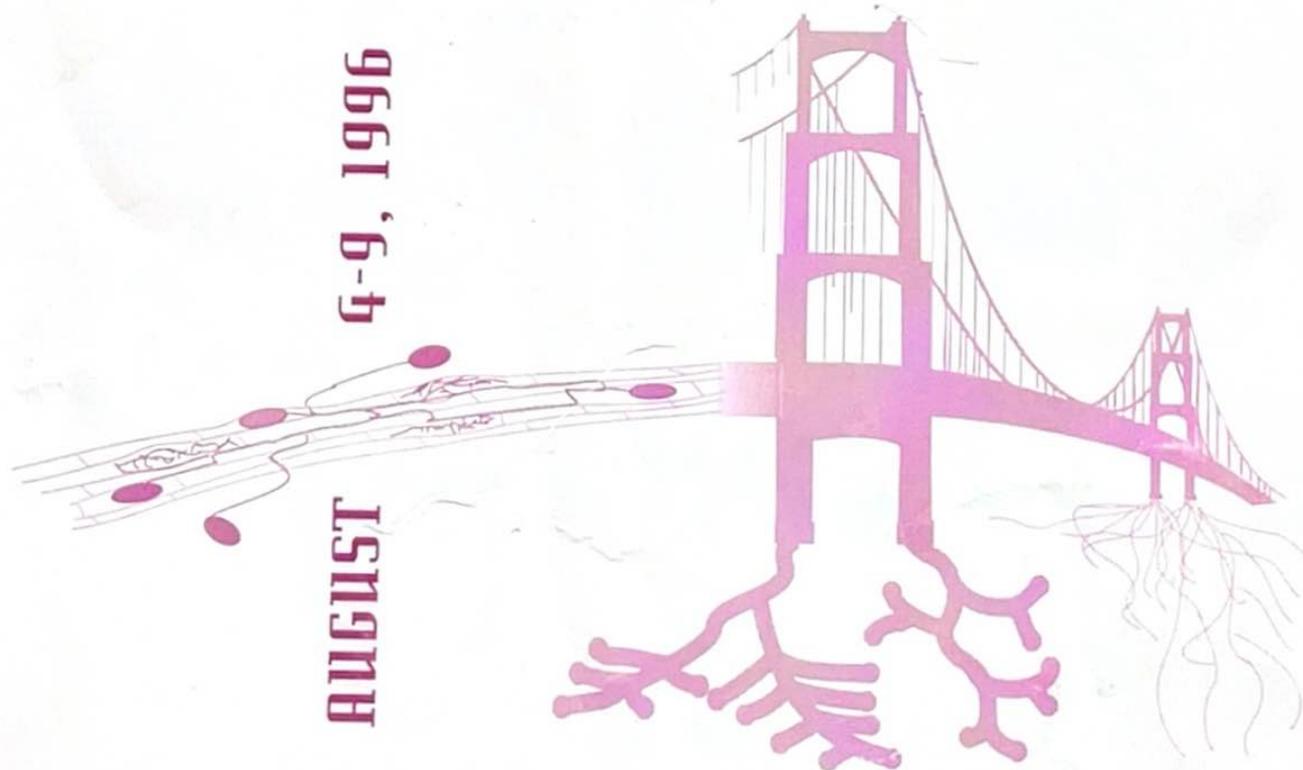


ICOM 1

*First International
Conference on Mycorrhizae*

4-9, 1996

AUGUST



UNIVERSITY OF CALIFORNIA
AT BERKELEY
USA



Program and Abstracts

Program and Abstracts of the

First International Conference on Mycorrhizae

August 4 - 9, 1996
University of California
Berkeley, California USA

COMPILED AND EDITED BY: TIMOTHY M SZARO and THOMAS D BRUNS

CONFERENCE SUPPORTED IN PART BY:

College of Natural Resources and
Department of Environmental Science, Policy and Management
University of California at Berkeley

College of Agriculture and Environmental Sciences
University of California at Davis

New Phytologist

Springer-Verlag

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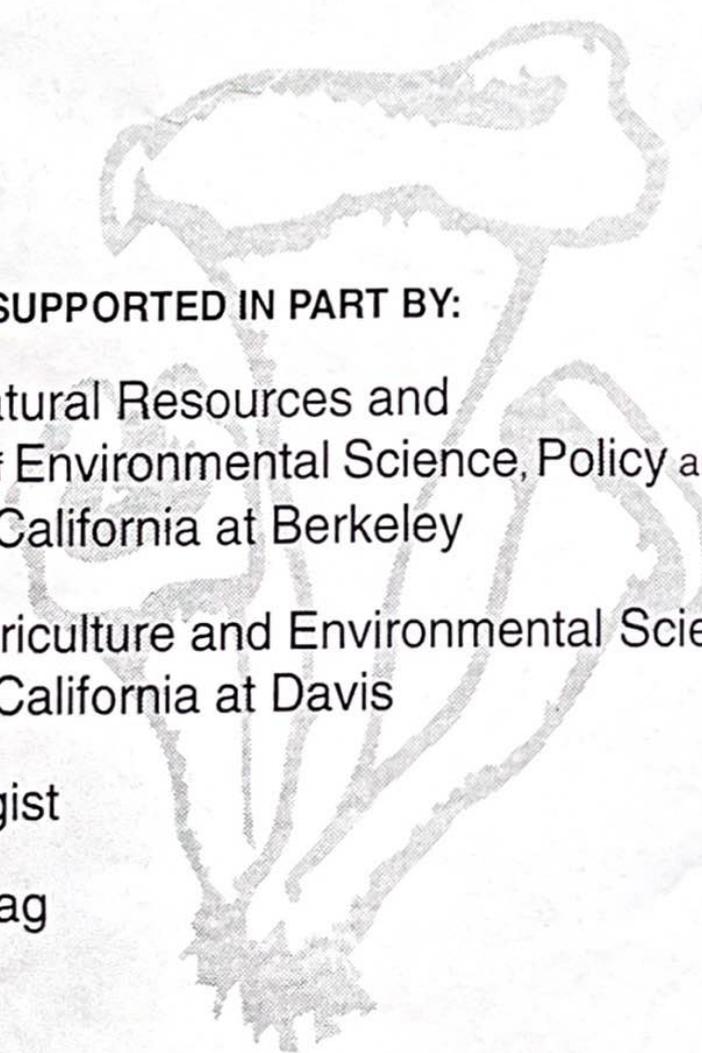


