isolated from *Pinus* root system in order to be able to follow the cell cycle regulation during formation of ectomycorrhiza. From a cDNA library of *S. bovinus* vegetative hyphae cDNAs encoding actin, Gα1 subunit of the heterotrimeric G-protein, and small GTPases Rac1, Cdc42 and Ras1 have been isolated (1). These proteins are known to play a central role in yeast and animal cells in signal transduction pathway leading to reorganization of actin during cell morphogenesis. The preliminary work (2) suggests that this is also the case at formation of ectomycorrhiza when the hyphal growth pattern changes from the vegetative tip extension growth to the branched and swollen one typical to symbiotic hyphae. *Raudaskoski et al. 2001,Cell Biology of Plant and Fungal Tip Growth. Nato Science Series 328:123-136. Gorfer et al. 2001: MPMI 14: 135-144.*

P1 126

Cytoskeltal reorganization in *Medicago truncatula* root cells during development of an arbuscular mycorrhizal symbiosis

L. Zhao, E. B. Blancaflor, and M. J. Harrison. Plant Biology Division, The Samuel Roberts
Noble Foundation, Inc., 2510 Sam Noble Parkway, Ardmore, Oklahoma, USA

The cytoskelton is involved in a variety of cellular functions in plants including establishment of cell polarity, cell shape, cell division, wall deposition and organelle transport. The colonization of plants by arbuscular mycorrhizal fungi has been shown to induce changes in cytoplasmic organization and morphology of root cells. Therefore, it is likely that cytoskeleton is involved either in the signaling events leading to the establishment of the symbiosis, or in changes in host cell morphology and cytoplasmic architecture. To probe further into the potential role of the cytoskeleton in the establishment of arbuscular mycorrhizal symbiosis, we examined the three dimensional arrangement of actin microfilaments and microtubules in roots of the model legume *Medicago truncatula* colonized by the arbuscular mycorrhizal fungus *Glomus versiforme* using indirect immunofluorescence and confocal microscopy. Our data show extensive remodeling of the microtubule cytoskeleton from the early stages of arbuscular development until arbuscule collapse and senescence. While confirming some of the microtubule patterns shown in other mycorrhizal systems, our results also reveal that cortical cells adjacent to those containing arbuscules or adjacent to intercellular hyphae, reorganize their microtubules. This indicates that cortical cells initiate modification of their actin microfilament and microtubule cytoskeleton prior to entry of the fungus and is consistent with signal exchange between the symbionts prior to fungal penetration of the cells. This detailed identification of cytoskeletal reorganization will provide a foundation for the further studies of the molecular mechanism of cytoskeletal involvement during the establishment of arbuscular mycorrhizal symbiosis and in particular for the analysis of mutants defective in development of an AM symbiosis.

P1 127

The role of the motile tubular vacuole system in mycorrhizal fungi

W G Allaway[1] and A E Ashford[2] [1] Biological Sciences, The University of Sydney, Australia
[2] Biological Science, UNSW, Sydney, Australia

Motile tubular vacuoles are widespread and have been demonstrated in a range of basidiomycetes including several ectomycorrhizal fungi. We have used colonies of an Australian *Pisolithus* isolate as a model system to study the cell biology of these vacuoles in the hyphal tip region. Here the vacuole system has two distinct components: less mobile spherical compartments linked to the plasma membrane and wall, and mobile tubular compartments which interconnect them and move vacuolar content between them. Form and motility of motile vacuole systems are modulated by external conditions, and the integrity of tubular components is dependent on microtubules but not actin microfilaments. Tubulation is controlled by GTP-binding proteins. These vacuoles are known to be rich in P and K but if they are to act as conduits for longitudinal transport they must be present in ectomycorrhizal systems. We have visualised motile tubular vacuoles by fluorescence microscopy throughout the extraradical mycelium in ectomycorrhizas synthesised between *Pisolithus tinctorius* and *Eucalyptus pilularis* after loading hyphae with Oregon Green carboxylic acid diacetate. However we could not demonstrate transfer of fluorochrome from labelled to unlabelled regions. Some superficial hyphae of ectomycorrhizas became labelled although the majority of the sheath did not. This is consistent with their relatively low permeability. Sheath and Hartig net contained tubular vacuoles that were rich in P, K and S, and they stained for polyphosphate but did not contain granular material in frozen anhydrously processed specimens.

P1 128

Microtubule arrangement of fungal hyphae and root cells in the *Entoloma - Rosa* mycorrhiza

H. Kobayashi[1] and K. Hatano[2]. [1]Osaka Museum of Natural History, Osaka 546-0034, JAPAN. [2]Faculty of Integrated Human Studies, Kyoto University, Kyoto 606-8501, JAPAN

Mycorrhiza between entolomatoid fungi and rosaceous plants is characterized by disappearance of the apical root cells, invasion by the fungal hyphae into these regions and collapse of the hyphae outside of the stele. As part of an effort to elucidate this mycorrhizal relationship, the intracellular arrangement of microtubules in the fungal hyphae and the root cells of mycorrhizas of *Entoloma clypeatum* f. *hybridum* and *Rosa multiflora* in the field was investigated by immunofluorescence microscopy. Microtubule bundles were longitudinally oriented in the hyphae of outer sheath, whereas a network of those was observed in the hyphae of inner sheath, where the rearrangement of microtubular system might start. Microtubule bundles branched at the branching hyphae adjacent to endodermal cells and were not visible in undulate and collapsing hyphae. On the other hand, helical bundles of cortical microtubules were observed in the intact root cells, but disappeared in the ones compressed by the fungal hyphae.

P1 129

Microtubules and tubulin dynamics of *Glomus intraradices* and tomato in arbuscular mycorrhizal symbiosis

S. Timonen[1,2], F.A. Smith[1] and S.E. Smith[1] [1]Department of Soil and Water, The University of Adelaide, South Australia. [2]Dept. of Biosciences, Div. of General Microbiology, University of Helsinki, Finland.

Cytoskeleton in plants and fungi consists of microtubules, actin filaments and associated proteins. Together these elements regulate much of the transport and morphogenesis of both fungal and plant cells. Regulation of microtubules takes place in transcriptional, translational, post translational and cellular levels. Both the associating proteins and other regulating factors such as calcium levels are of primary importance in how many microtubules are formed in a cell and how they are oriented. However, in long term development, regulation on the mRNA and protein levels also have significance. In this study the microtubules of an arbuscular mycorrhizal fungus *Glomus intraradices* were visualised for the first time in active symbiosis with a host plant. The structure and organisation of fungal microtubules were observed in extraradical mycelia, intraradical hyphae and arbuscules. Vesicles appeared devoid of microtubules. Plant microtubules reacted strongly to the presence of arbuscules in the cells as shown previously in other arbuscular mycorrhizal symbioses. Upregulation of plant tubulin was observed during mycorrhization of the tomatos. In the roots studied, the amount of fungal tubulin increased as colonisation grew more intense. However, it did not exceed plant tubulin even in the most heavily colonised roots.

P1 130

Proteomics of *in vitro* grown Ri T-DNA transformed carrot roots (*Daucus carota* L.) associated with the arbuscular mycorrhizal fungus *Glomus intraradices*

R.S. Saravanan[1], G. Bestel-Corre[1], B. Fontaine[2], S. Gianinazzi[1], M. St-Arnaud[2], E. Dumas-Gaudot[1].
[1]INRA-Université de Bourgogne, Dijon, France, [2]I.R.B.V. Jardin botanique de Montréal, Canada.

Only recently have proteomics studies been conducted for the identification of arbuscular mycorrhiza-related proteins using the combination of two-dimensional gel electrophoresis (2-DE), mass spectrometry and bioinformatics tools [1]. This approach allowed to identify several proteins however it was impossible to define whether they originated from the fungus or the host plant. In the present study, we have begun 2-DE analyses of polypeptide patterns of *in vitro* grown mycelium of *Glomus intraradices* N.C. Schenck & G.S. Sm. (DAOM 181602) and mycorrhizas of Ri T-DNA transformed carrot roots. This was carried out by using immobilized pH 3-10 gradient in the first dimension (IEF) and 12% SDS-PAGE in the second dimension. Gels were stained with either silver nitrate or SYPRO Ruby stains for analytical and mass spectrometry analyses, respectively. In all the cases about 400 spots were detected. 2-DE comparison between 19 weeks old non-mycorrhizal transformed carrot roots and 17.8 weeks old mycorrhizal roots revealed some differentially expressed symbiosis-related polypeptides, among which 10 polypeptides were also found in external hyphae from the two-compartment system [2], indicating their fungal origin. The first results involving protein identification by tandem mass spectrometry will be presented. Combination between proteomics and the use of *in vitro* mycorrhizas is promising to open new perspectives so as to increase our understanding of the arbuscular mycorrhizal symbiosis.

[1] Bestel-Corre, G., Dumas-Gaudot, E., Poinsot, V., Dieu, M., Dierick, J.F., van Tuinen, D., Remacle, J., Gianinazzi-Pearson, V., and Gianinazzi, S. 2001. Identification of symbiosis-related proteins from Medicago truncatula Gaertn. by two-dimensional electrophoresis and mass spectrometry. *Electrophoresis* submitted.

[2] St-Arnaud, M., Hamel, C., Vimard, B., Caron, M., and Fortin, A. 1996. Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an *in vitro* system in the absence of host roots. *Mycological Research* 100: 328-332.

P1 131

Molecular cloning and expression analysis of an ammonium transporter and other nitrogen-regulated genes in the ectomycorrhizal fungus *Tuber borchii*

B. Montanini, E. Soragni, N. Moretto, A. Bolchi, R. Percudani and S. Ottonello Dipartimento di Biochimica e Biologia Molecolare, Università di Parma, Italy

In fungi, nitrogen starvation is a prime environmental cue that leads to changes in nutrient foraging capacity, as well as to important morphogenetic transitions. Here, we report on the isolation of *TbAMT1*, an ammonium transporter from *T. borchii*, the first to be described in an ectomycorrhizal fungus. *TbAMT1* is a single copy gene encoding a 52 kDa membrane protein with 11 predicted transmembrane domains that is phylogenetically classified as a class I transporter. It is 53% identical to the MEP2 ammonium permease of *S. cerevisiae* and complements yeast Δ*mep* mutants for growth on 1 mM ammonium with an apparent K_M of 3 μM.. Similar to *MEP2*, *TbAMT1* enables yeast cells to grow in the presence of methyl ammonium, but does not rescue the lack of pseudohyphal growth of nitrogen-starved Δ*mep2* mutants. As revealed by expression studies conducted on *T. borchii* mycelia, the *TbAMT1* mRNA is specifically upregulated (~7-fold) upon nitrogen deprivation and downregulated by either glutamine or ammonium. Contrary to what commonly occurs in other fungi, and to what we have observed using a nitrate transporter (*TbNT*) specific probe, no *TbAMT1* upregulation takes place upon growth on "poor" nitrogen sources, such as nitrate or proline. These results will be compared with expression data recently obtained with the metabolically related enzymes glutamine synthetase (*TbGS*) and glutamate dehydrogenase (*TbGD*). A most peculiar finding that will also be presented is the dramatic upregulation (>100-fold) of surface protein mRNAs that concomitantly occurs under conditions of persistent nitrogen deprivation.

P1 132

Proteomics as a tool to study plant microbe interactions

U. Mathesius, G. Keijzers, S. Natera, J.J. Weinman, M.A. Djordjevic, B. G. Rolfe
Australian National University, Canberra, Australia

We have used proteome analysis of the model legume, *Medicago truncatula*, to study differential protein expression in roots in response to soil microbes to identify proteins involved in root symbioses. *Medicago truncatula* has been chosen as a model legume for the study of *Rhizobium*- and mycorrhiza-induced symbioses, both of which do not occur in *Arabidopsis thaliana*. We have established a reference map of *M. truncatula* root proteins using two-dimensional gel electrophoresis combined with mass spectrometry to display and identify proteins. The newly established *M. truncatula* EST database was used to identify proteins by peptide mass fingerprinting. We have so far identified 180 proteins, including hormone-regulated proteins and nodulins which could be used as markers to follow *Rhizobium*- and mycorrhizae infection, because in both cases, similar changes in hormone regulation and gene expression have been postulated. Proteome analysis promises to be a powerful tool to gain a global picture of gene and protein expression during the establishment of root symbioses, especially in combination with nodulation and mycorrhizae-deficient plant mutants.

P1 133

Differential expression of defence-related genes in wild-type and a mycorrhiza-defective mutant tomato

Ling-Ling Gao, Sally E. Smith, Wolfgang Knogge, Gabriele Delp, F. Andrew Smith.
Adelaide University, Australia

Previous studies have shown that species of arbuscular mycorrhiza (AM) fungi vary in their colonisation patterns in wild-type cv 76R and a mycorrhiza-defective mutant, *rmc*, of tomato (*Lycopersicon esculentum* Mill.). In 76R, *Glomus intraradices, G. mosseae,* and *G. versiforme* form *Arum*-type mycorrhizas, whereas *G. coronatum, Gigaspora margarita* and *Scutellospora calospora* form the *Paris*-type. In *rmc*, with the exception of *G. versiforme* which forms relatively normal colonisation, the other species abort either on the root surface or in the epidermis.

We compared the expression of defence-related genes (*PR-P6*, *GLUBAS*, *GLUAC*, *CHi3*, *CHi9* and *PAL5*) in 76R and *rmc* in response to the different fungal species. Interestingly, in both 76R and *rmc*, the fungi forming *Paris*-type AM frequently induced higher mRNA levels than those forming the *Arum*-type. However, irrespective of mycorrhizal morphology, the fungi failing to colonise *rmc* induced a higher expression of genes *PR-P6, CHi3, GLUBAS* and *PAL5* in *rmc* compared to 76R. In contrast, with *G. versiforme* transcript levels for these genes were lower in *rmc* than in 76R. In the case of *PR-P6* and *CHi3*, mRNA accumulation was even lower than in non-mycorrhizal plants. These results suggest that plant defence reactions play a role in preventing mycorrhiza formation in *rmc* and that suppression or delay of defence reactions is required for the successful colonisation of the mutant by *G. versiforme.*

P1 134

Mapping the *rmc* locus: Towards characterisation of the mutation

N.J. Larkan[1], S.E. Smith[2] and S.J.Barker[1]. [1]The University of Western Australia, Australia; [2]The University of Adelaide, Australia.

RFLP mapping techniques are being employed in order to define the locus of the *rmc* (reduced mycorrhizal colonisation) mutation in a fast neutron mutagenised tomato (*Lycopersicon esculentum*) line with a view to map-based cloning. Using tomato as a model plant for mutation studies allows for the utilisation of well developed molecular tools as well as overcoming the tri-species complications inherent with legumes. Using *Lycopersicon pennellii* as the wild parent both BC1 and F2 mapping populations have been constructed. The results of population screening with *Glomus mosseae* and the likely chromosomal position of the locus will be discussed along with future experiments.

P1 135

The role of plasma membrane H+-ATPases in arbuscular mycorrhizal roots

GM Rosewarne [1], SE Smith [2], FA Smith [2] and DP Schatchman [1]
[1] CSIRO, Australia [2] University of Adelaide, Australia.

The uptake of phosphate (P) by plants from the soil is an energetically unfavorable process that is facilitated by H+-ATPases. The ATPases generate a proton motive force across plant membranes enabling the co-transport of phosphate and protons into the plant cell. A similar process is presumed to occur in arbuscular mycorrhizal plants, with P transfer to the plant taking place in the arbuscular structure. This study investigates the role of various plant ATPases in this process.

The expression patterns of seven *Lycopersicon esculentum* H+-ATPases (LHA1-7) were investigated in plants having different stages of mycorrhizal colonisation and P nutrition. Only 3 of the ATPases (LHA1, 2 and 4) had significant levels of expression in roots. P nutrition had no effect on expression of these genes. Similarly, LHA1 and LHA2 had little change in expression in response to mycorrhizal colonisation. However, LHA4 expression was decreased in mycorrhizal plants, particularly those that had high levels of arbuscules. *In situ* hybridisation showed that LHA1, 2 and 4 were expressed at high levels in the epidermal cell layer of both mycorrhizal and non-mycorrhizal plants, particularly in the root tip. Furthermore, LHA4 was expressed in arbuscule containing cortical cells, indicating its involvement in nutrient acquisition from the fungus. This work identifies the role specific ATPases play in nutrient transfer between symbionts and a model that explains the ATPase expression patterns in relation to P uptake and transfer to the plant is presented.

P1 136

Expression of two phosphate transporters in barley plants colonised by mycorrhizal fungi.

D. Glassop[1], P.H.D. Schünmann[3], Frank.W. Smith[1] and S.E. Smith[2]
[1,2]CSIRO Plant Industry, Brisbane; [2]Adelaide University, Australia; [3]CSIRO Plant Industry, Canberra.

We are investigating the interaction of barley (*Hordeum vulgare* L.) phosphate transporters and the mycorrhizal fungi *Glomus intraradices* and *Glomus versiforme*. Of the eight barley phosphate transporters isolated to date, *HvPT1* and *HvPT2* have been shown to be strongly expressed in roots and induced by phosphate starvation (Smith et al. 1999). In order to facilitate expression studies, the promoter sequences of these two transporters have been coupled to the reporter gene GFP and the constructs introduced into barley (cv. Golden Promise). As shown on this poster, these transgenic plants are being used to determine whether either *HvPT1* or *HvPT2* are expresseed at the peri-arbuscular membrane of barley plants colonised by *Glomus versiforme* or *Glomus intraradices*.

Reference:
Smith, FW, Cybinski, DH. and Rae, AL. 1999 Regulation of expression of genes encoding phosphate transporters in barley roots. eds. Gissel-Nielsen, G and Jensen, A. Plant Nutrition - Molecular Biology and Genetics. Netherlands: Kluwer Academic Publishers. p 145-150

P1 137

Investigations of phosphate transporter expression in the soybean AM symbiosis

D.S. Bougoure, J.D.W. Dearnaley. The University of Southern Queensland, Toowoomba, Australia

Increased phosphate uptake is one of the key benefits of the plant arbuscular mycorrhizal (AM) symbiosis. We are currently studying genes that are potentially involved in this process in soybean (*Glycine max* L.). We have extracted mRNA from soybean roots both colonised and uncolonised by compatible AM fungi using biotinylated poly-T probes and streptavidin-coated magnetic particles. The total mRNA extracted by this method was reverse transcribed into cDNA with poly-T primer and reverse transcriptase. cDNA was next PCR-amplified with primers for previously published soybean root, stem, and leaf phosphate transporter genes. To identify genes which were upregulated in roots colonised by AM fungi, amplified sequences were visualised on silver-stained acrylamide gels. *In situ* hybridisation studies using cryosectioning, labelling with fluoroscein-conjugated antisense probes, and alkaline phosphatase visualisation of bound probes were carried out to determine the location of phosphate transporter gene transcripts in both colonised and uncolonised roots. Results of these analyses are presented here and we discuss the importance of the arbuscule as the major site of phosphate exchange in the soybean AM symbiosis.

P1 138

Functional characterization of *Mt4*-like genes, a common component of the phosphate-starvation signaling pathways in higher plants

Heungsop Shin and Maria J. Harrison, The Samuel Roberts Noble Foundation, Ardmore, Oklahoma, USA

In response to low phosphorus (P) availability in soils, most land plants form associations with arbuscular mycorrhizal (AM) fungi. It is well known that AM fungi can enhance plant growth by transferring P from the soil to the plant; however, the molecular mechanisms of P-starvation signaling in plants, and how these signaling pathways overlaps with signaling for the development of the arbuscular mycorrhizal symbiosis are largely unknown. Previously, we have cloned a cDNA (Mt4) from *M. truncatula*, which is induced in roots in response to P-starvation. Interestingly, Mt4 is composed of numerous short, overlapping open reading frames (ORFs) and is similar in structure to *enod40*, a plant gene that may encode a peptide signal involved in nodule development. In this presentation, we summarize our recent data on the functional characterization of the *Mt4*-like genes in plants. First, sequence and expression analysis of *Mt4* homologs from various plants, including *Medicago sativa* and *Arabidopsis thaliana*, suggest that *Mt4*-like genes are a common component of the phosphate-starvation signaling pathways in plants. Second, *in vitro* translation experiments with *Mt4*-like sequences demonstrate the translatability of the potential open reading frames of the *Mt4*-like genes. Finally, a reverse genetic approach to study the knock-out mutants of the *Arabidopsis At4* gene is described. The aim of this analysis is to reveal the roles of the *Mt4*-like genes in the phosphate-starvation signaling pathways in plants.

P1 139

Molecular cloning of an LTR-retroelement, *marY1*, from the ectomycorrhizal homobasidiomycete *Tricholoma matsutake*.

H. Murata[1], Y. Miyazaki[1], K. Babasaki[1] and A. Yamada[2]. [1]Forestry and Forest Products Research Institute, Japan, [2]Shinshu University, Japan.

Retroelements are DNA parasites present in the genome of many eukaryotes, which replicate through an RNA intermediate and integrate into other genetic loci. Retroelements are useful as markers for the study of molecular evolution of host organisms, and as tools for the construction of mutants and recombinants. We have cloned a retroelement designated as *marY1*, which is a member of *gypsy*-group LTR retroelements, from the ectomycorrhizal homobasidiomycete *Tricholoma matsutake*. *marY1-lacZ* gene fusions are expressed in the budding yeast *Saccharomyces cerevisiae*. Nucleotide sequences present in the LTR of *marY1* are ubiquitous in higher fungi. Data suggest that the LTR sequence of *marY1* originated early in the evolution of higher fungi and has become widely distributed. Therefore, it may be useful for the construction of LTR-mediated transformation system in basidiomycetes.

P1 140

Molecular characterization of Amino Acid transporters in *Hebeloma cylindrosporum / pinus pinaster* ectomycorrhizae

Daniel Wipf, Mariam Benjdia, M. Tegeder and Wolf B. Frommer
The University of Tübingen, ZMBP, Tübingen, Germany

In forest soils, ectomycorrhizal fungi are able to assimilate organic nitrogen, especially in the form of free amino acids. The host plant acts as strong sink for the amino acids available from the fungus. In this work we used the *Hebeloma cylindrosporum/Pinus pinaster* ectomycorrhizal association to investigate amino acid transport at the soil-fungus and at the fungus-plant interface. We identified amino acid transporter genes of mycorrhizal partners.

For the isolation of amino acid transporters from *Hebeloma* and *Pinus,* two different approaches have been used. (I) PCR was performed using degenerate primers designed from conserved regions of known plant and yeast amino acid transporter genes. (II) The second approach involved the cloning of genes by functional complementation of a yeast mutant deficient in amino acid uptake systems. This method has been successful for the isolation of transporter genes in herbaceous plants. An expression library was constructed by cloning *Hebeloma* cDNA into a yeast expression vector. Putative amino acid transporters were isolated by selection of transformed yeast on amino acids as sole nitrogen source.

Using yeast complementation a *Hebeloma* cDNA with strong homology to amino acid permease of yeast was identified (*HcBAP1*). We propose that HcBAP1 plays a role in importing amino acids into *Hebeloma* for hyphal growth. In the *Hebeloma/Pinus* symbiosis, *HcBAP1* could be a good candidate for uptake of amino acids from the soil and their transfer to the host plant.

P1 141

Increased arbuscule colonization in roots of *Lotus japonicus* hypernodulating mutants

K. Senoo[1], M.Z. Solaiman[2], S. Tanaka[1], M. Kawaguchi[3], H. Imaizumi-Anraku[4], S. Akao[4], A. Tanaka[1] and H. Obata[1] [1]Mie University, Tsu, Japan [2]University of Western Australia, Australia [3]University of Tokyo, Tokyo, Japan [4]National Institute of Agrobiological Resources, Tsukuba, Japan

The colonization of *Lotus japonicus* hypernodulating mutants (*Ljsym78-1* and *78-2*) by the arbuscular mycorrhizal (AM) fungus *Glomus* sp. was characterized. The mutants are defective in systemic autoregulation of nodulation and nitrate inhibition, and form an excess of nodules and lateral roots. The percent root length colonized by the AM fungus was significantly higher for the mutant than wild-type roots. Detailed assessment of the colonization indicated that the percentage of colonization by arbuscules was increased, but that by external hyphae, internal hyphae and vesicles was decreased, in the mutant roots compared with the wild-type. The succinate dehydrogenase activity of arbuscules, external hyphae and internal hyphae showed similar trends. The majority of individual arbuscules that formed in the mutant roots had a well-developed and seemingly tough morphology. The results suggest the presence of common genetic mechanisms between autoregulation of nodulation and regulation of arbuscule formation. Due to the unique morphology of the arbuscules, it was possible to separate and purify the arbuscules from the root tissue by a simple procedure. The metabolic activity of the arbuscules was retained after the isolation process. The simple and rapid method for the isolation of metabolically active arbuscules from the mutant roots should help to clarify the biochemical processes occurring in nutrient exchange at the arbuscules.

P1 142

An approach to elucidate molecular events in the recognition phase of ectomycorrhiza formation.

S. Herrmann, A. Krüger, T. Peškan, R. Oelmueller, F. Buscot. Friedrich-Schiller-University of Jena, Germany.

In an *in vitro* system with micropropagated oaks (*Quercus robur* L.) and *Piloderma croceum*, we could monitor and characterize plant development and morphological events during a period of several weeks (8-9) prior to formation of ectomycorrhizal symbiosis. In this prolonged pre-mycorrhizal phase, we first noticed an enhanced root development with large stimulation of lateral root elongation, followed by an increase of leaf area development. In situ-measurements of chlorophyll fluorescence quenching on such inoculated but non-mycorrhizal oaks, showed that photosynthetic effectiveness was maintained compared to a decrease in non-inoculated control plants.

The processes of recognition prior to EM formation are still poorly understood at the molecular level. The *Quercus-Piloderma* system with delayed mycorrhiza formation and usable morphological or physiological markers offers a suitable model to investigate the molecular events in the plant, that precede the mycorrhization of the roots. We have used a modified cold-plaque screening procedure to identify the transcripts which are up-regulated before the EM formation. For this purpose, the cDNA population from non-infected oak-roots was used as a probe to screen the cDNA library of inoculated, but still non-mycorrhizal roots. Non-hybridizing clones were selected and their differential expression was confirmed by northern-analysis.

P1 143

The influence of different arbuscular mycorrhizal fungi and phosphorus on gene expression of invertase and sucrose synthase in roots of maize

S. Ravnskov,[1] Y. Wu[2] and J.H. Graham[2] [1]Danish Institute of Agricultural Sciences, Flakkebjerg, Denmark, [2]University of Florida, Lake Alfred, USA.

The symbiosis between arbuscular mycorrhizal (AM) fungi and plants is functionally characterised by the bi-directional flow of nutrients between the symbionts. The nutrients of major importance are carbon (C) from plants to AM fungi, and phosphorus (P) from AM fungi to the plants. The outcome of the symbiosis measured as plant growth depends on the balance of these flows. Different combinations of plant varieties or species, and AM fungal isolates as well as external physical conditions as P content in the soil can influence reciprocal nutrient transport in the AM symbiosis, and thereby growth of the plant. Invertase and sucrose synthetase are the only enzymes, that catalyze the first step of sucrose utilization in carbon-importing tissues. By measuring the production of these enzymes in mycorrizal and non-mycorrhizal plants in time-course studies, it is possible to measure how the AM symbiosis influences carbon import to root cells. The objective of this study was to evaluate the effect of three different AM fungal isolates on the expression of invertase and sucrose synthase in maize roots. Maize plants were inoculated with either of two *Glomus intraradices* isolates, with *G. caledonium* or left uninoculated as a control. Control plants were grown at three different P levels in soil. Plants were harvested 3, 6, 9, 13, 18 and 25 days after emergence. Gene expressions of invertase and sucrose synthetase were measured by northern blot analysis. Dry weights and AM colonization of plants were measured. In general, both the different AM fungal isolates and P content in soil influenced sucrose synthetase activity in the roots and no invertase activity was found in older roots.

P1 144

Cloning of β-1,3-glucanase genes from mycorrhizal olive plantlets

C. Azcón-Aguilar, J.M. Barea, N. Ferrol and R. Calvente
Estación Experimental del Zaidín, CSIC, Granada, Spain.

As most mediterranean fruit trees, olive plants (*Olea europeae* L.) exhibit a highly mycotrophic character. In previous studies using some olive varieties of regional interest ("Arbequina" and "Picual") it was shown that arbuscular mycorrhizal (AM) endophytes isolated from the rhizosphere of mature, field-grown, olive trees, namely *Glomus intraradices* BEG 123 and *Glomus viscosum* BEG 126, were very effective in promoting plant growth. Considering the increasing evidence of the role of AM fungi at improving resistence of plants to soilborne pathogens, the induction of certain enzymatic activities involved in plant defence responses by the most effective AM fungi used (BEG 123 and BEG 126) was studied. It was shown that β-1,3- glucanases were differentially induced in mycorrhizal plants.

The aim of our study was to get further insights into the significance of these enzymes in plant defence and/or regulation of mycorrhizal colonization. It was decided to clone olive β-1,3-glucanase genes by RT-PCR using RNA from mycorrhizal olive roots and degenerated primers. Two partial clones encoding putative undescribed β-1,3- glucanases were isolated. Full-length clones are currently being obtained by rapid amplification of cDNA ends (RACE). The expression of these genes will be investigated and their possible involvement in plant protection evaluated.

P1 145

Antagonistic interactions between symbiotic and saprophytic microbes in birch rhizosphere - evidence from manipulating carbon availability

S. Stark and M.-M. Kytöviita, Department of Biology, University of Oulu, Finland

We studied the role of carbon (C) availability on the relationship between soil saprophytic microbes and mycorrhizal fungi in a greenhouse experiment. The microbial C availability was manipulated in a fully factorial experiment with defoliation of birch (*Betula pubescens*) seedlings, glucose addition, and fungicide/antibiotic addition as treatments. By reducing the photosynthetic capacity of birch, defoliation was expected to decrease the plant root exudation, which constitutes an important C source for the soil heterotrophic microflora, as well as reduce the amount of symbiotic fungi on roots. Glucose addition was expected to enhance microbial activity. Ectomycorrhizal symbionts of the birch seedlings, *Telephora terrestris* and *Laccaria laccata*, were able to grow in pure culture when exposed to the fungicides, and toxin addition was thus aimed to alter the balance between saprophytic and symbiotic microbes. Defoliation significantly reduced soil respiration and amount of ergosterol, a fungus specific sterol, in the birch roots, indicating C deficiency by defoliation in both saprophytic and mycorrhizal microbes. Soil respiration was considerably increased by glucose addition. Glucose addition counteracted the negative effects of defoliation on respiration resulting in no reductions in defoliation treatments when extra C was added. Birch stem and root biomasses were significantly reduced by defoliation, but were not affected by the glucose and the fungicide/antibiotic treatments. Interestingly, the amount of ergosterol in birch roots was significantly reduced by glucose addition, and significantly increased by fungicide/antibiotic addition. These results indicate that the relationship between saprophytic and mycorrhizal microbes in the birch rhizosphere is strongly antagonistic.

P1 146

Comparison of the activities of isocitrate dehydrogenase and isocitrate lyase involved in isocitrate metabolism in *Laccaria amethystea* during symbiosis with *Pinus densiflora*

T. Hattori[1], M. Itaya[1], A. Ohta[2] and M. Shimada[1] [1]Wood Research Institute, Kyoto University, Kyoto, Japan [2]Shiga Forest Research Center, Shiga, Japan

Metabolism of isocitrate in microorganisms, including wood-rotting fungi, is regulated by the activities of isocitrate dehydrogenase (ICDH) in TCA cycle and isocitrate lyase (ICL) in glyoxylate cycle in response to a carbon source, because isocitrate is a branch point of both cycles. However, the regulation of both enzymes in ectomycorrhizal fungi has not been elucidated yet. Thus, we have compared the activities of ICDH and ICL in the cell-free extracts from *Laccaria amethystea* grown symbiotically with *Pinus densiflora*. First we established *P. densiflora* dependent sandwich symbiotic culture of *L. amethystea* by use of sucrose as a carbon source. Free-living *L. amethystea* did not grow well in sucrose medium. However, the fungus grew well around the lateral roots and the hyphae penetrated between epidermal cells during the early stage of the cultivation. The weight of mycelia in the symbiotic culture reached 2.6 times that in the free-living culture. The typical ectomycorrhizae were formed during the cultivation of 45 days. NADP but not NAD dependent ICDH activity was detected, but ICL activity was not detected in *L. amethystea* grown in the symbiotic culture. Similarly NADP-ICDH activity was 10 times that of ICL in the free-living *L. amethystea* grown in a liquid medium. Thus the results suggest that NADP-ICDH rather than ICL play an important role in isocitrate metabolism in *L. amethystea* grown in the cultures under the symbiotic and free-living conditions, in contrast to wood-rotting fungi in which ICL play greater role than NADP-ICDH.

P1 147

Anatomy of carbohydrate utilization, metabolism, and transfer in an AM fungus

P.E. Pfeffer[1], B. Bago[2] D.D Douds[1] J. Jun[3], J. Abubaker[3], J.W. Allen[3], P.J. Lammers[3], Y.Shachar-Hill[3]
[1]US Department of Agriculture, Wyndmoor Pennsylvania, USA, [2] Departmento Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, CSIC, Spain USA [3]Dept Chem & Biochem, New Mexico State University, Las Cruces NM

This presentation will describe our work in recent years to elucidate the forms and pathways by which carbohydrates are taken up, transformed, transferred, stored and turned over by AM fungi. As well as acting as substrates for catabolism, different carbohydrates are crucial to the transfer of carbon from host to fungus (hexose), to structural integrity and growth (chitin, glucans), to carbon storage (trehalose, glycogen), to the production of reducing equivalents (pentose phosphate pathway), and to information storage and utilization (nucleic acids). It also appears that carbohydrates and the lipids derived from them have a role in carbon export from the root to the extraradical mycelium. For example, some of this transferred carbon has been found to reside within the roots of a second colonized host plant. The question remains whether this second host plant can utilize this transferred carbon. Our data give evidence on this and other questions concerning plant to plant carbon transfer. Insights gained from isotopic labeling experiments and NMR and MS analysis will be presented together with recent progress in identifying and quantifying gene expression related to carbohydrate metabolism.

P1 148

Cloning of *Suillus bovinus* GS/GOGAT genes and localisation of expression in the Scots pine mycorrhizosphere

J.T. Juuti, S. Jokela, S. Timonen, M. Hess and R. Sen University of Helsinki, Finland.

In boreal forest soils, nitrogen is a key plant growth limiting nutrient. Nitrogen is typically sequestered in high molecular weight organic detritus making up the humus/mor horizon. Microbial activity releases ammonium, a major source of available nitrogen in these soils. In order to study ammonium uptake in mycorrhizal Scots pine roots we have cloned the glutamine and glutamate synthetase (GS/GOGAT) genes of *S. bovinus*. Both cDNA and corresponding genomic library clones have been isolated. The cDNAs were used in Northern hybridizations and *in situ* probing to analyse gene expression in fungal pure cultures and different compartments of the pine mycorrhizosphere developed on humus. Additional isozyme and Western blotting analyses have further confirmed tissue location of GS activity. The data highlights extramatrical mycelium as the site of active fungal ammonium assimilation in the studied Scots pine - *S. bovinus* root system.

P1 149

Regulation of NO_3^- uptake in the symbiotic association *Pinus pinaster - Rhizopogon roseolus*

C. Plassard and A.M. Gobert UMR Sol et Environnement, INRA, 34090 Montpellier, France.
Many studies showed that coniferous species are less efficient than herbaceous plants to use NO_3^- as a sole mineral N source. This low capacity of nitrate utilization is reflected by (i) a long time to get the maximal induction of nitrate uptake and (ii) low nitrate uptake rates when measured on the basis of the whole root system. On the other hand, in natural conditions, the association of conifer species with ectomycorrhizal fungi is the rule and many ectomycorrhizal species grown in pure culture exhibit different properties from those of their host-plant, especially in their great ability to use NO_3^-. The aim of our work was to determine NO_3^- transport operating at low NO_3^- concentrations ($<$ 100 µM) in *P. pinaster* roots and *R. roseolus* thalli. Their regulation were studied in the presence of NO_3^-, NH_4^+ and glutamate in the solution. Our data provided evidence that in non mycorrhizal pine roots, NO_3^- uptake measured at KNO_3 50 µM was slowly inducible by NO_3^- as the net NO_3^- fluxes increased with the duration of exposure to nitrate (50 µM) and was maximal after 3 days of treatment. In contrast, NO_3^- uptake was not inducible with exposure to NO_3^- (1 mM) in *R. roseolus* thalli. Similar results were obtained when NO_3^- net fluxes were measured at a local scale with NO_3^--selective microelectrode at the surface of mycorrhizal short roots (MSR) compared to non-mycorrhizal short roots (NMSR). When NH_4^+ or Glu were added in pretreatment solution, NO_3^- uptake was strongly inhibited both in pine roots, fungal hyphae, NMSR and MSR. In conclusion, *P. pinaster* presents constitutive high affinity transport system (HATS) and NO_3^- inducible HATS inhibited by NH_4^+ and Glu. The mycorrhizal fungus possesses a different NO_3^- transport system expressed in N-free and NO_3^- solution but strongly inhibited by NH_4^+ and Glu. NO_3^- uptake in MSR was the only consequence of the fungal NO_3^- transport system.