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PROGRAM COMMITTEE

Caroline Bledsoe, Chair; Mike Allen; Tom Bruns; Randy Molina; David Read; Jane Smith; Sally Smith; Torgny Unestam.

MORNING AND AFTERNOON SESSION ORGANIZERS

Eddie Allen, Isabel Alvarez, Susan Barker, Paola Bonfante, Tom Bruns, Carlos Colinas, Cathy Cripps, Ken Cullings, Roger Finlay, Jean Garbaye, Monique Gardes, Manuela Giovannetti, Nada Gogala, Nancy Johnson, Ari Jumpponen, Ingrid Kottke, Jonathan Leake, Dan Luoma, James Merryweather, Mike Miller, Joseph B. Morton, Thom O'Dell, Tom Parker, Larry Peterson, Paul Rygielwicz, Ian Sanders, Sally Smith, Jane E. Smith, Denis Tagu, Jim Trappe, Yun Wang, Adolphe Zeze.

AFTERNOON DISCUSSION/WORKSHOP ORGANIZERS

Reinhard Agerer, Bob Auge, Jacqueline Baar, D. J. Bagaraj, Efrén Cázares, E. Cordell, Eric Danell, John Dighton, John Dodd, Dan Durrall, Jean Garbaye, Jim Graham, B.L. Jalali, Nancy Johnson, Roger Koide, F. Lapeyrie, Thom O'Dell, David Pilz, Andrew Smith and James Trappe.

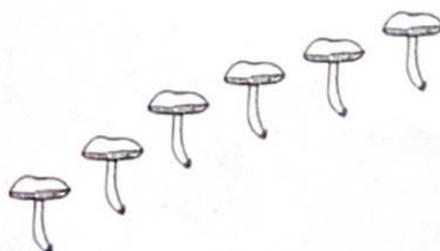
LOCAL ORGANIZING COMMITTEE

Tom Bruns, Chair; Zaphod Beeblebrox; Martin Bidartondo; Enrico Bonello; Tom Horton; Annette Kretzer; Tim Szaro; Lee Taylor.

PREFACE

Welcome to the First International Conference on Mycorrhizae and to the University of California at Berkeley. We are happy that you could make it to the meeting. Your contributions will make "bridging the gaps" in mycorrhizal research a reality.

The program is given chronologically, the abstracts are listed alphabetically by first author and all other authors are indexed. The abstracts are as submitted by the authors. The organizing committee did not edit them. We hope to have the complete set of abstracts available as a file that can be downloaded from the ICOM web site at <http://mendel.berkeley.edu/boletus/icom.html> by the end of September. This latter set may include additional abstracts that were not available at the time this program was printed and it will exclude any that were not actually presented at this meeting.



A NOTE OF THANKS

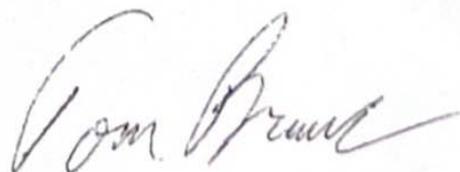
Many people have worked very hard to put this meeting together. The program committee was responsible for deciding the format of the meeting and in selecting the session organizers. The session organizers invited the morning speakers, reviewed abstracts and selected speakers for the afternoon sessions. The latter task was particularly hard for the Ecology-related sessions, which represented the overwhelming majority of the submitted abstracts. Cathy Cripps, Nada Gogala, James Merryweather and Jane Smith deserve special thanks for the work they did sorting through these abstracts. The Physiology section also contained a large number of abstracts; these were handled very efficiently by Paola Bonfante and Paul Rygielwicz. The workshop/discussion leaders selected the topics and decided on the formats; all this was done with much input and guidance from Randy Molina. Joe Morton and Reinhardt Agerer deserve special thanks for their pre-conference workshops, which were extremely popular and undoubtedly contributed to the large turn-out at this meeting.

Early financial support was obtained from sources on both the Berkeley and Davis campuses of the University of California; without this money, this meeting would not have been possible. We are also indebted to the *New Phytologist* for their donation, which was used to support invited speakers that were in financial need; and to Springer-Verlag, which donated 500 copies of the International Directory of Mycorrhizologists. These donations were facilitated through the efforts of Sally Smith and Valentin Furlan, respectively.

The local organizing committee dealt with all the mundane, but important aspects of the meetings such as space, budgets, field trips, T-shirts, mailings, printing and many other details. Tim Szaro deserves special recognition for his creation and maintenance of the ICOM web site, for his work on all other computer-related aspects of the meeting, for his correspondence and for his work on this program and abstracts booklet. Tom and Michael Horton deserve special credit for the "Bridging the Gaps" logo and Tom for his translation of it into the distinctive tie-dye T-shirts. We were helped with our early mailings and correspondence by Benita Smith, an administrative assistant in the Department of ESPM; with arrangements at Foothill by Mary Saito of conference services and by the veteran staff at Foothill; and with registration and housing by Krebs Conference Services.

Thank you one and all, participants, committee members, organizers, staff and financial supporters, for making this meeting possible. May our collective knowledge of mycorrhizae be the beneficiary of your efforts!

Sincerely,



Tom Bruns

SCHEDULE OF EVENTS

First International Conference on Mycorrhizae August 4-9 1996

SUNDAY

- 1:00 PM Registration - Foothill Dorms
 2:00 PM Editorial Meeting for *Mycorrhiza* in Classroom A - Foothill Dorms
 5:30 PM Evening Social - Foothill Dorms
 8:00 PM

MONDAY MORNING SESSION - ZELLERBACH AUDITORIUM

- 8:45 AM Welcoming Remarks and Notices
 9:00 AM VIVIENNE GIANINAZZI-PEARSON - Current challenges in mycorrhizal research: getting to the cellular, molecular and genetic roots of the symbioses.
 9:30 AM DAVID READ - Mycorrhizas in the Real World: the Past and the Future.
 10:00 AM Coffee

10:30- 12:00 Microbial Interactions in the Mycorrhizosphere. Organizers: Mike Miller (USA), Jean Garbaye (France)

- 10:30 AM JULIE JASTROW - Contributions of mycorrhizae to the development of soil aggregate hierarchy.
 11:00 AM ROBIN SEN - Bioremediation of petroleum derived hydrocarbons in the plant mycorrhizosphere.
 11:30 AM JEAN GARBAYE - New results and hypotheses about mycorrhizal helper fluorescent pseudomonads.

12:00 PM Lunch

MONDAY AFTERNOON CONTRIBUTED SESSION 1 BIRGE 50 CONTRIBUTED SESSION 2 LECONTE 4 CONTRIBUTED SESSION 3 LECONTE 1

Physiology

Organizers: Paola Bonfante-Fasolo, Paul Rygielwicz

1:10 PM PHILIP PFEFFER. Metabolism of Glucose, its Uptake and Translocation in Mycorrhizal Leek and Transformed Carrot Roots.

Ecology

Organizers: James Merryweather, Jane E. Smith

NO TALK SCHEDULED. SESSION BEGINS AT 1:30 PM

Mycorrhizae in Stressed Ecosystems

Organizers: Nada Gogala, Cathy Cripps

NO TALK SCHEDULED. SESSION BEGINS AT 1:30 PM

1:30 PM MICHEL CHALOT. Kinetics, energetics and specificity of a general amino acid transporter from the ectomycorrhizal fungus *Paxillus involutus*.

RASMUS KJØLLER. Arbuscular mycorrhizal fungi in roots from a Danish pea field determined by polyacrylamide gel electrophoresis of specific fungal enzymes.

REGINA LUIZÃO. Effects of selective logging on the spore populations and infectivity rate of AM fungi in the Amazonian lowland evergreen rain forest.

- 1:50 PM GERHARD HEBE. Signal transduction in spruce cells triggered by chitin elicitors released from ectomycorrhizal fungi.
- ✓ 2:10 PM IVER JAKOBSEN. Competition for soil P between roots and hyphae of arbuscular mycorrhizas.
- ✓ 2:30 PM STEVEN MILLER. Differential utilization of cellulose and protein reveals competitive advantage of ectomycorrhizal fungi over saprotrophs for complex organic substrates.
- 2:50 PM R. MICHAEL MILLER. Mycorrhizal effects on carbon gain in facultative and obligate ecotypes of *Andropogon gerardi*.
- 3:10 PM DANIEL MOUSAIN (first author: Corinne Conjeaud). Effects of ectomycorrhizal infection with *Hebeloma cylindrosporum* and *Rhizopogon rubescens* on the carbon balance in *Pinus pinaster* seedlings.
- ✓ 3:30 PM PAUL RYGIWICZ. Global climate change: Bridging the gap between the above- and below ground.
- 4:00-5:30 PM **WORKSHOP 1** **LeConte 1** Ecology, physiology, and fruitbody formation of edible ectomycorrhizal fungi. Discussion leaders: Eric Danell and David Pilz.
- ✓ **WORKSHOP 2** **LeConte 2** Identification of signal molecules in developing mycorrhizae. Which research strategies work best and how can we collaborate? Discussion leader: F. Lapeyrie.
- ✓ **WORKSHOP 3** **Birge 50** Developing a World Atlas of Ectomycorrhizae. Discussion leaders: Reinhard Agerer and Dan Durall.
- ✓ **WORKSHOP 4** **Lewis 100** Problems and potential ways forward to routine identification and characterization of VAM fungi - bringing together the European working group (European Bank of Glomales, BEG) with INVAM (Morton group). Discussion leader: John Dodd.
- ✓ PHILLIP VANDENKOORNHUYSE. Impact of heavy metals on the diversity and genetic variability of AM fungi in soil.
- KATIA VAN TICHELEN. Reclamation of a bare industrial area contaminated by non-ferrous metals: occurrence of the AM infection, physico-chemical and biological evaluation of the durability of soil treatment and revegetation.
- ✓ MIROSLAV VOSÁTKA. Extra- and intra-radical organs of AMF in acid rain stressed ecosystems.
- CATHY CRIPPS. Ectomycorrhizal communities with aspen on smelter-impacted sites: how ecosystems recover from airborne pollution.
- ERIK LILLESKOV. Patterns of ectomycorrhizal diversity over an atmospheric nitrogen deposition gradient near Kenai, Alaska.
- SUSANNE BECKMANN. Deposition of nitrogen in ectomycorrhizae of Norway spruce (*Picea abies* (L.) Karst.) on NH₄⁺-fertilized sites in the Black Forest, Germany.