P1 150

Nitrogen (N) Allocation Patterns of Mycorrhizal Fungi and N Supply Rate Control N Isotope Patterns in *Pinus sylvestris*

Erik A. Hobbie,[1] Jan V. Colpaert[2]. [1]Max Planck Institute for Biogeochemistry, Jena, Germany. [2]Limburgs Universitair Centrum, Diepenbeek, Belgium

Recent field studies suggest that nitrogen (N) isotope signatures ($\delta^{15}N$) may reveal plant-mycorrhizal N dynamics. Mycorrhizal fungi appear to produce ^{15}N-depleted N for transfer to host plants, creating lower plant $\delta^{15}N$ at lower N availability. We cultured *Pinus sylvestris* at high and low N supply to investigate how fungal N dynamics influence plant $\delta^{15}N$. Plants were inoculated with *Suillus luteus*, *Thelephora terrestris*, or grown without fungi. We predicted: 1) mycorrhizal plants will have lower $\delta^{15}N$ than non-mycorrhizal plants, 2) mycorrhizal plants will decline in $\delta^{15}N$ at low N supply, and 3) $\delta^{15}N$ of non-mycorrhizal plants will not change with N supply. Results agreed with all three predictions, confirming that mycorrhizal fungi can alter plant $\delta^{15}N$. N budgets indicated greater allocation below-ground at low N supply. Patterns of N retention in fungi between two N supply rates differed, with *Thelephora* retaining equal amounts of N at both N supply rates whereas *Suillus* retained twice as much N at low than at high N supply. These N retention patterns were also reflected in fungal $\delta^{15}N$, with *Suillus* increasing at low N whereas *Thelephora* did not change. Because of ^{15}N-enriched fungal matter on fine roots, fine root and needle $\delta^{15}N$ were negatively correlated ($r^2=0.69$, $n=29$, $p<0.0001$), suggesting that mycorrhizal transfer processes simultaneously created opposite isotopic patterns in mycorrhizal fine roots and foliage. $\delta^{15}N$ appears to be a particularly useful marker of plant-mycorrhizal N partitioning and could be used to examine species-specific responses to shifts in N supply.

P1 151

Effect of ectomycorrhiza of Scots pine on dissolved nitrogen compounds in peat and nitrogen uptake and polyamines of host plant.

T. Sarjala. The Finnish Forest Research Institute, Parkano Research Station, Finland.

Nitrogen compounds in peat are mainly organic in drained peatland forests in Finland. The dissolved inorganic fraction, which is composed almost totally of ammonium, varies according to the microbial activity and is often much lower than the dissolved organic nitrogen (DON) pool especially under low temperature regions. Scots pine (*Pinus sylvestris*) seedlings were inoculated with different fungal species, *Lactarius rufus*, *Piloderma croceum*, *Cenococcum geophilum*, *Suillus variegatus* and *Paxillus involutus* and grown in natural peat substrate with different total N content. Control peat samples without seedlings were also incubated under the same conditions. After removing the seedlings from the peat substrate the formation of dissolved N compounds was measured in the peat, as well as the total N uptake by the seedlings during the experiment. Great differences in the amounts of dissolved N compounds were found especially between the control samples of pure peat and the peat occupied earlier by mycorrhizal fungi and pine seedlings. There were differences between the fungi in the effect on the N uptake and polyamine concentrations of the seedlings. Some species decreased (e.g. *L. rufus*) the polyamine concentrations whereas some species (e.g. *S. variegatus*) increased them both in the roots and needles. This preliminary experiment showed that on lower total N level (1.6%) in the peat the N uptake by the pine seedlings exceeded in some cases over 40 % of the formation of inorganic N in the peat.

P1 152

The impact of peaty substrate on mycorrhization of Scots pine (*Pinus sylvestris* L.) seedlings and containerised stock

T. Gaitnieks, Latvian Forestry Research Institute “Silava”, Salaspils, Latvia

The afforestation of former grass fens and flood-plain meadows often has failed, despite agrochemical indices characterising eutrophic growing conditions (GC). The impact of peaty substrate (S) on mycorrhiza (M) development of *P.sylvestris* seedlings and nursery grown containerised seedlings was analysed under laboratory and field conditions. Four different S, representing dissimilar GC (total height and annual increment of planted Norway spruce) were tested. Thus, 2 S represented rich and 2 poor GC. The impact of degree of peat decomposition, their botanical compositions, agrochemical properties and number of cellulose-decomposing microflora (CDM) on 1-year old *P.sylvestris* seedlings M frequency (MF) and M morphological characteristics were investigated. In the S representing poor GC, the MF was 61-87% (the share of short roots with yellow thick mantle 25-63%), number of M per 10 mm roots 1.6-2.4 pieces, M relative frequency 293-520 pieces/g. In the S representing rich GC the MF 98-100% (yellow M type 71-90%), number of M per10 mm roots 4.4-5.0 pieces, M relative frequency 775-1016 pieces/g. The agrochemical properties of peat did not exibit any difference of MF. The decomposition degree (over 60%) and the proportion of wood peat (esp. *Alnus sp.*) were higher for the S representing rich GC. In peaty soils, an increase in the amount of CDM fungi (esp. *Fusarium sp.*) has negative impact on seedlings mycorrhization. The MF of 2-year-old containerised seedlings was compared in cultivated and uncultivated soil. The height of containerised seedlings correlated with total number of M ($r^2=0.25$, $p=0.003$). Soil cultivation significantly ($P<0.05$) affects the performance of M resulting in a 2-4 fold increase of the MF of fine roots.

P1 153

Diversity of ectomycorrhizal fungi and ectomycorrhizae in forests of *Pinus culminicola* Andersen & Beaman in the state of Nuevo León, México

F. Garza Ocañas & R. Arias Mota. Universidad Autónoma de Nuevo León, Facultad de Ciencias Forestales, Nuevo León, México. fortunatogarza@infosel.net.mx

Pinus culminicola is an endemic species from Northeastern México. In the state of Nuevo León this pine is located in a mountain known as "cerro El Potosí" where is distributed in altitudes ranging from 3200-3650 m.a.s.l; trees are 1.5 – 2.5 m tall, they grow very close together forming compact forests. Distribution of forests in this location has been reduced by almost 60% due to wild fires (1972 & 1998). This study was carried out from 1996-2000 and deals with identification of ectomycorrhizal fungi, ectomycorrhizal morphotypes, isolation of cultures, mixed inoculation of seedlings in greenhouse as well as reafforestation of burned areas. Results showed that 23 species of ectomycorrhizal fungi and 74 ectomycorrhizae morfotypes from 8 categories were identified, 27 pure cultures were obtained from ectomycorrhizas and twenty thousand seedlings were inoculated either with single or mixed micelia of *Cantherellus cibarius*, *Pisolithus tinctorius* and *Scleroderma verrucosum* in the nursery. Mixed inoculated seedlings showed the best grow responses to inoculation for all parameters measured. Reafforestation of burned areas with inoculated seedlings was carried out in September 2000.

P1 154

The role of mycorrhization in the reafforestation of agricultural soils with *Pinus halepensis*

G. Díaz; A. Gutiérrez and M.Honrubia
Dpto. Biología Vegetal, Universidad de Murcia, Spain

A field experiment was carried out to asses the survival anf growth response of outplanted *P. halepensis* seedlings. Two experimental plots were set up, in Albacete (1997) and in Murcia (1998), both from the South-East of Spain but with different climatic and soil characteristics.

Seedlings for Albacete were inoculated with *Rhizopogon roseolus, R. rubescens, Suillus mediterraneensis, S. collinitus and Pisolithus tinctorius* and produced in nursery under two methods differing in container, substrate and fertilization. Seedlings for Murcia were inoculated with *R. rubescens, S. collinitus and P. tinctorius*, and produced under two different fertilization regimes.

The differences among inoculation treatments, plant production technique and field site, survival rates etc. are discussed.

P1 155

The potential for woody understory plants to provide refuge for ectomycorrhizal inoculum at an Interior British Columbia Douglas-fir forest after clear-cut logging

D.M. Durall , S.M. Hagerman and S.M. Sakakibara Okanagan University College, Kelowna, Canada

Clear-cut logging can result in a decrease in the amount of inoculum of some ectomycorrhizal fungi. Ectomycorrhizal plants that are not selected for harvest (refuge plants), may therefore, be important for the maintenance of ectomycorrhizal fungal inoculum in clear-cuts following logging. The purpose of this study was to identify refuge plants that could provide ectomycorrhizal fungal inoculum for outplanted seedlings. The ectomycorrhizal status of selected plants was assessed in 1.6 ha clear-cuts, in the adjacent forest and in a greenhouse bioassay. There was no difference in mycorrhizal richness between *A. uva-ursi* plants sampled from the clear-cut and forest in the latter two years of the study. Conversely, the richness of ectomycorrhizas associated with Douglas-fir sampled from the forest was significantly greater than for seedlings sampled from the clear-cut. In the green-house bioassay, inoculation with mycorrhizal root fragments of *A. uva-ursi*, *Salix commutata*, and advanced regenerating Douglas-fir resulted in relatively high percent colonization and ectomycorrhizal richness on Douglas-fir bioassay seedlings.

P1 156

The effects of disturbance (post-logging) on sequestrate fungal diversity.

T. Lebel, Royal Botanic Gardens Melbourne, Victoria.

Recent studies in East Gippsland and New South Wales have revealed an extremely high diversity of species of sequestrate fungi, many of which are undescribed (some 170 new species). However we have little knowledge of the effects of disturbance on diversity and abundance of these fungi. Logging practices in the South East Forests National Park have created a mosaic of forest patches of different regeneration ages and vegetation types in fairly close proximity. Providing an ideal opportunity to more closely examine the effects of disturbance on sequestrate fungal diversity, species composition, and relative abundance post logging. Three different regeneration age classes (5-10 yrs, 15-20 yrs and +100 yrs) and two vegetation types (Silvertop Ash and Monkey-Gum dominated) will be examined. Preliminary results from the first spring and autumn sampling are presented.

P1 157

Pruning effects on root length density, root biomass, and arbuscular mycorrhizal colonization in two shrubs in a simulated xeric landscaped yard

Sean A. Whitcomb and Jean C. Stutz, Arizona State University, USA.

Although shoot pruning is a common landscape practice, little research has focused on its effects on the roots and mycorrhizal associations of woody landscape plants. In this study, we examine the effects of shoot pruning on root length density, root biomass, and arbuscular mycorrhizal (AM) colonization of two woody shrubs commonly used in xeriscape^{TM} landscapes in the Phoenix metropolitan area, *Nereum oleander* and *Leucophyllum frutescens*. Seven experimental plots were established using landscape practices typical of arid, urban environments including drip irrigation and decomposed granite mulch and three pruning treatments initiated (2 plots per treatment + 1 unpruned control plot). These treatments included 1) shearing every 6 weeks, 2) heading back every 6 months and 3) rejuvenation pruning (cutting back to 1 m) every year. Roots were sampled at the base of three plants of each species by soil coring to three depths: 1-20 cm, 20-40 cm, and 40-60 cm. The first root sampling occurred in the late winter, after the shearing and heading treatments had been imposed, but prior to the first rejuvenation pruning. A second root sampling will occur during active growth in the late spring. At the 1-20 cm depth, shearing and heading reduced root biomass but had no effect on root length density (RLD) or percent AM colonization in *Leucophyllum*. For *Nereum* at the same depth, percent colonization was positively affected by shearing and heading, but there was no effect on biomass or RLD. Final results from all coring depths at both sampling times will be reported.

P1 158

Inoculation of Australian native seedlings with arbuscular mycorrhizal fungi improved growth in the field

G.S. Pattinson, K.A. Hammill, B.G. Sutton & P.A. McGee

The University of Sydney, Australia

The difficulties of revegetating highly disturbed sites are associated with low soil nutrient status, absence of appropriate soil microflora and structural instability of the soil. Procedures to improve establishment of plants in disturbed sites may include inoculation of plants with arbuscular mycorrhizal (AM) fungi. Seedlings (*Dodonaea triquetra, Callitris rhomboidea, Leptospermum polygalifolium, & Eucalyptus gummifera*) inoculated or not with arbuscular mycorrhizal fungi (*Glomus atrouva* and *G. pellucidum*), matched in size by fertilisation, were planted in highly disturbed sandstone soil at Lucas Heights Waste Disposal Centre, Sydney, Australia. The height and survival of plants and spread of fungi in soil were monitored over an 18-month period. The field soil was successfully inoculated by sowing seedlings colonised by AM fungi. The fungi spread through the soil, with the density increasing over the duration of the experiment. Plant survival was not influenced by inoculation. Plant growth response to inoculation varied according to the mycorrhizal dependency of the species. Inoculation of *C. rhomboidea* and *D. triquetra* seedlings resulted in significantly ($P<0.05$) greater plant heights at 18 months, while inoculation of *L. polygalifolium* and *E. gummifera* did not significantly influence plant height, though these plants became ectomycorrhizal. This study demonstrated that AM fungi can be successfully reintroduced into disturbed soil and can improve plant growth to the extent attained by moderate fertilisation.

P1 159

Effect of metallic trace elements on AM fungi diversity within roots of *Trifolium subterraneum* colonised with AM spores isolated from a polluted soil : application to soil remediation

C.Tonin, P.Vandenkoornhuyse, E.J.Joner, J.Straczek, C.Leyval Centre de Pédologie Biologique, CNRS Vandoeuvre-les Nancy, France

To consider potential applications of arbuscular mycorrhizal (AM) fungi in soil remediation, interactions between fungi, host plants and metallic trace elements (MTE) have to be better understood, including effect of MTE on AM fungi diversity, and effect of these fungi on transfer of MTE from soil to plants. Spores of AM fungi were isolated from the rhizosphere of *Viola calaminaria*, a metallophyte plant, on a heavy metal contaminated site and some of them characterized (Tonin et al. 2000, Mycorrhiza, 10). Subterranean clover was inoculated with this consortium of spores, and was grown on a soil spiked with different concentrations either of Zn and Cd for 62 days or of Zn for 62 and 120 days. At harvest, plant biomass, MTE uptake and AM colonisation were estimated. Diversity of AM fungi in clover roots was explored using T-RFLP and through sequences of the nuclear rDNA ITS and the SSU rDNA regions. After 62 days, Zn and Cd were more accumulated in roots of mycorrhizal clover than in non-mycorrhizal clover, without plant biomass reduction. T-RFLP showed that only one *Glomus* colonised clover roots grown on Zn and Cd spiked soil. Further results will be presented and discussed.

P1 160

Effect of native mycorrhizas on the growth and uptake of heavy metals and nutrients by native plant species growing on lateritic nickel soil.

F.M. Boulet[1] and D. A. Jasper[1] [1]Centre for Land Rehabilitation - Soil Science and Plant nutrition, Faculty of Agriculture. The University of WA, Nedland, Western Australia.

Lateritic soils associated with ultramafic parent material are characterized by extreme soil conditions, such as very low nutrient content and high concentration of some toxic heavy metals, in particular nickel. To deal with these hostile conditions, native plant species have adapted. Most plant species also associate with mycorrhizal fungi and these symbioses have been shown to be beneficial to plants in previous research. In the context of lateritic nickel soils, there are few studies investigating the contribution of mycorrhizal symbiosis to plant nutrition and tolerance to nickel. In this study, 3 native plant species were used (*Eucalyptus flocktoniae*, *Melaleuca coronicarpa*, and *Hakea verucosa*). These species originated from a lateritic outcrop associated with a nickel deposit in Western Australia. A non-adapted plant species (*Trifolium subterranean* clover) was also included in the experiment. Plants were grown in soil collected from the site where the native plants were originating. Some pots were inoculated with a mycorrhizal inoculum (a combination of arbuscular, AMF, and ecto-mycorrhiza, ECM) isolated from the same site. The effect of both native AMF and native ECM on plant growth and uptake of nickel and nutrients by these plants were assessed.

P1 161

Molecular responses of ericoid mycorrhizal fungi to heavy metals

S. Perotto[1,2], E. Martino[3], M. Vallino[3], L. Lanfranco[3] and P. Bonfante[2,3] - [1]Istituto Meteorologia e Oceanografia–I.U.N Naples, Italy; [2]Centro Studio Micologia del Terreno-C.N.R. and [3]Dipartimento Biologia Vegetale, Turin, Italy

Ericaceous plants can be dominant species in low mineral acidic soils enriched in toxic metal ions, thanks to their association with ericoid mycorrhizal fungi (Bradley *et al.* 1981; Sharples *et al.* 2000). To investigate the molecular bases that regulate interactions between ericoid fungi and heavy metals, isolates from polluted and non polluted sites were analysed in relation to their ability to grow in the presence of zinc, and to influence mobility of this metal. Expression of two chitin synthase genes was found to be differentially regulated by zinc in a sensitive strain, and may explain the slower growth rate in the presence of metal ions. Strains of *Oidiodendron maius* from polluted soil showed an increased ability to survive on media containing high concentrations of soluble and insoluble zinc. Their strong tendency to reduce metal solubilization, and a significant decrease in the accumulation of this metal ion in their mycelial mass when compared to strains from non polluted sites, may explain the higher tolerance. Decreased metal mobilization from insoluble zinc compounds was achieved by a reduced production of organic acids and by limited acidification of the culture medium. Several extracellular proteins were produced specifically in the presence of heavy metals (Zn, Cd, Cu). Among these proteins are two superoxide dismutases, which are known to be involved in oxidative stress response. By random sequencing of a cDNA library, we have identified several genes involved in oxidative stress responses and a clone with high homology with an ABC transporter involved in cadmium tolerance in *S. pombe.*

P1 162

Proteomics for studying cadmium-induced modifications in mycorrhizal pea roots

O. Repetto[1], G. Bestel-Corre[2], S. Gianinazzi[2], V. Gianinazzi-Pearson[2], E. Dumas-Gaudot[2], G. Berta[1]
[1]Università del Piemonte Orientale "Amedeo Avogadro", Alessandria, Italy [2]UMR INRA/Université de Bourgogne BBCE-IPM, INRA-CMSE, Dijon, France

Arbuscular mycorrhizae (AM) have a potential role in contributing to biological solutions proposed to restore soil fertility in heavy metal (HM) polluted ecosystems, because of their capacity to increase plant tolerance to HM toxicity. Our studies focus on the effect of cadmium (Cd) on the AM symbiosis with *G. mosseae* at two different developmental stages in three pea genotypes stressed or not with Cd 100 mg kg-1. Mycorrhization was affected by Cd when the Cd stress was applied the same day as *G. mosseae* inoculation, a Cd-sensitive genotype being more affected than a tolerant one, suggesting an inhibitory effect of Cd during the early steps of root colonization. In spite of this, mycorrhization had a positive effect on the biomass of Cd-sensitive and moderately tolerant genotypes, while no protective effect was observed in the Cd-tolerant one. When Cd was added 15 days after *G. mosseae* inoculation, a positive effect of the AM symbiosis was observed on the biomass of the Cd-tolerant pea genotype, while growth of the Cd-sensitive one did not seem affected. Analyses of total soluble proteins of pea roots were carried out to identify proteins induced by Cd stress and modulated by mycorrhization. Total 1D SDS-PAGE protein profiles and 2D-PAGE analyses revealed the synthesis of a subset of polypeptides at 30 kDa in response to Cd, the induction of which depended on pea developmental stages and was down regulated by mycorrhization. These proteins were purified by micro-preparative pH 4-7 IPG, 2D-PAGE and Coomassie blue staining, and the spots were excised for further analysis by tandem mass spectrometry. Their identification will contribute to evaluating the physiological role of AM fungi in detoxification and/or tolerance of Cd in pea roots.

*This work is part of an EU INCO Copernicus project (IC15-CT98-0116)

P1 163

Molecular diversity of mycorrhizal fungi on the roots of trees grown with contaminated soil inoculum

K.P. Ridgway, L.A. Marland, J.P.W. Young, A.H. Fitter. Department of Biology, University of York, U.K.

Soil contamination can greatly reduce the diversity of key microbial taxa. Since revegetation of contaminated land often depends upon the presence of symbiotic microorganisms for plant health a study was undertaken to assess the diversity of these organisms in urban post-industrial soils with varying levels of compaction, disturbance, heavy metal and organic pollution. Four tree species were used to trap fungal root symbionts from ten differently contaminated soils. A molecular strategy was used to identify arbuscular mycorrhizal fungal (AMF) taxa colonising the roots of *Robinia pseudoacacia*, *Alnus incana* and *Acer pseudoplatanus*. AMF populations within roots varied markedly with soil type and to a lesser extent with host plant but were dominated by *Glomus* spp. Evidence of host preference was detected between *A. incana* and an Acaulospora sequence type. A second experiment to test the ability of mycorrhizal inoculum to survive and persist in a gasworks soil demonstrated that introduced inoculum can compete with indigenous strains and cause shifts in AMF community structure. The abundance and diversity of ectomycorrhizal fungi on the roots of *Betula pendula* were described using both morphological and molecular techniques. Fifteen different fungal types were identified by ITS/RFLP analysis and an average of four fungal types occurred on each root system. These results demonstrate the unexpectedly large reservoir of symbiont diversity within some contaminated soils. Implications for revegetation will be discussed.

P1 164

Cadmium tolerance and mechanisms of detoxification in mycorrhizal pea genotypes*

F. Rivera-Becerril[1,3], D. van Tuinen[1], K. Turnau[2], S. Gianinazzi[1], V. Gianinazzi-Pearson[1]. [1]UMR INRA/Université de Bourgogne, BBCE-IPM, Dijon, France; [2]Jagiellonian University, Krakow, Poland; [3]Universidad Autonoma Metropolitana-Xochimilco, Mexico City, Mexico

Contamination of soils with heavy metals (HM) such as cadmium (Cd) affects plant productivity and sustainable soil management. Arbuscular mycorrhiza (AM) can reduce plant susceptibility to environmental stresses induced by HM, including Cd, and the combinations of HM-tolerant plant genotypes with beneficial soil microorganisms appears a promising approach for phytostabilisation of HM-polluted soils. Plants respond to HM toxicity through different ways such as cell wall binding or chelation by metallothionein (MT) and phytochelatin (PC) peptides, and we are investigating the possible involvement of these mechanisms in Cd tolerance of AM plants. Plant biomass and pod production of four genotypes of *Pisum sativum* (cv. Frisson, VIR4788, VIR7128 and VIR3429) were decreased when plants were grown in the presence of Cd, but inoculation with the AM fungus *Glomus intraradices* attenuated this negative effect in all the genotypes. Cd accumulated from 20 to 50 fold more in roots than shoots, and overall concentrations of the HM in roots were greatest in VIR7128 and VIR3429 pea genotypes, although cv. Frisson accumulated the highest concentrations in pods. Cell wall deposits of Cd increased greatly in roots of plants treated with the HM but AM development had no consistent effect on cell binding. Expression profiles of one stress-related gene (*pI206*), and of genes encoding MT or enzymes involved in the metabolism of glutathione, a precursor of PCs, have been analysed in roots of cv. Frisson. Transcript analyses indicate increased expression of *pI206*, MT22, *gsh1* (γ-glutamylcysteine synthetase), *hgsh2* (homoglutathione synthetase), and *gr* (glutathione reductase) genes in roots of Cd-treated mycorrhizal and non mycorrhizal plants.

*Research from an EU INCO-Copernicus project (IC15-CT 98-0116).

P1 165

Arbuscular mycorrhizae and restoration of endangered plants in subtropical Florida

Jack B. Fisher and K. Jayachandran
Fairchild Tropical Garden and Florida International University, Miami, FL, U.S.A.
jfisher@fairchildgarden.org

Arbuscular mycorrhizal fungi (AMF) are reported in the roots of many common and rare plants in the Anacardiaceae, Arecaceae (Palmae), Cactaceae, Convolvulaceae, Cycadaceae, Euphorbiaceae, Fabaceae, Lauraceae, Rubiaceae, and Simarubaceae that grow in the coastal maritime and inland hammocks (= tropical forests) of South Florida. Greenhouse experiments were carried out with seedlings of species in the following genera: *Amorpha; Coccothrinax; Gymnanthes; Hamelia; Jacquemontia; Licaria; Nectandra; Opuntia; Picramnia; Psychotria; Rhus; Sabal; Serenoa;* and *Zamia*. Inoculation with native mixed AMF showed variable enhancement of growth and phosphorus uptake on local sandy, nutrient poor soils. Most native species depend on AMF under natural conditions. Variable levels of AMF colonization were observed. Restoration projects are now planned for endangered species of: *Amorpha* (Fabaceae); *Jacquemontia* (Convolvulaceae); *Optunia* (Cactaceae); and *Pseudophoenix* (Arecaeae). Horticultural methods are being developed to propagate species with AMF in the nursery to improve survivorship after out planting in the field.

P1 171

Ectomycorrhizal and microfungal diversity on Norway spruce stands with different degrees of decline in the Krkonose Mountains (CZ).

Egli, S.[1], Gonthier, P.[2], Nicolotti, G.[2], Peter, M.[1], Varese, G.C.[3], Voyron, S.[3]

[1]Swiss Federal Research Institute WSL, Birmensdorf, Switzerland, [2]University of Torino, Torino, Italy, [3]University of Torino, Grugliasco, Italy

Since the late 1970s the forests of Northeastern Bohemia (CZ) have shown a severe decrease in vitality, caused by air pollution. In the Krkonose Mts. a forest dieback, followed by a lack of forest regeneration, has occurred due to industrial emissions of SOx and NOx in combination with various other types of stress.

Investigations on the microfungal and mycorrhizal communities associated with three spruce stands with different degrees of decline were carried out. Mycorrhizal diversity was desribed using morphological and molecular techniques (ITS-PCR-RFLP). Rhizospheric and rhizoplanic microfungi were isolated using the soil dilution plate and the serial washing technique, respectively. The microfungal communities of the three stands show differences in species composition, which are not correlated to the degree of decline. However, mycorrhizal diversity decreased with increasing degrees of decline in old trees, whereas these differences were much less pronounced in seedlings. Even on the site with the highest decline the roots of the seedlings were completely mycorrhized. These results suggest that, even if site conditions affect mycorrhizal and microfungal communities, a deficiency in these fungi is not the primary cause for the observed lack of natural regeneration at these sites.

P1 172

Soil microbial population and activity affected by mycorrhizal and non-mycorrhizal winter cover crops in Southern Brazil

Paulo Emílio Lovato and Alceu Kunze, Universidade Federal de Santa Catarina, Brazil.

Cover crops are increasingly used in agricultural systems, but little is known about their effects on soil activity of microbial communities, especially those related to mycorrhizal and non-mycorrhizal plant species. That raises the question whether microbial populations and activities related to soil P cycling are different in the rhizosphere of plants with different mycorrhizal status. With the aim of evaluating the effect of mycorrhizal and non mycorrhizal winter cover crops on soil properties related to phosphorous, a field experiment was carried out in Southern Brazil(27° S), on a sandy soil (Typic Quartzament, pH 4.8, $P = 10\ \mu g.L^{-1}$). Mycorrhizal species were black oat (*Avena strigosa* Schreb) and annual ryegrass (*Lolium multiflorum* Lam.), and non mycorrhizal ones were oilseed radish (*Raphanus sativus* L. var. *oleiferus* Metzg) and corn spurry (*Spergula arvensis* L.). Resident vegetation, mostly grasses, served as the control. Plots were 1 x 2 m, with 8 replications in a randomised complete block design. After 19 weeks of crop growth, soil samples were collected to evaluate extractable P (Mehlich 1), biomass-P, alkaline and acid phosphatase activity (incubation with P-nitrophenyl phosphate, pH 6.5. and 11.0), total fungal and bacterial numbers, as well as mycorrhizal colonisation of all roots found in the soil. Total bacterial numbers, alkaline phosphatase activity and extractable P had higher values in soils with non mycorrhizal cover crops, whereas none of the variables decreased in the presence of non mycorrhizal plants. These results suggest that the mycorrhizosphere is an environment where the biological activity related to P cycling is lower than in soil or non mycorrhizal rhizosphere.

P1 173

Arbuscular mycorrhizal infection changes the bacterial 16S rDNA community composition in the rhizosphere of maize

P. Marschner[1], D.E: Crowley[2] and R. Lieberei[1] [1] Institute for Applied Botany, Hamburg, Germany [2] Department of Soil and Environmental Sciences, University of California, Riverside, USA

Mycorrhizal and non-mycorrhizal (NM) maize plants were grown for 4 and 7 weeks in an autoclaved quartz sand-soil mix. Half of the NM plants were supplied with soluble P (NM-HP) while the other half (NM-LP) received, like the mycorrhizal plants, poorly soluble Fe and Al phosphate. The mycorrhizal plants were inoculated with *Glomus mosseae* (GM) or *Glomus intraradices* (GI). Soil bacteria and those associated with the mycorrhizal inoculum were reintroduced by adding a filtrate of a low P soil and of the inocula. At 4 and 7 weeks, plants were harvested and root samples were taken from the root tip (0-1 cm), the subapical zone (1-2 cm) and the mature root zone at the site of lateral root emergence. DNA was extracted from the roots with adhering soil. At both harvests the NM-HP plants had higher shoot dry weight and shoot P content than the plants grown on poorly soluble P. Mycorrhizal infection of both fungi ranged between 78 and 93% and had no effect on shoot growth or shoot P content. Eubacterial community compositions were examined by PCR-DGGE of 16S rDNA, digitisation of the band patterns and multivariate analysis. The community composition changed with time and was root zone-specific. The differences in bacterial community composition in the rhizosphere between the NM plants and the mycorrhizal plants were greater at 7 than at 4 weeks. The two fungi had similar bacterial communities after 4 weeks while they differed after 7 weeks. The observed differences are probably due to changes in substrate composition and amount in the rhizosphere.

P1 174

Detection of Archaea in Scots pine mycorrhizospheres and boreal forest humus

Malin Bomberg, German Jurgens, Aimo Saano, Robin Sen, Sari Timonen University of Helsinki, Finland

Archaea have traditionally been associated with extreme environments. Yet recently, these microorganisms have been identified, via 16S rDNA analysis, in e.g. agricultural soils and freshwater bodies. Members of the Crenarchaeota were first described from Finnish boreal forest soil in 1997 and in 2000 were identified colonising terrestrial plant roots. Our work continues this theme, in the detailed mapping of Archaea-specific niches in Finnish forest soils. Archea were found in the different habitats provided by Scots pine-*Suillus bovinus* and -*Paxillus involutus* mycorrhizospheres developed on humus. Crenarchaeota were identified in the close vicinity of mycorrhizas and at the margins of extramatrical hyphae and adjacent uncolonised humus. The Finnish forest soil Archaea form a specific grouping that strongly differs from other soil Crenarchaeota detected around the world. Though their functioning in boreal forest soils has yet to be clarified, Archaea are known to synthesize methane (methanogens), withstand extreme environmental conditions (halophiles, thermophiles) and even enter into endosymbiotic relationships with eukaryotic organisms.

P1 175

Biological Activity in the mycorrhizosphere of *Trachypogun plumosus* NESS, dominant species in venezuelan acid savannas

M. Toro[1], J.C. López-Gutiérrez[1], I. Hernández-Valencia[1], & D. López-Hernández[1]
Instituto de Zoología Tropical, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela.

Savannas are tropical ecosystems widely spreaded in the tropics. In Venezuela, they constitute the lands to be potentially used for agricultural purposes. Most of them have low fertility and acid soils. In these ecosystems a native grass, *Trachypogun plumosus,* grow spontaneously suggesting it may have adaptive mechanisms to grow in such soils. We studied arbuscular mycorrhiza root colonization (%AMRC), P fractions, enzyme activities (phosphatase and deshidrogenase) and phosphate solubilizing microrganisms in the rhizosphere of *T. plumosus* growing in different savanna locations. Our aim is to characterize biological mechanisms to support *T. plumosus* in such poor soils. Total P soil content was 134 µg/g.P. P fractions showed that approximately 62 % of soil P is hardly available to the plant. Only a small fraction of P, 2 % is considered to be available to the plant. 30 % of P is in mineralizable forms as organic phosphates. *T. plumosus* showed variations in %AMRC, the values being between 80 and 20 %. Phosphatase activity values indicated relevant phosphate mineralizing activity in savanna soils. Different phosphate solubilizing capacities (calcium, aluminum and iron phosphates) where monitored in microorganisms. Interesting relations between phosphate solubilizing fungi and bacteria, phosphate fractions, mineralizing activity and AM seem to work in the mycorrhizosphere of *T. plumosus*, in order to moderate P cycling in savanna ecosystems.

P1 176

Mycorrhiza-regulated plant genes common to beneficial rhizobacteria interactions*

S. Weidmann, L. Brechenmacher, M. Batoux, D. van Tuinen, P. Lemenceau, and V. Gianinazzi-Pearson.
UMR INRA/Université Bourgogne BBCE-IPM, INRA-CMSE, Dijon, France

Arbuscular mycorrhizal fungi and rhizobacteria are amongst the most frequent rhizosphere microorganismes which stimulate plant growth. Their common ability to establish symbiotic associations with plant root tissues may imply shared molecular programmes. Previous work has in fact shown up-regulation of some nodulin-encoding genes in arbuscular mycorrhiza. In order to gain more insight into plant genes which may be common to beneficial rhizosphere interactions, we are comparing expression profiles in *Medicago truncatula* roots inoculated with *Glomus mosseae*, *Pseudomonas fluorescens* C7R12 or *Sinorhizobium meliloti*. ESTs from a suppressive subtractive hybridization library, established to identify genes that are up-regulated in *M. truncatula*/*G. mosseae* mycorrhizal roots, are being screened for differential gene expression during mycorrhization, nodulation or root interactions with *P. fluorescens*. Out of the first 100 ESTs to be analysed, two correspond to plant genes induced in all three root-microbe associations, eight are overexpressed in both mycorrhizal and nodulated roots, and eight represent genes which are up-regulated during *G. mosseae* and *P. fluorescens* interactions. This screening programme is on-going and further results will be presented, together with sequencing data, for plant genes which indicate that common molecular events are involved in the compatible interactions in the three symbiotic associations.

* This research is partly supported by the EU project QLG2-CT-2000-30676

P1 177

To which extent observed changes on plant $\delta^{15}N$ values are due to the symbiosis with arbuscular mycorrhiza fungi?

H.M.A.C. Fonseca[1], R.L.L. Berbara[2].

[1] *Universidade de Aveiro, Centro de Biologia Celular, 3810-193 Aveiro, Portugal.*

[2] *Universidade Federal Rural do Rio de Janeiro, Departamento de Solos, Seropédica, Itaguaí, RJ, CEP 23851-970, Brazil.*

Abstract

Most if not all controlled experimentation on the effect of arbuscular mycorrhizal fungi (AMF) on plant carbon and nitrogen isotopic discrimination describes the observed changes of plant $\delta^{13}C$ and $\delta^{15}N$ as attributable to the symbiosis between hosts and AMF.

A glasshouse pot-experiment confronting the effect of *Glomus etunicatum* inoculum on two contrasting plants (non-host, *Brassica rapa*; host, *Sorghum bicolor*) showed significant changes of shoot $\delta^{15}N$ values on both plant species, even though no colonisation was observed for inoculated *B. rapa*.

Our data raises a still unresolved question about the extent that observed isotopic changes are due alone to the symbiotic interaction between host and AMF. We speculate that part of the observed effects may be due to extra-AMF microbe populations selectively enhanced by the mycorrhizal rhizosphere.

P1 178

Effects of hardened wood ash on microbial activity, plant growth and nutrient uptake by ectomycorrhizal spruce seedlings

S. Mahmood[1], R.D. Finlay[2], A.-M. Fransson[1] and H. Wallander[1]. [1] *University of Lund, Sweden;* [2] *Swedish University of Agricultural Sciences, Uppsala, Sweden*

Application of hardened wood ash (HWA) has been recommended as a method to supplement nutrient loss resulting from harvesting of forest residues for bioenergy production. In the present study, we have investigated the role of ectomycorrhizal mycelia in mobilisation of nutrients in HWA and their translocation to plants in intact symbiotic associations. Plant growth, nutrient uptake, microbial biomass and activity were examined in pot systems containing spruce seedlings colonised with three different ectomycorrhizal fungi (*Piloderma* sp. 1, *P. croceum*, Ha-96-3) originating from an ash fertilised forest. The seedling root systems were enclosed in mesh bags inside an outer compartment containing HWA (+HWA; or no ash, –HWA). When nutrients were in high supply (+HWA) the ectomycorrhizal fungi had a small positive influence on the growth of the host plants but one species (*Piloderma* sp. 1) had a significant effect on release of P from HWA to the soil solution. 5-6.7% of the total P (contained in HWA) was solubilised, with 0.9-1.5% in soil solution, 3.6-4.6% in the plants and 0.5-1.5% in microbial biomass. Total plant contents of K, Ca, Mg and P were significantly increased in the +HWA treatment. In –HWA treatment, mycorrhizal inoculation significantly increased the contents of these elements in all cases. Bacterial activity was significantly greater in +HWA treatments than in –HWA controls and showed higher variation between different mycorrhizal treatments than in –HWA controls. Principle component analysis of phospholipid fatty acids showed a clear difference in bacterial community structure between samples collected from +HWA treated pots and –HWA controls. Ectomycorrhizal fungi were found to be very important in transporting K and P to the host plants when these elements were limiting plant growth in –HWA treatment.

P1 179

Bacteria associated with *Glomus clarum* spores influence mycorrhizal activity

J.J. Germida and L.J.C. Xavier. The University of Saskatchewan, Canada

Arbuscular mycorrhizal fungi (AMF) produce resting spores that persist in soil. However, these spores are colonized and form intimate relationships with a variety of soil bacteria. This study assessed the effects of bacteria associated with *Glomus clarum* NT4 spores on spore germination, growth *in vitro* and the pea (*Pisum sativum* cv. Trapper) - *G. clarum* NT4 symbiosis. *Glomus clarum* NT4 spores were recovered from soil and decontaminated with 5% chloramine-T for 30 to 60 minutes. Spore wall associated bacteria were then recovered from untreated and decontaminated spores using nutrient rich and nutrient poor media. Both gram positive and gram negative bacteria were recovered from untreated NT4 spores, whereas only gram positive bacteria were isolated from decontaminated spores. An *in vitro* assessment of the effect of spore-associated bacteria on *G. clarum* NT4 spores revealed that (i) bacteria isolated from untreated spores did not significantly alter spore function, (ii) bacteria isolated from decontaminated spores inhibited or stimulated spore germination, (iii) stimulation of spore germination occurred only when bacteria were in contact with spores, and (iv) inhibition of spore germination was the result of volatile bacterial metabolites. *Bacillus pabuli* LA3, a stimulatory bacterial isolate, significantly ($P<0.05$) enhanced the shoot growth, AMF colonization, shoot N content and P use efficiency of *G. clarum* NT4-inoculated 6 week-old pea plants compared to that of plants co-inoculated with *G. clarum* NT4 and an inhibitory bacterial isolate, *Bacillus chitinosporus* LA6a. Our results demonstrate the importance of spore wall associated bacteria on mycorrhizal activity.