TUESDAY MORNING SESSION - WHEELER AUDITORIUM

- 8:30-10:00 "Advances in Molecular Ecology". Organizers Monique Gardes (USA), Ian Sanders (Switzerland)
- 8:30 AM IAN SANDERS - Genetic and functional diversity of arbuscular mycorrhizal fungi in natural communities.
- 9:00 AM OLA KÁRÉN - Community structure of ectomycorrhizal fungi - a molecular approach.
- 9:30 AM TOM WHITHAM - Using molecular techniques to understand community structure from the bottom up: interactions of environmental stress, pinyon pine, mycorrhizae, soil microbes and insect herbivores at Sunset Crater.
- 10:00 AM Coffee

10:30-12:00 "Biodiversity of Mycorrhizal Fungi: Problems of Measurement and Meaning".

Organizers: Thom O'Dell (USA), Adolphe Zeze (Ivory Coast)

- 10:30 AM BRIAN BAINBRIDGE - Fungal Biodiversity: origins, estimation and significance.
- 11:00 AM KEITH EGGER - Molecular Biodiversity: What are we measuring?
- 11:30 AM JOE MORTON - Measurement and Interpretation of Arbuscular Fungal Species Diversity at Different Scales.
- 12:00 PM Lunch

TUESDAY CONTRIBUTED SESSION 4 LECONTE 1 CONTRIBUTED SESSION 5 BIRGE 50 CONTRIBUTED SESSION 6 LECONTE 4 afternoon Biodiversity Gene Expression Physiology, Structure, & Anatomy

Organizers: James Merryweather, Jane E. Smith Organizers: Susan Barker, Denis Tagu Organizers: Ingrid Kottke, Larry Peterson, Paola Bonfante-Fasolo, Paul Rygielwicz

- 1:30 PM THOM O'DELL. Diversity and abundance of ectomycorrhizal fungi: scaling in space and time. FRANCIS MARTIN. Signaling in the ectomycorrhizal symbiosis. The tryptophan betaine, hypaphorine, produced by *Pisolithus tinctorius* stimulates the expression of an auxin-regulated gene in roots of *Eucalyptus globulus*.
- 1:50 PM KARIN PRITSCH. Diversity of black alder ectomycorrhizae - anatomical and molecular investigations. PHILIPP FRANKEN. RNA accumulation and gene expression in arbuscular mycorrhizal fungal spores.
- 2:10 PM SILVIA PEROTTO. Identification of introns in the nuclear ribosomal genes of ericoid mycorrhizal fungi. YORAM KAPULNIK. (first author: Hanan Itzhaki). Differential expression of tobacco genes in response to VA mycorrhizal fungi colonization.
- 2:30 PM JAMES MERRYWEATHER. The diversity of AM fungi associated with *Hyacinthoides non-scripta* (L.) Chouard ex Rothm. in English woodland. FABRICE MARTIN-LAURENT. PSEMYC1, a plant gene induced during early stages of arbuscular mycorrhiza development.
- YUKARI UETAKE. Changes in microtubule arrays in symbiotic orchid protocorms during fungal colonization and senescence.
- ARMELLE GOLLOTTE. Immunological and molecular biological studies of interactions between *Glomus* and *Gigaspora* in roots of leek and tomato.
- ANNE ASHFORD. Fungal vacuoles, endosomes and transport.

2:50 PM MARK BRUNDRETT. The ecology of VAM fungi in undisturbed and disturbed habitats in tropical Australia.

Overlap in induced gene expression sets for early VAM mycorrhizal symbiosis and root knot nematode feeding sites in tomato.

3:10 PM LENA JONSSON. Ectomycorrhizal succession during establishment of *Pinus sylvestris* seedlings in virgin forest.

The cloning of two genes involved in the *Medicago truncatula*/*Glomus versiforme* mycorrhizal symbiosis.

3:30 PM MELANIE JONES. Silvicultural practices and ectomycorrhizal diversity in British Columbia.

Quantitative and structural variations in host cell nuclei of arbuscular mycorrhizal and/or pathogenic systems.

MARTIN GUTTENBERGER. Carbon exchange between mycorrhizal symbionts: Are carbohydrates the whole story?

MARIO JOLICOEUR. Measurement of endomycorrhizal fungi intracellular pH.

4:00-5:30 PM WORKSHOP 5 LeConte 1
 Estimating biodiversity and monitoring target species. Discussion leader: Thom O'Dell. - Ecto?

LeConte 4
 Saprotrophic capabilities of mycorrhizal fungi. Discussion leaders: John Dighton and Jacqueline Baar.

Birge 50
 VAM symbioses & plant drought resistance - Do AM fungi really enhance host stress resistance? Discussion leader: Bob Auge.

WORKSHOP 8 LeConte 3
 Dark-septate root endophytes: are they mycorrhizal? Discussion leaders: Efrén Cázares and James Trappe.

7:30- 10:00 PM POSTER SESSION 1
 # category
 1 Applications 1
 2 Ecology - Biodiversity, Community Ecology
 3 Ecology - Stressed Ecosystems
 4 Gene Expression, Manipulation and Characterization
 5 Physiology

Foothill Dorm - Hillside Area
 Social with snacks and beverages

room	session
RR	1: Sun - Tues
SH	1: Sun - Tues
SH	1: Sun - Tues
AR	1: Sun - Tues
AR	1: Sun - Tues

AR = Assembly Room
 RR = Recreation Room
 SH = Stern Hall

WEDNESDAY FIELD TRIPS

- 9:00 AM Field Trip Number 1: Guided San Francisco City Tour
 - 9:00 AM Field Trip Number 2: Wine Country Tour
 - 9:00 AM Field Trip Number 3: Mount Tamalpais, Muir Woods & the Pacific Ocean
 - 9:00 AM Field Trip Number 4: Elephant Seals and Tidal Pools
 - 9:00 AM Field Trip Number 5: Point Reyes Hikes & Strolls
- busses load at 8:45 am at the Foothill dorm - Hillside parking lot

THURSDAY MORNING SESSION - WHEELER AUDITORIUM

- 8:30-10:00 "Mycorrhizal Linkages between Plants". Organizers: Sally Smith (Australia), Roger Finlay (Sweden)
- 8:30 AM SUZANNE SIMARD - Ecological significance of carbon transfer in ectomycorrhizal tree species mixtures.
- 9:00 AM ALASTAIR FITTER - Quantifying carbon transport between mycorrhizally-linked plants using natural abundance ¹³C measurements.
- 9:30 AM ANDREW SMITH - Selectivity and control: the role of membranes in inter-plant transfer of nutrients.
- 10:00 AM Coffee

10:30-12:00 "Mycoheterotrophy". Organizers: Dan Luoma (USA), Jonathan Leake (UK)

- 10:30 AM JONATHAN LEAKE - Myco-heterotrophy as a way of life.
- 11:00 AM LEE TAYLOR - Myco-heterotrophy in North American orchids: Specificity and Trophic Links.
- 11:30 AM CARLA ZELMER - Getting to the root of the matter: Mycoheterotrophy in the Orchidaceae and Ericaceae from a fungal perspective.
- 12:00 PM Lunch

BIRGE 50

THURSDAY CONTRIBUTED SESSION 7 ~~LECONTE 7~~ Systematics, Specificity & Evolution

Organizers: Joseph B. Morton, Tom Bruns, Manuela Giovannetti and Ken Cullings

- 1:30 PM HUBERT DULIEU. (first author: Michel Hosny). Molecular genetics as a unique tool for identification of biodiversity and evaluating genetic exchanges within endomycorrhizal species.

- 1:50 PM ANNETTE KRETZER. Internal transcribed spacer sequences from 41 recognized species of *Suillus* sensu lato: phylogenetic and taxonomic implications.

- ✓ 2:10 PM KATHERINE LOBUGLIO. Phylogenetic origins of the asexual mycorrhizal symbiont *Cenococcum geophilium* Fr. and other mycorrhizal fungi among the ascomycetes.

- ✓ 2:30 PM GERALD NAGAHASHI. A rapid micro-injection technique allows for the sensitive detection of root exudate signals which stimulate the branching and growth of germinated VAM fungus spores.

LECONTE 1

THURSDAY CONTRIBUTED SESSION 8 ~~LECONTE 8~~ Applications

Organizers: Carlos Colinas-Gonzales and Wang Yun

- 1:30 PM IAN HALL. The New Zealand Périgord black truffle programme - the first decade.

- CÉLINE DI BATTISTA. Monitoring the persistence of the ectomycorrhizal basidiomycete *Laccaria bicolor* S238N on Douglas-fir and Norway Spruce in nursery and forest sites by PCR.

- XIAOMEI HUA. A technique for mycorrhizal inoculation: the effect and field performance.

- ✓ M. RANGARAJAN. Synergistic effects of *Glomus fasciculatum* and *Azospirillum brasilense* on the production of *Morus alba* and economic characters of silk worm larvae.

CONTRIBUTED SESSION 9 ~~ARCTIC & ALPINE/REGIONAL ECOLOGY~~

Organizers: Ari Jumpponen, James M. Trappe, James Merryweather, Jane E. Smith

- ✓ DOT HELM. Mycorrhizal and plant community development on recently deglaciated terrain, Exit Glacier, Alaska.

- ✓ HENRY VARE. Mycorrhizas in *Fennoscandian oroarctic*.

- ✓ DAVID JANOS. Arbuscular mycorrhiza limitation of tropical rainforest host community diversity.

- ✓ ELISABETH W van der HEIJDEN. Dual mycorrhizal infection, a matter of priority or selection?

- 2:50 PM CASSANDRA McLEAN. Use of PCR to determine relationships among root isolates of Epacridaceae and the endophyte of Ericaceae, *Hymenoscyphus ericae*. THOMAS R. HORTON. Ectomycorrhizal community structure in Andean Montane pine plantations, Ecuador.
- 3:10 PM FRANCOIS BUSCOT (first author: Sylvie Herrmann). Differential effects of mycobionts with different ecological strategies on oak morphology. DAN LUOMA. Fifteen years amongst the Snow Plants.
- 3:30 PM THIERRY BEGUIRISTAIN. Host plant stimulates the tryptophan, betaine, hypaphorine accumulation in *Pisolithus tinctorius* hyphae during ectomycorrhizal infection. Fungal hypaphorine controls K⁺ uptake, H⁺ extrusion and root hair development. GISELA CUENCA. Arbuscular mycorrhizae in the rehabilitation of tropical fragile degraded lands. JOHN DODD. The detection of arbuscular mycorrhizal fungi (AMF) in root systems of different plants using fungal/mycorrhizal-specific isozymes as molecular markers.

- 4:00-5:30 PM **WORKSHOP 9** LeConte 1 Can mycorrhizae be parasitic? Re-addressing our definition of mycorrhiza: structure vs function. Discussion leaders: Nancy Johnson, Jim Graham and Andrew Smith. **WORKSHOP 10** LeConte 4 Mycorrhiza associated bacteria. Discussion leader: Jean Garbaye. **WORKSHOP 11** Birge 50 Current applications of mycorrhizae in forestry, agriculture and restoration/reclamation: problems and solutions. Discussion leaders: E. Cordell, B. L. Jalali and D. J. Bagaraj. **WORKSHOP 12** LeConte 3 Culturing VAM fungi - why have we failed? Discussion leader: Roger Koide.