POSTER
SESSION
TWO

Wednesday & Thursday
11 & 12 July

P2 01

Do plants selectively associate with species of ectomycorrhizal fungi that support highest plant growth?

M. Kummel University of Michigan, USA

The hypothesis of this research is that plants, as a part of their foraging strategy, selectively associate with the species of ectomycorrhizal fungi (ECMF) that support the highest plant growth This hypothesis has three assumptions: 1) ECMF species differ in their effect on plant growth and thus the fungal species can be arranged in a growth-effect hierarchy, 2) the growth effect hierarchy changes between environments, and 3) the composition of ECMF is under at least partial control of the plant. These assumptions lead to the following predictions: 1) Each plant should selectively associate with the species of ECMF that supports the highest plant growth; and 2) the plant should selectively associate with different fungi in different environments, leading to 3) variation of ECMF with environmental conditions. Assumptions 1 and 2 and prediction 3 of the hypothesis are supported by the available published variation of ECMF with environmental conditions. Assumptions 1 and 2 and prediction 3 of the hypothesis are supported by the available published evidence. Predictions 1 and 2 have never been tested before, but are supported by my research on naturally occurring seedlings of *A. balsamea* along a light availability gradient in northern hardwood forests. Three of the four most abundant morphotypes of ECMF have distinct distributions along a light-availability gradient with the two most abundant morphotypes switching dominance along the gradient. As predicted, each of the two dominant morphotypes was dominant in the portion of the light gradient where it had a positive effect on *A. balsamea* growth.

P2 02

Influence of plant community composition and diversity on ectomycorrhizal diversity in boreal mixed-woods

G. Kernaghan[1,2], S. Légaré[1,3], P. Widden[1,4] and Y. Bergeron[1,2,3]
[1]Groupe de recherche en écologie forestière interuniversitaire [2] Université du Québec à Montréal [3]Université du Québec en Abitibi-Témiscamingue [4] Biology Dept., Concordia University

As part of a larger study on microbial diversity in boreal mixed-wood forests, we are comparing ECM diversity with plant community characteristics on twelve stands in the Lake Duparquet Research and Teaching Forest, in the Abitibi region of North-Western Quebec. Stands are of similar age and soil type, but support varying proportions of *Abies, Betula Picea, Pinus* and *Populus*. Diversity and composition of overstory trees and understory vegetation were assessed and ECM sampled by removing forest floor material in such a way that exploratory root systems of host trees remained intact. Exploratory roots were then separated into species on the basis of wood anatomy. Diversity of ECM colonizing each host species was determined on the basis of anatomical characters so that the influence of individual tree species on the ECM community can be determined. ECM diversity based on anatomy is currently being confirmed by PCR/RFLP analysis. Preliminary analysis of the data indicates that both plant diversity and species composition are important determinants of ECM diversity.

P2 03

Influence of habitat on diversity of putative mycorrhizal endophytes of *Epacris microphylla*(Ericaceae).

A.F.Williams, S.M. Chambers and J.W.G. Cairney.
Mycorrhiza Research group, University of Western Sydney, Australia.

Plants of *Epacris microphylla*(Ericaceae) were collected from Australian coastal heathland, subalpine grassland and alpine feldmark communities. Putative mycorrhizal endophytes were isolated from hair roots and genomic DNA was extracted from cultured mycelia. One hundred and forty endophyte isolates were obtained from plants from the coastal heathland site and 172 and 174 from the plants from the subalpine and alpine sites respectively. These were grouped according to culture morphology and ITS-RFLP patterns were determined for representative isolates from each site by digestion of the ITS product with the endonucleases *RSA I*, *Hinf I* and *Hae III*. Isolates were subsequently grouped according to RFLP patterns and ITS sequences were obtained for each RFLP type. Endophyte diversity will be disscussed in relation to habitat and known endophytes of other Ericaceae taxa.

P2 04

Spatial relations of ectomycorrhizae and their relations to soil heterogeneities in natural soil of a Norway spruce stand as studied by the new method ‚Micromapping' (McMp)

R. Agerer[1], S. Raidl[1], R. Grothe[2] and A. Göttlein[3]
[1]The University of München, Germany [2,3] The Technical University of München, Germany

Ectomycorrhizae are not evenly distributed. Their occurrence is depending upon the distribution of roots, availability of fungal inocula and soil conditions. Spatial heterogeneity in the soil may create a mosaic of fungal colonization. Coexisting species vice versa may influence soil patches to a different degree.

We applied McMp to map ectomycorrhizae in their exact natural position and analysed their spatial relations with the 'growing grid method'. Due to this method all co-occurring species could be compared separately and their relations were categorized by an index. The results suggested that *Russula ochroleuca* and *Piceirhiza internicrassitunicatihyphis* exclude another, *Cortinarius* cf. *obtusus* and *Piceirhiza internicrassitunicatihyphis* appear to be associated. Many combinations indicate an uninfluenced occurrence.

Ectomycorrhizae of *Lactarius* cf. *theiogalus* were significantly and positively correlated with concentrations of NH_4^+, K, Na, Mg, Fe+Mn and with pH. Those of *Cortinarius* cf. *obtusus* were only significantly correlated with concentrations of NH_4^+ and Mg. *Russula ochroleuca* showed no significant correlations with any of the tested ions.

To estimate the possible zone of influence within the soil, it was attempted to evaluate the amount of extramatrical mycelium of synthesized ectomycorrhizae by picture analysis. Significant differences in densities and growth radius were apparent and indexed by %-coverage.

P2 05

The effects of long-term field fertilization on mycorrhizal fungal communities in a temperate oak savanna

P.G. Avis, D. McLaughlin, P. Reich, and I.D. Charvat. University of Minnesota Plant Biological Sciences Graduate Program, St. Paul, Minnesota, USA. Since the growth of mycorrhizal fungi (MF) depends on the availability of soil nutrients and root carbohydrates, these two factors should be important in structuring MF communities. Events such as fertilization that change soil nutrient and root carbohydrate availability should dramatically alter the composition of MF communities and their ecosystem function. We examine the response of MF communities in a temperate oak savanna in Minnesota subject to 15 years of experimental field fertilization to address this hypothesis. Results to date indicate that nitrogen mineralization increases and fine root biomass decreases in fertilized plots relative to control plots. Furthermore, in 1999 and 2000, arbuscular mycorrhizal root colonization, inoculum potential, and host plant presence was lower with fertilization. However, it is not clear how EMF respond -- fertilization decreases the abundance and diversity of fruitbodies but not EMF colonization in 2000. While morphotype richness remains relatively constant among treatments, particular dominant morphotypes such as *Cenococcum geophilum* are less abundant in fertilized plots suggesting that EMF communities differ among the treatments. RFLP-type richness is high across the treatments and to date few matches have been made among treatments as well as to identified fruitbodies. How this work will be used to examine potential functional differences between EMF communities will be discussed.

P2 06

Conservation of ectomycorrhizal fungi: Green-tree retention preserves species diversity. Eberhart, J.L. and D.L. Luoma. Department of Forest Science, Oregon State University, Corvallis, Oregon, USA.

The DEMO project is a large, interdisciplinary study designed to test the biological and silvicultural effects of green-tree retention in Douglas-fir forests. Six treatments were replicated on six blocks in Washington and Oregon, USA: no harvest, 75% aggregated, 40% (dispersed and aggregated), and 15% (dispersed and aggregated) green tree retention. We have studied treatment effects on ectomycorrhizal fungi in three of the study blocks. Ectomycorrhizae (EM) were sampled by use of 350 cc soil cores. Prior to tree felling, one spring and one fall set of cores were collected from each study unit. After the trees were cut, soil cores were again collected the fall a full year after cutting, and the following spring. A total of 432 cores have been assessed. To test for treatment effects, mean number of EM morphotypes in cores, relative frequency, and species accumulation curves were compared amongst pre-treatment, control, moderate thin, heavy thin, clearcut, and leave groups. All methods of measure show a reduction of EM in clearcut and heavy thin treatments. The dispersed moderate thin treatment (40% green tree retention) showed less reduction in EM types, except in one block, where compaction during logging may have contributed to root mortality. Most of the 188 morphotypes described are rare and are not taxonomically identified. Work is ongoing to identify morphotypes using molecular techniques; successful matches are presented.

P2 07

Preliminary data about how the ectomycorrhizal community changes in space and time in an evergreen oak forest.

E. Sarrionandia, M. Gartzia and I. Salcedo
Lab de Botánica, Dpto. Biología Vegetal y Ecología. Fac. Ciencias. Universidad del País Vasco/EHU. Spain

Fructifications of ectomycorrhizal species are changing along the year and year after year. However, although it is said that ectomycorrhizae are not evenly distributed in space, little is known about their timescale distribution. In order to know how the distribution of the ectomycorrhizal community works a permanent plot of 600 m^2 was delimited on an evergreen oak forest (*Quercus rotundifolia*) and divided into 1x1 subquadrats. Every week five soil cores were randomly taken and the coordinates of each sample were noted down to see spatial distribution of morphotypes. Soil moisture and temperature were also registered every three hours. Data presented here correspond to 2 and a half months of sampling.

A total of 54 morphotypes were found in the first 50 soil samples that correspond to October, November and the first fortnight of December. It averaged out at 4 morphotypes per soil core. The data were analysed through CCA using CANOCO for windows being space and time taken as variables. In accordance with the results, time makes ectomycorrhizal community change, but the variability of the data is not explained by space. Anyway, these results are preliminary and the data taken during the year will be essential to re-evaluate these influences.

P2 08

Below ground community structure of ectomycorrhizas; roots and external mycelia.

S. Erland, S.Mahmood and J. Rahm. Microbial Ecology, Lund University, Lund, Sweden.

For the last decade molecular methods have been applied in studies of the ectomycorrhizal (EM) communities on the roots in forest ecosystems. All these studies have been conclusive in that other mycorrhizal species have been determined to be frequent and abundant on the roots compared to results based on above ground sporocarps. Management and pollution often result in a shift in the EM communities on the roots and there is data showing functional differences between species that increase and decrease in response to e.g. N-deposition and ash-treatments. There is also data showing that the growth of the external mycelia can be differently affected depending on species and treatment. We expect the external mycelial phase to be the most dynamic part of the EM community and thus the part that first would respond to alterations in the environment. Little is known about how the community on the roots (frequency and abundance) is reflected in the external mycelial phase. Currently we are working on different methods to compare community structure on roots and in the mycelial phase. Since both extraction of fungal hyphae and DNA from humus is very difficult we work with EM mycelia that have colonised sand-meshbags burried in the humus layer of spruce forests. Other studies in our laboratory show that the mycelia colonising such bags are predominantly (85-100%) of EM-origin.

P2 09

Mycorrhiza Fungal Diversity as Impacted by the Loss of Endemic, Rainforest Plants in Madagascar.

Erick C.M. Fernandes[1], Erika Styger[1], Harivelo 'Mparany' Rakotondramasy[2] [1]Cornell University, NY, USA. [2]LDI International, Madagascar.

We are evaluating the impact of slash and burn agriculture on vegetation succession and the diversity of arbuscular mycorrhizal fungi in the rainforest region of eastern Madagascar. High rates of deforestation and repeated burning are rapidly converting the rainforest that is characterized by very high endemic species diversity to scrub forest and degraded lands dominated by introduced weeds such as *Lantana camara*, *Pteridium aquilinum* and *Imperata cyclindrica*. We hypothesize that the loss of endemic plant species results in a corresponding and significant loss of mycorrhizal fungi. The Oxisols and Ultisols commonly found in this region are acid (pH 4.5 -5.5), have very low fertility and exchangeable P levels of <1 mg kg^{-1}. Given the low levels of available P in local soils, mycorrhizal associations are likely to be vital for the successful regeneration of rainforest species on deforested land. This paper presents the first results of the mycorrhizal fungi encountered in the rainforest, and in secondary succession dominated by (a) *Trema orientalis* and *Harungana madagascariensis*, (b) *Psiadia altissima*, (c) *Lantana camara* and *Rubus mollucana*, and (d) *Imperata cylindrica* and *Pteridium aquilinum*. The sequence from forest to *Imperata* represents increasing levels of degradation and loss of native plant species.

P2 10

An investigation of arbuscular mycorrhizas in a South African coastal sand dune system.

Dames, J. F. & Haller, A.H.A.
Botany Department, Rhodes University, P.O. Box 94, Grahamstown, South Africa.

Arbuscular Mycorrhizas (AM) have been investigated in many coastal sand dune systems worldwide, but little has been known of their role in South African dunes. This study aimed to investigate the presence and extent of AM colonisation of a coastal sand dune at Old Woman's River in the Eastern Cape province of South Africa. The roots of five plant species (*Scaevola plumieri, Arctotheca populifolia, Ipomoea pes-caprae, Ehrharta villosa* and *Chrysanthemoides monilifera*) were sampled along a foredune profile and were assessed for the percentage mycorrhizal colonisation they supported. Spores were extracted from the rhizosphere sand of each plant species. These were counted and identified to genus level. Results showed that all the plant species were mycorrhizal and that colonization and spore numbers were related to seasonality and the position of the plants along the dune profile. Percentage colonization ranged from 0-92% and was generally highest in the winter months, particularly in *I. Pes-caprae* and *E. villosa*. Spore numbers ranged from 0-48 spores 100g^{-1} sand, with highest numbers also in winter months. *S. plumieri* and *A. poplulifolia* were associated with the greatest spore abundance. Four fungal genera were identified: *Glomus, Acaulospora, Scutellospora* and *Gigaspora*. This study gives an insight into the AM component of South African sand dunes and paves the way for more in-depth ecological and physiological research.

P2 11

Below-ground ecology of Scottish heathlands: the role of mycorrhizas

D.R. Genney[1], I.J. Alexander[1] and S.E. Hartley[2] [1]University of Aberdeen, UK [2]CEH Banchory, UK

The upland regions of Scotland are dominated by a contrasting mosaic of ericoid mycorrhizal (ErM) dominated heath and arbuscular mycorrhizal (AM) dominated grassland communities. Both mycorrhizal types improve host plant nutrition in pot studies and may therefore affect species interactions in the field. The ErM shrub *Calluna vulgaris* and invasive AM grass *Nardus stricta* were used as model species to investigate the potential role of mycorrhizas in the observed replacement of heathland by grassland. In a pot study, *Calluna* was the superior competitor over *Nardus* for nutrients irrespective of mycorrhizal status. This was due to the ability of *Calluna* to proliferate roots in, and exclude *Nardus* roots from, organic surface substrate layers. The presence of ErM colonisation in *Calluna* resulted in a further reduction in *Nardus* root growth in the organic layer but did not reduce *Nardus* shoot nutrient content, suggesting that ErM suppression of *Nardus* growth was due to non-nutritional interference. In a field study, *Nardus* root length colonised by AM fungi (%RLC) was assessed in relation to proximal *Calluna* and *Nardus* density and host plant nutrient status. Correlation of %RLC with *Nardus* density was demonstrated but this relationship was masked where *Nardus* tissue phosphorus concentrations were high. Bioassay *Nardus* transplants within *Calluna* swards failed to become colonised by AM. Suppression of *Nardus* root growth in surface organic horizons by ErM colonised *Calluna*, and lack of AM inoculum availability are suggested as potential below-ground constraints to the rate at which *Nardus* invades *Calluna* heath.

P2 12

Established vegetation negates mycorrhizal benefit in seedlings – a case study of subarctic perennial herbs

M.-M. Kytöviita[1], M. Vestberg[2] and J. Tuomi[1] [1]Department of Biology, 90014 Oulu University, Finland. [2] MTT-Agricultural Research Centre of Finland, Laukaa FIN-41330 Vihtavuori, Finland

Subarctic plant communities are characterized by long-lived plants that form intensive mycorrhiza. We tested the role of arbuscular mycorrhizal fungi (AMF) and established vegetation on seedling growth as seedling establishment is critical life history trait for persistence of populations. The perennial, subarctic herbs *Antennaria dioica, Campanula rotundifolia, Sibbaldia procumbens* and *Solidago virgaurea* were germinated solitary or in the vicinity of an established *Sibbaldia* plant. The seedlings were inoculated by spores or by hyphal network connected to established *Sibbaldia* plant by one subarctic strain of the three AMF species *Glomus claroideum*, *G. hoi*, *G. boreale* or left non-mycorrhizal in a fully factorial greenhouse experiment. The results show that (i) seedlings were unable to establish themselves as sinks for symbiosis mediated benefits in presence of the established *Sibbaldia*, although they were able to do so when grown solitary and (ii) plant-fungus relationship varied with the life stage of the plant. Although heavily infected, seedling growth was not improved by symbiosis in presence of neighbouring plant, but was greatly improved in absence indicating that (iii) infection intensity does not necessarily indicate functionality of the association and (iv) function of the symbiosis is conditional even after formation of the symbiotic structures. In the light of the current results, recruitment of mycorrhiza-dependent species from seedlings may be possible only in gaps of established vegetation.

P2 13

Diversity and host preference of ectomycorrhizal fungi in arctic heath communities

B.Moyersoen, O. Knox, I Shand & I.J.Alexander. Department of Plant & Soil Science, University of Aberdeen, Scotland.

What determines the species richness and relative abundance of ectomycorrhizal fungi in plant communities? This question is particularly interesting where more than one host species is present, because host specificity or host preference may influence the composition and structure of the fungal community. Arctic *Salix/Dryas* communities are a good model in which to study these questions because they are small in stature, simple in above ground community structure, relatively easy to sample, and there are steep environmental gradients over short distances.

Ectomycorrhizas were sampled by coring in a mixed *Dryas/Salix* community, and an adjacent pure *Salix* community, on Svalbard (78°N). Over 40,000 tips in 120 cores were classified into 43 morphotypes. The validity and identity of the morphotypes was confirmed using ITS PCR/RFLP, and ITS1/4 or ML5/6 sequences, by reference to a collection of basidiomes and to published databases. Russulaceae and Thelephoraceae were the dominant families.

Species richness was greater in the mixed *Dryas/Salix* community. In both communities there were a few abundant species and many rare ones, but species composition, the species-area relationship and species rank abundance were markedly different between the two communities. In the mixed *Dryas/Salix* community the most abundant ectomycorrhizal fungi showed strong host preference, and some of the less common ones were host specific.

The presence of *Dryas* altered the relative abundance of ectomycorrhizal fungi associated with *Salix*.

P2 14

Ecology and Mycorrhizae of *Craterellus tubaeformis* in the Pacific Northwestern United States.

M.J. Trappe, Oregon State University, Corvallis, Oregon, USA

Craterellus tubaeformis is a small to medium-sized mushroom that is common in temperate forests of the northern hemisphere. Synonyms have included *Cantharellus tubaeformis* and *Cantharellus infundibuliformis*. In the coniferous forests of the Pacific Northwestern United States its productivity is closely associated with the abundance of well-rotted (class 4 & 5) coarse woody debris. While it is not strictly dependent on old-growth stand conditions, the likelihood of its occurrence increases proportionally with stand age. The mycorrhizal association of *Craterellus tubaeformis* with *Tsuga heterophylla*, *Pseudotsuga menziesii*, and *Picea sitchensis* in the Pacific Northwestern United States has been confirmed by molecular analysis. When *Tsuga heterophylla* is available in a stand it appears to be the host of preference. *Craterellus tubaeformis* will occasionally occur in monoculture *Pseudotsuga menziesii* or *Picea sitchensis* stands but it is relatively rare. Molecular evidence is accumulating that the Pacific Northwestern variant of *Craterellus tubaeformis* may be a distinctly different species than the variants found in the eastern United States or Europe. This molecular evidence is supported by apparent differences in regional microhabitat associations and possibly their cohort of mycorrhizal hosts.

P2 15

Does man indirectly responsible in the dissemination of AMF spores

H.C.Lakshman

Post-graduate Studies in Botany (Microbiology Laboratory)
Karnatak University Dharwad – 580 003 India

Depending upon the season's Indian farmers adapted varied types of agricultural practices, and he himself disseminates AMF spores through his foot without his knowledge. Eight different AMF spores were recovered from the foot mat used at the door entrance. Twelve different AMF spores were isolated from spade samples while transported by farmer from soil deposited in the dried pond to agricultural fields. Twenty-four AMF spores were recovered from four agricultural fields during slit blade hoeing. Comparative distance of spore dissemination was measured in soil samples, which were adhered to wooden and iron ploughing after cropharvest. *Glomus fasciculatum* and *G.mosseae* from footmat and adhered soils of wooden plough were inoculated to *Eleusine coracana* Gaertn. *Sorghum vulgare* Pers to determine the effectiveness of spores. These spores which induce infection on the plants had competitive advantage probably due to its dispersal indirectly by man.

P2 16

P2 17

Moisture retention properties of a mycorrhizal soil

R.M. Augé, A.J.W. Stodola, J.E. Tims, A.M. Saxton. Tennessee Agricultural Experiment Station, University of Tennessee, USA.

The water relations of arbuscular mycorrhizal plants have been compared often, but virtually nothing is known about the comparative water relations of mycorrhizal and nonmycorrhizal soils. Mycorrhizal symbiosis typically affects soil structure, and soil structure affects water retention properties. Therefore, it seems likely that mycorrhizal symbiosis may affect soil water relations. We examined the water retention properties of a Sequatchie fine sandy loam subjected to three treatments: seven months of root growth by (1) nonmycorrhizal *Vigna unguiculata* given low phosphorus fertilization, (2) nonmycorrhizal *V. unguiculata* given high phosphorus fertilization, (3) *V. unguiculata* colonized by *Glomus intraradices* and given low phosphorus fertilization. Mycorrhization of soil had a slight but significant effect on the soil moisture characteristic curve. Once soil matric potential (Q_m) began to decline, changes in Q_m per unit change in soil water content were smaller in mycorrhizal than in the two nonmycorrhizal soils. Within the range of about -1 to -5 MPa, the mycorrhizal soil had to dry more than the nonmycorrhizal soils to reach the same Q_m. Characteristic curves of nonmycorrhizal soils were similar, whether they contained roots of plants fed high or low phosphorus. The mycorrhizal soil had significantly more water stable aggregates and higher extraradical hyphal densities than the nonmycorrhizal soils. Importantly, we were able to factor out the possibly confounding influence of differential root growth among treatments. Mycorrhizal symbiosis affected the soil moisture characteristic and soil structure, even though root mass, root length, root surface area and root volume densities were similar in mycorrhizal and nonmycorrhizal soils.

P2 18

MYCORRHIZAL DIVERSITY AND NICHE REQUIREMENTS IN RELATION TO N-DEPOSITION AND DRYNESS

J. Wöllecke[1)], B. Münzenberger[2)], R. F. Hüttl[1)]
[1)] BTU Cottbus, Germany; [2)] Centre for Agricultural Landscape and Land Use Research Müncheberg (ZALF), Germany.

Mycorrhizal parameters are highly sensitive to environmental influences. In two Scots pine stands impacted by different atmospheric nitrogen deposition in the lowlands of northeastern Germany edaphic dryness caused different reactions of the mycorrhizal coenosis. In relation to dryness seasonal fluctuations of mycorrhizal frequency or Shannon-diversity were more pronounced at the high-N site with drastical decreases down to a mycorrhizal frequency of 22 %. Additionaly, the mycorrhizal coenosis of the high-N site was absolutely dominated by euryoecious species. The different reaction of both mycorrhizal coenosis suggest a reduced ability of the pine trees to withstand natural stress at the high-N site. Compared with the low-N site, the high-N site showed a reduced number of ectomycorrhizal types, a different species composition, a lower amount of the below-ground fungal mycelium and a reduced fruitbody diversity.

The micro-niche requirements of different functional groups of ectomycorrhizal fungi are shown exemplarily. This kind of investigation can give hints at the ecological strategies of the ectomycorrhizal fungi. The occurance of euryoecious species like *Xerocomus badius, Cenococcum geophilum* or *Lactarius rufus* showed no dependence to specific micro-habitat conditions, whereas distribution of *Russula ochroleuca* correlates with different parameters like composition of floor vegetation.

P2 19

Uptake of organic nitrogen in Dutch heathland ecosystems

J.D. Zijlstra, F.Berendse Wageningen University, The Netherlands.

During the last decades species replacement has taken place in many heathlands and nutrient-poor grasslands. The increased atmospheric nitrogen deposition may have decreased the degree of mycorrhizal infection in ericoid plants reducing the competitive ability of these species. Ericoid mycorrhiza have a major role in the uptake of organic nitrogen and the production of polyphenolic compounds in the leaves and roots. Negative effects of plant litter with high concentrations of phenolics on the ratio between the release of inorganic and organic nitrogen may favour species that are able to absorb organic nitrogen (e.g. ericoid plants with ericoid mycorrhizal fungi) relative to species that are not (e.g. grasses with arbuscular mycorrhizal fungi). Field observations showed that in Dutch heathland ecosystems plant species showed great differences in their ability to produce polyphenolic compounds which is related to organic nitrogen uptake. In *Vaccinium myrtilis, V. vitis-ideae* and *Deschampsia flexuosa* growing under reduced light conditions levels of total polyphenolics and condensed tannins were lower compared to plants without shaded conditions. In young plants of *Calluna vulgaris* on recent sod cutted soil total polyphenolics contents were higher compared to plants on sites from an older succession stage. Fungal isolates from *Ericaceae* roots showed great variety of dark, slow growing fungi. Mycorrhizal infection still has to be analysed.

P2 20

Phylogenetic and Environmental Influences on Nitrogen Stable Isotope Ratios in Sporocarps of Ectomycorrhizal Fungi

Steven A. Trudell[1], Paul T. Rygiewicz[2], Robert L. Edmonds[1]. [1]Ecosystem Science Division, College of Forest Resources, University of Washington, Seattle, WA, USA. [2]National Health and Environmental Effects Research Lab, US Environmental Protection Agency, Corvallis, OR, USA.

It has been suggested that nitrogen stable isotope ratios (expressed as $\delta^{15}N$) of fungus sporocarps, in conjunction with $\delta^{15}N$ data from other ecosystem compartments, can be used to elucidate key processes in forest N-cycling. Although results of previous studies generally support this idea, they also show that the large number of factors that influence $\delta^{15}N$ can complicate interpretation of the data. To date, there has been no integrated study of the relative degree to which the different sources of variation contribute to the observed $\delta^{15}N$ of fungus sporocarp samples. Here, we report initial $\delta^{15}N$ measurements on a large number of sporocarp collections from two conifer forests located in contrasting environmental zones on the Olympic Peninsula, Washington. Based on these data, we provide estimates of variance in $\delta^{15}N$ for individual species and genera of ectomycorrhizal fungi and assess the degree to which phylogenetic and environmental factors appear to influence $\delta^{15}N$ values. The results of our overall study, of which this work is one component, will increase the usefulness of stable isotope ratios in understanding N-cycling and, hopefully, draw greater attention to the critical ecosystem roles played by ectomycorrhizal fungi.

P2 21

Nitrogen fertilization promotes homogeneity in Glomalean communities

L.M. Egerton-Warburton, N.C. Johnson and E.B. Allen
University of California, Riverside, CA, and Northern Arizona University, Flagstaff, AZ.

Diversity, the synergy of species richness and evenness, is declining rapidly in response to human impacts such as nitrogen deposition. Such declines traverse temporal and spatial scales and phylogenetic boundaries, yet the contribution of mycorrhizal fungi to the global biodiversity debate has been largely overlooked. We evaluated the impact of nitrogen (N) fertilization (>100 kg N ha^{-1}) on arbuscular mycorrhizal (AM) fungi in five grasslands within mesic and semi-arid regions of the U.S. Two distinct lines of evidence from our study lends support to the hypothesis that N enrichment leads to homogeneity in AM communities. Firstly, in four out of five localities, N fertilization was associated with a consistent loss of species richness, especially in the Gigasporaceae, and/or replacement of formerly diverse AM communities by a few small-spored *Glomus* species that may be less effective mutualists. Secondly, N fertilization results in little community variation across localities. Spore communities in non-fertilized plots showed striking differences between mesic and semi-arid localities while in N fertilized plots there was no distinction between localities due to the prevalence of certain *Glomus* species. Conversely, one grassland site with high soil N:P ratio demonstrated an increase in species richness following N fertilization thereby indicating that N:P ratios are potentially part of the mechanism for N-induced loss of diversity. Our results suggest that N enrichment alters the diversity and mutualistic functioning of AM communities that, in turn, may influence the plant community.

P2 22

N fertilization results in reduced growth of ectomycorrhizal (EM) mycelium in the field

L.O. Nilsson, H. Wallander and E. Bååth, Lund University, Sweden

Mesh bags filled with sand were buried in forest soils and used to estimate the production of EM mycelium. The amount of EM in relation to total fungal mycelium was calculated by subtracting values from root-free (trenched) plots from adjacent plots with roots. The majority of the mycelia colonizing the mesh bags were of EM origin, which was confirmed by analysis of the $\delta^{13}C$ values of the mycelia. We found that EM mycelial growth peaked during autumn, which coincided with periods of maximal root-growth. EM mycelial production was almost insignificant during spring. In a Norway spruce forest in SW Sweden the growth of EM mycelium in mesh bags was reduced from 210 kg ha^{-1} in control plots to zero in nitrogen treated plots (100 kgN ha^{-1} yr^{-1}) during 1998 and from 190 kg ha^{-1} to 110 kg ha^{-1} during 1999. EM biomass in humus samples (including EM mycelium and mantles) decreased from 900 kg ha^{-1} in control plots to 50 kg ha^{-1} in N fertilized plots during 1998. A comparison with previous studies of the same forest, reveal that about 85% of the EM biomass is present as mycelium, 15% as fungal biomass in root tips and less than 1% as fruit bodies. Our field data also confirm earlier results from laboratory experiments showing that the mycelium is more sensitive than mycorrhizal root tips to N fertilization. In four natural nutrient gradients in N Sweden we have studied the growth of fungal mycelia. Here we also found reduced amounts of EM mycelium produced with increased soil nutrient availability. The poster will present results about the growth of mycelia from both arbuscular and EM fungi along these gradients, based on analyses of the biochemical markers PLFA 16:1ω5 (AM) and PLFA 18:2ω6,9 (EM).

P2 23

Seasonal and host specific responses of AM fungi to elevated carbon dioxide and nitrogen.

J. Wolf, N.C. Johnson, and D.L. Rowland. Northern Arizona University, Flagstaff, AZ, USA.

Elevated atmospheric carbon dioxide (CO_2) can increase net carbon fixation; nitrogen (N) availability may mediate this response. Arbuscular-mycorrhizal fungi (AMF) are expected to be sensitive to anthropogenic enrichment of CO_2 and N because they are obligate biotrophs and facilitate plant nutrient uptake. We report the first three years of data from a long-term field experiment at Cedar Creek, Minnesota, USA, in which factorial combinations of CO_2, N, and monocultures of eight perennial plant species (including forbs, legumes, C3 and C4 grasses) were arranged in a split-plot design. Extraradical AMF hyphal length was measured repeatedly during the growing seasons, and AMF spore communities were quantified at the end of each growing season. Extraradical hyphal length increased significantly with elevated CO_2, but only in legumes and C3 grasses and only at the height of the growing season. Nitrogen enrichment decreased AMF hyphal length under forbs, legumes, and C3 grasses. Spores of sixteen AMF species were identified. Only *Glomus clarum* responded significantly to elevated CO_2, with two- to four-fold increases in spore populations. Nitrogen significantly reduced spore populations of *Glomus fasciculatum*, and had erratic effects on several other AMF species. Spore production of all species varied greatly with year. These results indicate that 1) AMF response to CO_2 and N enrichment varies greatly with year, season and plant host; this could account for the range in responses reported in similar published studies, 2) elevated CO_2 may alter AMF community structure and potentially mutualistic functioning, and 3) mycorrhizal feedbacks may influence plant community responses to anthropogenic enrichment of CO_2 and N. Studies are underway to examine community-scale responses to elevated CO_2 and N.

P2 24

Determination of the carbon and nitrogen sources for arbuscular mycorrhizal fungi using stable isotopes

A. Nakano[1], K. Takahashi[2], R.T. Koide[3] and M. Kimura[4] [1]McGill University, Canada [2]Toyota Biotechnology and Afforestation Laboratory, Japan [3]Pennsylvania State University, USA[4]Nagoya University, Japan

The sources of carbon and nitrogen for the arbuscular mycorrhizal (AM) fungus *Gigaspora margarita* were determined by measuring ^{13}C in spores and hyphae in cocultures of C_3 and C_4 plants and by differential ^{15}N labeling. C_3 and C_4 plants, which have different $\delta^{13}C$ values, were grown in the same pot separated by a series of hyphal compartments. In the first experiment, N was applied as coated urea to the soil and in urea solution to plant shoots. Soil-applied fertilizer had a significant effect on spore % ^{15}N ($P<0.01$), with a 24 to 75 % contribution to spore N. Fertilizer applied to either alfalfa shoots or bahia grass shoots had little effect on spore % ^{15}N, accounting for 0 to 14 % or 1 to 9 % of spore N, respectively. In the second experiment, ^{14}N- and ^{15}N-urea were applied as coated urea to the soil in the C_3 and C_4 plant compartments, respectively. After clipping of the C_3 shoots, spore $\delta^{13}C$ gradually approached that of the C_4 roots. Hyphal $\delta^{13}C$ paralleled that of spores. Spore % ^{15}N was similar to that of mineral N in the C_4 plant compartment. These results indicate that spore C in AM fungi comes from living plants and spore N mostly from the soil.

P2 25

In situ dynamics of carbon transfer through AM mycelium in an upland grassland

D. Johnson[1], J. R. Leake[1], N. Ostle[2], P. Ineson[3] & D. J. Read[1]. [1]Department of Animal and Plant Science, University of Sheffield, UK., [2]CEH-Merlewood, Grange-over-Sands, Cumbria, UK., [3]Department of Biology, University of York, UK.

Arbuscular mycorrhizal (AM) associations are ubiquitous in grasslands being found in the majority of the dominant plant species in these communities (Sparling & Tinker, 1978). Our knowledge of the rates and quantities of C transfer through AM mycelium is based largely on inference from pot experiments, often using soils which differ markedly (e.g. low organic matter content and neutral pH) from those typically found in nature. We have developed novel mesh core systems to control the colonisation of defined soil volumes by native AM mycelia and which enabled us to provide both qualitative and quantitative data on the dynamics of C transfer through these fungi in control and limed grassland.

In the first experiment, mesh cores filled with non-sterile soil were inserted into control plots of an upland grassland and after 10 weeks the vegetation was exposed to $^{13}CO_2$ at ambient CO_2 concentrations using a novel stable isotope delivery system. In a second experiment, identical cores were inserted into control and limed (0.6kg lime m^{-2}) plots prior to exposure to $^{13}CO_2$.

Release of $^{13}CO_2$ from colonised cores peaked 10-14h after labelling and declined within 24h after severance of mycelial connections to roots. Between 5-8% of carbon lost by plants was respired by AM mycelium during 21h post labelling. Liming increased the amount of carbon fixed by plants and subsequently allocated to fine roots and AM mycelium. The results demonstrate for the first time under field conditions that AM mycelium provide a rapid and important pathway of carbon flux to the atmosphere.

P2 26

Element transport from minerals by the ectomycorrhizal mycelium in forest of different nutrient status.

D. Hagerberg and H. Wallander
Lund University, Sweden

Deposit of acidifying substances and forestry practices like whole tree harvesting for bioenergy purposes might result in loss of base cations and phosphorus from forest soil. Ectomycorrhizal (EM) fungi may compensate the loss of base cations and phosphorus by an increased weathering of soil minerals. Five forests in southern Sweden with varying K-status in the needles were selected for the present study. Mesh bags with sand or sand amended with 2 % biotite or 1 % apatite were buried in the forest soils to estimate the production of EM mycelium. The mesh bags were harvested after 1 and 2 years. The K-status of the trees had no influence on the EM colonisation of the mesh bags. Amendment with apatite or biotite stimulated the colonisation by EM mycelium. Apatite amendment resulted in increased amounts of mycorrhizal root tips surrounding the mesh bags. These roots contained large amounts of rare earth elements originating from the apatite, which indicates that EM fungi had released the elements from the apatite and transported them to the roots. The amount of rare earth elements that had accumulated in the roots, corresponds to a solubilisation rate of 1% apatite per year, which means that EM fungi can have an important role in releasing P from apatite in the field.

P2 27

P2 28

P2 29

Distribution and persistence of Australian *Pisolithus* spp. genets at native sclerophyll forest field sites.

I. C. Anderson, S. M. Chambers & J. W. G. Cairney
Mycorrhiza Research Group, School of Science, Food & Horticulture, University of Western Sydney, Australia.

One hundred and fifteen sporocarps of a *Pisolithus* species were collected from a *ca* 2500 m^2 Australian sclerophyll forest site during 1997 and 1999 while 18 sporocarps of a second *Pisolithus* species were collected from a further *ca* 150 m^2 site during 1999. Inter-simple sequence repeat (ISSR) PCR was conducted on DNA extracted from each sporocarp using the primers 5'BDB(ACA)$_5$, 5'DDB(CCA)$_5$ and 5'DHB(CGA)$_5$. Thirty-seven genotypes of *Pisolithus* species I were detected at the North Wilberforce site, with eight genotypes present during both 1997 and 1999. All other genotypes were observed during only one year. Mapping genotype distribution according to location of sporocarp collection at the site suggested that most genotypes were present as small ($<$ 2.0 m^2) below-ground mycelial genets, however several larger ($>$ 4.0 m^2) genets (including one *ca* 120 m^2 genet) were also present. All *Pisolithus* species II sporocarps at the North Turramurra site were of common genotype, suggesting the presence of a single large (*ca* 69 m^2) below-ground mycelial genet of this taxon at the site. Both *Pisolithus* species thus appear to be capable of producing large long-lived soil-borne mycelia, however, the establishment of large genets may be restricted under some circumstances.