7:5:30-8:30 PM **POSTER SESSION 2** Foothill Dorm - Hillside Area. Social with snacks and beverages. Wines of the world contest

#	category	room	session
6	Applications 2	AR	2: Wed - Fri
7	Applications - Edible Fungi	AR	2: Wed - Fri
8	Ecology	AR	2: Wed - Fri
9	Ecology - Arctic/Alpine	SH	2: Wed - Fri
10	Ecology - Microbial Interactions	SH	2: Wed - Fri
11	Ecology - Molecular Ecology	RR	2: Wed - Fri
12	Physiological Ecology	SH	2: Wed - Fri
13	Structure, Anatomy and Morphology	SH	2: Wed - Fri
14	Systematics	RR	2: Wed - Fri
15	Systematics - Molecular Systematics, Population Genetics	RR	2: Wed - Fri

AR = Assembly Room
RR = Recreation Room
SH = Stern Hall

FRIDAY MORNING SESSION - WHEELER AUDITORIUM

8:30-10:00 **"Mycorrhizal Mediation of Plant Dynamics". Organizer: Nancy Johnson (USA), Tom Parker (USA)**

- 8:30 AM DAVID HARTNETT - Mycorrhizal mediation of plant competition, demography, and species diversity in grasslands.
- 9:00 AM ^{JIM HENDRIX} SHIVCHARN DHILLION - Mycorrhizal relationships with plant diversity and elevated CO₂ conditions. *Plant-Mycorrhizal Interactions in Grasslands, habitat shifts*
- 9:30 AM BERNIE DELL - A role for Australian ectomycorrhizal fungi in the rehabilitation of denuded landscapes in Asia.

10:00 AM Coffee

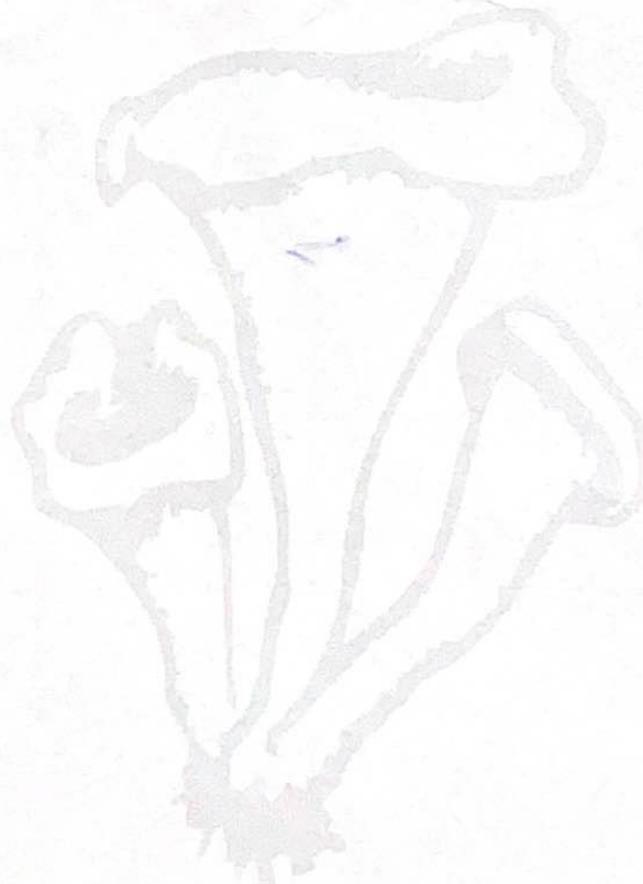
10:30-12:00 **"Advances in Applications and Restoration Ecology". Organizers: Isabel Alvarez (Spain), Edie Allen (USA)**

- 10:30 AM FRANÇOIS LE TACON - Nursery and field response to ectomycorrhizal inoculation of forest trees in western Europe.
- 11:00 AM DAVID JASPER - AM fungi in land restoration - we can apply the principles, what about a product?
- 11:30 AM TO BE ANNOUNCED
- 12:00 PM Lunch

FRIDAY AFTERNOON SESSION - WHEELER AUDITORIUM

- 1:30 PM Business meeting - all are welcome
- 2:00-4:00 PM Workshop Summaries

FINAL PARTY Faculty Club 7:00 pm



POSTER SESSIONS

There are two poster sessions. Session 1 will run from Sunday August 4 through Tuesday August 6. Session 2 will run from Wednesday August 7 to Friday August 9. Session 1 consists of 5 sections; Session 2 has 10 sections.