P2 30

Hypogeous fungal diversity on Rottnest Island, Western Australia

S.R. Thomas[1], B. Dell[1], N. Malajczuk[1] and J.M. Trappe[2]
[1]Murdoch University, Western Australia. [2] Oregon State University, USA.

The mycophagous relationship between the small mammal the Quokka (*Setonix brachyurus*), hypogeous ectomycorrhizal (ECM) fungi and *Eucalyptus/Melaleuca* stands on Rottnest Island, south-western Australia, was investigated in 1999. As a result, collections of hypogeous sporocarps were made during the fungal fruiting seasons of 1999 and 2000. Hypogeous fungal diversity appears to be high (3 classes, 8 families, 12 genera and 29 species, 23 (79 %) undescribed). Two undescribed genera including 7 species were identified, along with the first known collections in Western Australia of species in the genus *Dingleya* (2) and *Leucogaster* (1). Species from other known Australian mycorrhizal fungal genera including the genera *Descomyces* (3), *Endogone* (2), *Glomus* (1), *Gymnomyces* (3), *Hymenogaster* (1), *Pisolithus* (1), *Redellomyces* (4) and *Scleroderma* (4) were collected. Although Rottnest Island is described as being nutritionally and environmentally hostile (low rainfall, fragmented natural stands, a single terrestrial mammal species and few ECM host species) the number and diversity of fungal taxa collected can be described as abundant. Tree species and ECM fungi both rely on the dispersal range of *S. brachyurus* and possibly a pre-treatment value for spores to initiate germination. Maintaining the fungal diversity on Rottnest Island is imperative to maintaining the fungal diet of *S. brachyurus* and the sustainability of the few remaining natural vegetation stands. Subsequently, these stands should be actively maintained for their conservation value in supporting and stimulating fungal production and conserving the current fungal diversity on Rottnest Island.

P2 31

Tomentella and related genera: ectomycorrhiza forming resupinate basidiomycetes occurring in forests and woodlands of south-western Australia.

Bill Dunstan[1], Urmas Kõljalg[2] and Bernie Dell[1]
[1]School of Biological Sciences and Biotechnology, Murdoch University, Perth, Western Australia; [2]Institute of Zoology and Botany, Estonian Agricultural University, Tartu, Estonia.

A rich and largely undescribed suite of ectomycorrhizal fungi occurs in Western Australia. Taxonomic effort has been directed toward the larger epigeous fungi and hypogeous false-truffles, whereas ECM fungi with resupinate basidiomata have been overlooked. We conducted a survey of tomentelloid fungi in south-western Australia. Collections included 16 species of *Amaurodon*, *Pseudotomentella*, *Tomentella* and *Tomentellopsis* that are morphologically similar to described species from the Northern Hemisphere, and 6 undescribed species, including *Pseudotomentella larsenii* Kõljalg & Dunstan ined. and *Tomentella kidjawurria* Kõljalg, Dunstan & Dell ined., that are apparently endemic to Western Australia. The ECM habit of 5 taxa was confirmed by direct sequencing of ITS rDNA of ectomycorrhizas.

At most sites, basidiomata of tomentelloid fungi were more common than sporocarps of other epigeous ECM fungi, and probably at least as abundant as sporocarps of hypogeous ECM fungi. Under the strongly Mediterranean climate prevailing in the south-western Australia, litter and soil in *Eucalyptus* woodlands dry rapidly in spring and early summer, and drying can also occur in winter. The abundance of sporocarps suggests that tomentelloid fungi are well adapted to such conditions and can revive readily from desiccated hyphae and/or spores. A provisional list of tomentelloid fungi and associated vegetation types is provided.

P2 32

Size and distribution of genets of five *Amanita* spp. native to Australia.

N.A. Sawyer, S.M. Chambers and J.W.G. Cairney
Mycorrhiza Research group, University of Western Sydney, Australia.

Sporocarps of five native Australian *Amanita* spp. were collected from field sites in New South Wales during March-May, 2000. These included sporocarps from two populations of *A. ochrophylla*, and single populations of *A. pyramidifera*, *A. conicoverrucosa*, *A. punctata* and *A. alboverrucosa*. The size and distribution of genets belonging to each of these populations have been estimated using inter-simple sequence repeat PCR (ISSR-PCR) with the primers (GTG)$_5$ and (GACA)$_4$. Results indicate that populations of some *Amanita* species are characterised by large genets (<60 m in diameter), while others comprise of many small genets. The results will be discussed in terms of the ecology of Australian *Amanita* spp . and their abilities to utilise different nitrogen sources.

P2 33

P2 34

Impact of elevated CO_2 and nitrogen fertilization on fungal biomass of ectomycorrhizae of *Piloderma croceum* and *Tomentellopsis submollis*

S. Raidl and R. Agerer
The University of Munich, München, Germany

Ectomycorrhizae are generally accepted to be the primary nutrient and water absorbing organs of the common forest trees of boreal and temperate forests. In most species of ectomycorrhizal fungi, nutrient uptake is performed by the extramatrical mycelium consisting of single hyphae or strands of bundled hyphae usually called rhizomorphs. Depending on the organisation of the rhizomorphs, several morphotypes or exploration types of ectomycorrhizal mycelia can be distinguished, which may differ strongly in structure and extension as well as in biomass.

In rhizotrone experiments, spruce seedlings inoculated with the ectomycorrhizal fungi *Piloderma croceum* and *Tomentellopsis submollis* were exposed to different CO_2 concentrations and nitrogen fertilization. Elevated CO_2 combined with N-amendment showed in both the fungal species an increase in their biomass: In *Piloderma* the biomass of the mycorrhizal mantle (measured by the length of the mycorrhizal systems) increased nearly 25 % and the biomass of the extramatrical mycelium (measured by the total hyphal length) about 50 %; in *Tomentellopsis* the mantle biomass increased about 60 %.The possible conversion of mycorrhizal and hyphal length into real biomass data (fungal dry weight in mg) may allow a rough estimation about the carbon need of symbiontic ectomycorrhizal fungi.

P2 35

Genet size analysis of selected late-stage and multi-stage fungi.

D. Redecker[1], T. M. Szaro[2], R. Bowman[2], J. Tan[2], and T.D. Bruns[2], [1]University of Basel, Switzerland; [2]Univ. California, Berkeley, CA, USA,

Genet sizes were determined in *Russula silvicola/cremoricolor*, *R. densifolia*, *R. brevipes*, *Lactarius xanthogalactus*, *L. fragilis*, *Amanita francheti*, and *Suillus pungens* by spatially mapping basidiocarps and genotyping with amplified fragment length polymorphisms (AFLP). The first six species were mapped in undisturbed mature forest settings, while *Suillus* was mapped at a three to four year-old post-fire site, where genet structure in the pre-fire forest previously had been previously determined. We found that none of the species mapped in the undisturbed forest sites had large genets. From this result we infer that spore establishment, rather than vegetative growth, is responsible for most of the observed fruiting in these "late-stage" fungi. In addition we found that the red and white pileus *Russula* species, which we had tentatively identified as *R. silvicola* and *R. cremoricolor*, are two different color morphs of a single population. The post-fire population of *Suillus pungens* was also found to be composed of small genets. This is in contrast to the situation in the mature forest prior to the fire, where a single genet, which covered more than 300 m^2 accounted for most of the fruiting. None of the genotypes found after the fire were identical to those found in the pre-fire forest. Thus, most of the post-fire fruiting is the result of new establishment by spores. This confirms our previous assumption that genet establishment starts no earlier than the establishment of the new forest, and it means that our prior estimate of a 0.5 meter/year expansion rate for *Suillus* remains a conservative one.

P2 36

Tracking the role of arbuscular mycorrhizal fungi in vegetation dynamics by molecular community analysis

G.A.Kowalchuk[1], F. A. de Souza[1,2], R. M. van der Kaaij[2], W.H. Van Der Putten[2]. [1]Netherlands Institute of Ecology (CTO-NIOO), The Netherlands. [2]Embrapa Agrobiologia (CNPAB), Brazil.

A PCR-DGGE approach for the detection and characterization of arbuscular mycorrhizal (AMF) 18S rDNA was developed and applied to the study of AMF communities. This approach was applied to the study of AMF associated with the main sand-stabilizing plant species of the Dutch sand dunes, marram grass (*Ammophila arenaria*, L), and native as well as invasive plant species of the Kalahari savanna in Botswana. DNA was extracted directly from plant roots, soil or isolated AMF spores, and prominent bands resulting from AMF-specific DGGE profiles were excised for sequence analysis. This strategy provided a robust means of detecting and identifying AMF-like species without the use of trap plant cultivation methods. In the coastal dune systems, clear shifts in the total AMF infection level and AMF community structure were correlated with the health of A. arenaria stands. AMF infection level and species composition may therefore affect plant vigor either via differential uptake of nutrients and/or defense against soil-borne pathogens in this system. Different savanna plants showed distinct patterns in the capture of AMF from the native soil spore bank, as well as distinct mycorrhizal infections in the field. The invasive plant species, *Cenchrus biflorus*, was highly capable of capturing a variety of AMF species, suggesting that this species is not limited by AMF infection in this foreign habitat. In addition, there were discrepancies observed between the AMF-like groups detected in spore populations versus direct 18rDNA analysis of root material, corroborating previous suggestions that spore inspection alone may poorly represent actual AMF population structure.

P2 37

Establishment of mycorrhizas on *rolC*-transgenic Aspen in a field trial

F. Buscot[1], M. Kaldorf[1,2], M. Fladung[2], H.-J. Muhs[2], [1] University of Jena, Germany, [2] Federal Research Centre for Forestry and Forest Products, Grosshansdorf, Germany

As genetically engineered trees are likely to be introduced in forestry, it is essential to assess their ability to form mycorrhizas, which concerns both mycorrhization rate and mycobiont diversity.

The establishment of AM and EM on roots of genetically transformed aspen lines carrying the *rolC* gene from *Agrobacterium rhizogenes* was investigated in a field release experiment. AM were rare, while fully developed EM were found in all samples. Although *rolC* modifies the hormonal balance in the trees and could have affected their mycorrhization ability, no significant difference in the mycorrhization degree was observed in the transformed aspen. The structural diversity of EM was monitored over three years by morphotyping and PCR/RFLP plus sequencing of the ITS region in the rDNA. From 22 characterized EM types, 4 dominated, representing respectively 96% of the EM in 1998, 93% in 1999 and still 78% in 2000.

The structure of the EM community of the different aspen lines was similar. However, over the three years, one morphotype formed by *Phialocephala fortinii* appeared to be significantly less represented on the transgenic line (E2/5) compared to all other transgenic and control lines. This depressed compatibility for one mycobiont represents the first example of a clone specific effect concerning mycorrhization of transgenic trees. This modification seems not to be in relation with the insertion site of the *rolC* construct in E2/5, but possibly reflects an indirect effect of the transformation on the regulation of the plant genome expression.

P2 38

Effect of fermentation of organic matter on changes in growth stimulatory and inhibitory substances for mycorrhizal fungi

T. Ishii[1], H. Kitabayashi[1], Y. Yachi[1], J. Okuda[1], Y. Yang[2], S. Kirino[3] and K. Kadoya[2] [1]Faculty of Agriculture, Kyoto Prefectural University, Kyoto, Japan, [2]Faculty of Agriculture, Ehime University, Ehime, Japan, [3]Ehime Chuo Agricultural Cooperative Association, Ehime, Japan

We reported that although the application of unfermented organic matter to the soil severely inhibited vesicular-arbuscular mycorrhizal (VAM) development, fermented organic matter stimulated VAM development and growth of citrus seedlings (Ishii and Kadoya, 1996). In this study, we examined the effect of fermentation of organic matter on changes in growth stimulatory and inhibitory substances for mycorrhizal fungi. By using a flash chromatograph, we fractionated the 80% MeOH extracts of fermented and raw organic matter. The organic matter used was the stems and leaves of *Artemisia princeps*, *Cayratia japonica*, *Paspalum notatum*, *Stellaria media* and *Trifolium repens*, and rice straw. Every raw organic matter contained both growth stimulatory substances and growth inhibitory substances. After fermentation, however, the inhibitory effects on 100% MeOH fraction containing a great amount of growth inhibitory substances disappeared and the stimulatory effects on 25% MeOH fraction containing a great amount of growth stimulatory substances remained. When the liquid obtained from the fermented organic matter was applied to the trifoliate orange-planted soils, every liquid treatment promoted root colonization and growth of trifoliate orange seedlings.

P2 39

Ectomycorrhizal weathering of soil minerals

Ellis Hoffland[1], Toine Jongmans[1], Reiner Giesler[2] and Nico van Breemen[1] [1]Wageningen University, The Netherlands [2]Swedish University of Agricultural Science(Umeå), Sweden

In 1997 a new type of weathering was discovered: tunnel-like holes inside feldspar grains. Tunnel formation was attributed to ectomycorrhizal (EcM) hyphae, exuding organic anions and thereby dissolving the Ca- and K-containing feldspars. We studied EcM tunneling of feldspars in two forest productivity gradients and in a soil chronosequence in North Sweden. Nitrogen bioavailability decreased upslope in both productivity gradients. EcM density in the O horizon in both gradients increased with decreasing nitrogen availability (five and twofold, respectively). Tunnel frequency in feldspars from the upper mineral soil appeared to increase with EcM density. This is circumstantial evidence that EcM may be responsible for tunneling. EcM tunneling of minerals may represent a so far unknown strategy to provide Ca and K for plant growth. The chronosequence showed an exponential increase of tunneled minerals with soil age. Tunnels were found in soils aged older than 2000 y. After 8000 y of soil development, at least 25% of the feldspars were tunneled, indicating that EcM-mediated weathering probably plays a significant ecological role. The lag phase of 2000 y can be explained (1) as a consequence of higher availability of K and Ca in the younger soils. This would mean that EcM tunneling of minerals is stimulated by K and Ca limitation. Alternatively, (2) prior chemical weathering of mineral grain surfaces is necessary to keep organic anions exuded by EcM concentrated long enough to further dissolve the mineral, ultimately resulting in tunneling. In the latter case, tunneling might be a side effect of organic anion exudation due to excess carbon availability in boreal forest ecosystems.

P2 40

PIXE analysis to estimate the elemental composition of ectomycorrhizal rhizomorphs grown in contact with different minerals in forest soil.

H. Wallander[1], L. Johansson[2] and J. Pallon[3] Department of Microbial Ecology[1], Department of Geology[2], Department of Nuclear Physics[3], Lund University, Sweden

Ectomycorrhizal (EM) mycelia have been suggested to play an important role in solubilizing minerals in the soil although the contribution of EM fungi to this process has never been quantified. In this study we have used particle induced X-ray emission (PIXE) to quantify elemental composition of EM rhizomorphs growing in contact with apatite and biotite both in experimental systems and in the field. Molecular methods were used to identify rhizomorphs from the field. Elemental maps of the rhizomorphs were produced and the concentrations of P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr were quantified. It was found that *Rhizopogon* rhizomorphs connected to apatite sources in laboratory systems transported large amounts of Ca originating from the apatite. Calcium was deposited as calcium oxalate crystals on the surface of the rhizomorphs and SEM analysis demonstrated that apatite particles were eroded on the surface. In samples collected from the field, rhizomorphs connected to apatite sources contained elevated concentrations of P, Ca and in some cases K. It was calculated that some species of EM fungi could solubilize up to 7 mg P from apatite per g mycelium and year. EM weathering of apatite in the field was calculated to 1.7 μg P per g soil^{-1} yr^{-1}, which is in the same range as other estimate of apatite weathering. Addition of biotite did not influence the elemental composition of rhizomorphs but some rhizomorphs contained high concentrations of K (range: 0.3-10 mg g^{-1}). It is suggested these EM rhizomorphs may have a significant role in transporting K to trees.

P2 41

Importance of mycorrhizal symbiosis for tolerance to mowing in the grasses *Brachypodium pinnatum* and *Bromus erectus*

B. R. Kahlert, P. Ryser and P. Edwards; ETH Zürich; Switzerland

Regularly mown middle-european limestone grasslands are dominated by *Bromus erectus*. Cessation of mowing leads to an increasing dominance by *Brachypodium pinnatum*. These grasslands are poor both in available N and P, and nutrient acquisition capacity is likely to play an important role in plant regrowth after mowing. As *B. erectus* and *B. pinnatum* are known to differ in their dependency of arbuscular mycorrhizal fungi (AMF), we investigated the role of mycorrhizal symbiosis in species ability to recover after mowing.

We conducted a greenhouse experiment with seedlings of the two grasses with P-fertilisation (high/low), cutting treatment (with/without), and the AMF infection (with/without) in a full factorial combination. The plants were harvested after ten weeks. The results of total plant biomass and total P amount in the plants confirm the assumption that AMF support the recovery after mowing and that *B.* pinnatum is more dependent on AMF for P uptake. *B. pinnatum* can hardly at all recover after mowing without AMF. But the interspecific difference in allocation pattern of biomass and P indicate that *B. erectus* uses the symbiosis more efficiently for recovery.

P2 42

Plant responses to earthworms and arbuscular mycorrhizas

J. Scullion[1], F. Tuffen[1] and W.R. Eason[2] [1]University of Wales, Aberystwyth, UK. [2]Institute of Grassland and Environmental Research, Aberystwyth, UK.

Arbuscular mycorrhizal fungi (AMF) and earthworms occupy similar soils and zones within these soils. They have been implicated in similar processes, such as aggregate stabilisation and nutrient acquisition by plants. Although earthworms affect the distribution of AMF propagules, there has been little research on the influence of earthworm activity on AMF-plant relationships. A series of pot trials was carried out to investigate these inter-relationships using a common, host plant (*Allium ameloprasum*) grown in several nutrient poor media. In some trials earthworms occupied root zones; in others, earthworms were confined to a hyphal compartment. Comparisons included γ-irradiated and AMF (indigenous or single species) re-inoculated soils, control and earthworm (mainly *Aporrectodea caliginosa*) under different nutrient input regimes. Individual trials gave plant responses to either earthworms or to AMF, but never both. There were few direct AMF-earthworm treatment interactions. However, across a wide range of root infection levels, correlations between plant growth and %AMF infection were significant only in the presence of earthworms. There was also evidence that these relationships changed with varying earthworm abundance and nutrient availability. These findings suggest a) that gross plant AMF and earthworm responses may be independent of one another for many soils and b) that earthworms may have a role in regulating the plant-AMF symbiosis.

P2 43

Interactions of mycorrhizal symbiosis and herbivory in tallgrass prairie.

G. W. T. Wilson, D. C. Hartnett, T. C. Todd. Kansas State University, Manhattan, Kansas, USA.

Experimental microcosms (40 x 52 x 32 cm) containing an assemblage of eight tallgrass prairie grass and forb species in native prairie soil were maintained under mycorrhizal and nonmycorrhizal conditions, with and without native soil nematodes, and with and without aboveground herbivory (clipping treatments and grasshopper herbivory) to elucidate the interactions and effects of these key species interactions. Microcosms inoculated with mycorrhizae exhibited increased growth and relative abundance of warm-season, C_4 grasses, whereas cool-season grasses accumulated more biomass and were a significantly greater proportion of total community biomass in nonmycorrhizal microcosms. Forbs showed variable species responses to mycorrhizal treatment. Nematode herbivory reduced biomass production of mycorrhizal C_4 grasses, and nonmycorrhizal C_3 grasses, while having variable effects on the forb species. Greater compensatory above- and belowground biomass production was observed in defoliated plants under mycorrhizal conditions than under nonmycorrhizal conditions, whether the removal was a consequence of clipping or grasshopper herbivory. These results underscore the importance of above-and belowground linkages and indicate that alterations in mycorrhizal and rhizosphere processes can have large effects on plant responses to consumers.

P2 44

Chinese truffles (*Tuber*): Diversity and their Geographical Distribution

Y. L. CHEN[1], B. DELL[2], F. LE TACON[3] *[1]Research Institute of Tropical Forestry, Chinese Academy of Forestry, Guangzhou, China; [2]School of Biological Sciences and Biotechnology, Murdoch University, Perth, Australia; [3]Equipe de Microbiologie Forestiere, INRA – CRF Champenoux, France*

More than 60 species belonging to the genus *Tuber* Micheli ex F. H. Wigg. have been reported around the world, but most occur in Continental Europe where the commercial cultivation of a few species has been developed. In contrast, the truffle fungi in China are poorly known. Since 1985, over 20 *Tuber* species have been recorded, including *T. taiyuanense* Liu, *T. sinense* K. Tao et Liu, *T. gigantosporum* Y. Wang, *T. himalayense* Zhang et Minter, *T. liui* A. S. Xu, *T. xizangense* A. S. Xu, *T. pseudoexcavatum* Wang et Moreno and *T. liaotongense* Y. Wang. Although, these fungi are widely distributed across China in a variety of habitats covering 9 provincial regions (Yunnan, Sichuang, Tibet, Xinjiang, Shanxi, Liaoning, Jilin, Fujian and Taiwan), most occur in the south-western highlands. The symbiotic relationship of some species has been verified. Species of *Quercus* and *Pinus* are the most common hosts. Some species, such as *T. indicum*, *T. pseudohimalayense*, and *T. pseudoexcavatum* from Sichuang Province have been introduced to Spain, France and Italy. The main discriminating morphological characters of key species collected from mainland China are described for comparison with those found outside China. Questions on the taxonomy of some previously mis-identified species are raised and discussed.

P2 45

Characteristics of hypogeous fungal colony of *Sarcodon aspratus*

H.-E. Kim, C.-D. Koo, J.-I. Park, J.-S. Kim, W.-S. Shin, C.-S. Shin
Department of Forestry, Chungbuk National University, South Korea

Abstract

Sarcodon aspratus known as the best in flavor in Korea has been used as a traditional digestant for meat food. This fungus is ectomycorrhizal with oak trees. Its habitat is fertile and moist sites, very often valley with oak trees as main woody species. The oak species are *Quercus mongolica, Q. serrata* and *Q. variabilis*. The fruiting body has a rigid, thick and dark brown mycelial bundle connected to white hypogeous fungal colony. The shape of the colonies are oval to irregular or strip with 1.1 to 5.9 m long, 0.3 to 2.0 m wide. In soil profile the hypogeous colonies grew into 30 cm deep. The colonies were highly compact with soil particles, mycelia and oak's ectomycorrhizas. The hardness of the colony soils ranged 2.0 to 20.0 kg/ and the soil moisture was 15.7 to 21.1%, by about 2% lower than in the non-colony. Ergosterol content in the colony was almost as twenty times, 17.3 ug/g dry soil, as that of the non-colony. Its mycorrhizas were light brown covered with copious white cottony hyphae. The hyphae were hyaline, smooth but stiff clamp connections. The fungal mantle was 10 - 25 thick and Hartig net developed into one or two epidermal cell layers. The process of the hypogeous colony development is waiting for our further studies.

P2 46

Tricholoma matsutake: a highly coveted ectomycorrhizal mushroom

A. Guerin-Laguette[1], L.M. Vaario[1], W.M. Gill[1], N. Matsushita[1], F. Lapeyrie[2] and K Suzuki[1]. [1]The University of Tokyo, Japan [2]INRA Nancy, France

Tricholoma matsutake (Matsutake) is an ectomycorrhizal fungus of great repute in Japan. Successful artificial cultivation of Matsutake has never been reported. Moreover, all attempts to introduce Matsutake in the field as mycorrhizal partner of its main host *Pinus densiflora* failed so far. Recently, we reported the ectomycorrhizal status of Matsutake, at least during part of its life cycle. This has been fully evidenced using both morphological and molecular analyses. Moreover, significant progresses have been made to synthesize *T. matsutake* / *P. densiflora* mycorrhizae under aseptic conditions. Therefore, the possibility to enhance the production of Matsutake by transplanting colonized pine seedlings is gaining new consideration. However, so far, despite Hartig net occurrence, the symbiotic mycelium of Matsutake could not be acclimatized to non sterile environments, suggesting that Matsutake trophic status is still only partially understood. Our recent results suggest that the saprotrophic abilities of Matsutake and their improvement by suitable substrates may be crucial in order to successfully manipulate Matsutake / *P. densiflora* symbiosis for forestry applications.

P2 47

A sampling technique for counting VAM fungal spores extracted from soil

W. Wangiyana and P.S. Cornish. Key Centre for Landscape and Ecosystems Management, Hawkesbury Campus (Bld. J4), The University of Western Sydney, Australia.

Previous research suggested that VAM populations were depleted in rice-based farming systems. This may have implications for VAM-dependent rotation crops. A field survey of VAM population dynamics was conducted at 45 sites representing various combinations of rice-based cropping systems and soil types, in Lombok island (Indonesia), from July 1999 to March 2000. The survey produced 900 samples for analysis. With this number, a fast counting technique was needed. Spores were extracted from 50 representative samples using the wet sieving and decanting technique of Brundrett *at al.* (1996), and captured on 5mmx5mm stamp gridded filter papers (Whatman no. 40, ϕ 90 mm). To sample grid cells for spore counting, 5 lines of grid cells were numbered upwards (H5 to H1) and downwards (H6 to H10) from the diameter line passing through the centre point of the filter papers. Spore number and the time needed to count them, for each line of grid cells, including the upper and lower unnumbered parts of filter papers, were recorded. Data were analysed using regression analysis between spore number from 3, 4, 5 or the 10 lines and the total spores. The best results of 3, 4 and 5 lines, in terms of highest R^2 and lowest standard error (s), were from H248, H2468 and H14689 (H=horizontal), with regression equations of $Total = 20.7+3.14\ H248$ (s=91.60, R^2=0.984, time=9 min), $Total = 18.2+2.44\ H2468$ (s=74.87, R^2=0.989, time=12 min), and $Total = 14.0+2.09\ H14689$ (s=54.36, R^2=0.994, time=14 min), respectively. The 10 lines (H1-10) actually produced a standard error of s=59.21 (R^2=0.993, time=28 min), while combination of these 10 lines and upper unnumbered parts of filter papers produced the lowest standard error (s=8.309, R^2=1.00, time=31 min).

P2 48

Effects of greenhouse environment on assessment of arbuscular mycorrhizal fungal diversity in trap cultures

J. C. *STUTZ*[1] & J. B. MORTON.[2] [1]Arizona State University, USA. [2]West Virginia University USA

Assessing community structure of arbuscular mycorrhizal fungi (AMF) is complicated by reliance on sporulation to identify and quantify species. Greenhouse pot cultures are useful in detecting non-sporulating species, especially in arid and semi-arid habitats. The purpose of this study was to determine the extent to which differences in greenhouse environment impacted on species composition of pot cultures grown in parallel at two locations: Arizona and West Virginia, USA. Cultures were started from soil samples collected from the rhizosphere of sacaton grass (*Sporobolus wrightii*) in a semiarid riparian community in Arizona. Field soil samples yielded only six AMF species. Seventeen AMF species sporulated in trap cultures, indicating that more than half the species present were nonsporulators in the field. Eleven AMF species produced spores at both locations, but abundance varied with location. Four species consistently sporulated abundantly at both locations and were likely aggressive colonizers regardless of location. In contrast, species unique to each location (four in WV and two in AZ) were distributed sporadically among pots and were weak sporulators. Parallel cultures of each soil sample did not differ significantly in species richness, but similarity in species composition averaged only 53%. Aggressive AMF species appeared to colonize and sporulate well regardless of location, but detection of less aggressive fungi (35% of the species in this case) was location-dependent. A second propagation cycle might have reduced this location effect. Results suggest that pot environment mediates colonization and sporulation behavior to a greater extent than greenhouse location.

P2 49

ECOLOGY OF THE AM FUNGI IN A STAR OF AFRICA(*Cynodon pletos*tachyus)PREIRI WITH DIFFFERENT RESTING CYCLES.

L. Lara-Capistrán., Y. Tablada-Aguilar., and D. Trejo. Agriculture Science School Universidad Veracruzana, México.

The purpose of this work was to determine the effect of the resting cycle of the AM fungi symbiosis on the star of Africa preiri. Three resting cycles were evaluated: longest (60 days),medium (15 days) short(0 days).The experiment consisted of three random groups with four repetitions. The measured variables were :the quantity of forage ,the quality of forage(protein), colonization percentage and the amount of spores. The 60 days resting cycles showed the greatest amount of forage(5012 kg MS/Ha),the largest percentage of colonization, the least amount of spores and intermediate values in the forage quality as related to the other two treatments ,this was probably due to the existance of the flowering cycle ;the 15 days (2769 kg MS/Ha)) and 0 days(2199 kg MS/Ha) resting cycles showed respectively 45% and 56% less($P<.05$) forage than the 60 days resting cycle. The percentage of colonization was least in the parcel with 0 days resting cycle ,but the greatest amount of spores was found. The treatment in the 15 days resting cycle showed intermediate colonization with the largest quantity of protein, this latter due perhaps that in this moment the greatest growth activity was taking place. The conclusion is that the shorter resting cycles affect the mycrobiological (symbiosis-mycorriza) and productive characteristics of the soil.

P2 50

What are the attributes of ectomycorrhizal fungi to be managed for future health of energy and nutrient flows in remnants of Australia's temperate tall tree woodlands?

I.C. Tommerup and N.L.Bougher CSIRO Forestry and Forest Products Perth, Western Australia

Woodlands once covered much of temperate Australia but they are now highly fragmented. Secondary salinisation has developed in much of this region. The remnants provide the genetic resources of plants and fungi for future agroforestry crops and land remediation. Plant diversity in western Australian woodlands is comparable to wet tropical rainforest. The woodlands are characterised by spatial changes in soil and plants over short distances and slower biogeochemical process than wet forests, due to a dry climate. The diversity spectrum of ectomycorrhizal fungi is equivalent to wet forests, e.g. up to 250 species per hectare. Spatial changes in fungi reflect changes in soil and plants over short distances. Trees and shrubs are characterised by few fine roots and strong development of ectomycorrhiza soon after seedling emergence through to old age. Plants, fungi and animals in the woodlands are highly interdependent but energy and nutrient flows between them have been disrupted or diminished due to reduction and/or absence of key conduits. Management options to maintain healthy, self-perpetuating woodland remnants and their expansion on former farmland for agroforestry, land remediation and conservation need to focus on redeveloping energy and nutrient flows. A primary strategy is to maintain or restore environmental attributes which encourage survival of physiologically diverse fungi with strategies coping for disturbance and climatic perturbations. Examples include re-establishing diverse woodland plant communities having the N-fixing ectomycorrhizal species, and re-introducing mammal vectors of Australia's mega-diverse truffle fungi.

P2 51

Diversity and species distribution in orchid-fungal symbioses

McCormick, M. K., D. F. Whigham, and J. P. O'Neill. Smithsonian Environmental Research Center, Edgewater, MD 21037 USA.

Fungal specificity of orchids may reflect adult fungal dependence, but may also affect their distribution across habitats and tolerance of environmental change. We examined fungal diversity of four terrestrial orchids in the eastern U.S. with varied life history strategies from non-photosynthetic (*Corallorhiza odontorhiza*), to seasonally photosynthetic (*Liparis lilifolia* and *Tipularia discolor*), to evergreen (*Goodyera pubescens*). We used DNA sequencing to evaluate fungal diversity of each orchid throughout its range and evaluated whether fungal diversity reflected photosynthetic status and/or breadth of habitats occupied by these orchids. We predicted that widely distributed orchids would associate with a broader range of fungi than those more narrowly distributed across habitats. In the laboratory we investigated the effect of diverse fungi on growth of *G. pubescens* and *L. lilifolia* across a range of media. We also examined potential for protocorms to switch among fungal associates. We found that diversity of fungal associates did not reflect photosynthetic status, but may be associated with breadth of habitat distribution in these four species. In the lab, a narrow range of fungal associates limited growth of *L. lilifolia* to a few media types while the greater diversity of fungi associated with *G. pubescens* allowed it to grow well across all media types. Although *G. pubescens* protocorms were able to change fungi, conditions required to do so are unlikely to occur in natural environments. These results suggest that fungal diversity may affect orchid distribution across habitats, but is unlikely to affect protocorm-stage tolerance of environmental change.

P2 52

Characterization of ascomycetous fungi colonizing the liverwort *Cephaloziella exiliflora* in the Antarctic using direct PCR and cloning

A. Jumpponen[1], K.K. Newsham[2] and Neises, D.J.[1] [1]Kansas State University, Manhattan, Kansas, U.S.A.; [2]Biosciences Division, British Antarctic Survey, Cambridge CB3 0ET, U.K.

Recent studies have indicated that fungal symbionts frequently isolated from ericaceous species may be an important component in fungal communities colonizing the rhizomes of liverworts. We used PCR to directly amplify ascomycetous fungi from surface sterilized *C. exiliflora* rhizome tissues collected in the Antarctic to assess the fungal community composition. The target-sized PCR products were excised, cloned, RFLP-phenotyped, and representatives for each RFLP-type sequenced. Six distinct groups of ascomycetes were included in our clone libraries. One of these clustered with *Hymenoscyphus* sp. Another RFLP-type formed a well-supported clade with Trichocomaceae. The four remaining types remain unidentified. Our results suggest that *C. exiliflora* tissues are colonized by a variety of ascomycetes, most of which are not related to taxa often isolated from the tissues. We are currently expanding our sampling and comparing the direct cloning method with isolation techniques.

P2 53

Diversity of fungi forming ericoid mycorrhiza in the Australian family Epacridaceae

C.B. McLean[2], R.A. Collins[1], N. McDonald[1], M. Ramsperger[1] and A.C. Lawrie[1]
[1]RMIT University, Melbourne, [2]Burnley College, University of Melbourne, Australia

Fungi isolated from ericoid mycorrhizal roots of epacrid plants in Victoria showed large diversity on both molecular and physiological criteria. All fungi were tested for their ability to produce typical ericoid mycorrhiza on *Epacris impressa in vitro*. When ITS (internal transcribed spacer) sequences of rDNA (ribosomal DNA) were compared, fungi isolated from most Victoria epacrids diverged significantly from *Hymenoscyphus ericae* and *Oidiodendron maius*, the common endophytes of the Ericaceae. Some alpine isolates, however, grouped strongly with *H. ericae* isolates, and also showed preferences for ammonia-N rather than nitrate-N, as might be expected in their habitat. One isolate from *Astroloma humifusum* from inland sandy heathland was 95% similar to *O. maius*, but differed in some traditional characters from the type culture. Two other isolates from the same species and site formed gymnothecia on isolation and infected *E. impressa*, but were not *Oidiodendron* species. One formed both phialides and arthrospores in culture and the other only arthrospores and their ITS regions most closely resembled ascomycetes not previously associated with ericoid mycorrhiza. Neither was matched closely by sequences currently in databases.

P2 54

Mycorrhizal endophyte of medicinal plant *Homanotica aromatica (roxbo)* Schott *(Araceae)*

Nguyen Ngoc Cuong*, Hoang Hoa Long*, Le Nhu Kieu*
Nguyen Hong Ha†, Vu Thi Nhung† and Le Thi Minh Thanh†

PCR-RFLP analysis applied to ITS region of endophytic fungus SII-4 generated from surface-sterilized feeder root samples taken from medicinal plant *Homanotica aromatica* within a natural accession in the North of Vietnam has the ITS-RFLP pattern that is more similar to the ITS-RFLP pattern of taxon 4 ericoid mycorrhizae fungal strain D33 associated with *Woollsia pungens* in Australia (S. M. Chambers et al 2000) then PCR-RFLP patterns of 2 other members of the taxon - strains A49 and B17 - do. Particularly, 7 among 8 restrictases and restrictase combination applied to amplified ITS region of the endophyte SII4 give the same results as they do when being applied theoretically to ITS region of the ericoid mycorrhizae fungus D33. Basing on this result authors suggest that there may be association between taxon 4 ericoid mycorrhizas described firstly in Australia and *Homanotica aromatica (roxbo)* Schott *(Araceae)* of natural forest in the North of Vietnam.

*: Aricultural Genetic Institute, Hanoi-Vietnam
†: Institute of Biotechnology, Hanoi-Vietnam

P2 55

P2 56

GINCO : Glomales in vitro collection

Y. Dalpé[1] and S. Declerck [2] [1]Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada. [2] MUCL, Mycothèque de l'Université catholique de Louvain, Belgium.

During the last decade, a growing interest was devoted to the *in vitro* propagation of arbuscular mycorrhizal fungi (AMF) on root-organ culture. As a result, several species are now routinely cultivated allowing the development of pure culture collections available to the scientific community. **«GINCO» Glomales IN vitro COllection** is the first available international culture collection of AMF based exclusively of in vitro propagated fungal strains. **«GINCO»** traces its origin in the close scientific collaboration between the Mycothèque de l'Université catholique de Louvain (MUCL) and the Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada (ECORC) in charge of the National Mycological Herbarium (DAOM) and the Canadian Collection of Fungal Cultures (CCFC). This unique trans-continental collection has been developed in order to provide the scientific community and industrial sectors with high-quality, contaminant-free in vitro-produced AMF strains. **«GINCO»** is supported by research teams having a huge know-how in isolation, cultivation, propagation and conservation of monoxenically produced AMF. Together, more than forty AMF strains, covering at least twelve species and four genera, have been cultivated successfully and four of them officially offered to the scientific community for research and/or industrial purposes. Informations on **«GINCO»** are online on the respective addresses :
Canada - http://res2.agr.ca/ecorc/ginco-can Belgium - www.mbla.ucl.ac.be/ginco-bel

P2 57

Infrequent recombination in the supposedly asexual AM fungi

G. Kuhn, I. R. Sanders, Institute of Ecology, University of Lausanne, Switzerland

AM fungi are thought to be ancient asexuals and one predicted consequence of this is the accumulation of deleterious mutations. According to evolutionary theory this should lead to extinction. As recombination is thought to be a mechanism to purge deleterious mutations we have looked for evidence of recombination events in the genomes of AM fungi.

AM fungi are multinucleate and are highly polymorphic within individuals. Due to this unusual genetic variation, we propose that conventional population genetics-based methods, such as Hardy-Weinberg equilibrium and index of associations are unlikely to be sensitive enough to detect recombination. Assuming an asexual lifestyle, the genome of AM fungi should accumulate mutations sequentially. Therefore, the evolution of polymorphic sequences is expected to be the progeny of one original sequence. As a consequence, the accumulation of mutations in a clonal genome would allow the easier construction of a phylogenetic tree from the sequence data than if the sequence variation had resulted from random rearrangements as a result of recombination.

We present a phylogenetic method that allows a comparison of sequence variation and the detection of possible recombination events in AM fungi. We also present data that suggest that these recombination events occur in low frequency. Our data have important implications for understanding how AM fungi have evolved.

P2 58

Scutellospora castanea spores as microbial communities

M. Hijri[1], D. Redecker[2] and I.R. Sanders[1].

[1]Institute of Ecology, University of Lausanne, Switzerland [2]Botanical Institute, University of Basel, Switzerland.

Previous phylogenetic analysis based on 5.8S rDNA genes had shown clearly that some sequences that were isolated from spores of *Scutellospora castanea* clustered strongly within ascomycetes. What is the true origin of these sequences? Spores of *S. castanea* were harvested, placed and crushed on a V8 medium. Two fungi with different phenotypes grew out from surface sterilized crushed spores but not from intact surface sterilized spores. The ITS regions were amplified by PCR and sequenced. One sequence showed 98% of identity with the previously published ITS sequences T1 and T3 (previously reported from *S. castanea*) and the 5.8S gene of this sequence showed a high homology with *Leptosphaeria* species. Previous experiments using type-specific PCR from single spores showed that the *Leptosphaeria* sequence was present in 83% of spores tested. The other sequence showed 98% identity with *Nectria haematococca. Nectria* only occurred in spores that have a cloudy appearance and are easily identifiable. *Leptosphaeria* occurs in apparently healthy spores that can germinate. Electron micrographs of their contents will be shown.

Leptosphaeria sp. can be pathogens of non-mycorrhizal plants, such as *Brassica sp. Leptosphaeria sp.* isolated from *S. castanea* spores was compared with other *Leptosphaeria* species by RAPD-based molecular probes, and the analyses show that this fungus is different from plant pathogens known at the present time. The role of this fungus in *S. castanea* spores remains unclear but the results serve as a warning that sequences obtained from apparently healthy AM fungal spores cannot be presumed to be of glomalean origin.

P2 59

Community structure of AM fungi colonizing roots of rare and common *Pulsatilla* spp. in boreal forest and meadow habitats

M. Öpik[1,2], M. Moora[1], M. Zobel[1], and R. Sen[2] [1]University of Tartu, Estonia [2]University of Helsinki, Finland

Composition of arbuscular mycorrhizal fungal communities has been shown to influence the coexistence of plant species through differential plant-fungus relationships. The studied plants, rare *Pulsatilla patens* (L.) Mill. and common *P. pratensis* (L.) Mill. (*Ranunculaceae*), are ecologically compatible in Estonia, being found in dry sandy boreal-nemoral forests and meadows. The identity of AM fungi in roots of the target species were determined by PCR, DGGE, cloning and sequencing of SSU rDNA. Two-month-old bait seedlings grown in excavated soils hosted up to 5 different AM fungal taxa per root system. Distinct AMF communities were detected in seedling roots of both target species grown on soils from sites that naturally support the rare or only the common plants, respectively. More AMF taxa were found in seedling roots of both plants grown in soils where the two *Pulsatilla* spp. co-exist. Established plants of both species from the different habitats were almost exclusively colonized by a single endophyte showing high sequence homology to *Glomus mosseae.* Root colonizing AMF taxa on seedlings were not represented as spores in the soils. Our results suggest that the presence of particular AM fungi or specific combinations may be important for seedling establishment of rare plant species.

P2 60

Defoliation effects on the community structure of arbuscular mycorrhizal fungi based on 18S rDNA sequences

K. Saito[1], S. Sato[1] and K. Sugawara[1] [1]Tohoku University, Japan

Defoliation and grazing influence the carbohydrate balance of host plants. Such disturbances are crucial for arbuscular mycorrhizal fungi (AMF), which obtain carbohydrates from their host plant. In a field experiment, the effects of defoliation on AMF were investigated in terms of the community structure of colonizing fungi. DNA was extracted from the mycorrhizal roots of grazing-sensitive (*Miscanthus sinensis*) and grazing-tolerant (*Zoysia japonica*) dominant plant species that were or were not defoliated. The 18S rDNA of AMF was amplified by nested PCR. The amplified products were cloned and sequenced. Seventy-eight sequences were determined, and about one thousand clones were analyzed by PCR-RFLP (MboI, Hsp92II, and HpyCH4IV). Seventy-five sequences were clustered into a Glomales clade and were classified into 6 groups based on phylogenetic analysis. In the grazing-tolerant plant, AMF of one group in the *Glomus* clade dominated, and the frequency of RFLP types in its group did not change with defoliation treatment, except at the end of the plant growing season. In the grazing-sensitive plant, AMF of three groups in the *Glomus* clade were frequently detected. The frequency of two groups decreased and that of one group increased with defoliation treatment. These results show that the community structure of AMF and the effect of defoliation on its structure differ among plant species.

P2 61

Diversity of deeply branched arbuscular mycorrhizal fungi, *Archaeospora* spp., and their detection with PCR

H. Sawaki#, T. Kojima, N. Shinozaki*, M. Saito National Institute of Livestock and Grassland Science, #BRAIN, *Univ. of Tokyo, Japan

Current phylogenetic analysis showed that arbuscular mycorrhizal fungi, Glomales, Zygomycetes, were highly divergent. The fungi in Archaeosporaceae, a newly created family, were described to form dimorphic (glomoid and acaulosporoid) or monomorphic (acaulosporoid) spores in the protologue. We found 2 isolates which exclusively formed glomoid spores. The 18S rRNA gene sequences of these isolates were almost identical to those of the previously reported *Ar. leptoticha* and *Ar. gerdemannii*. The phylogenetic analysis of the internal transcribed spacers showed that these species including our isolates was divided into two clades, which were comprised of *Ar. leptoticha* subgroup and *Ar. gerdemannii* subgroup. The present isolates were within the clade of *Ar. gerdemannii*. In spore morphology, however, the glomoid spores in these isolates were distinct in size from those of *Ar. gerdemannii* and were nearly identical to those of *Ar. leptoticha*. This inconsistency between molecular and morphological characteristics in the *Archaeospora* species suggested the necessity of revision in the taxonomy. We designed a PCR primer specific to 18S rRNA gene in *Ar. leptoticha* and the related species. The specific primer enabled to identify the fungi with a small amount of spore or mycorrhizal DNA. The application of the PCR detection to field collected samples demonstrated that this fungal group was commonly found in wild grasses in Japan.

P2 62

Molecular identification of arbuscular mycorrhizal fungi (Glomales) in roots from natural and agricultural settings

I. Alie[1], F. Oehl[1], K. Ineichen[1], P. Mäder[2], E. Sieverding[3], A. Wiemken[1], D. Redecker[1]
[1]Institute of Botany, University of Basel, Switzerland; [2]Institute of Organic Agriculture (FIBL), Frick, Switzerland; [3]Institute of Plant Production and Agroecology in the Tropics and Subtropics, Hohenheim, Germany

The diversity of arbuscular mycorrhizal (AM) fungi within roots was studied using specific primers targeted at the rDNA of major subgroups of glomalean fungi. A nested PCR approach was used to specifically amplify sequences of the glomalean 18S subunit and the Internal Transcribed Spacers. Soil and roots were sampled from field sites under organic or conventional agricultural management widely differing in input intensity as well as from semi-natural grassland. These samples were used to set up trap cultures with a combination of three different host plants. AM fungi were characterized in the roots of these trap cultures. The results were compared with the data of the AM spore population from the respective soils. The variation of ribosomal DNA sequences within single spores and isolates of AM fungi was studied using Single Strand Conformation Polymorphisms.

P2 63

Detection and quantification of the arbuscular mycorrhizal (AM) fungi *Glomus mosseae* [1]and *Gigaspora margarita* using species - specific PCR primers.

Zaida I. Antoniolli[1], Daniel P. Schachtman[2,3], Kathy Ophel-Keller[2,4], Herdina[4] and Sally E. Smith[2] Universidade Federal de Santa Maria - RS, Brazil and [2]Adelaide University, [3]CSIRO Plant Industry, [4]South Australian Research and Development Institute, Australia.

Species of AM fungi are difficult to identify and quantify at species level during symbiotic growth, when they influence plant nutrition and community interactions. We have developed a sensitive molecular method for identification and quantification of two species of AM fungi. PCR primers for *Glomus mosseae* and *Gigaspora margarita* were designed from ITS regions of field-collected spores. The specificity of the primers was assessed by PCR amplification of genomic DNA extracted from spores of 12 species of AM fungi, from mycorrhizal roots of a range of host plants and from several root inhabiting fungi which may interfere with mycorrhizal assessment in the field. The primers for *G. mosseae* and *Gi. margarita* gave amplification products from spores of the same fungal species and roots colonised by them, either in the presence or absence of other AM fungi but not from other AM fungi. The *G. mosseae* primers did not amplify from other root infecting fungi, but the *Gi. margarita* primers gave products (of different sizes) from *R. solani* spp.. A DNA slot-blot hybridisation assay was tested for potential quantification of the AM fungal DNA. This method could be used to estimate amounts of DNA of specific fungi in colonised roots.

P2 64

Polypeptides comprising a mycorrhizal specific esterase band on maize roots inoculated with four different AMF

R.P. Sancayaningsih[1], Y. Setiadi[2], W.T. Artama[3], H. Hartiko[4], S. Moeljopawiro[4], and J. Soedarsono[3] [1]Dept. of Biology UGM, Yogyakarta, Ind.; [2] IUC-Biotechnology IPB, Bogor, Ind. ; [3]IUC-Biotechnology UGM, Yogyakarta, Ind.; [4]Dept. of Biology UGM/IUC-Biotechnology UGM, Yogyakarta, Ind.

Isozymes analysis in spores are used to determine inter and intra-generic, or even isolates of AMF species. Detection of mycorrhiza in planta of some AMF species was proved by performing a mycorrhizal specific isozymes (MSI) of malate dehydrogenase (MDH), esterase (EST), and glutamat oxaloacetate transferase (GOT) on mycorrhizal plant roots. MSI of esterase bands of AMF species in roots may vary depending on plant age.

Maize inoculated with each of four AMF species, *Glomus manihotis*, *Glomus etunicatum, Gigaspora rosea* and a local isolate of *Glomus* sp., were used to detect MSI esterase in comparation to an uninoculated plant which were grown for 11 weeks. Esterase staining following native PAGE electrophoresis was performed initially to localize the esterase MSI band. The MSI gel bands were separated using SDS-PAGE after electroelussion to detect their comprising polypeptides.

Similar Rf value (0.72) of esterase MSI band of maize roots inoculated with each AMF species are shown. These consist of four to six polypeptides with combination of 50.5, 52.7, 62.8, 65.6, 74.8, 78.2, 88.9, 110.9, 126.5, 131.8, 150.3, and 171.4 kDa.

P2 65

Preliminary study on the arbuscular mycorrhizae of Namibia

Elisabeth Uhlmann[1], Claudia Goerke[1], Ben Strohbach[2], Franz Oberwinkler[1]
[1]University of Tuebingen, Germany [2] National Botanical Research Institute, Windhoek, Namibia

Within the framework of the "BIOTA Southern Africa" Project, VA-mycorrhizae are studied in three locations in Namibia. The BIOTA Southern Africa Project is a multidisciplinary project contributing to the analysis of biodiversity in the Republic of South Africa and Namibia. A further aim of the project is the monitoring and realistic modelling of change in biodiversity. As biodiversity and land use are strongly controlled by environmental parameters, sampling sites are located along the most important climatic gradient, the humidity gradient. One sampling site is located in the arid Nama Karoo near the Fish River Canyon, another in the savannas surrounding Windhoek and the third site in the dry woodlands of the North. The precipitation increases from approx. 60 mm/year in the south of Namibia to approx. 400 mm/year in the north. As land tenure systems and land use practises have a great impact on the change of biodiversity, communal and commercial farms with different land use practises are compared at each research site. This might eventually lead to an assessment of the effect of different land management practices on diversity of VA spores. VA spores were extracted from soil by wet sieving and the mycorrhization rate of dominating plant species was estimated.

P2 66

Fatty acids of total lipids from AM fungal spores and host roots can detect, identify and differentiate AM species.

R. Madan[1], S.E. Smith [1], C.E. Pankhurst[2], B. Hawke[3],
[1]Adelaide University, Adelaide, Australia [2]CSIRO Land & Water Aitkenvale, Australia [3]CSIRO Land & Water Glen Osmond, Australia.

Fatty acid methyl ester (FAME) analysis of total lipids was performed on spores, roots of host plant and soils under five arbuscular mycorrhizal (AM) fungal species to check its usefulness in identification and differentiation between species. Fatty acid 16:1ω5c and 18:1ω9c were detected from spores, roots and soils of all AM treatments, but not the non-mycorrhizal controls, confirming their use as general bio-markers to detect the AM species in roots or soils. Fatty acids 20:1ω9c, 22:1ω9c, 22:0, and 20:0 ISO were identified as markers with potential to differentiate AM species. Fatty acids 22:0 and 21:02 OH could differentiate mycorrhizal roots from non-mycorrhizal roots. Fatty acid 20:1ω9c differentiated species of *Gigaspora* from the other species and 22:1ω9c differentiated species of the family Gigasporaceae from species in the family Glomaceae. Fatty acid 18:1ω9c was significantly higher in the spores of the species of family Gigasporaceae, but this difference in amount was not reflected in the roots or soils.

The extent of root colonisation and the number of spores per g soil both showed similar trends to the amount of FAME 16:1ω5c from the two sources, but not a significant linear relationship. No significant effect of P was observed on the fatty acid profiles of non-mycorrhizal control plants, although slightly better plant growth was observed with additional P. The method showed potential to detect AM fungi in roots and soils, identify AM fungi and differentiate them at family level.

P2 67

Glomus spinosum sp. nov. in the Glomaceae from Taiwan and its mycorrhizal associations

H. T. Hu Department of Forestry, College of Agriculture, National Taiwan University, Taiwan, ROC.

Glomus spinosum, a yellowish brown to dark brown, vesicular-arbuscular species with yellow-greenish, blunt spine covered by subhyaline mucilage adherent on the outermost layer. The chlamydospores are 40-90 m in diam, and have a 4-10 m thick wall which consists of a thin pale yellow-brown outer wall, a thick dark brown middle wall with warts on the surface, and a thin yellowish brown inner wall in one wall group. *G. spinosum* forms typical vesicular-arbuscular mycorrhizae with *Cunnighamia lanceolata* and has different compatibility with plant species.

P2 68

Detection of introduced AM fungi in plant roots using nested PCR

B. Zhao, X.L. Dong and S.X.Zheng
Laboratory of Agricultural Microbiology, Huazhong Agricultural University

The ability of three arbuscular mycorrhizal fungi – *Glomus intraradices*, *Glomus mosseae* and a local isolate *Glomus sp.*was assessed for their competitiveness with indigenous mycorrhizal fungi in a glasshouse experiment. The plant of *Astragalus sinicum* grown in a untreated field soil was inoculated with three *Glomus* species. After trypan blue staining treatment, the introduced mycorrhizal fungi in plant roots were detected by nested PCR using species specific molecular probes. The three inoculated AM fungi shown different behavour in competition with indigenous AM fungi and *G. Mosseae* and *G.intraradices* presented as high as 60 – 72% in root fragments. Development of *Glomus* sp. Was limited in *Astragalus* roots measured in this study, implicating that this local isolate lacks of ability for infection competition. Nested PCR offers a useful method in monitoring the introduced AMF species in the host roots and ecosystem.

P2 69

P2 70

A novel method for extraction and purification of amplifiable DNA from ectomycorrhizas

R.T. KOIDE and I.A DICKIE. Department of Horticulture, The Pennsylvania State University, University Park, PA USA

A common method to identify an ectomycorrhizal fungus colonizing a root involves extraction of its DNA, purification, selective amplification of a specific region of the genome by PCR, and either sequencing or analysis of restriction fragments of the amplicon. DNA from the ectomycorrhiza is then compared to DNA from tissues of identified fungi, often sporocarps. The methods used to obtain amplifiable DNA from ectomycorrhizas, as commonly published, may require many hours. Extraction may involve manual grinding of the ectomycorrhiza, freezing and thawing, and incubations at elevated temperatures. Purification may involve extraction with chloroform mixtures and precipitation of DNA by alcohol. We describe here a new, simple and rapid method for extracting and purifying readily amplifiable fungal DNA from ectomycorrhizas of red pine (*Pinus resinosa*), which requires none of the steps described above. This method is based on a commercially available kit originally designed for the extraction of DNA from liquid-cultured microbes. The method requires only a common laboratory mixing apparatus (vortex), a freezer, and a microcentrifuge. DNA extraction occurs at room temperature with the aid of sharp mineral particles that finely abrade the mycorrhiza when shaken in a microcentrifuge tube on the laboratory mixer. A unique solution is added at the extraction step to reduce the negative effects on subsequent PCR of phenolic substances extracted from the root. Purification does not require chloroform but does include precipitation of proteins with acetate in the freezer, binding the DNA to a silica column, washing the DNA with ethanol, and elution from the column. The result is DNA that is ready for PCR.

P2 71

Characteristics of surface carbohydrates of hyphae in ectomycorrhizal fungi using fluorochrome labeled lectins and their diagnostic application to detect their ectomycorrhizas and external hyphae in soils

T. Tateishi[1,2], A. Kohzu[3], E. Wada[3], T. Marumoto[2] [1]BRAIN, Japan. [2]Dep. Agric., Yamaguchi University, Japan. [3]CER, Kyoto University, Japan

Characteristics of surface carbohydrates of vegetative mycelia of ectomycorrhizal fungi were analyzed using FITC labeled lectins and two fluorochromes in order to investigate their availability for selective diagnostic detection of ectomycorrhizas and external hyphae in soils. All hyphae in cultured aerial mycelia of ectomycorrhizal fungi were found to be basically composed of ß-1,4-glucan, ß-1,3-glucan and side residues of oligomer of N-acetyl-glucosamine by binding assay with calcofluor white M2R, aniline blue and FITC-labeled wheat germ agglutinin (WGA). Binding assay with FITC-labeled peanut agglutinin (PNA) showed that side residues of ß-galactosamine and its derivatives existed on the cell walls of both vegetative and aggregated mycelia of Sect. *Amanita* in Amanitaceae, but did not in other ectomycorrhizal fungi except some strains. This suggested that PNA was available to detect the external hyphae and mycorrhizas of Sect. *Amanita* in Amanitaceae in soils. Binding assay with FITC-WGA and FITC-PNA was undertaken with the ectomycorrhized root tips and hyphae collected from the soils where fruiting bodies of *A. pantherina* appeared. FITC-WGA could bind the hyphae on the mantle surface of some mycorrhized root tips examined. FITC-PNA did not bind the hyphae on the mantle surface of many mycorrhized root tips. Although hyphae bound with FITC-PNA were detected, the proportion of these hyphal lengths to total hyphal lengths in the soil was very low.

P2 72

P2 73

P2 74

Ectomycorrhizal Species Richness and Composition in Old Growth Mixed Conifer and Adjacent Stands of Lodgepole Pine (*Pinus contorta*) in Yellowstone National Park, U.S.A.

V.T. Parker[1], R.B. Douglas[1], and K.W. Cullings[2]. [1]San Francisco State University, San Franciso, CA, USA, [2]NASA-Ames Research Center, Moffett Field, CA, USA.

We compared below-ground ectomycorrhizal (ECM) species richness and composition between adjacent pure lodgepole pine (~100 yr old) and mixed conifer stands (250+ yr old) in Yellowstone National Park. A total of 27 soil cores were collected in each forest type, roots tips were washed and sorted, and identified using ITS-RFLP methods. Although no statistical difference was observed for the mean number of ECM root tips per core between stand types, the total number of species identified (83 versus 35) and the mean number of species per core (8.8 versus 2.5) were significantly higher in the pure lodgepole pine. Additionally, we conducted a soil survey for both stands and found that mixed conifer stands were significantly lower in pH, but higher in OM, X-K, Bray-P, and N-NH_4 than lodgepole pine. Furthermore, temperature and moisture regimes were slightly different between stand types but followed similar patterns throughout the growing season. The species distribution patterns observed in this study are consistent with past studies that observed declines in ECM fungal diversity within older, late-successional forest stands. The soil survey data suggests that pH, nutrient availability, temperature and moisture regimes correlate with the differences observed in ECM community structure within this system. Specific field experiments will be necessary to determine the exact causal mechanisms generating these patterns.

P2 75

Characterization of *Pinus* ectomycorrhizas from mixed conifer and pygmy forests using morphotyping and molecular methods

N. Wurzburger[1], M.I. Bidartondo[2], C.S. Bledsoe[1], University of California, Davis[1], Berkeley[2], USA.

We used morphotyping and molecular methods to characterize ectomycorrhizas of Bishop pine (*Pinus muricata* D. Don) and Bolander pine (*Pinus contorta* ssp. *bolanderi* (Parl.) Critchf.) from mixed conifer and hydric pygmy forests on the northern California coast. Sixteen ectomycorrhizal morphotypes produced 15 ITS-RFLP types; 12 were identified via ITS sequencing. From a given site, all root tips of a specific morphotype produced identical ITS-RFLP patterns. However, sometimes two morphotypes produced the same ITS-RFLP type, and sometimes samples of the same morphotype from two different sites produced two different ITS-RFLP types. These results indicate that surveys of ectomycorrhizal fungi based on morphology alone are not sufficient; however grouping morphotypes prior to molecular analysis could expedite the process. Ectomycorrhizas from mixed conifer included Russuloid sp., *Tomentella sublilacina, Tuber* sp., and two Thelephoroid species. Ectomycorrhizas from hydric pygmy included two *Dermocybe* spp., a *Cortinarius* sp. two Thelephoroid spp. and *Suillus tomentosus.* Both plant communities contained *Cenococcum geophilum.* The hydric pygmy sites were more similar to each other than to the mixed conifer site (Jaccard similarity). Ectomycorrhizal taxa may respond to biotic (host specificity) or abiotic (soil fertility or hydrology) factors and be adapted to one plant community type over another.

P2 76

Effect of soil moisture on mycorrhizal symbiosis in *Pinus sylvestris* L. nursery seedlings

D. Hilszczaska', Z. Sierota' 'Forest Research Institute, Warsaw, Poland

Mycorrhizal development is likely to be influenced by edaphic and climatic factors such as soil moisture, texture and temperature. Seedlings of Scots pine (*Pinus sylvestris)* raised in nursery on loamy sand and litter (pine and spruce needles). Moisture gradient was different, from 44 % to 51 % field water capacity on loamy sand, and from 63 % to 75 % on litter. After 5 months, higher percentage of mycorrhizal associations on pine roots was on loamy sand and litter with higher moisture, respectively 28 % and 6 %. To identify mycorrhizal species morphotyping based on colour and external anatomy of mycorrhizal roots were used. In this way 5 morphotypes on pine roots on loamy sand and 6 on litter were distinguished. The morphotypes were further divided using restriction length polimorphism (RFLP) analysis of the internal transcribed spacer (ITS) region of the ribosomal DNA extracted and amplified from single mycorrhizal roots. The RFLP data revealed a total of 3 ITS types. One of these was identified as *Cenoccocum geophilum.* There was not difference between numbers of morphotypes on different level of moisture. Positive correlation between mycorrhizal total number and upturn of moisture were found.

P2 77

Molecular identification of ectomycorrhizae of *Hydnum albidum* Peck. on Austrian pine *Pinus nigra* Arnold.).

T. Grebenc[1], M. P. Martin[2], H. Kraigher[1] [1]Slovenian Forestry Institute, SI-1000 Ljubljana, Slovenia, [2]Real Jardin Botanico, C.S.I.C., Plaza de Murillo 2, 28014 Madrid, Spain.

Ca 220 fruitbodies and types of ectomycorrhizae from forest research plots from the Alpine and Dinaric regions in Slovenia have been used for establishment of a PCR-ITS-RFLP database (ITS1f and ITS4b after 1, described in 2, database after 3, described in 4 & 5). Two different restriction patterns for *Hydnum rufescens* were obtained. Sequencing of the ITS regions revealed several differences in the sequences. One of the genotypes could belong to *H. umbilicatum* Peck, although we could not compare them with any sequences in the GeneBank (www.ncbi.nlm.nih.gov). Or, the difference might result from the intraspecific variation in ITS (6). Equal comparisons of the types of ectomycorrhizae on Austrian pine with the database revealed a match of the restriction pattern of type of ectomycorrhiza with the restriction pattern of *H. albidum* Peck. Type exicates are being searched for in Herbaria for further analysis.

Acknowledgements: We would like to thank A. Piltaver for determination of fruitbodies and the financers of the programme PS404/501 and project no. L4 1254,.MESS and MAFF of RS.

1. Gardes, M., Bruns, T.D. (1993) *Mol. Ecol.* **2**, 113-118.
2. Kraigher, H., Agerer, R., Javornik, B. (1995) *Mycorrhiza* **5** 175-180.
3. Karen, O., Hogberg, N., Dahlberg, A., Jonsson, L., Nylund, J.-E- (1997) *New Phytol.* **136**, 313-325.
4. Grebenc, T., Piltaver, A., Kraigher, H. (2000) *Phyton* **40 (4)**, 79-82.
5. Grebenc, T., Kraigher, H. (2000) In (Eds. H. Kraigher, I.Smolej): The Rhizosphere. PSW 118. SFI, pp. 179-190, Ljubljana..
6. Agerer, R., Kraigher, H., Javornik, B. (1996) *Nova Hedwigia* **63 (1/2)**, 183-194.

P2 78

High species and intraspecific diversity in ectomycorrhizal fungi in Eucalyptus forest

M. Glen[1], N. L. Bougher[2], I. C. Tommerup[2] & P. A. O'Brien[1]
[1]Murdoch University, Murdoch, Western Australia. [2]CSIRO Forestry and Forest Products, Perth, Western Australia.

PCR-RFLP analysis of ectomycorrhiza from a 3 ha study site in *Eucalyptus marginata* forest revealed the presence of 150 basidiomycete fungal taxa. An additional 95 species have been recorded as sporocarps from this site but have not yet been found on roots. Members of the Russulaceae, Amanitaceae and Cortinariaceae but not the Thelephoraceae are prominent as root associates in this forest type. Molecular analysis of sporocarps from this small area showed a high level of intraspecific variation in nrDNA ITS and mtLSU sequences. Fire history affects the presence and abundance of some species and sub-species. Intraspecific variation may represent taxonomic complexes in some groups, particularly the Russulaceae and Cortinariaceae. Sampling of more than one collection of each morpho-species is therefore a necessity, with the retention of good collections and vouchers. The many co-occurring species of Cortinariaceae (over 40 species recorded), which have low interspecific variation in the ITS region, need a higher level of molecular analysis than digestion of the ITS with two restriction enzymes. For many species in this family, PCR-RFLP of a mtLSU fragment provides greater resolution than PCR-RFLP of the ITS, in contrast to many other families. Using molecular analyses of sporocarps and roots, we are currently investigating the variation among a series of forest areas with a range of understorey vegetation types, and also the development of ectomycorrhizal communities in minesites rehabilitated with forest vegetation.

P2 79

Diversity of mycobionts of *Pisonia Grandis* on coral cays in the Great Barrier Reef

S. M. Chambers, C. J. Hitchcock, J. W. G. Cairney
Mycorrhiza Research Group, University of Western Sydney, Australia.

An isolate of the *Pisonia grandis* mycobiont from Heron Island, Great Barrier Reef, Australia, was obtained in a previous study. This isolate was identified (using rRNA ITS sequencing) as a basidiomycete belonging to the Thelephoraceae, probably a *Tomentella* or related species, and was thought to be the sole mycobiont of *P. grandis*. In the present study, rRNA ITS-RFLPs and sequencing of the ITS region were used to determine the identity of mycobionts from *P. grandis* on other coral cays in the Capricornia bunker group. Mycorrhizal roots were collected along transects on six cays including Heron Island. DNA was isolated and subjected to rRNA ITS-RFLP analysis and ITS sequences were obtained for selected samples. ITS-RFLP analysis identified a single fungal RFLP type to be present across all six coral cays. This RFLP type was different to the mycobiont originally isolated from Heron Island. The mycobiont was identified using sequence analysis as belonging to the Thelephoraceae, with > 79% sequence identity to the previously isolated mycobiont. The results will be discussed in relation to the ecology of *P. grandis* on coral cays.

P2 80

Nitrogen addition changed macromycete sporocarp production and belowground ectomycorrhizal species composition in a Norway spruce stand

M. Peter, F. Ayer, S. Egli, Swiss Federal Research Institute WSL, Birmensdorf, Switzerland

The effects of simulated N deposition on the ectomycorrhizal and total macrofungal community in a subalpine *Picea abies* stand were investigated by adding 150 kg N $ha^{-1}year^{-1}$ as ammonium nitrate as a long-term fertilizer. Ectomycorrhizal diversity was studied 1) above-ground in terms of sporocarp production, including three years of recording before N addition, and 2) below-ground by PCR-RFLP analysis of the ITS region of rDNA extracted from single mycorrhizal root tips before and after one and two years of fertilization in treated and control plots. Sporocarp surveys revealed a strong impact of increased N input on the ectomycorrhizal community by drastically decreasing species diversity one year after the start of N addition, whereas the saprobic fungal community was not affected. The impact of N addition on below-ground ectomycorrhizal diversity was less pronounced. No change in the number of ectomycorrhizal taxa and Simpson's index of diversity was found. However, ordination analysis as well as comparison of similarities among control and treated plots indicated a change in below-ground species composition two years after N addition. Significant changes in abundances of single species were observed in fertilized plots. Eleven out of 25 species which produced conspicuous sporocarps in the six years of survey were identified on the root system. These species accounted for approx. 25% of all sampled root tips. At least 44% of all ectomycorrhizas, comprising nine different RFLP-types, were formed by species belonging to the Thelephoraceae and Corticiaceae, taxa which produce inconspicuous sporocarps. Our data indicate that the abundance of these species on the root system increases at higher N concentrations, whereas the abundance of species which produce fewer sporocarps after N input decreases.

P2 81

Community structure of ectomycorrhizal fungal sporocarps among successional forest age classes of *Pseudotsuga menziesii*

J.E. Smith[1], R. Molina[1], M.M.P. Huso[2], D.L. Luoma[2], D. McKay[1], M.A. Castellano[1], T. Lebel[3], and Y. Valachovic[4]. [1]USDA Forest Service, Pacific Northwest Research Station, Corvallis, OR, USA, [2]Oregon State University, Corvallis, OR, USA, [3]Royal Botanic Gardens Melbourne, Victoria, Australia, [4]University of California, Eureka, CA, USA

Host plant species composition and habitat conditions associated with forest stand age influence the community structure of ectomycorrhizal (EM) fungi. Results of our four year study in the Cascade Range of Oregon, USA show high species richness (48 hypogeous, 215 epigeous) of EM fungal sporocarps in mesic *Pseudotsuga menziesii* associations. Data from 3 replicate stands of 3 forest age classes suggest that EM species richness is relatively stable from canopy closure through late-succession. Even though many EM species occur at multiple forest successional stages, 36% were unique to a particular forest age class. There was a greater likelihood for species unique to an age class to occur in old-growth stands. Seventeen species (8 hypogeous, 9 epigeous) accounted for 79% of the total sporocarp biomass. Patterns of EM species dominance and occurrence of some EM species were most similar between managed young and rotation-age stands, and differed from old-growth stands. Average sporocarp biomass in stands of young and rotation-age compared to old-growth was about 3 times greater for hypogeous and 6 times greater for epigeous sporocarps. Our results suggest that all age classes of forests are important for maintaining the biological diversity of EM fungi.

P2 82

P2 83

Terfezia species (desert truffles): ITS and 25S rDNA analyses.

V. Kagan-Zur[1], S. Aviram[2], Y. Ferdman[2] and N. Roth-Bejerano[2]. [1]Inst. Appl. Res. And [2]Dept Life Sci. Ben-Gurion Univ. POB 653, Beer-Sheva 84160, Israel.

Desert truffles of the genus *Terfezia* (Terfeziaceae, Pezizales, Ascomycetes) may be found in different parts of the old world, mainly in deserts. We have been studying the molecular variability of these truffles using both the Internal Transcribed Spacer (ITS) and the 5' variable region of the 25S rDNA gene cluster. Phylogenetic relations between four terfezia species were analyzed using *Choiromcyes echinulatus* and *Mattirolomyces terfezioides* (formerly *Terfezia terfezioides*) ITS sequence (the latter sequence obtained from GenBank) as out groups. *Terfezia pfeilii* and *Terfezia leptoderma* were revealed to be the out groups, pointing to the need for redefinition of the *Terfezia* genus. The molecular analysis of *Terfezia boudieri* revealed a significant difference between certain types of isolates, which could be interpreted as representing two different species, although no morphological differences could be discerned. The significance of this finding remains open to discussion. This study enabled to determine that nearby truffle initiations fuse to form a lobed fruit body. It also revealed a normally proliferating *T. boudieri* genet which has been supporting the coexistence of two ITS forms for the last 5 years. Is it a viable Ascomycetous heterokaryon, or are both forms included in one haploid nucleus?

P2 84

Genet distribution of two related ectomycorrhizal fungal species: *Tricholoma scalpturatum* and *T. populinum* in a black poplar stand

H. Gryta H., F. Carré, J.Y. Charcosset and M. Gardes. CESAC, CNRS - Université Paul Sabatier, Toulouse, France

We collected fruitbodies of *Tricholoma scalpturatum* and *T. populinum* over two years in a black poplar stand located near the Garonne river. The genet distribution was analyzed using RAPD markers and RFLPs in the IGS. In *T. scalpturatum*, 60 genets were identified from 63 fruitbodies collected in 1998. 92% of the genets (i.e. 58 out of 60) were represented by individual fruitbodies. Only two IGS-RFLP types were detected, one was represented by 9 fruitbodies located within a small area. In 1999, twelve new genets were observed from 12 fruitbodies. No new IGS or RAPD fragments were found, which suggests a high level of inbreeding. These results are indicative of a population mainly structured by sexual reproduction. Contrasting results were observed in the *T. populinum* population colllected in 1998. One genet was represented by 18 fruitbodies and covered an area of 2,5 m2. The 6 remaining genets comprised one fruitbody. This population appeared to be structured both by vegetative growth and sexual reproduction. The observed differences between the two species will be discussed.

P2 85

Population genetics of *Boletus edulis* in Western North America: genets and gene flow.

Francisco J. Camacho and Tom Bruns University of California, Berkeley, USA

Boletus edulis is an economically important ectomycorrhizal mushroom in the coniferous forests of the Western United States. Our research goal is to learn more about the basic biology of this species. Genet size can reveal features of colonization strategy and growth; many small genets indicate multiple spore establishment events and large genets reflect vegetative spread. Comparisons of alleles within and between populations can provide estimates of gene flow, suggesting frequency of dispersal. We have mapped and sampled fruitbodies from two montane populations one in the Sierra Nevada and an other 637 km away in the Oregon Cascades. We are using anonymous dominant genetic loci, amplified fragment length polymorphism (AFLP), and two co-dominant loci, internal transcribed spacer rDNA and chitin synthase II, to determine genet sizes in these populations and gene flow between populations. We found that the greatest distances separating genetically identical fruitbodies was over 10 m, but many closely spaced fruitbodies were genetically unique. From these data we infer that vegetative spread occurs over at the scale of several meters but many new individuals continue to establish. This is a somewhat unexpected finding because it has generally been assumed that vegetative spread accounts for most of the colonization in undisturbed forest settings. The Gst value is 0.2066 translating to a gene flow value of Nm = 1.92. This low value of Nm suggests that there is little gene flow between these two populations. This is contrary to the current dogma that mushroom spores are readily dispersed by wind.

P2 86

A PCR-based method for identifying genets (=genetic individuals) of ectomycorrhizal fungi on ectomycorrhizal root tips

Jesús Díez, and François Le Tacon. UMR INRA-UHP, Interactions Arbres Microorganisms (IaM), I.N.R.A.-Nancy, France

Several PCR-based methods allow identification of genets (=genetic individuals) among fruit bodies and isolates of ectomycorrhizal fungi; e.g Random Amplified Polymorphic DNA - RAPD- and microsatellite-primed DNA analyses. These methods are based on short non-specific primers. Over the last years, such approaches were used to study the temporal/spatial structure of populations of several ectomycorrhizal fungi on the base of fruit bodies surveys. These methods, however, are not suitable for mycorrhizal typing. As fungal specific primers are not used, plant and bacterial DNA are amplified from mycorrhizal root tips. As a consequence, ectomycorrhizal underground populations were rarely studied. But fruiting genets might not truly reflect the structure of the populations of ectomycorrhizal fungi and there is hence an increasing interest in studying the underground root-associated genets. The methodological difficulties can be solved by the use of fungal specific primers. RAPD primers can be transformed into longer fungal specific primers by using Sequence Characterized Amplified Regions (SCAR) from cloned RAPD fragments, obtained from fungal pure cultures or fruit bodies. The identification of strains-specific SCAR markers is possible in some cases. Alternatively, a short set of SCAR primer pairs can be used to obtain multilocus fingerprints, which allow the identification of ectomycorrhizal genets on mycorrhizal root tips. In the present work, we analyze such approach for studying the underground populations of ectomycorrhizal fungi.