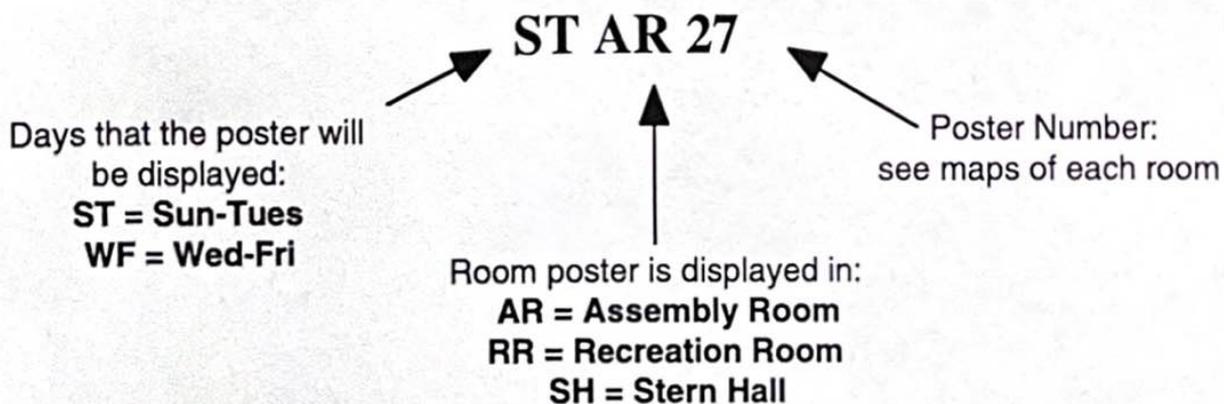
#	section	room	session
1	Applications 1	RR	1: Sun - Tues
2	Ecology - Biodiversity, Community Ecology	SH	1: Sun - Tues
3	Ecology - Stressed Ecosystems	SH	1: Sun - Tues
4	Gene Expression, Manipulation and Characterization	AR	1: Sun - Tues
5	Physiology	AR	1: Sun - Tues
6	Applications 2	AR	2: Wed - Fri
7	Applications - Edible Fungi	AR	2: Wed - Fri
8	Ecology	AR	2: Wed - Fri
9	Ecology - Arctic/Alpine	SH	2: Wed - Fri
10	Ecology - Microbial Interactions	SH	2: Wed - Fri
11	Ecology - Molecular Ecology	RR	2: Wed - Fri
12	Physiological Ecology	SH	2: Wed - Fri
13	Structure, Anatomy and Morphology	SH	2: Wed - Fri
14	Systematics	RR	2: Wed - Fri
15	Systematics - Molecular Systematics, Population Genetics	RR	2: Wed - Fri

HOW TO FIND A POSTER

All posters will be displayed in the Foothill dorm complex. The poster display rooms should be open for viewing throughout the day and evening. If a poster room is locked, inquire at the Foothill dorm main desk for a key. It would be a good idea to take advantage of these informal viewing times because the formal poster sessions are likely to be crowded.

To locate when and where a poster is to be presented, look up the abstract in this program; abstracts are alphabetized by first author. The poster number is located on the top right hand side of the abstract.

Each poster has a unique number. The number identifies which session the poster will be displayed (session 1: Sunday through Tuesday or session 2: Wednesday through Friday), the room in which it will be displayed (the Assembly Room, the Recreation Room or Stern Hall) and the location of the poster in the display room. The example below shows how to read it:



FOR THOSE PRESENTING A POSTER

If you are presenting a poster, **please make sure you set your poster up on the correct days.** ST posters can go up anytime Sunday. This will allow your poster to be viewed informally during the opening social on Sunday night (August 4). The formal poster session for ST posters will be Tuesday night (August 6); at that time presenters should stand by their posters to facilitate discussions. **ST posters should be taken down immediately after the Tuesday night poster session;** posters remaining after that time will be removed by others and may be thrown away. Please leave the push pins in the poster boards for the next presenter to use. WF posters can go up as soon as ST posters are down on Tuesday night (August 6) or anytime on Wednesday (August 7). The formal poster session will be on Thursday night (August 8); at that time presenters should stand by their posters to facilitate discussions. WF posters should be taken down no later than 1:30 PM Friday. Please remove all push pins at this time.

FOR THOSE WHO ARE PRESENTING TALKS

Slide carousels will be available at each session. **Please arrive 20 minutes early if you need to load your slides on site.** Classroom B in the Foothill Dorms has two slide projectors available for slide previewing. In the same room there are also extra carousels that you may borrow on the night before or the morning of your talk. Please do not borrow them earlier, as we have a limited supply, and do not remove the last one from a projector, otherwise no one after you will be able to practice their talk or check their slides. If you borrow a carousel, leave it with the projectionist at the conclusion of your session.

LOOKING FOR A MEETING ROOM?

Classroom A in the Foothill dorm is set up for informal meetings. It is reserved for a *Mycorrhiza* editorial board meeting on Sunday afternoon, but at all other times it is open for use on a first-come first-serve basis. You can reserve it for a specific time by posting a note on the door.