P2 87

Spatial Distribution of the Subterranean Mycelia and Ectomycorrhizae of *Suillus grevillei* Genets

Z. Zhou, M. Miwa, Y. Matsuda, and T. Hogetsu. The University of Tokyo, Symbiotic Function Research Unit, Tokyo, Japan

Increasing number of reports indicated that the aboveground ectomycorrhizal population and community do not correspond to the subterranean ones and emphasized the necessity of direct studies on subterranean genets and communities. To analyze the distribution of subterranean parts of ectomycorrhizal genets, species-specific polymorphic markers are necessary. We developed several SSR markers of *Suillus grevillei* DNA and found a polymorphic and *S. grevillei* specific marker among them. The horizontal and vertical distributions of the subterranean parts of several *S. grevillei* genets in a *Larix* forest were analyzed by using the species-specific marker. The spatial distribution of *S. grevillei* genets in the soil demonstrated that the development of *S. grevillei* sporocarps is correlated with that of extra-radical mycelia and ectomycorrhizae of the same genet, which are distributed in a narrow area. However, sporocarps are not always centered over the subterranean parts and the amount of the subterranean mycelia and mycorrhizae is not always correlated to the number of sporocarps formed on them. No *S. grevillei* mycelia and mycorrhizae were detected beneath the positions where *S. grevillei* sporocarps emerged in the previous year. The observation indicates the rapid alteration of the subterranean parts of *S. grevillei* genets, and suggests that *S. grevillei* genets may change location instead of only extending their habitat year after year. As a possible factor influencing the small size of *S. grevillei* genets, competition of the *S. grevillei* mycelium with other ECM fungal mycelia could be assumed. Thus, investigation of species compositions of subterranean ECM communities around those *S. grevillei* genets is in progress.

P2 88

Pisolithus : a worldwide ectomycorrhizal partner.

M. Ducousso[1],Y. Prin[1] and D. Thoen [2]. [1]Laboratoire des Symbioses Tropicales et Méditerranéennes, TA10J, 34398 Montpellier Cedex 5, France; [2]Fondation Universitaire Luxembourgeoise, 185 avenue de Longwy, B-6700 Arlon, Belgium.

Pisolithus is a worldwide spread ectomycorrhizal genus. Until now, its systematic remains unclear and quite confuse. Recently, progresses in molecular biology, in ordination techniques, in scanning electronic microscopy led to a better understanding of the *Pisolithus* complex. More datas concerning the world distribution and the tree host range have conducted to a better ecological knowledge of the complex. Features such as color of mass spores deposits, ornementation types of spores, peridium color of young sporocarps, together with the linkage to peculiar host tree, are important for the characterization of « small » species of the *Pisolithus* complex. Efforts are now to be made for the typification of these species. At least 10 groups of *Pisolithus* species are distinguishable by coupling ecological (host specificity) and morpho-anatomical characteristics. Short descriptions of these ten "species" are given as well as worldwide maps of their potential distribution related to native forests extents and kinds of exotic tree plantations (ecological datas).

P2 89

Species and world wide evolutionary lineages within the ectomycorrhizal fungus *Pisolithus*

Bernie Dell[1], Jesús Díez[2] Christine Delaruelle[2] & Francis Martin[2]
[1]School of Biological Sciences and Biotechnology, Murdoch University, Murdoch WA6150, Australia, [2]UMR INRA-UHP "Interactions Arbres/Micro-Organismes", Centre INRA de Nancy, F-54280 Champenoux, France

Sequences of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA) were obtained from 100 isolates of the ectomycorrhizal fungus *Pisolithus.* They were analysed with distance and cladistic methods along with 30 more sequences from the GenBank database. The phylogenetic analyses resolved isolates into ten terminal clades, in two major lineages, that may represent ten phylogenetic species within this species complex. Several isolates and pairs of isolates at the bases of the ten terminal clades may represent a further ten species. Some terminal clades can be clearly ascribed to described species of *Pisolithus.* A strong pattern was evident in the data in regard to the biogeographic origin of the hosts. Each species associated with host plants from a single biogeographic region. Isolates of *Pisolithus* found in eucalyptus plantations worldwide, and in plantations of Australasian species of *Acacia* in western Africa (Senegal), were co-introduced along with their hosts.

P2 90

Genetic diversity in *Tuber uncinatum* in plantations and woodlands of Northeastern France

P. Luis, C. Delaruelle, C. Dupré*, G. Chevalier* & F. Martin

*INRA-Nancy & *INRA-Clermont-Ferrand, France*

To address the possibility of gene fluxes between populations of wild and inoculated Burgandy Truffle (*Tuber incinatum*), we investigated levels of genetic diversity among populations of Northeastern France (Lorraine and Burgandy). We used high stringency RAPD technique to type fruiting bodies collected from various plantations and woodlands. Diversity levels among populations were relatively high. The estimated pairwise distance among genotypes ranged from 3 to 50%. Individuals of the the largest clone were found in plantations and woodlands over a 300 km^2 area. However, most genotypes were limited to a single or a few sites. Though *T. uncinatum* has the capacity for vegetative persistence, the present study indicates that its populations maintain a genetic structure more consistent with a high frequency of sexual reproduction.

P2 91

Morphological and molecular characterisation of the mycelium of different *Tuber* species in pure cultures

M. Iotti[1], A. Amicucci[2], V.Stocchi[2,] A. Zambonelli[1]
[1]Diparimento di Protezione e Valorizzazione Agroalimentare , University of Bologna, Italy
[2]Istituto di Chimica Biologica "Giorgio Fornaini", Università degli Studi di Urbino, Urbino (PU), Italy

Truffles are ectomycorrhizal ascomycetes belonging to the genus *Tuber*. Truffle cultivation involves planting infected plants in suitable sites. The infected plants are usually obtained by spore inoculation, due to the difficulties of obtaining their mycelium in pure culture. In the past many attempts have been made to isolate truffle species mycelium but the mycelia obtained grew too slowly for a mycelial inoculation methods. Moreover, these mycelia were very often isolated from mycorrhizae and tools were not available to verify whether the mycelia isolated belonged to the *Tuber* genus. Only *T. borchii* cultures are currently available for research purposes and they have undergone molecular and morphological characterisation. This has also made it possible to perfect a model for *in* vitro mycorrhizal synthesis with *T borchii* mycelium. In this study we isolated the mycelium of T. *maculatum, T. aestivum, T. macrosporum, T. rufum,* and *T. brumale* in pure cultures. Molecular identification of the mycelia isolated was performed using RFLP and/or sequence analyses of the ITS region. The mycelia of the different *Tuber* species showed several common morphological features such as anastomosis and vesicle formation and hyphal granulation. Differences were found in the frequency of these morphological features and in the hyphal pattern. Mycelial growth was of 3- 6 mm week on the selected medium depending on the *Tuber* species.

P2 92

Taxonomical re-evaluation of the mycorrhizal fungus "Matsutake-modoki" (*Tricholoma robustum* s. auct. In Japan)

K. Iwase[1] and C. Tanaka[2] [1]Biological Environment Institute, KANSO Co., Ltd., Japan [2]Lab. Env. Mycosci., Kyoto University, Japan

Matsutake-modoki, literary meaning matsutake look-alike, distributes almost throughout Japan, and mainly associates with *Pinus densiflora*. This fungus has been regarded as one of the matsutake related species based on the fruitbody morphology, but fruits a little later than matsutake. Matsutake-modoki was first described by Kawamura as *Armillaria caligata*, but later changed into *A. robusta* by Imazeki with uncertainness. We collected some specimens of matsutake-modoki in Japan and *Tricholoma robustum* in Sweden, and compared their characteristics mainly with a partial rDNA analysis. Swedish *T. robustum* generally has a thicker stipe than Japanese matsutake-modoki, though one specimen has thinner stipe than usual. From the DNA analysis, the three type specimens, one Japanese and two Swedish, thinner and thick ones, formed one cluster with some difference in their profile. One thinner Swedish specimen was found to be *T. focale* based on the comparison with other available data. Swedish thicker type showed some difference and could be the real *T. robustum*, while Japanese matsutake-modoki was more different. Thus, they are likely to form a complex species, but there are some distinct differences. Further studies are required for the detailed characterization.

P2 93

Molecular analyses of inter- and infrageneric relationships within the agaricoid Russulaceae (Basidiomycetes): Correspondence with mycorrhizal anatomy

Ursula Eberhardt, Spezielle Botanik & Mykologie, Universitaet Tuebingen, Germany

Ectomycorrhizal features are not commonly used in fungal systematics, even though the anatomical structure of the sheath is known to be a constant character, not modified by host tree, environmental factors or developmental stage. The Russulaceae illustrate how mycorrhizal features can successfully be used for the definition and limitation of systematic groups. Analysing ITS and LSU sequences of predominantly European *Russula* and *Lactarius* species, it was shown that similarities in the cellular structure of the outer regions of ectomycorrhizal mantles agreed well with the results of phylogenetic analyses of DNA sequence data. *Lactarius* species formed a single clade. Moreover, the analyses largely confirmed the allocation of the majority of European *Lactarius* species to three subgenera, *Piperites*, *Russularia* and *Plinthogalus*. While all known *Lactarius* mycorrhizae are distinguished by the occurrence of lactifers, each of the subgenera named above can be characterised its mantle anatomy. For *Russula*, phylogenetic analyses resulted in four paraphyletic clusters, splitting the *Compactae* as well as the *Genuinae* in two distinct clades, *Plorantinae* and *Nigricantinae*, and *Heterophyllidia* including *Foetentinae* and a clade carrying the remaining species of the *Genuinae*, respectively. Only species of this latter clade possess mantles lacking cystidia. Namely the division of the *Genuinae*, supported by sequence analyses and mycorrhizal characters, is a challenge to the current infrageneric classification of Russula.

P2 94

Anatomical and molecular characterization of Ramaria ectomycorrhizae.

E. Nouhra[1], T. Horton[1], E. Cazares[1] and M. Castellano[2]. [1]Oregon State University, Corvallis, Oregon, USA and [2]Forestry Sciences Laboratory, Corvallis, Oregon, U.S.A.

Ramaria species are conspicuous mycorrhizal symbionts of conifers in the Pacific Northwest region. Several species and mycorrhizal root tips were collected from mats directly below the fruiting bodies. By comparing polymerase chain reaction amplified ribosomal DNA (internal transcribed spacer) of conifer mycorrhizal root tips and sporocarp tissue, we found that all ectomycorrhiza were colonized by the associated fungal species *Ramaria acrisiccescens*, *R. cyaneigranosa*, *R sandaracina*, *R. celerivirescens*, *R. flavobrunnescens* var. *aromatica* and *R. leptoformosa*. Restriction fragment length polymorphism (RFLP) analysis, using, *Hinf* I and *Alu* I enzymes provided a distinctive profile for each of the Ramaria collections and mycorrhizal morphotypes, facilitating the identification. Mycorrhizae of these Ramaria species (sub.genus Laeticolora), exhibited similar macro- and microscopic characteristics, such as aggregation of mycelia and mycorrhizae, ramification pattern, general appearance and coloration, abundant mycelial strands and emanating hyphae, mantle morphology and chemical reactions with KOH, Fe SO_4 and Melzer's reagent. Because of the similarity in EM morphology, the RFLP analysis is critical to identify these closed related species in the absence of sporocarps.

P2 95

Molecular phylogenetics of *Gomphus* and related genera inferred from nuclear large and mitochondrial small subunits ribosomal DNA sequences

A.J. Giachini, J.W. Spatafora, E. Cazares and J.M. Trappe
Oregon State University, Corvallis, Oregon, USA

Phylogenetic relationships of the Gomphales *sensu* Jülich and closely related genera were estimated via parsimony analysis of nuclear large and mitochondrial small subunits ribosomal DNA sequences. Although some nodes deep in the tree could not be fully resolved, some well-supported lineages were recovered, and the interrelationships among *Gomphus* and *Ramaria* are better understood. *Gomphus* and *Ramaria* were both recovered as paraphyletic lineages within the Gomphales. Three separate lineages of gomphoid fungi were inferred and were consistent with the presence of clamps and fruiting body morphology (branched vs. infundibuliform). These three lineages included: 1) the *G. floccosus* complex which consisted of clamped, trumpet-like species *G. bonarii* (and varieties), *G. floccosus* (and varieties), *G. fujisanensis, G. kauffmanii,* and *G. thiersii*, 2) *G. clavatus* which includes branched forms with clamps, and 3) *G. purpurascens* which lacks clamps and have been classified in a separate genus, *Gloeocantharellus*, by some authors. The sequestrate genus *Gautieria* was recovered as a paraphyletic group nested within *Ramaria*, agreeing with previous observations showing common traits shared between these two ectomycorrhizal fungi. The placements of *Clavariadelphus* and *Kavinia* were ambiguous and no final conclusions could be presented. In general these results corroborate prevailing hypotheses that there has been extensive convergence in fruiting body morphology in this closely related group of fungi.

P2 96

Identification of the ectomycorrhizae of *Helvella leucomelaena* on *Pinus sylvestris* confirmed by the analysis of the ITS sequence

A. Urban[1] [1]Department of Cryptogamic Botany and Mycology, Institute of Botany and Botanical Garden of the University of Vienna, Rennweg 14, A-1030 Wien, Austria.

The ectomycorrhizal association of *Helvella leucomelaena* with *Pinus sylvestris* was investigated. Among several ectomycorrhizal morphotypes found below sporophores of *H. leucomelaena*, those which are formed by this ascomycete were identified by the comparison of ITS (Internal Transcribed Spacer) rDNA sequences obtained from ectomycorrhizae and sporophores. The ITS sequence of *H. leucomelaena* was compared to published sequences by NCBI Blast search and was most similar to a sequence of *H. solitaria*.

The ectomycorrhizae of *H. leucomelenae* are rarely ramified and irregularly beaded. The tips are orange brown and smooth, older parts are dark brown and wrinkled. The mantle is rather thin and consists of a plectenchymatous outer layer and an irregularly lobed to pseudoparenchymatic inner layer which is not thicker than one cell layer. The Hartig net is broadly lobed and reaches the endodermis. By these criteria, the ectomycorhizae of *H. leucomelenae* can be differentiated from hitherto described ectomycorrhizae assigned to the Helvellaceae, like the ectomycorrhizae of *Balsamia alba* on *Pinus jeffreyi* and those of *Leucangium carthusianum* on *Pseudotsuga menziesii*, both associations being recently described by PALFNER & AGERER.

P2 101

P2 102

The status of arbuscular mycorrhizal fungi in soil: a potential indicator of the sustainability of farming systems

L. K. Abbott,[1] J. Graham, J.[1], L. Edwards,[2] I. Djuuna[1] and J. Delaney,[2] [1]Soil Science & Plant Nutrition and [2]Department of Geography, The University of Western Australia, Australia.

A GIS-based model was used to predict the status of arbuscular mycorrhizal (AM) fungi in agricultural soils using data provided by land managers including fertilizer use, cropping and pasture history, yield and soil tests. Although AM fungi are generally considered to be important in farming systems, there is little practical information available to land managers that allows them to make management decisions to maximise contributions of AM fungi. We investigated the possibility that a simple model could be developed for use by land managers within a GIS format based on existing knowledge of the impact of management practices on the formation of mycorrhizas in farming systems in south-western Australia. Information from the land managers was collated and ranked. Direct measures of AM fungi within roots of plants grown in current rotations and bioassays of infectivity of AM fungi in soil were used to ground-truth the GIS output for the current year. Digitised coverages created in ARCINFO and ARCVIEW geographical information programs provided a visual template of the current and predicted mycorrhizal status on a paddock basis. This study demonstrated that it was indeed practical to estimate a longer-term status of AM fungi in agricultural soils based on information already available to land managers. Current levels of colonisation of roots by AM fungi did not necessarily reflect the longer-term mycorrhizal status of a soil. Some farming practices have considerable detrimental effects on AM fungi that affected assessment of the mycorrhizal status of soil. The model is now being evaluated on a wider scale in terms of the sustainability of farming systems.

P2 103

P2 104

Effects of mycorrhizal colonization on growth and phosphorus uptake by *Trifolium alexanderium* as affected by the size of soil aggregates

H. Nadian. The University of Shahid Chamran, Ahvaz, Iran

It has been shown that root growth in an aggregated soil is influenced by aggregate size. The lower root growth and phosphorus (P) uptake in beds of large compared with small aggregates has been attributed to greater mechanical impedance to root penetration and lower availability of P. Since the diameter of external hyphae of vesicular arbuscular mycorrhizal (VAM) fungi is much less than impeded roots, it was proposed that the external hyphae of VAM fungi can penetrate the large aggregates and exploit them more effectively than impeded roots. For the purpose of this study non-mycorrhizal clover plants (*Trifolium alexanderium* L.) and mycorrhizal clover plants (colonized by *Glomus intraradices*) were grown in pots containing aggregates of 6 different size in the range of 2-19mm. The results of this study indicated that shoot dry weights of both mycorrhizal and non-mycorrhizal clover plants decreased with increase in aggregate size. However there was a significant difference between shoot dry weights of mycorrhizal and non-mycorrhizal clover plants at any size of soil aggregates. Although total root length of both mycorrhizal and non-mycorrhizal plants decreased. with increase in aggregate diameter, the length of living external hyphae was not decreased as the size of soil aggregates was increased. In fact, the hyphal biomass per unit root length colonized increased with increase in the size of soil aggregates. This resulted in higher tissue P concentration and consequently greater mycorrhizal growth response in beds of large aggregates than in beds of small aggregates. In conclusion, the beneficial effects of mycorrhizal colonization increased as the size of soil aggregates was increased.

P2 105

Growth and function of AM external hyphae in sands of varying pore sizes

E.A. Drew[1], I. Jakobsen[2], R.S. Murray[1] and S.E. Smith[1] [1]Adelaide University, Australia, [2]Risø National Laboratory, Roskilde, Denmark

Research with arbuscular mycorrhizas (AM) has mainly focused on the root-fungus interface and less attention has been given to the growth and functioning of external AM hyphae in the bulk soil. The behaviour of external hyphae may differ in unfavourable soil environments, such as compacted soils. The effects of pore size on the growth of two AM fungi (*Glomus intraradices* and *mosseae*) and their ability to transport ^{33}P from the bulk soil to the host was investigated.

Trifolium subterraneum L. plants were grown individually in single arm cross-pots with and without AM fungi. The side arm was separated from the main compartment by nylon mesh to prevent root penetration. It contained three media zones: 5 mm of soil:sand mix, 25 mm of media treatment (HC2) and 20 mm of ^{33}P-labelled soil. There were four media treatments; soil and three types of quartz sands with most common pore diameters of 100 μm, 38 μm and 26 μm.

Production of external hyphae in HC2 was influenced by the interaction of fungal species and media treatments. Both fungi produced maximum amounts of external hyphae in the soil medium. Sand pore size affected hyphal growth and diameter distribution of *G. intraradices* but not *G. mosseae*. AM plants had similar growth and total P uptake. Plants grown with *G. intraradices* contained twice the amount of ^{33}P as those grown with *G. mosseae*, however, spatial differences in P acquisition were not correlated with production of external hyphae. The results suggest that different AM fungal species not only acquire P from different soil zones, but can also alter their morphology in response to different soil environments.

P2 106

The ectomycorrhizal fungi of four North American forest ecosystems: a cross-site comparison of EM diversity and community structure and the effects of nitrogen fertilization

M.F. Allen[1] and J.L. Lansing[1] [1]University of California, Riverside, CA, USA

The ectomycorrhizal (EM) fungal communities of four North American forest vegetation types were assessed to gain basic knowledge of the below-ground EM community and diversity. A manipulative nitrogen fertilization field experiment on mature stands was performed to investigate the effects of fertilization. The four sites *Populus balsamifera*, AK; *Quercus rubra*, GA; *Pinus resinosa*, MI; and *Pinus edulis*, NM were fertilized with 100kgN/ha/yr and the EM community was assessed using a combination of morphotyping and RFLP analysis of the rDNA ITS (internal transcribed spacer) region. EM percent colonization of live short roots was approximately 90% at all sites with sites differing in live EMs per meter root (*P. balsamifera* > *Q. rubra* = *P. edulis* > *P. resinosa*). No fertilization effect was observed on EM colonization. At all sites, we found that alpha diversity is not a good indicator of the larger scale beta diversity. All of the sites had a similar alpha diversity of 5-9 species but differed greatly in beta diversity (*P. balsamifera*, 55 EM species > *P. resinosa*, 40 > *P. edulis*, 25 > *Q. rubra*, 20). Fertilization did not affect EM richness but did result in community differences and shifts in the dominance of certain EM species.

P2 107

Models of Nutrient Partitioning by Fungal-Plant Interactions in Arbuscular Mycorrhizal Ontogeny

D.W.Graves[1], P.G.Long[2], A.N.Macgregor[2], H.A.Outred[1]. P.E.Jameson[1]. Institute of Molecular Biosciences[1], and Institute of Natural Resources[2], Massey University, New Zealand.

In soil and root ecosystems the partitioning of plant carbon assimilates is ubiquitously affected by interactions with heterotrophic rhizosphere micro organisms, including mutually beneficial soil fungi such as arbuscular mycorrhizal (AM) fungi, (Smith & Read, 1997). Odum (1983) analysed two-species population interactions that may infer a two-way continuum between beneficial and detrimental outcomes, a 'grey-scale' gradation between bipolar opposites, positive (+/+) and negative (-/-).

Our modified model is a venn diagram with three entities depicting interaction types between AM fungi and plants, including mutual benefits from bidirectional exchange of mineral and carbon nutrients (+/+), neutralism (0/0), and competition (-/-). Intersecting sets of these entities depict a three-way continuum of interactions: parasitism or predation (+/-), and escape (-/+); amensalism (-/0, 0/-); and commensalism (0/+, +/0). The modified model can be interpreted in the context of a continuum over time and space, during the development of AM symbioses.

AM fungi are obligate biotrophic symbionts, and are parasitic (+/-) on plant carbon sources during early root colonisation stages, and in intercellular hyphae, where plant carbohydrates are actively taken up from root cortical and epidermal intercellular spaces. The model set containing (+/-) also includes opposite outcomes of fungal-plant interactions, (-/+), representing anti-fungal plant defence, or mycorrhizal incompatibility mechanisms where fungi are excluded from, or inhibited by, non-mycorrhizal species.

P2 108

P2 109

Biodiversity of arbuscular mycorrhizal fungi from rhizosphere of *Macaranga denticulata* (Bl.) Mull. Arg. in Mae Hong Son province, Thailand

S. Youpensuk[1], S. Lumyong[2], B. Rerkasem[3], B. Dell[4]
[1]Biotechnology, Graduate School, Chiang Mai University, Thailand
[2]Department of Biology, Faculty of Science, Chiang Mai University, Thailand
[3]Department of Agronomy, Faculty of Agriculture, Chiang Mai University, Thailand
[4]School of Biological Science and Biotechnology, Murdoch University, Australia

Macaranga denticulata (Bl.) Mull. Arg. is a fast-growing, small to medium-sized, evergreen tree and an open crown. *M. denticulata* is known as a common pioneer species in moist open areas and secondary forests. It produces paniculate flowers after 3 years. The hill tribe (Karen) in Sop Moei district of Mae Hong Son province in Thailand have believed in beneficial effects of *M. denticulata* on rice yields in their traditional shifting cultivation. Karen farmers in Sop Moei district have been able to manage the establishment of *M. denticulata* canopy in fallow plots of their rotational shifting cultivation. Therefore, we are interested in studying arbuscular mycorrhizal (AM) fungi in these plants. Evaluation of AM colonization of seedling roots of *M. denticulata* revealed approximately 82%. Numbers of AM spores in rhizospheres of *M.denticulata* seedlings were about 20-45 spores/g fresh soil. Thirty species of AM fungi were found. They were belonged to genera of *Glomus, Acaulospora, Gigaspora, Scutellospora,* and *Paraglomus* in number of 19, 6, 2, 2 and 1 species respectively. We believe that it would be beneficial to naturally inoculate AM fungi in the plots before new crops are planted.

P2 110

Biodiversity of Mycorrhizae on *Quercus garryana* in Southern Oregon

D. Southworth, L.L. Valentine, T.L. Fiedler, S.R. Haney, H.K. Berninghausen, Southern Oregon University, Ashland, OR, USA

Quercus garryana, Garry oak or Oregon white oak, is the dominant vegetation on the western edge of the Agate Desert, an alluvial fan capped with shallow clay loam over a cemented hardpan. The landform exhibits patterned ground with mounds and vernal pools. The oaks are associated with *Ceanothus cuneatus* and with native and exotic grasses. In preparation for a study of biocomplexity in common mycorrhizal networks among oaks and grasses, we examined the morphotypes of ectomycorrhizae on *Q. garryana*. Soil cores were taken at distances half way to the canopy edge, at the canopy edge, and outside the canopy. Forty ectomycorrhizal morphotypes were distinguished. Six morphotypes occurred in >5% of soil samples with *Cenococcum geophilum* present in 78% of samples. Diversity of ectomycorrhizal morphotypes correlated inversely with distance of the tree to its nearest neighbor and with tree size. We compared DNA of ectomycorrhizae via PCR using the fungal specific primers ITS1F and ITS4 and the restriction enzymes *Hin*fI and *Taq*1. RFLPs showed that small differences in mycorrhizal morphology correlated with differences in restriction fragment patterns and most likely indicated different species. In addition, endomycorrhizae with intraradical hyphae and vesicles, but no arbuscules, also occurred in oak roots. Funded by NSF Grant DEB-9981337.

P2 111

Structural diversity of arbuscular mycorrhizal fungi in a disturbed grasland

C. Renker, M. Kaldorf, F. Buscot
Institute of Ecology, University of Jena, Germany

Industrial waste deposites is a major concern in restoration ecology. The field site of the following study is located close to a former fertilizer factory, which emitted high quantities of phospate, cadmium etc. for more than two decades. Consequently, plant phosphate availability at the experimental site is extremely high, reaching up to 4 g/kg soil.

Despite this high P concentration, AM colonization rates up to 90 % were observed on some plant species. Spores isolated along a pollution gradient on a hill slope were characterized by ITS-PCR/RFLP and sequence analysis. Additionally, fungal diversity was investigated on maize roots inoculated with roots from the field site. The highest sequence homology between AM fungi from the field site and the NCBI-database was 76% identity to the complete *Glomus coronatum* ITS sequence. Since the identity of ITS sequences is usually over 90% within *Glomus* species, the spores from the phosphate contaminated field site do not belong to any of the AM species with published ITS sequence. This might indicate that phosphate tolerant or even phosphate dependent AM species could have been selected, which are rare at non-contaminated sites and therefore poorly characterized so far. In addition to AM fungi, different soil fungi like *Mrakia* and *Lepista* were found to be associated with mycorrhizal plant roots.

Further studies will clear the functional processes and show which factors lead to the strong mycorrhization observed under high phosphate conditions at the experimental site.

P2 112

High species richness an dominance of *Cortinarius* taxa in belowground communities of ectomycorrhizal fungi from the Canadian Arctic.

Anders Dahlberg[1] , Monique Gardes[2] and Jean-Yves Charcassot[2], [1]Swedish University of Agricultural Sciences, SWEDEN, [2]CESAC/CNRS Université Paul Sabatier/Toulouse FRANCE,

In 1999, we participated to a a ship based research expedition to the Canadian arctic archipelago arranged by the Royal Swedish Academy of Sciences. The expedition, Tundra NorthWest 1999 (http://www.polar.kva.se), was focused on the tundra's ecology. Seventeen study sites were selected to represent a longitudinal and latitudinal gradient of the area and to encompass vegetation within the Low to High Arctic zones. We collected mycorrhizas from the two most abundant and widely spread ectomycorrhizal plants in the Arctic: *Salix arctica* and *Dryas integrifolia*. PCR-based molecular methods were used to identify the mycobionts and to provide an assessment of diversity patterns at different spatial scales. The mycobiont species richness was high, approaching that in boreal forest ecosystems. In total more than 130 taxa were encountered. Species of *Cortinarius* dominated, comprising more than 20% of the abundance and constituted 4 of the 6 most frequently encountered taxa. *Salix* and *Dryas* to a large extent shared the same mycobiont taxa. The characteristics and tentative mechanisms shaping the Arctic ectomycorrhizal commmunities will be discussed and related to patterns found in forest ecosystems:

P2 113

Pterospora andromedea: Morphological and molecular assessment of mycorrhizae from plants growing in a sub-boreal forest ecosystem in central British Columbia.

J.M. Catherall, H.B. Massicotte, B.W. Young, L.E. Tackaberry, K.N. Egger. University of Northern British Columbia, Prince George B.C., Canada.

Pterospora andromedea is an achlorophyllous, mycoheterotrophic species in the Monotropoideae that is believed to receive its photosynthates from neighboring trees via shared fungi. To explore the mycorrhizal associations within its northern range, plants were sampled from 4 sub-boreal forest sites in central BC. Site-associated trees included *Pinus contorta*, *Picea* spp., and *Populus tremuloides*. Root clusters (5 per site) were collected during July and August 1999. Sporocarps of the *Suillus-Boletus-Rhizopogon* group occurring on the sites were also collected. Both morphology (light microscopy) and molecular analysis (PCR-RFLP and DNA sequencing) showed that mycorrhizae from individual root clusters (often several hundred tips) consisted of fungal monocultures. Only one fungal morphotype was characterized for all root tips; the mycorrhizae produced a white to mauve to darker blue mantle, short bristle-like verrucose cystidia, no clamps, and rhizomorphs. The restriction endonucleases *Alu*I, *Hinf*I and *Rsa*I used to cleave the PCR product for RFLP analysis showed no variation between the fragment patterns for the samples. DNA sequencing of the 5' end of the 28S region of the ribosomal RNA gene of the *P. andromedea* and *P. contorta* root tips, and a hypogeous sporocarp, resulted in a highly similar partial sequence alignment. Submission of the fungal sequences to GenBank suggests that these fungi all closely resemble *Rhizopogon subcaerulescens* (I.D. #AF071534), extending the specialization of *P. andromedea* with the *R. subcaerulescens* group well into its northern range in western North America.

P2 114

Ectomycorrhizae between *Gyrodon monticola* Singer and *Cortinarius* sp. and *Alnus acuminata* H.B.K. (Argentina) characterized by anatomical and molecular tools

A.G. Becerra[1], G. M. Daniele[1], L.S. Domínguez[1], E.R. Nouhra[2] and T.R. Horton[2]
[1]Instituto Multidisciplinario de Biología Vegetal (CONICET), República Argentina. [2]USDA Forest Service, Forest Science Laboratory, Corvallis, USA.

Natural ectomycorrhizae between *Gyrodon monticola* and *Cortinarius* sp. and *Alnus acuminata* ("andean alder", "aliso del cerro") are described by morphological and anatomical characteristics. Ectomycorrhizal roots were sampled from underneath sporocarps of both fungi. The samples were taken from four plots in two homogeneous *A. acuminata* sites (Tucumán and Catamarca Provinces, Argentina). The ectomycorrhizae were described morphologically and by molecular tools using polymerase chain reaction (PCR) amplification of the fungal DNA. The most important features of *Gyrodon monticola* ectomycorrhizae are: highly differentiated rhizomorphs with central hyphae, inflated cells on the surface mantle with brown pigments, hyaline and brown emanating hyphae with dolipores and a plectenchymatous mantle on the mycorrhizae with rounded apex. *Cortinarius* sp. ectomycorrhizae showed a thick blue, white to beige mantle, with abundant hyphal bundles and milky exudates when injured. The ectomycorrhizae presented in this work are part of a project where the morphotypes diversity associated with *Alnus acuminata* are being studied.

P2 115

Characterization of growth, nutritional, and arbuscular mycorrhizal colonization behaviours of *in vitro* tomato hairy roots clones

K. Labour[1,2], M. St-Arnaud[1], M. Jolicoeur[2] [1]I.R.B.V., Jardin botanique de Montréal, Canada [2]Dept. of Chemical Engineering, École Polytechnique de Montréal, Canada

The arbuscular mycorrhizal (AM) symbiosis induces important modifications in the telluric microflora, and significantly reduces diseases and stresses of host plants. However, the regulation mechanisms involved are still not completely understood. In fact, as those fungi are obligate biotrophs, it is difficult to study their metabolism and physiology because they can not be cultivated axenically, without a host plant. Four tomato cultivars (*Lycopersicon esculentum*) were transformed using strains of *Agrobacterium rhizogenes* (A4, ATCC 15834 and R1000). 48 clones were obtained from which four stable ones were chosen considering parameters as their geotropism, ability to grow on minimal medium *in vitro* and potential for mycorrhization. The four transformed isolates along with the non-transformed isolate of the cultivar Cobra and carrot (*Daucus carota*) hairy roots were inoculated or not with the AM fungus *Glomus intraradices* and compared during a 4 months growth period. The fresh weight, dry weight, root length, nutritional kinetics and the mycorrhizal affinity of the different clones differed significantly. The non transformed cultivar Cobra, the clone 4C9 and the carrot hairy roots have a slow development compared to the clones AJ3, 4C4 and 4C7. Moreover, only the carrot hairy roots, 4C4 and 4C9 developed the symbiosis with *Glomus intraradices*.

P2 116

Lactarius deliciosus culture conditions and *in vitro* mycorrhization of *Pinus pinaster* plants raised from seeds

J.L. Manjón[1] , M. Bernedo[1], M. Pérez[1], C. Santamaría[2], M. Camacho[2], A. Aguilar[2], L. Romero[2] and A.Daza[2].

[1] *Dpto. Biologia Vegetal, Universidad de Alcalá, Alcalá de Henares (Madrid), Spain.*
[2] *CIFA Las Torres, Junta de Andalucía. Alcalá del Río (Sevilla), Spain.*

Strains of ectomycorrhizal fungi isolated from sporocarps of *Lactarius deliciosus* associated with *Pinus pinaster* and *Pinus pinea* coming from the Sierra of Aracena (Huelva, Spain) have been cultured. These strains and the Spanish type culture collection (CECT 2975) were grown on nutrient agar media containing different carbon and nitrogen resources in order to determine their miceliar biomass (radial growth rates during cultures, the final colony dry weight, ergosterol, etc.). Influence of pH and temperature was also studied.

Finally, it has also been carried out the *in vitro* mycorrhizal synthesis of *Lactarius deliciosus* associated with *Pinus pinaster* axenic seedlings. *L. deliciosus* ectomycorrhizae were identified by morphological, anatomical characters and molecular analysis.

P2 117

Influence of temperature and light on the functioning of arbuscular mycorrhizal (AM) and on the extraradical hyphae growth: implications of climate change

A. Heinemeyer' and A.H. Fitter' The University of York, Great Britain

The biology of the fungi (Glomales) in the arbuscular mycorrhizal (AM) symbiosis is barely known. Our lack of understanding of the impact of environmental factors such as temperature and PAR on this ubiquitous symbiosis prevents predictions of AM response to environmental change. Yet global mean temperature is expected to rise by c. 3°C over the next century and significant changes in global albedo are also likely. Because AM fungi require their carbon supply from the host plant, it is known that the symbiosis functions poorly in weak light. Recently it has been shown that the growth of roots is more strongly affected by PAR than by temperature, probably because respiration and growth acclimate to fluctuating temperatures. Root growth is therefore controlled directly by the supply of assimilates from the photosynthesising shoot, and root and fungal growth must compete for assimilate supplies. We have measured AM fungal response to different temperature and light regimes in garden and growth room experiments. Growth room experiments showed that AM fungi are directly influenced by soil temperature, independently of any effect on the host plant, as well as indirectly by PAR via photosynthesis. Shading drastically reduced both root colonisation and the amount of extraradical mycelium. However, in a long-term garden experiment, involving combinations of shading and soil heating, root colonisation by AM fungi was significantly affected by shading but only marginally by soil heating. The results show a clear down-regulation of carbon allocation to the AM fungal partner under carbohydrate-limiting conditions in the host plant. These results indicate firstly, that PAR is a dominant factor influencing the growth and activity of AM fungi and secondly, that fungal species show species-specific responses to temperature that may influence AM fungal community structure as climates change.

P2 118

Some ultrastructural aspects of the Anadenanthera falcata Speg. mycorrhizae.

E. Gross, L. Cordeiro and F. H. Caetano UNESP, Rio Claro, Brazil

Anadenanthera falcata Speg. (Mimosoideae), is a woody leguminous tree of Brazilian savanna that presents symbiotic association with arbuscular mycorrhizal fungus. In this environment, poor in some nutrients, mycorrhizae can contribute to the nutrition of the host leguminous improving nutrient uptake and helping in plant adaptation. Morphology and structure of *Anadenanthera falcata* mycorrhizae were observed by transmission and scanning electron microscopy in this study. Samples of roots were collected from pot cultivated plants and fixed in glutaraldehyde 2.5% in 0.1M phosphate buffer. For TEM the samples were, post-fixed in 1% osmium tetroxide and embedded in Spurr resin for a extended period, since these roots are relatively difficult to infiltrate properly. Ultrathin sections were stained and observed in a Philips CM 100 transmission microscope. For SEM the nodules fixed in glutaraldehyde 2.5% were dehydrated in graded ethanol series, critical point dried, coated with gold and then examined with Philips 505 scanning microscope. On the root surface several hyphae of varying size were observed sometimes close to epidermic papillae. These external hyphae possessed small vacuoles and some formed appressoria penetrating in epidermic cells. Arbuscules in different stages of development presented in their dense cytoplasm organelles like mitochondria, nuclei and small vacuoles with osmiophilic material inside. Cytoplasm of cortical cell with numerous organelles like mitochondria and rough endoplasmatic reticulum surrounded these arbuscules. However great part of volume of these cells was occupied by vacuole. General appearance and ultrastructure of these *Anadenanthera falcata* mycorrhizae were in agreement with pattern observed in arbuscular mycorrhizae of several plants.

P2 119

Formation of cross walls in trunk hyphae of AM fungal arbuscules and their relationship to metabolic activity

S. Dickson and S. E. Smith, Adelaide University, Australia

The regular occurrence of cross walls in trunk hyphae of arbuscules formed by five species of *Glomus* in two species of *Allium* was investigated. The cross wall examined using confocal microscopy showed that the structure was within the hypha and was intact with no central pore.

A time-course of *A. porrum* colonised by *Glomus coronatum* was conducted to relate presence of cross walls to the development and senescence of arbuscules. Mycorrhizal structures visualised using nitroblue tetrazolium as a vital stain indicated metabolic activity with counterstaining by acid fuchsin. Results showed cross walls were often formed at sites between metabolically active and inactive fungal regions. Cross wall presence was correlated with the metabolic status of the mycorrhizal unit (i.e. arbuscule branches, arbuscular trunk hypha and intercellular hypha) using an interdependence magnified intersects technique (IMIT) (Cavagnaro *et al.* 2001). IMIT analysis indicated the incidence of cross walls increased with the loss of metabolic activity.

Cross wall formation is likely to influence transfer of nutrients by allowing the delivery of P to the plant only by young metabolically active arbuscules or by preventing the possible flow of C derived from the plant out of the arbuscule. This may require a reconsideration of the previously proposed mechanisms of nutrient transfer between fungus and plant.

P2 120

Tomato: a model plant to unravel the functional implications of AM structural diversity

T.R. Cavagnaro and F.A. Smith. Adelaide University, Australia

It has long been accepted that the morphology of arbuscular mycorrhizas (AM) is under the control of the plant. However, the morphology of AM formed by *Lycopersicon esculentum* (tomato) has been found to be controlled by fungal identity. That is, the morphological type of AM formed by tomato is different when colonised by different fungal species. To date most research has focused on the development of the different morphological types, and possible physiological differences between the types have been largely ignored. Structural diversity within the one plant species (tomato) provides an exciting opportunity to gain insight into functional differences between the two morphological types. Tomato was grown in association with a range of AM fungal species using a nurse-pot system. The growth of the plants and development of the symbiosis were investigated. The results and their potential implications, focusing on structural diversity, are discussed here. Future directions for this research are also considered.

P2 121

Do arbuscular mycorrhizal fungi determine the *Arum*- or the *Paris*-type morphologies seen in different host roots?

M. Kubota’, M. Hyakumachi’ and T. P. McGonigle’ ‘Gifu University, Japan ‘Idaho State University, USA

Arbuscular mycorrhizal (AM) fungi are known to have two structural types, which were first described by Gallaud (1905). These are: (1) the *Arum*-type, defined on the basis of intercellular longitudinal hyphal growth in the root cortex; and (2) the *Paris*-type, defined by cell-to-cell growth of intracellular hyphal coils. The anatomical characteristics of host roots are thought to influence these structural types. In a previous study, we determined that a deciduous tree, *Clethra barbinervis* Sieb. et Zucc. (Ericales, Clethraceae), has an AM status with the *Paris*-type morphology. In this study, we confirmed that artificially inoculated AM fungi, *Glomus* spp. and *Gigaspora margarita,* which are known to form the *Arum*-type structure in cucumber and tomato, also exhibited the *Paris*-type structure in *C. barbinervis*. On the other hand, we found not only the *Arum*-type, but also the *Paris*-type morphology in cucumber and tomato roots grown in soils collected from around *C. barbinervis* trees. These results suggest that AM fungi themselves may also determine the hyphal structures in different host roots.

P2 122

Asphodelus fistulosus and *Glomus coronatum:* physiological studies of a *Paris*-type AM

F.A. Smith[1], T.R. Cavagnaro[1], S.M. Ayling[1,2] and S.E. Smith[1]
[1]Adelaide University, Australia [2]Cross, United Kingdom

There are two main morphological types of arbuscular mycorrhizas (AM), the *Arum*-type and the *Paris*-type. Although the *Paris*-type is reported to occur in more plant families than the *Arum*-type, most research has focused on the latter, probably because it is formed by most crop species. Given this lack of knowledge about what is potentially the more common type, there is a need for physiological studies of *Paris*-type AM.

The work presented here investigated the physiology of the *Paris*-type AM formed by the common weed *Asphodelus fistulosus* (‘onion-weed’) and *Glomus coronatum*. Plants were grown in a range of soil P concentrations and harvested after six and nine weeks. The morphological development of colonisation and growth of the plants were assessed. With low soil P, mycorrhizal plants grew better than nonmycorrhizal plants. With higher soil P, nonmycorrhizal plants were larger. The implications of the results for the functionality of this *Paris*-type AM association are discussed in terms of costs and benefits to the partners, and especially with respect to the weedy life-style of *A. fistulosus.*

P2 123

The interface between the arbuscular mycorrhizal fungus *Glomus intraradices* and root cells of ginseng: a *Paris*-type mycorrhizal association.

R. Larry Peterson and Lara Armstrong Department of Botany, University of Guelph, Guelph, Ontario, Canada

Two major types of arbuscular mycorrhizal associations, the *Arum*-type and the *Paris*-type have been described based on morphological features. Although the *Paris*-type is the most common, it is the *Arum*-type that has been most intensively studied in terms of structure/function because of its prevalence among important crop plants. The interface between root cell cytoplasm and the extensive hyphal coils and the arbuscules that develop from them that typify the *Paris*-type arbuscular mycorrhiza was studied. Using immunofluorescence techniques combined with laser scanning confocal microscopy, dramatic changes in the cytoskeleton were observed as hyphal coils and arbuscules developed. Both microtubules and actin filaments were closely associated with hyphal coils and the fine arbuscule branches and both may be involved in the organization of a perifungal membrane and interfacial matrix material. Using various affinity methods, an interfacial matrix consisting of cellulose and pectins was demonstrated between the perifungal membrane and the fungal cell wall of hyphal coils. There was less matrix material around the arbuscule branches. The deposition of interfacial matrix material results in an apoplastic compartment, similar to that shown for *Arum*-type mycorrhizas, between host cell cytoplasm and fungus. It remains to be shown, however, what role this apoplastic compartment plays in *Paris*-type mycorrhizas and if fungal coils are involved in nutrient transfer between the symbionts.

P2 124

Characterization of the ectomycorrhizal (*Paxillus involutus*) plant/fungus interface

H. Rouhier and B. Söderström, Lund University, Sweden

Most forest tree species of temperate and boreal regions form ectomycorrhizal associations. One characteristic of association is the Hartig net, a region of intimate contact between fungal mycelium and root epidermis and cortex cells. Nutrient exchange between the ectomycorrhizal symbionts must occur across the interfaces in this Hartig net region. The aim of the presented work is to describe and quantify the area of interface shared by ectomycorrhizal symbionts. For this purpose, pine (*Pinus contorta*) and birch (*Betula pendula*) seedlings were grown in association with the ectomycorrhizal fungus *Paxillus involutus* in dual culture systems. Mycorrhizal root tips were cut into thick sections (25-30 μm) and stained with chlorazol black E for light microscopical examination. Linear measurements of features were made on light microscopical images using image analysis software. Based on these linear measurements the potentially active area of the plant/fungus interface was calculated for different zones within a mycorrhizal root tip. Differences between the two ectomycorrhizal associations in the structure and area of interface are presented.

P2 125

Visualisation of Ectomycorrhizal Rhizomorph Structure Using Laser Scanning Confocal Microscopy

Peter Schweiger[1], Hervé Rouhier & Bengt Söderström; Microbial Ecology, Lund University, Sweden; [1]Chemical Physiology of Plants, Vienna University, Austria.

The extramatrical mycelium of many ectomycorrhizal fungi aggregates into linear, multi-hyphal organs. These rhizomorphs are an effective means of inoculum spread through soil. They also are organs of carbon, nutrient and water translocation. Their structure ranges from undifferentiated, with loosely interwoven hyphae of equal diameter, to highly differentiated, with centrally arranged thicker hyphae. Since rhizomorph structure is considered a fairly conserved taxonomical feature it is one of the most valuable anatomical characters for the classification of ectomycorrhizas.

A method for the observation of the 3D structure of intact ectomycorrhizal rhizomorphs was developed. The method is based on a combination of clearing the material with KOH followed by staining with congo red and subsequent imaging under a laser scanning confocal microscope. The images obtained are of a much higher 3D resolution than than those obtained previously by use of conventional light microscopical techniques. Structural observations from highly differentiated rhizomorphs formed by *Paxillus involutus* and from undifferentiated rhizomorphs formed by an unknown pink isolate are presented. Applications of the method are briefly discussed.

P2 126

The morphology and anatomy of two mycoheterotrophic species in central British Columbia: Fungal specificity north of 50!

H.B. Massicotte[1], L.E. Tackaberry[1], L.H. Melville[2] and R.L. Peterson[2]
[1] University of Northern British Columbia, Prince George, B.C., Canada
[2] Department of Botany, University of Guelph, Guelph, Ont., Canada

Monotropa uniflora and *Pterospora andromedea* were sampled from a sub-boreal forest ecosystem in central B.C. In both *Monotropa* and *Pterospora*, mycorrhizal root clusters belonging to the same plant were morphologically and anatomically similar. Scanning electron microscopy of the root surface of *M. uniflora* showed an abundance of mantle cystidia and the presence of intra and extramatrical crystals, presumed to be calcium oxalate, in the fungal mantle. In *P. andromedea*, surface hyphae of the mantle were extensively covered with globular accumulations, also presumed to be calcium oxalate. Light microscopic examination of sectioned material of both revealed intracellular penetration of epidermal cells via a penetration peg, characteristic of monotropoid mycorrhizas. These structural aspects were similar to those described in the literature. Phylogenetic analysis suggested all *M .uniflora* mycorrhizae nested within the Russulaceae. *P. andromedea* clustered uniquely with the *Rhizopogon subcaerulescens* group. Structural and molecular evidence suggest that *M.uniflora* and *P. andromedea* form mycorrhizal associations with a limited number of fungal species well into their northern range in western North America.

P2 127

Mycorrhizas of the Ladyslipper Orchids (*Cypripedium*), Orchidaceae)

L. Melville[1], Carla D. Zelmer[2] and R. Larry Peterson[1] [1]Department of Botany, University of Guelph, Guelph, Ontario, Canada [2]Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada

There has been debate regarding the mycorrhizal status of the Ladyslipper Orchids (*Cypripedium spp.*). Like all known orchids, *Cypripedium* species are dependent upon symbiosis with particular soil fungi for their early growth in nature. This dependency has been said to decline during maturation of plants, such that mature *Cypripedium* specimens are rarely mycorrhizal. We examined and quantified the mycorrhizal status of wild plants of four *Cypripedium* species (*C. reginae, C. acaule, C. parviflorum, C. arietinum*) collected in Ontario and Manitoba, Canada. Our results indicate that most roots of these *Cypripedium* species are mycorrhizal and are frequently heavily colonized by mycorrhizal fungi. There is a diversity of symbiotic fungi participating in the symbiosis with *Cypripedium*, including *Rhizoctonia s.l.* and unidentified clamp-connection bearing fungi. Evidence for sampling error as the source of the apparent decline in mycorrhizal frequency with plant maturity for *Cypripedium spp.* is presented. These findings have important implications for the conservation of *Cypripedium spp.*

P2 128

Morphological classification of Monotoropoid mycorrhizas on *Monotropastrum globosum*

Y. Matsuda1, A. Yamada2, 1 Mie University, Japan, 2 Shin-shu University, Japan

The achlorophyllous plant *Monotropastrum globosum* is a mono-specific species in the Monotropoideae distributed in East Asia from the Himalayas to Japan and must depend on fungal root symbionts for the supply of carbon sources. This study was conducted to identify the fungal symbionts associated with *M. globosum.* A total of 29 individuals were collected at 5 different forests in the central Japan. The root system of *M. globosum* was intermingled within and among individuals forming root balls. The pattern of root branching was simple and found to be less than third order lateral roots. On a light microscopy, fungal penetration pegs were frequently observed at the outer cortical cells as well as the presence both of fungal sheath and Hartig net. This confirmed that *M. globosum* forms monotropoid mycorrhizas. Ten mycorrhizal types were classified based on morphological differences observed on the surface layer of fungal sheaths. Among them, fungal symbionts of 3 types possessed net prosenchyma- or felt prosenchyma-like fungal arrangements with characteristic shapes of cystidia and were considered belonging to the genus *Russula*. In addition, one type may also be colonized by *Russula* sp. because of a distinctive hyphal arrangement forming regular synenchyma with intracellular latex. Laticiferous hyphae were distributed over the fungal sheath of one type suggesting that causal fungus would be a member of *Lactarius*. These results indicate that fungal symbionts associated with *M. globosum* are partly specialized in members of the Russulaceae.

P2 129

A mycorrhiza-defective mutant tomato (*Lycopersicon esculentum Mill.*) forms functional mycorrhizas with *Glomus versiforme*

K. H. Poulsen[1], L-L. Gao[2], , S. E. Smith[2] and F. A. Smith[2].
[1]Aarhus University, Denmark and [2]Adelaide University, Australia

An arbuscular mycorrhiza-defective mutant (*rmc*) in tomato, excludes most species of AM fungi tested. However, *Glomus versiforme* can partially overcome the resistance and colonise relatively normally, including production of arbuscules. We investigated whether the mycorrhiza formed between *rmc* and *G. versiforme* could promote the phosphorus (P) uptake and growth of the plants at two P levels and at two harvest times. Wild-type tomato cv. 76R was included as a control plant genotype and *G. intraradices* as a fungal species which fails to colonise *rmc*. Non-mycorrhizal wild-type and *rmc* tomato responded to added P similarly. Colonisation of wild-type tomato by the two fungi was extensive (~90% at 9 weeks), structurally normal and reduced at the higher P supply, as expected. At low P supply, P uptake was increased, accompanied by a positive mycorrhizal growth response for both fungi by 9 weeks. G. *intraradices* failed to colonise *rmc* and had no effect on P uptake or plant dry weight. *G. versiforme* colonised *rmc* more slowly than wild type (40% at 9 weeks). P uptake by *rmc* was unaffected by *G. versiforme* at 6 weeks and slightly increased at 9 weeks. There was no effect on plant growth at low P and a marked depression at high P. The results suggest that the mycorrhiza formed by *G. versiforme* is capable of increasing P uptake but that the benefits in terms of plant growth may be reduced compared with the wild-type interaction.

P2 130

P2 131

Macro (N, P, K, Ca and Mg) and micronutrient (Fe, Mn, Cu and Zn) content of mycorrhizal and nonmycorrhizal micropropagated *Castanea sativa* Mill. plants along three months of *in vitro* growth.

Anabela Martins*, Paula Baptista, M: J. Sousa and M. S. Pais**. *Escola Superior Agrária de Bragança. Qta. de Sta. Apolónia. 5300 - 855 Bragança. Portugal. ** Laboratório de Biotecnologia Vegetal - ICAT, Campo Grande, 1749 - 016, Lisboa, Portugal.

Castanea sativa Mill. micropropagated plants were inoculated *in vitro* with *Pisolithus tinctorius*. Macro (N, P, K, Ca and Mg) and micronutrient (Fe, Mn, Cu and Zn) contents in the plants were analysed separately in roots, stems and leaves (every 10 days) from 10 to 90 days.

In all plant organs, total N content is higher in mycorrhizal plants (M) since the 20th day of mycorrhizal synthesis. Total and inorganic P is higher in M plant organs after 20 days. Organic P is higher in M roots and stems after 40 days and in leaves after 60 days of symbiosis. K content is higher in M roots since 10 days of mycorrhizal synthesis and in M stems and leaves after 20 days. Ca content is higher in M roots and stems but in leaves there is no difference in Ca content between M and NM plants. Mg is higher in M roots and leaves after 30 and 40 days of symbiosis respectively but in stems there is no differences along period of trial.

Micronutrient contents present a different pattern. Fe is higher in M plant organs since 20 days of trial, while Mn and Zn contents just show increased values in roots. Cu is higher in M roots and leaves, but lower in stems, resulting in an increased Cu content of M plants.

The differences in plant nutrient content are discussed in order to evaluate the advantage of mycorrhizal inoculation for micropropagated plant production.

P2 132

Nitrogen transfer between Australian native *Eucalyptus* and *Casuarina*: ^{15}N labelling experiments

Xinhua He[1], George Stewart[2], Hock Ng[1] & Christa Critchley[1]
[1]The University of Queensland and [2]The University of Western, Australia

Plants make biomass from sunlight and carbon, but their roots often cannot access enough other nutrients for this. Symbiotic fungi can extract nutrients from soil, but cannot assimilate carbon. Plants and fungi collaborate mutualistically to form mycorrhization, the fungi gaining photosynthetically-fixed C from plants and pumping soil nutrients into the plants in return. Moreover, roots can be interconnected by a common mycorrhizal network for potential nutrient exchange. The general hypothesis is that N flows from N-rich N-fixer (source) to N-poor non-N-fixer (sink). N-transfer between Australian native plants has not been investigated.

N cycling and mycorrhization are substantial in Australian *Casuarina* and *Eucalyptus* communities. Could N be shifted from *Casuarina* to *Eucalyptus*, or vice versa? Surprisingly, we found that significant N-transfer occurred from *Eucalyptus* to *Casuarina*, but not from *Casuarina* to *Eucalyptus*, and that the transfer was affected by mycorrhization and/or N-fixation. We suggest that mycorrhizae associated with *Eucalyptus* can donate N to the N-fixer *Casuarina* because of different N requirements. It is the mycorrhization together with N availability and requirements, not the N-fixing nature of plants, that play a vital role in re-distribution and flux direction of N. Our report challenges our current view of nitrogen dynamics in plant communities.

P2 133

P2 134

Nitrogen assimilation in a mycorrhizal *in vitro* culture system

J.-P. Toussaint[1,2], M. St-Arnaud[1], C. Charest[2] [1]I.R.B.V., Jardin botanique de Montréal, Canada; [2]Dept. of biology, University of Ottawa, Canada

Arbuscular mycorrhizal fungi (AMF) are known for their beneficial effect in plant nutrition, disease resistance and physiological stress tolerance. Previous studies have shown the importance of AMF in nitrogen (N) uptake and assimilation. The difficulty of growing AMF aseptically has hindered the experimental characterization of N metabolism in mycorrhizal symbiosis. Our main objective is to analyze nitrogen metabolism in the AMF and host roots using a compartmented *in vitro* culture system which allows accurate control of experimental conditions. Key enzyme activities (GS, GDH and NR) involved in nitrogen metabolism were determined in roots of *Daucus carota* L. grown with or without *Glomus intraradices* Schenck and Smith. The results show that AMF colonization increased the GDH activity in host roots, but not GS and NR activities. However, the addition of NH_4NO_3 to the hyphal compartment (not directly available to the roots) induced the same level of GS, GDH and NR activities as in non-mycorrhizal roots which had direct access to the N source, and significantly higher level of enzyme activities than roots which received a control water treatment. The total N content in non-mycorrhizal roots was higher than in mycorrhizal roots but similar to extraradical hyphae. This suggests that AMF transfer nitrogen to host roots. Further studies on N transfer are presently in progress.

P2 135

Genetic variation in growth and nutrient uptake of mycorrhizal and non-mycorrhizal *Picea abies* seedlings.

S. Mari[1], A. Jonsson[1], G. Eriksson[1] and R. Finlay[2]. [1]Department of Forest Genetics, & [2]Department of Forest Mycology and Pathology. SLU, Uppsala Sweden.

Genetic variation in growth and nutrient uptake in mycorrhizal and non-mycorrhizal seedlings from 30 open-pollinated families of *Picea abies*, was investigated at two levels of nitrogen in two experiments. Six-week old seedlings were transplanted into 1.7-L pots filled with pumice and half of them inoculated with the ectomycorrhizal fungus *Laccaria bicolor*. The experimental design consisted of a 2x2 factorial combination of the presence or absence of the fungus and two levels of N availability (25 mg N/L and 200 mg N/ L) during the initial two weeks of establishment. Plants then received no additional water or nutrients for the following 10 weeks. At harvest (week 18) final height, shoot and root dry weight, amounts of N & P in the roots and shoots, and amounts of ergosterol in the roots were determined. There was a strong treatment effect on most growth traits at both levels of N availability. They ranked in increasing order: low-N, low-N+mycorrhiza, high-N, high-N+mycorrhiza. Lower ranking families were more positively influenced by mycorrhiza symbiosis than higher ranking families. Significant differences among families were found for most traits in treatments without mycorrhiza. Mycorrhiza strongly reduced family variance components for most traits. Despite large effects on plant growth there was no significant difference in N uptake between mycorrhizal and non-mycorrhizal seedlings at low N availability, suggesting that *Laccaria bicolor* was not efficient at transferring N to its host plant.

P2 136

Nitrate reductase regulation in the ectomycorrhizal fungus *Tuber borchii* Vittad. cultured on different nitrogen sources

M. Guescini, L. Vallorani, R. Pierleoni, C. Sacconi, S. Zeppa, F. Palma, A. Amicucci and V. Stocchi. University of Urbino, Istituto di Chimica Biologica "Giorgio Fornaini", Urbino - Italy.

Tuber borchii Vittad. is an ectomycorrhizal fungus able to establish symbiosis with roots of some trees and shurb species. Ectomycorrhizal fungi contribute to the nitrogen nutrition of their host plants, but not information is available on the molecular control of their nitrogen metabolism. Inorganic nitrogen is one of the main environmental factors limiting the growth of trees in many temperate forests. In order to investigate nitrogen regulation in symbiotic tissues and the possible influence of the host plant in this process, the cloning and pattern transcriptional regulation of *T. borchii* nitrate reductase gene are presented. Nitrate reductase (NR) catalyses NADPH-reduction of nitrate to nitrite and play a pivotal role in the integration of metabolism by governing flux of reduced nitrogen by several regulatory mechanisms. As in most other organisms this enzyme resulted to be substrate inducible. When nitrate was the sole nitrogen source, the level of NR enzymatic activity was 10-15 $nmol^{-1}mg\ protein^{-1}$,while no activity was observed when nitrogen source was ammonium. Nitrate reductase gene TbNR1 from *T. borchii* mycelium was isolated from a lambda EMBL4 genomic DNA library. As a probe 356 bp DNA fragment synthetized by PCR from *T. borchii* genomic DNA was used. The predicted protein presents high identity with nitrate reductases from other organisms. Further studies are in progress in order to understand the role of this enzyme in the mycorrhizal phase.

P2 137

Carbon uptake during the symbiotic, asymbiotic and presymbiotic stages of the AM fungus *Glomus intraradices*

Séjalon-Delmas N, Rogister I, Jauneau A, Roux C, and G. Bécard. University Paul Sabatier, Toulouse, France

AM fungi are obligate biotrophs unable to take up carbon source saprophytically in their environment. Labelling experiments with 13C-glucose and NMR spectroscopy (1) showed that intra- and extra-radical hyphae behave like a metabolic bipole (2). Intraradical hyphae are able to take up glucose, whereas extraradical one seem to be devoid of this function. Our studies on 14C-glucose labelling and on enzymatic activities (pyruvate kinase, glucose-6-phosphate isomerase, 6-phosphogluconate deshydrogenase) confirmed that extraradical hyphae cannot take up glucose from the medium (whatever the sugar concentration used), and that glucose is not used as a source of carbon and energy in this fungus compartment. These results suggest that during the symbiotic stage, sugar transport and metabolism in AM fungi are regulated by plant factors. In contrast, germinating spores exhibit natural ability to take up glucose at low external concentrations (3). However, we showed that this transport was inhibited with high glucose concentrations, suggesting a possible catabolic repression of the sugar transporters.

The glucose transport ability of *G. intraradices* during the asymbiotic stage was compared with this of a saprophyte (*Mucor mucedo*). It was also compared with this of the presymbiotic stage, in the presence of stimulatory root exudates.

1) Pfeffer P.E et al. 1999. Carbon uptake and the metabolism and transport of lipids in and arbuscular mycorrhiza. Plant Physiol. 120: 587-598.
2) Bago B. et al. 2000. Carbon metabolism and transport in arbuscular mycorrhizas. Plant physiol. 124: 947-959.

P2 138

A holistic view of chanterelle carbon transfer

J. Ignacio Rangel-Castro, Eric Danell
Department of Forest Mycology and Pathology, SLU, Uppsala, Sweden.

The photosynthetically produced sucrose is broken down into glucose and fructose in the mycorrhizal host-fungus interface. Our ^{1}H-NMR analyses of mycelia in liquid culture indicate that the chanterelle mycelium utilised glucose before fructose. Glucose was transformed into mannitol, trehalose, and amino acids. Using ^{13}C-NMR, mannitol and trehalose were also found in chanterelle exudates. A major obstacle in chanterelle isolation, is the constant presence of millions of bacteria in fruit body tissues. The basis for this association has been unknown. We found that bacteria isolated from the inside of fruit bodies and other biotopes were able to grow *in vitro* using mannitol and trehalose as the sole sources of carbon. We have found that *C. cibarius* is not a proteolytic fungus (sensu Abuzinadah & Read, 1986), but the bacteria associated with chanterelles have this ability. Bacteria associated with vegetative mycelium may therefore circumvent the disability of the chanterelle to use organic nitrogen. We can now propose one feasible explanation for the relationship between chanterelle and its associated fluorescent *Pseudomonas*.

P2 139

Respiratory metabolism of rhizosphere dissolved inorganic carbon in arbuscular mycorrhizal roots under P starvation

A. J. Valentine and M. D. Cramer Dept of Botany, Uni of Stellenbosch, South Africa

Inorganic C in the soil exists not only as gaseous CO_2 but also as a pH dependent combination of dissolved CO_2 (i.e. H_2CO_3), HCO_3^- and CO_3^{2-}, collectively referred to as dissolved inorganic C (DIC). Rhizosphere DIC concentrations play a vital role in root respiratory C metabolism. During P stress, a suite of respiratory bypass reactions are initiated, which includes a glycolytic step that favours DIC incorporation and the engagement of an alternative pathway of mitochondrial electron transport. The influence of AM colonization on DIC utilisation was studied in terms of P supply. Root respiration and DIC metabolism were measured in six week old P-sufficient (2 mM) and P-stressed (2 μM) arbuscular mycorrhizal (AM) tomato plants, grown in hydroponic culture. The roots were supplied with elevated root zone DIC, by aerating the nutrient solution with 5000 ppm CO_2. During P stress, AM roots had higher DIC incorporation than non-AM roots, while roots supplied with high P, showed no difference between AM and non-AM roots. In the AM plants under P stress, the majority of the incorporated DIC was in the organic acid fraction in the roots and after subsequent xylem translocation, also in the shoots. Under P stress, the higher % cytochrome pathway (Vcyt) and the lower % alternative pathway (Valt) activities in the AM roots relative to non-AM roots, indicate that AM colonisation can reduce the engagement of mitochondrial respiratory bypasses under P stress. It is concluded that the engagement of glycolytic and mitochondrial respiratory bypass reactions in P starved roots are strongly influenced by root AM status.

P2 140

A laboratory study of ectomycorrhizal fungi and belowground carbon transfer: problems and constraints.

Philip, L.J.[1], and M, Jones[1]. [1]Biology Department, Okanagan University College, 3333 College Way, Kelowna, British Columbia, Canada, V1V 1V7.

Mycorrhizal fungi are a large group of soil fungi that form symbiotic associations with plant roots. Since many mycorrhizal fungi have broad host ranges, these fungi can colonize and subsequently link the root systems of plants of the same (intraspecific) and different (interspecific) plant species. Mycorrhizal hyphal connections have been studied extensively in the context of belowground carbon, nitrogen and phosphorus transfer. Carbon transfer has been of particular interest. Although net belowground transfer between ectomycorrhizal plants has been demonstrated, the biological importance of transfer through ectomycorrhizal hyphae relative to transfer through soil pathways is uncertain. Root-box microcosms, isotope labeling and autoradiography have been applied to the study of carbon transfer in the laboratory and glasshouse while few field investigations of belowground carbon transfer have been completed. When, paper birch <Betula papyrifera Marsh.> and Douglas fir <Pseudotsuga menziesii Mirb. Franco.> were grown together in the laboratory no significant differences between carbon transfer through hyphal pathways and soil pathways were found. Prompted by this study, belowground carbon transfer between ectomycorrhizal paper birch and Douglas Fir seedlings is now being studied using root-box microcosms, root restrictive pouches and dual isotope labeling in a glasshouse setting. Preliminary data will be used to discuss positive and negative aspects of the use of root –boxes in the study of carbon transfer between these seedlings. Additionally, constraints related to visualizing hyphal connections and identifying ectomycorrhizal fungi will be considered for both morphotyping and molecular techniques.

P2 141

Growth depression of mycorrhizal *Citrus* seedlings grown at high P supply is mitigated by elevated CO_2

James H. Graham, John L. Jifon, Diana L. Drouillard, and James P. Syvertsen. *University of Florida, Citrus Research and Education Center, Lake Alfred, FL, USA*

Arbuscular mycorrhizal (AM) fungi enhance phosphorus (P) uptake and plant growth when soil P supply is limiting, but at high P, root colonization can result in growth depression. Effect of root colonization by *Glomus intraradices* at high soil-P supply and elevated CO_2 on shoot and root gas exchange and growth of sour orange (*Citrus aurantium* L.) and sweet orange *(C. sinensis* L. Osbeck) seedlings was studied over 11 weeks. Seedlings were either inoculated with *G. intraradices* or not inoculated (NM), and grown at elevated (eCO_2) or ambient CO_2 (aCO_2) partial pressures. Net photosynthetic rate (Pn) of both genotypes was enhanced by eCO_2. Pn of AM sour orange was higher than that of NM plants at eCO_2, but Pn of AM and NM sour orange was similar at aCO_2. Pn of sweet orange was not altered by AM colonization. At aCO_2, growth of AM sour orange was depressed (18%) compared to NM seedlings, but at eCO_2, AM plants were 40% larger than NM plants. Onset of growth depression of sour orange coincided with increase in AM root colonization, greater rhizosphere respiration rates, reduced starch concentration in roots, and lower plant relative growth rates. In contrast to sour orange, gas exchange and growth of sweet orange seedlings were largely unresponsive to AM colonization. Results confirm previous predictions that greater mycorrhizal dependency (MD) at low P is related to levels of growth depression at high P supply. Growth depression at aCO_2 in sour orange (the genotype of higher MD) is attributed to greater C utilization in roots of AM sour orange than AM sweet orange. Increased Pn of sour orange at eCO_2 more than compensates for the C expenditure on mycorrhizas. Different response of *Citrus* genotypes spp. suggests that C expenditure on AM symbiosis is more tightly regulated in citrus of low MD than in genotypes of high MD.

P2 142

Phosphorus uptake by a community of arbuscular mycorrhizal fungi from the jarrah forest

M. Z. Solaiman[1], L. K. Abbott[1], I. Jakobsen[2], and H. Lambers[3] [1]Soil Science & Plant Nutrition, The University of Western Australia, Australia, [2]Plant Biology and Biogeochemistry, Risø National Laboratory, Denmark., [3]Plant Sciences, The University of Western Australia, Australia.

Jarrah (*Eucalyptus marginata*) forest is unique to the south-western corner of Western Australia. The ecosystem is complex, having low soil fertility, in particular the limited availability of nutrients such as N and P. Arbuscular mycorrhizal associations are assumed to be important in natural ecosystems of this forest but it is difficult to assess. Communities of indigenous AM fungi are expected to alter phosphorus uptake and biomass productivity in relation to characteristics of their contrasting life-cycles and modes of interaction and to differential associations with host plants. This research investigated differences among three AM fungi from different genera isolated from the jarrah forest with respect to their colonisation, biomass production and P uptake over time. We examined differences in their capacity to access P from increasing distances from the root. Phosphorus sources in root-exclusion mesh bags were inserted 2, 4 and 6 cm from plants restrained in separate mesh bags. We grew an understorey plant, *Phyllanthus calycinus*, that has been shown to be extensively mycorrhizal. *Acaulospora laevis* colonised plants to the greatest extent, producing higher plant biomass and P content compared to the other two fungi. Functioning of natural communities of indigenous AM fungi in intact soil cores from the jarrah forest in relation to phosphorus uptake was also investigated. Plant biomass and P uptake were enhanced where P sources were closest to the root, as expected, but there were differences among fungi. The capacity of individual AM fungi and natural communities of AM fungi to access P sources at different distances from a highly mycorrhizal native forest understorey plant will be discussed.

P2 143

AM-P

G.T.S. Baylis, Department of Botany, University of Otago, P O Box 56, Dunedin, New Zealand

In 1959 *Griselinia littoralis* became the first plant known to depend on arbuscular mycorrhizas (AM) for phosphorus (P). Now it is accepted that in most natural vegetation the limiting nutrient is P and extraction is by root hairs or mycorrhizal hyphae. Mycorrhizal dependency is inversely correlated with root hair length. It is zero in families with the longest hairs and maximum in those without them. Only hairs and hyphae can penetrate the finest soil pores to reach immobile P and extending them is the only economical way of generating the length of root system required to pervade the soil completely. Extending even a hair-like root involves generating a cross-section of about 50 cells, but root texture probably influences the length of soil mycellum required and thus the relative effectiveness of endophytes. We have selected cultivars on enriched soils and they require internal levels of P for growth much higher than suffices in the wild. Reselection is necessary if full use is to be made of AM in cropping and deleterious effects avoided.

P2 144

Possible involvement of hyphal phosphatase in phosphate efflux from intraradical hyphae isolated from mycorrhizal roots colonized by *Gigaspora margarita*

T. Kojima and M. Saito. National Grassland Research Institute, Japan

We developed the method to isolate physiologically active intraradical hyphae. The enhancement of phosphate efflux form the intraradical hyphae was found with the addition of glucose (*New Phytol.* in press). In the present study, the phosphate efflux from the isolated intraradical hyphae was examined in relation to hyphal phosphatase activity. Arbuscular mycorrhizal onion plants (*Allium cepa*) inoculated with *Gi. margarita* were grown. Six weeks after transplanting, the intraradical hyphae were isolated using plant cell-wall digestion enzymes from the mycorrhizal roots. Then this hyphae was incubated at 25 with gently shaking in the buffer (50mM Tris / HCl, pH7.4) with various phosphatase inhibitors ($BeSO_4$, KCN, Na_2MoO_4). Phosphate efflux, phosphate released to the buffer, was analyzed by EnzChek phosphate assay kit (Molecular probe). Hyphal phosphatase activity was stained histochemically and a proportion of phosphatase active arbuscules was examined. The degree of inhibition in the arbuscular phosphatase was related to the decrease in the phosphate efflux. These results implied that hyphal phosphatase might be involved in the phosphate efflux process from intraradical hyphae at arbuscule.

P2 145

Phosphor imaging as a tool for non-invasive, real time studies of nutrient transport in arbuscular mycorrhizas

I. Jakobsen[1], J.S. Nielsen[1], E.J. Joner[2], S. Declerck[3] [1]Risø National Laboratory, Roskilde, Denmark, [2]Centre de Pédologie Biologique, Vandoeuvre-les-Nancy, France, [3]Mycothèque de l'Université Catholique de Louvain (MUCL), Louvain-la-Neuve, Belgium.

Studies of nutrient transport dynamics in arbuscular mycorrhizas using tracers and sequential, harvests, require many replicates to obtain an adequate time resolution. The mycorrhizas must furthermore be sub-sampled to study the spatial distribution of the tracer. These problems are overcome by using non-invasive techniques, which allow for real time studies of transport. Such non-invasive technology was applied to Petri plate-based, root-organ cultures with either of two *Glomus* isolates in order to study P translocation. The root-external mycelium grew into a root-free compartment and P was supplied as either $^{32}P_i$ or α^{32}P-CTP. Time sequences of digital autoradiograms were obtained by a phosphor imager and frames were assembled to create a time lapse animation of P translocation. Radioactivity was quantified by densitometry, and total transport from one compartment to the other at a certain time was used to calculate the P flux rates in living hyphae crossing the barrier. Hyphae and roots were harvested at the end of the experiment and results of scintillation counting corresponded to those obtained by densitometry. Radiograms were further compared to light-field scanning images of mycelium and roots in order to localise P spatially. Uptake of the inorganic P was fast enough to create depletion zones in the solidified synthetic growth medium and rate of P uptake appeared to differ between the fungi. However, both fungi took up only small quantities of ^{33}P from the organic source.

P2 146

Phosphate pools and metabolism in arbuscular mycorrhizal fungi studied by ^{31}P NMR spectroscopy

N. Rasmussen[1], P. E. Hansen[2] and I. Jakobsen[1] [1]Risø National Laboratory, Roskilde, Denmark. [2]Roskilde University, Roskilde, Denmark.

Knowledge about the pathways by which phosphate (P) is taken up and translocated towards the host plant by arbuscular mycorrhizal fungi (AMF) is limited. So far, only invasive biochemical and histochemical methods have been used to investigate the mechanisms. *In vivo* ^{31}P NMR may therefore provide new information since it is a non-invasive and non-destructive technique, which allows for real time studies of metabolic processes in a tissue. The ^{31}P NMR spectra of excised *Glomus intraradices* mycelia and of mycorrhizal cucumber roots contained signals from polyphosphate (polyP), which were absent in the spectra of nonmycorrhizal roots. This confirmed that the P taken up by the fungus to some extent was transformed into polyP. Results of a time course study will be presented. Its aim was to detect the location of the P species (cytoplasm/vacuoles) at different times after the supply of P to mycelia, which had previously received different amounts of P. We also present results on interfungal variation in polyP content as measured by ^{31}P NMR.

P2 147

Influence of early stages of arbuscular mycorrhiza on uptake of zinc and phosphorus by red clover from a low-phosphorus soil amended with zinc and phosphorus

Yinli Bi[1] and Peter Christie[2]
[1]China University of Mining and Technology(Beijing Campus), Beijing, 100083,China
[2]Queen's University of Belfast, UK

In a pot experiment, red clover (*Trifolium pratense*) was grown in sterilized Zn-amended low available P soil (0, 50 or 400 mg Zn kg^{-1}) with or without 100 mg kg^{-1} added P and with or without inoculation with the arbuscular mycorrhizal (AM) fungus *Glomus mosseae*. When the plants were harvested after 40 days, AM colonization of the roots was still at an early stage, with only 14-38% of total root length colonized on average. AM colonization was highest in low-P soil, and was lowest in soil amended with 400 mg Zn kg^{-1}. Shoot yields were higher in AM plants but root yields were unaffected by AM inoculation. Shoot and root yields were higher with 100 mg added P kg^{-1} soil, but lower with 400 mg Zn kg^{-1} than 50 mg Zn kg^{-1} or controls unamended with Zn. Shoot and root P concentrations were higher in AM plants, but shoot P offtakes were higher in AM plants. Concentrations of Zn and Cu were much higher in the roots than in the shoots. Shoot and root Zn and shoot Cu were lower, but root Cu was higher, in AM plants. Soil residual pH after plant growth was higher in AM treatments, and residual total Zn was also higher, indicating lower Zn uptake by AM plants. The results suggests improvement of host P nutrition was involved in resistance of plants to heavy metal.

P2 148

Studing Mg-transport across and via mycorrhiza of Norway spruce roots

A.J. Kuhn[1], G. Jentschke[2], A. Stettien[1], B. Brandes[2], D.G. Godbold[3] and W.H. Schröder[1]
[1]Research Centre Juelich, Germany, [2]Forest Ecosystem Research Centre, Goettingen, Germany, [3]University of Wales Bangor, Bangor, UK

Mg may enter roots from the rhizosphere (i) directly across the root cortex and/or mycorrhizal structures or (ii) from distant sources via fungal hyphae. The stable isotope tracer 25Mg was used to measure the uptake of Mg into roots of intact mycorrhizal and nonmycorrhizal Norway spruce seedlings. (1) We followed the kinetics of the direct Mg entry into fine roots at the cellular level using LAMMA (laser microprobe mass analyzer) and isotope-specific imaging by SIMS (secondary ion mass spectrometry). In mycorrhizal and nonmycorrhizal fine roots, Mg rapidly exchanged between the labeling solution and cortical cell walls with a half time of < 5 minutes. Mycorrhization (unspecific soil culture, or specific with *Laccaria laccata*) resulted in only a slight reduction of the apoplastic accessibility for Mg as compared to nonmycorrhizal roots. The extensive hyphal mantle of *P. tinctorius* mycorrhizae caused a short delay of a few minutes in the kinetics of Mg uptake into the cortex. The uptake beyond the endodermis, into the stele, was significantly slower with similar half-times of 60-120 min for both mycorrhizal and nonmycorrhizal finest roots. (2) Spruce seedlings, mycorrhizal with *Paxillus involutus* Batsch were grown in a two compartment sand culture system. The compartments were separated by a 45µm Nylon mesh that could be crossed by hyphae, but not by roots. Within a 11-week labelling period 25Mg was transferred from the fungal compartment amounting to 3-4% of the total Mg in the plant. The transfer was not observed in nonmycorrhizal control plants and was strongly dependent on the presence of P-supply to the hyphal compartment.

P2 149

Circadian cycles of water transfer between host plant and mycorrhizal extraradical mycelium.

Ignacio Querejeta, Louise Egerton-Warburton, Michael F. Allen. Center for Conservation Biology. University of California Riverside, U.S.

Hydraulic lift is the process of water movement from moist to dry soil layers using plant root systems as a conduit. During drought periods, this phenomenon might contribute to keep mycorrhizas functional in nutrient-rich but dry upper soil layers as long as water is available to deeper roots. To evaluate this hypothesis, mycorrhizal coast live oak (*Quercus agrifolia*) seedlings were planted in pots with three separated compartments for upper roots, tap roots and fungal growth. Air gaps were used to prevent bulk flow of water between compartments. Eight months after planting, drought conditions were simulated in the pots by discontinuing irrigation to the upper root and fungal chambers (watering was restricted to the tap root compartment only). After 3, 12, 70 and 80 days of drought, membrane-impermeant dyes were injected at dusk into the tap root compartment of the pots. Most ecto- and AM hyphae and spores in the fungal chamber were uniformly labeled with dyes the following morning, thus indicating significant apoplastic transfer of water from plant to extraradical mycelium overnight. The uniform presence of dye along the mycorrhizal hyphae points to cytoplasmic mass flow as the mechanism responsible for water distribution. Conversely, dye transfer from plant to fungal mycelium did not occur during daylight periods, suggesting water flow direction reversal (from fungus to plant) at that time. Dead plant tissue fragments in the fungal chamber were also labeled, probably due to nocturnal water exudation from the hyphae into the surrounding soil. Saprophytic fungal hyphae in the fungal chamber were not labeled.

P2 150

Effect of two species of arbuscular mycorrhizal fungi on growth, assimilation and leaf water relations in maize (Zea mays L.).

M.R.Amerian[1],W. S. Stewart[1], H.Griffiths[2],

1- Department of Agriculture & Environmental Science, University of Newcastle upon Tyne, U.K. 2-Department of Plant Sciences, University of Cambridge

Arbuscular mycorrhizal (AM) fungi are ubiquitous components of many ecosystems and can colonise the roots of approximately two thirds of all plant species. In a greenhouse experiment, the effect of AM fungi on drought stress and recovery from drought stress was investigated in maize (*Zea mays* L.). Plants were grown with and without the fungi *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe and *Glomus interaradices* Schenck & Smith, in sandy loam soil. After three months, water was withheld from all plants for six days. During the unwatered period and after rewatering, leaf water potential, assimilation rate and stomatal conductance was measured. During the unwatered period leaf water potential and assimilation rate of mycorrhizal plants were greater than in the non-mycorrhizal plants, particularly in plants infected by *G.mossea.* Mycorrhizal plants were able to postpone the onset of wilting.

To determine the effects of infection on recovery from drought, plants were rewatered. During the recovery period leaf water potential and assimilation rate of mycorrhizal plants were higher than those of non-mycorrhizal plants, particularly in those plants that were infected by *G.mosseae.* Leaf surface areas and specific leaf area of mycorrhizal plants were generally greater than in non-mycorrhizal plants.

P2 151

Characterization and histochemical localization of *Terfezia claveryi* tyrosinase

A. Morte[1], M. Pérez-Gilabert[2], M. Honrubia[1], F. García-Carmona[2].
1. Dpto. Biología Vegetal, 2. Dpto. Bioquímica y Biología Molecular-A, Universidad de Murcia, Spain.

A fully latent polyphenol oxidase (PPO) from desert truffle (*Terfezia claveryi* Chatin) ascocarps is described for the first time. The enzyme was partially purified by using phase partitioning in Triton X-114 (TX-114). The achieved purification was 2-fold from a crude extract, with a 66% recovery of activity. The interfering lipids were reduced to 13% of the original content. In addition, the purification reduced the phenolic compounds to only 37.5%, thus avoiding postpurification tanning of the enzyme. Latent PPO was activated by the anionic surfactant sodium dodecyl sulfate (SDS) or by incubation with trypsin. The amount of SDS necessary to obtain maximum activation was dependent on the nature of the substrate. The use of SDS also permitted the histochemical localization of the latent enzyme within the ascocarp. *Terfezia* polyphenol oxidase was kinetically characterized with two phenolic substrates (L-DOPA and tert-butycatechol). The latter substrate presented inhibition at high substrate concentration with a K_{si} of 6.3 mM. Different inhibiting agents (kojic and cinnamic acid, mimosine and tropolone) were also studied, tropolone being found the most effective.

P2 152

Monophenolase activity of *Tefezia claveryi* tyrosinase

M. Pérez-Gilabert[1], A. Morte[2], M. Honrubia[2], F. García-Carmona[1]
1. Dpto. Bioquímica y Biología Molecular-A, 2. Dpto. Biología Vegetal, Universidad de Murcia, Spain.

The monophenolase activity of partially purified *Terfezia claveryi* tyrosinase (EC 1.14.18.1) is described for the first time. This enzyme is fully latent and can only be detected if SDS is present in the reaction medium. Monophenolase activity was localized within the ascocarp using histochemical techniques. A detailed kinetic study of the parameters affecting this activity was carried out. Both the charactersitic lag period and the steady-state rate are affected by pH and the enzyme and substrate concentrations. The presence of catalytic concentrations of *o*-diphenols affected the lag period but not the steady-state rate. By increasing the concentration of *o*-diphenols, it was possible to evaluate the enzyme activation constant, $K_{act'}$, which showed a value of 7.2 μM. The experimental results are compatible with the mechanism previously described for tyrosinases from other sources.

P2 153

Electrophysiology of vesicular arbuscular mycorrhizal fungi

S.M.Ayling, T.Edmonds, S.E.Smith and F.A.Smith
The University of Adelaide, Australia

Spores of many species of vesicular arbuscular mycorrhizal (VAM) fungi will germinate *in vitro* but, in the absence of a host plant, growth will cease. We do not know whether this is because the germ tube is unable to take up nutrients until it has been stimulated by a signal from a host or whether some substance is lacking in the medium.

Transmembrane electric potential differences (p.d.) of ~ -40 mV were recorded from external hyphae of three species of VAM fungi and germ tubes of *Gigaspora margarita*.

Thermodynamic calculations showed that despite these low values of p.d. efficient high-affinity uptake of phosphate is possible. Therefore the effect of phosphate on germ tube p.d. was studied. Exposure to K^+ reversibly depolarised the p.d. of germ tubes. It seems unlikely that there are major physiological differences in the physiology between germ tubes and external hyphae of VAM fungi with respect to the parameters we measured.

The p.d. of germ tubes became more negative when plant root extract was added to the medium, showing that the early stages of interaction between plant and fungus occur via direct effects on the plasma membrane rather than via effects on gene expression.

P2 154

P2 161

Effects of arbuscular mycorrhizal fungi on damage by *Striga hermonthica* on sorghum, *Sorghum bicolor*

Th.W. Kuyper and V. W. Lendzemo; Wageningen University, the Netherlands and Institute of Agricultural Research for Development, Yaounde, Cameroon

Striga hermonthica is a major yield reducing factor of cereal crops such as maize, sorghum, and pearl millet in savannah regions in Africa. For integrated management of the hemiparasite, it is important to understand the interaction of the host plant with the biotic environment. A pot experiment was conducted as a full factorial with three factors, viz arbuscular mycorrhiza (present or absent), *S. hermonthica* (present or absent) and sorghum (a *Striga*-sensitive or tolerant cultivar). In the absence of AM fungi, *S. hermonthica* reduced sorghum biomass in both cultivars, and in the absence of *S. hermonthica*, AM fungi increased biomass in both cultivars. AM colonization was well over 70%. AM fungi cancelled out damage by *S. hermonthica* in the tolerant but not in the sensitive cultivar. The number of *S. hermonthica* emerged was significantly reduced in the AM fungal treatment in the tolerant, but not in the sensitive cultivar. Emergence time of *Striga* was increased in the tolerant cultivar in the presence of AM fungi. The results show that AM fungi have the potential to reduce damage by *S. hermonthica* in at least one cultivar. It would be worthwhile to compare sorghum cultivars that differ in *Striga* tolerance for mycorrhizal responsiveness and rate of mycorrhizal colonization.

P2 162

Changes in rhizopheric biological dynamics of *Trachypogon plumosus* Ness affected by phosphate fertilization in three acid savanna soils.

J.C. López-Gutiérrez[1], M. Toro[1], & D. López-Hernández[1]
Instituto de Zoología Tropical, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela

Phosphorus (P) is one of the most limiting macronutrients in savannas. Proccesses such as P mineralization, mycorrhization and microbial activity in the rhizosphere seem to be crucial for plant adaptation to such ecosystems. We study arbuscular mycorrhizae (AM), microbial and phosphatase activity in the rhizosphere of *Trachypogon plumosus* Ness in three acid savanna soils differing in taxonomic order and P content at Estación Experimental La Iguana, Guárico State, Venezuela. In addition, we study the effect of P fertilization by applying P in a readily soluble form ($KHPO_4$) and as rock phosphate. We determine acid phosphomonoesterase activity (APA) and dehydrogenase activity (DHA) in rhizospheric samples. We also study AM dynamics in *T. plumosus*. Our results show that the three soil orders have a very low fertility and small amounts of P fertilizer affects rhizosphere dynamics in *T. plumosus* depending on soil texture, P-content and P source. AM colonization, microbial activity and in APA seem to be important adaptive processes of *T. plumosus* to savanna ecosystems. Agricultural implications of our findings are discussed.

P2 163

Correlation of VA colonization with *Cercospora coffeicola* infection in leaves of coffee seedlings.

L. Lebrón[1], D.J. Lodge[2], R.P. Rodríguez[3]. [1]Institute for Tropical Ecosystem Studies, University of Puerto Rico-Rio Piedras, [2]Center for Forest Mycology Research, USDA Forest Service, Forest Products Lab. Luquillo, PR, [3]Crop Protection Department, University of Puerto Rico-Mayagüez, Puerto Rico.

Arbuscular mycorrhizal colonization was compared in nine pairs of coffee seedlings (*Coffea arabica* L., var. *catura*) with and without lesions of *Cercospora coffeicola.* Members of each pair were growing within 13 cm of each other to control for differences in soil nutrients, microenvironment and *C. coffeicola* inoculum under field conditions. The percentage of AM root colonization (MRC) was significantly higher ($P<0.05$) in plants with than in plants without *C. coffeicola* lesions (77.3% vs. 60.6%, respectively). The mean cotyledon area affected by lesions was also positively correlated with percentage MRC ($P=0.011$), but the number of lesions per seedling was not ($P=0.30$)($N=18$). The total mean cotyledon area was significantly lower in plants with than without *Cercospora* lesions (23.6 +/- 8.2 cm^2 and 27.3 +/-8.4, respectively; $P=0.027$), and lesions further reduced the green tissue area (mean of 2.8 cm^2 per seedling). Although this study is only correlative, it is possible that higher rates of colonization by mycorrhizal fungi increased the severity of *C. coffeicola* lesions in coffee seedlings. Our data suggest that high levels of AM colonization may have a negative effect on coffee seedlings in the field. These results are consistent with those of Shaul et al., 1999 who showed VA mycorrhizal fungal colonization in tobacco increased severity of disease in the leaves by suppression of PR-protein expression.

P2 164

Interactions of arbuscular-mycorrhizal fungi and wetland plant species in the everglades ecosystem

K. Jayachandran, S. Kemp, and R.D. Jones Florida International University, Miami, FL, USA

Sawgrass (*Cladium jamaicense* Crantz) is one of the dominant species of the South Florida's wetland ecosystems. Little is known of the occurrence of Arbuscular-mycorrhizal fungi (AMF) and its role on the growth, nutritional status, and the physiology of sawgrass plants. The objectives of this study are to investigate the mycorrhizal status of sawgrass under different environmental conditions and to determine the growth response due to AMF inoculation under different soil types such as the everglades peat, marl, and prairie soils. An initial survey revealed that the soils collected nearer to the root zone of sawgrass under prairie system, showed AMF spores and root colonization. Young roots collected from peat and marl soils also exhibited mycorrhizal colonization. Further research focuses on experiments under greenhouse conditions in order to assess the ecological significance of AMF to this important native species of tropical Florida.

P2 165

Host and environment controls on arbuscular mycorrhizal communities in a moist tropical forest

C.E. Lovelock[1] and J. Morton[2], [1]Smithsonian Environmental Research Center, USA, [2]West Virginia University, USA

Different arbuscular mycorrhizal (AM) fungal communities can have variable effects on plant growth, which could influence patterns of seedling recruitment and potentially affect the maintenance of tree diversity in tropical forests. In this study we investigated the composition and distribution of AM fungal communities in a moist tropical forest within the La Selva Reserve in North Eastern Costa Rica. Spores were extracted from rhizosphere soil of 14 tree species to test whether AM fungal communities were influenced by 1) life history strategy of the host tree species, 2) host tree species or 3) soil fertility. Eleven fungal species and two conglomerate species groupings were identified. *Acaulospora* species dominated, accounting for 97% of the 5000 spores examined. Life history of host species did not have a significant influence on the AM fungal community. Most AM fungal species were present under all host tree species, but relative abundance of AM species differed depending on the host. Over the two dominant soil types within the La Selva Reserve spore abundance was significantly higher in low fertility Ultisols compared to the higher fertility Inceptisols. However AM communities were not significantly different between the two soil types. Our results show that AM fungal communities in the forest vary depending on the host trees species with which they are associated. If differences in relative abundance of AM species within an AM community influences plant growth, then variability in AM fungal communities could be a factor in maintaining forest tree diversity.

P2 166

Arbuscular mycorrhizal fungi in rain forest fragments in Sergipe, Northeast of Brazil*

M.F. Landim[1,2] and W. Heyser[1] [1] University of Bremen, UFT, Germany, [2]Universidade Federal de Sergipe, Departamento de Biologia, Brazil,

The rain forest of North Eastern Brazil is certainly one of the most endangered Brazilian ecosystems. Only fragments of variable size and level of conservation can now be found. In order to establish plans of forest recovery, it is intended to understand the role of the arbuscular mycorrhizal (AM) interactions in this ecosystem. The present study was carried out in fragments of the Atlantic rain forest of Sergipe, North Eastern Brazil. Seasonal sampling (dry and rainy season) was done in areas of forest and cocoas plantations, which occupy nowadays much of the area originally covered by these coastal forests. Two sampling sites were chosen, differing in soil type. The soil physical and chemical parameters, distribution of the spores and the degree of roots colonization in soil profiles (0-30cm) were studied, as well as the presence of root colonization in seedlings of some selected species. No clear pattern was found regarding the distribution of the spores in the soil profiles, although some variation between sampling sites, time, depth and plant cover were found. Until now species of *Gigaspora*, *Scutellospora*, *Acaulospora*, and *Glomus* were found. *Gigaspora* and *Acaulospora* species dominated, presenting the broader spatial distribution. Trap cultures were also established, in which species absent from the previously collected soil samples were identified. The isolation and multiplication of the species found are being carried aiming to compare its capacity of promoting plant growth under conditions similar to the acidic and poor soils of Northeast of Brazil.

*This research has being supported by CNPq (Research council of Brazil).

P2 167

Do Native Trees Restore Mycorrhizal Communities to Eroded Tropical Pasture?

F.L. Carpenter[1], S. Palacios[2], and E. Gonzalez[2]. [1]University of California at Irvine, [2]Universidad Nacional Autonoma de Mexico, Mexico, D.F.

We are interested in using AMF to help restore deeply eroded pasture in the humid tropics. Our study site in Costa Rica has a chronosequence of natural succession from eroded pasture through 5- and 20-year abandoned pasture, to primary forest. We measured number of spores/100g dry soil (abundance), number of spore types spores (richness diversity), and % of total spores in one genus (dominance). Spore abundance and dominance decreased, and richness diversity stayed constant along the chronosequence. High forest humidity, SOM, and darkness may reduce the need for the fungi to form spores. We experimentally planted mixed species of native trees in pasture plots to test whether tree restoration would reestablish mycorrhizal characteristics of undisturbed forest. After 5 years we compared planted plots to the unplanted controls, and to nearby primary forest. Spore abundance did not differ between planted and control plots, but forest had significantly fewer. Richness diversity and dominance did not differ among any plots. Apparently 5 years of tree regeneration were inadequate to reestablish conditions characteristic of forest, and spore abundance remains high in planted areas. Among planted plots, some plots showed poor tree growth and others excellent tree growth. No abiotic soil factors correlated with growth. To determine if AMF could explain growth differences, we compared spores in the two areas. Neither abundance nor richness diversity significantly differed in the two areas, but *Glomus* was less dominant in the better growth area. Perhaps high relative abundances of the rarer genera aid tree growth through preferences of tropical tree species for rarer fungal species.

P2 168

Ectomycorrhizal fungi associated with eucalyps in Africa and Madagascar

D. Thoen[1], M. Abourouh[2] and M. Ducousso[3], [1]Fondation Universitaire Luxembourgeoise, Arlon, Belgium. [2]Direction des Recherches des Eaux et Forêts, Rabat, Morocco; [3]Laboratoire des Symbioses Tropicales et Méditerranéennes, Montpellier, France.

Since more than one century, eucalypts have been widely introduced in Africa. Nowaday, the number of eucalypts used in African plantation trials exceeds certainly 250 species. Among them some are subjected to an extensive use as pure species or as hybrids in industrial plantations. In South Africa, during the year 1918, Van der Bijl reported for the first time, the ability of Eucalypts to bear ectomycorrhizas formed by the fungus *Polysaccum crassipes* (now known as an early synonym of *Pisolithus arhizus*). Since this discovery, the rational use of ectomycorrhizal fungi in eucalypts plantations have been poorely developped. In Africa, most ectomycorrhizal fungi found associated with Eucalypts were introduced fortuitously from Australia but, perhaps, some of them are originating from neighbouring native African forests which may be rich in ectomycorrhizal fungi.

A check list of putative ectomycorrhizal fungi associated spontaneously with eucalypts in Africa is established. This list reports : the name of the fungus ; the name of the associated eucalypt(s); the country of record ; the origin of the datas (litterature or authors personnal observations) ; herbarium material ; etc. With the exception of eucalypts plantations in Madagascar, where the associated fungal diversity is high, the level of diversity is generally very low and, in most cases, eucalypts plantations are colonized by a single, dominating, ectomycorrhizal fungal species. This general lack of fungal diversity might affect plantation sustainability. Studies to set out the establishment and the importance of ectomycorrhizal fungus diversity for sustainable forestry are now under the way.

P2 169

Ectomycorrhizal mushrooms from the Guatemala highlands

R. Flores ,.[1,2] MC Bran.[1] and M. Honrubia [2]
[1]DIGI-Depto. Microbiología, Fac. CCQQ y Farmacia, Universidad de San Carlos de Guatemala, Guatemala. [2]Dpto. Biología Vegetal, Universidad de Murcia, Spain

Pine forests (*P. ayacahuite, P. rudis* and *P. hartwegii*) and fir woods (*Abies guatemalensis*) were studied in several areas of Guatemala at altitudes ranging from 2400 to 3600 m asl.

Seventeen genera were found from *Abies* forest, 23 from the association *P.rudis+P.hartwegii* and 23 from *P. ayacahuite.*

There were differences between the species for pines but the biggest differences are those found in fir woods. The fir mycoflora was similar to that found by other authors in Europe, Japan and USA.

Some species are reported for the first time from Guatemala and enlarge the area of distribution from North America and some show the characteristics of endemic species.

44 strains were isolated from one or more different species of *Alpova, Amanita, Boletus, Laccaria, Lactarius, Suillus, Rhizopogon* and *Tylopilus*, while some strains of *Amanita rubescens, Boletus edulis, Laccaria bicolor, Suillus bovinus, S. tomentosus and Rhizopogon aff vulgaris* and *Rh. aff roseolus* showed good growing potential.

Some mycorrhization tests in containers with pines were successful using mycelia of *Laccaria* and *Suillus* and with spores of *Inocybe* and *Rhizopogon.*

Information is provided about uses and the local names of many mushrooms.

P2 170

Is incorporation into an ectomycorrhizal hyphal network important for the growth of dipterocarp seedlings?

Francis Q. Brearley, Malcolm C. Press & Julie D. Scholes, University of Sheffield, UK

Dipterocarps are the most important tree family in the lowland evergreen rain forests of South-east Asia, both ecologically and economically. All dipterocarp species examined so far form ectomycorrhizal associations (EcMs) which are thought to improve their mineral nutrition and hence growth. In addition, it has been hypothesised that EcMs may benefit seedlings growing in the understorey of tropical rain forests by allowing transfer of carbon from adult trees through an EcM hyphal network. The influence of EcMs on the growth and the level of predation by insect herbivores on seedlings of two species of dipterocarp (*Parashorea tomentella* and *Hopea nervosa*) are being examined in a lowland evergreen rain forest in Sabah, Malaysia. These species differ in their ability to utilise light and in their responses to gap formation. Experimental treatments have been established in the field, using a series of fine diameter meshes, to simulate incorporation of seedlings into, or removal from, an EcM hyphal network. Results after 12 months of this 24 month study reveal that the two species differ in their responses to the treatments. The two species showed differing patterns of growth (allometry) and these are discussed in relation to their ecology.

P2 171

Competition for nutrients between Acacia mangium and Hopea odorata

R. Strömmer[1], H. Jokinen[1] and S.S. Lee[2], [1]The University of Helsinki, Finland [2]Forest Research Institute Malaysia, Malaysia

The Dipterocarpaceae is the most important family of timber trees in Malaysia and Southeast Asia. Trials of mixed plantations have been established in Malaysia where slow-growing, shade tolerant dipterocarps have been planted under the canopy of the fast-growing, exotic legume, *Acacia mangium* with encouraging results. However, until recently little was known of the associated soil microorganisms and interactions between the tree species and soil microorganisms. Cuttings of *Hopea odorata* (Dipterocarpaceae) and *A. mangium* were used in a green house experiment at the University of Helsinki for studying the competition for nutrients between the two species. Pots with the seedlings grown in sand were inoculated with microorganisms taken from the native soils of a mixed *Hopea-Acacia* plantation at Chikus, Perak, Malaysia. The study design consisted of bare soil, either of the tree seedlings of each species alone or seedlings of both species together. Nutrient levels in the soil were adjusted to two levels by watering the pots with nutrient solutions (nitrogen levels 5 and 15 mg/l; other nutrients relative to nitrogen as in Ingestad nutrient solution). The seedlings, starting from an initial height of about 20-30 cm, reached a final height of about 60 cm after 2.5 months when the experiment was completed. The microcosms were analysed for shoot and root biomass and N concentration, bacterial numbers in the rhizosphere, and soil nutrients. This study is part of the project '*Harnessing mycorrhizal symbiosis in mixed Dipterocarpaceae-Acacia mangium forest plantations in Malaysia and the Philippines*' (EU contract ERB1C18CT-98319).

P2 172

Arbuscular Mycorrhizas Fungi under Teak Clones Seed Orchard

Corry(1); Ferry Maryadi(2); Irmawati (3)

(1)Forest Resources Development Center, Perum Perhutani, Tromol Pos 6 CEPU, Central Java, Indonesia 58302, e-mail: corry0301 @hotmail.com; (2) and (3) Students of Faculty of Forestry, Bogor Agriculture University, Bogor.

Arbuscular mychorrizes fungi as the result research show four genuses, *Glomus*; *Acaulospora*; *Schlerocystis;* and *Gigaspora*. *Glomus* was showed more frequency than others are – the most abundance are and more adaptable in wide environment. An arbuscular mycorrhizes fungus that seen in plant roots on every site observed indicates teak roots have independence of mychorrizes symbioses. This thing was seen on young plants as well as older plants. In rainy season symbioses was happened in every site observed; but it was not seen in dry season. This case indicates, rainy environment was support enough to grow and to develop this fungus. Advance research to understand interdependency phenomena naturally is continued information that could be gained.

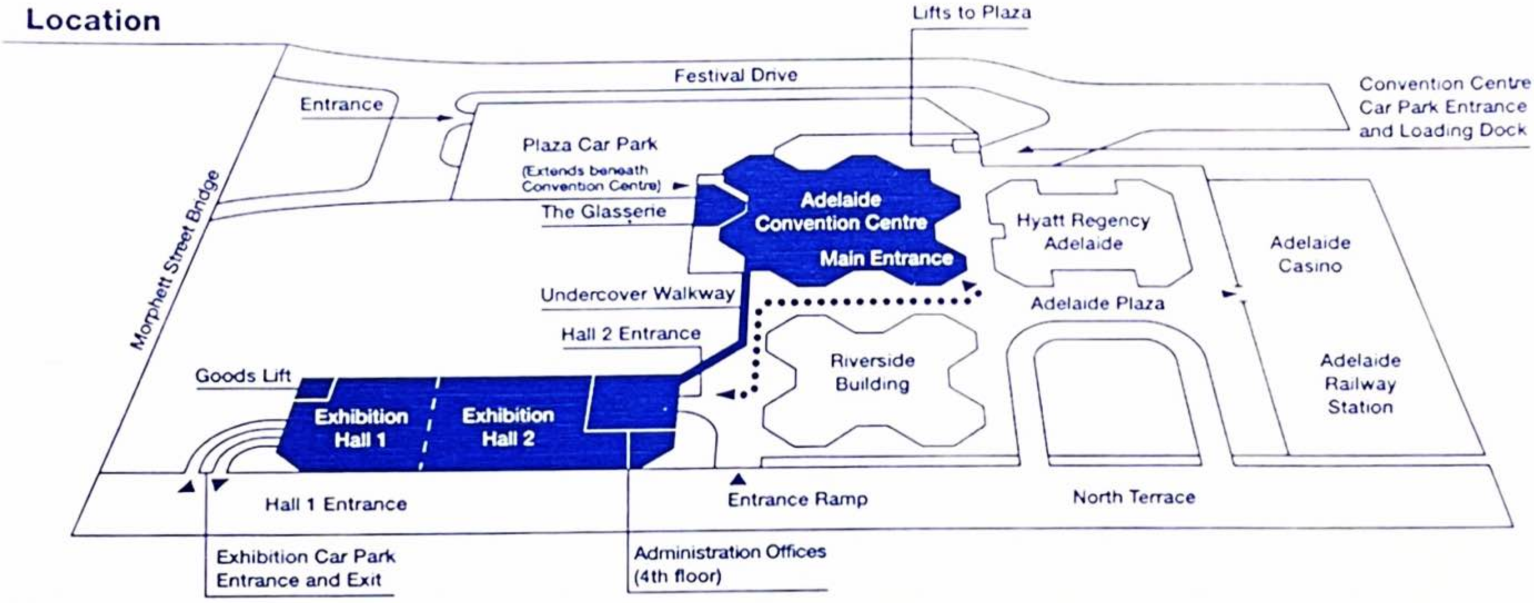
Keywords: Arbuscular Mycorrhizes Fungi, teak, root, spore abundance.

AUTHOR INDEX

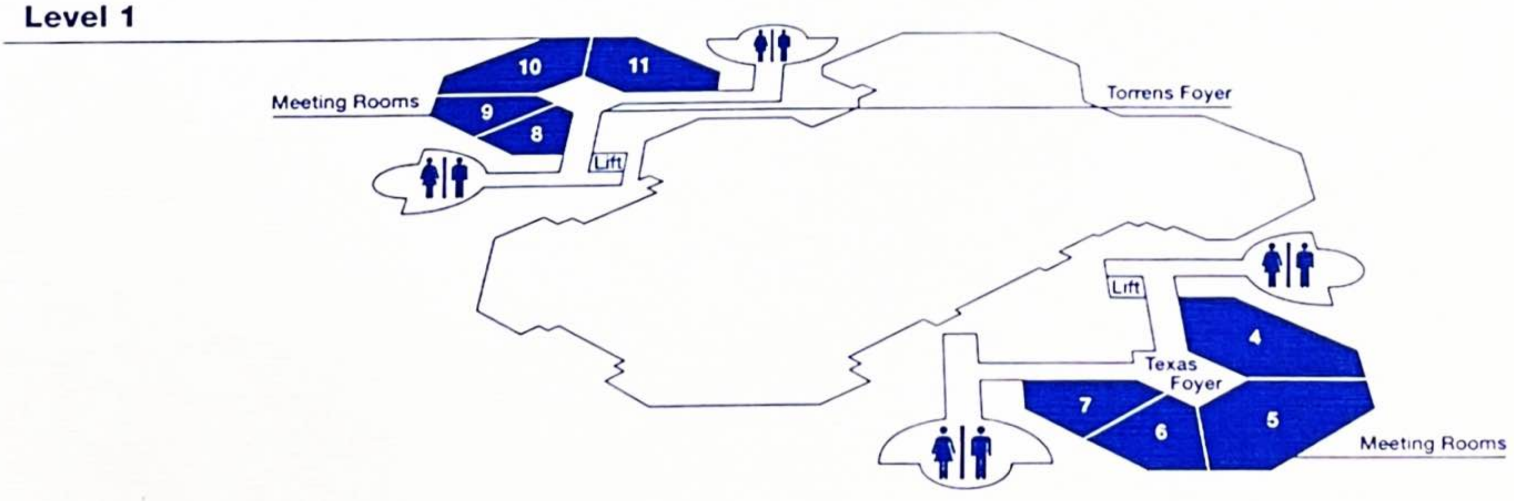
Adelaide Convention Centre

South Australia

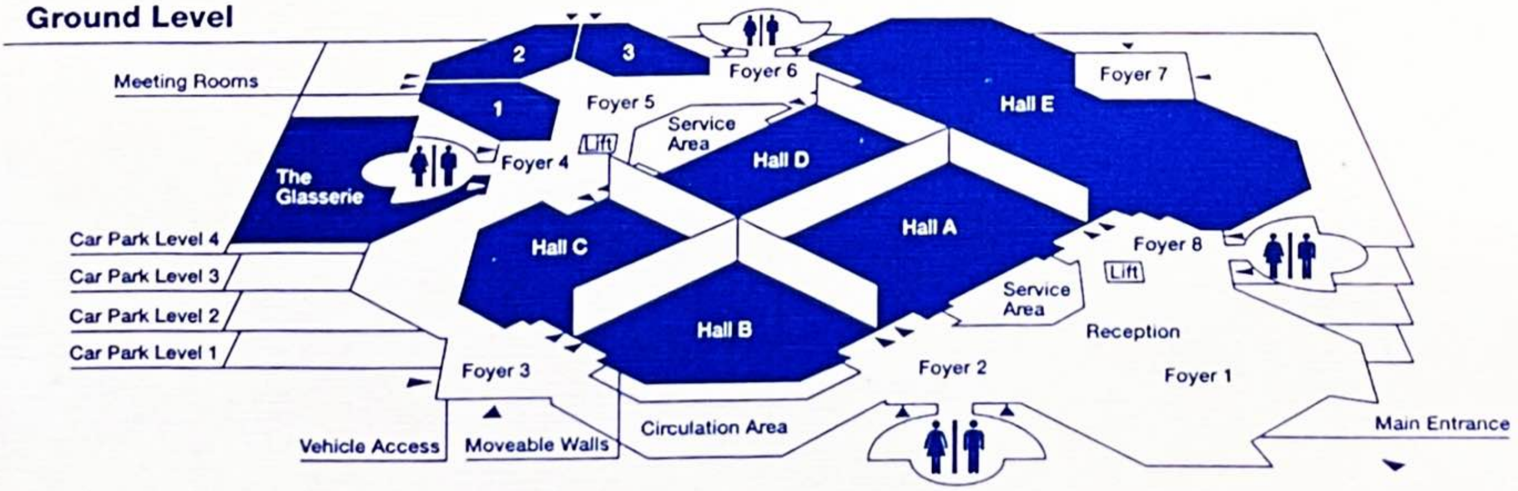
Location



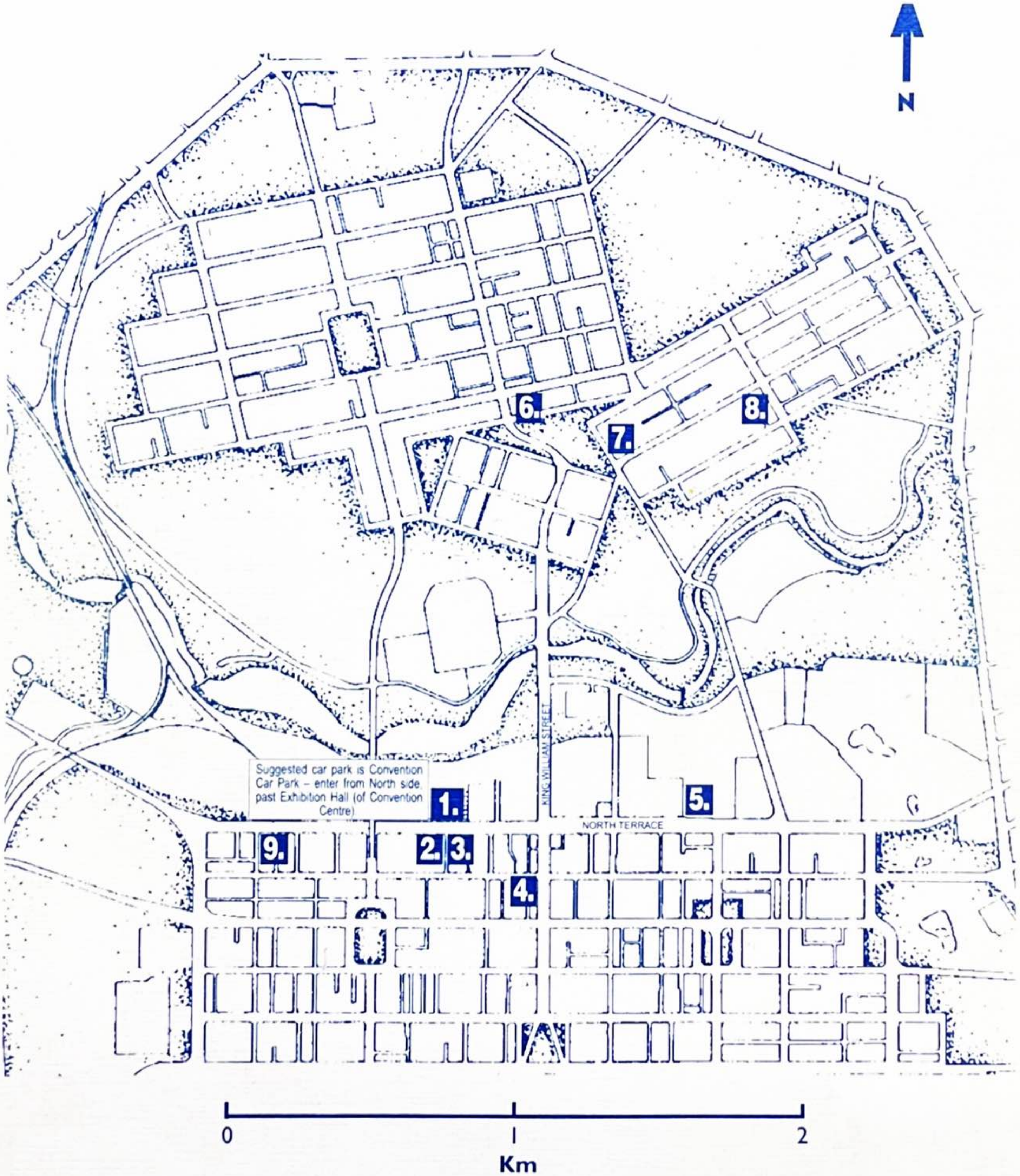
Level 1



Ground Level



MAP SHOWING LOCATION OF ADELAIDE CONVENTION CENTRE, ADELAIDE UNIVERSITY, HOTELS AND UNIVERSITY COLLEGES



Suggested car park is Convention Car Park – enter from North side, past Exhibition Hall (of Convention Centre)

1. Adelaide Convention Centre
2. Radisson Playford
3. Grosvenor Vista
4. Paringa Motel
5. Adelaide University
    - Wines of the World
    - Workshops
    - Students Resource Centre (Email access)
6. Lincoln College
7. St Ann's College
8. Old Lion Apartments
9. Riviera Adelaide

Manning Printers – 8277 9022