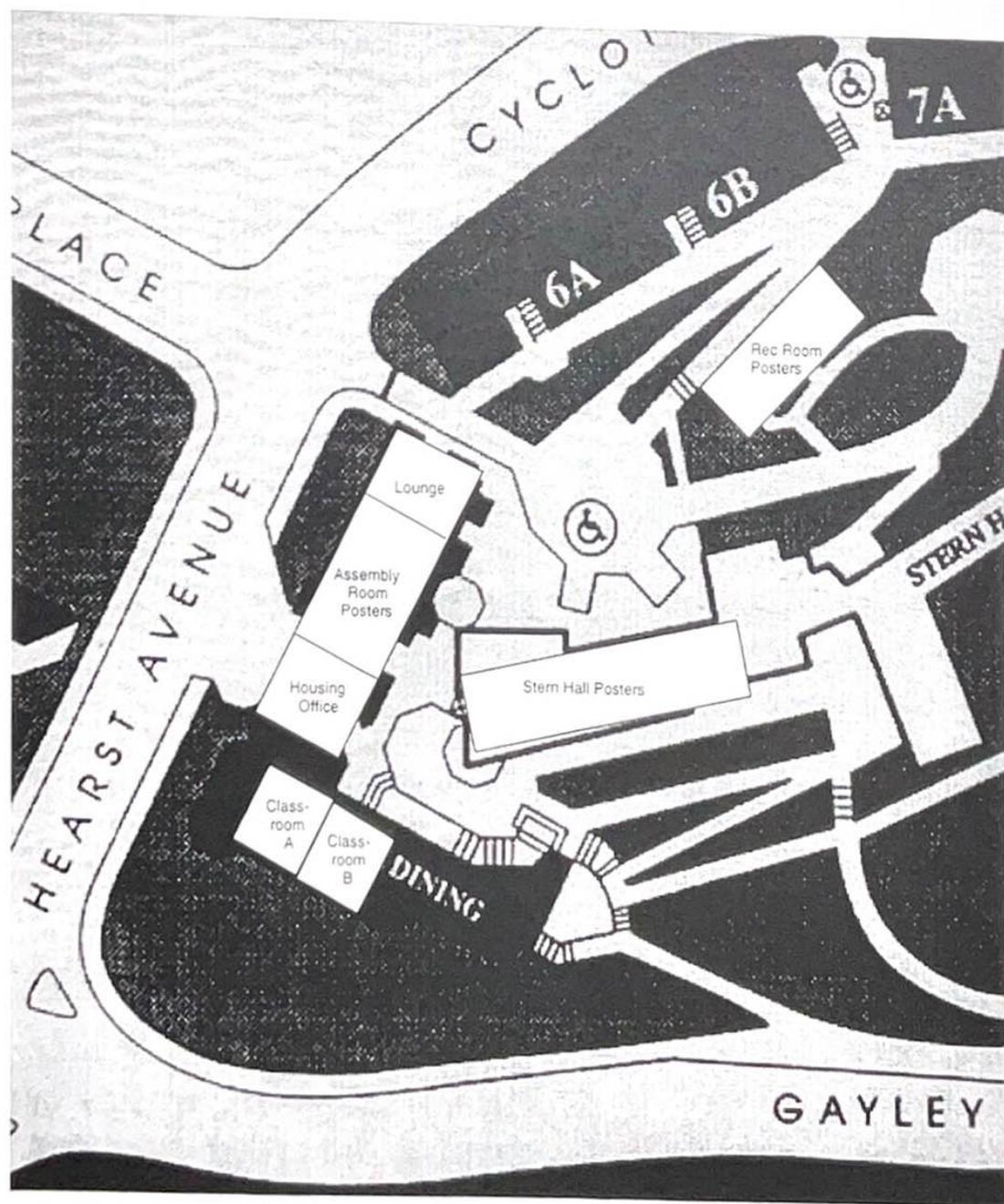
WEDNESDAY FIELD TRIPS

Busses for the Wednesday field trips will load promptly at 8:45 Wednesday morning. **PLEASE bring your ticket** and be careful to get on the correct bus. If you are going on the Muir Woods, Point Reyes, or Elephant Seal trip, please remember to bring a warm jacket or sweater; it can be cold.

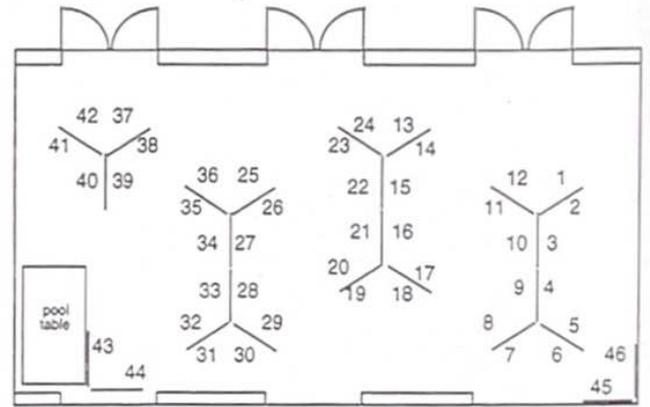
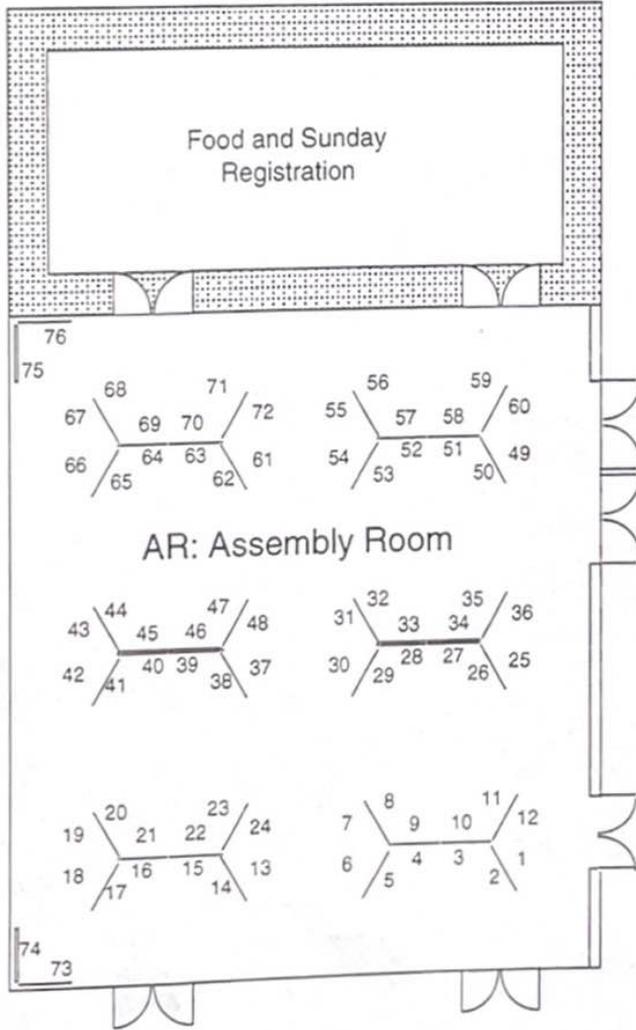


Foothill Dorm Hillside Area Map

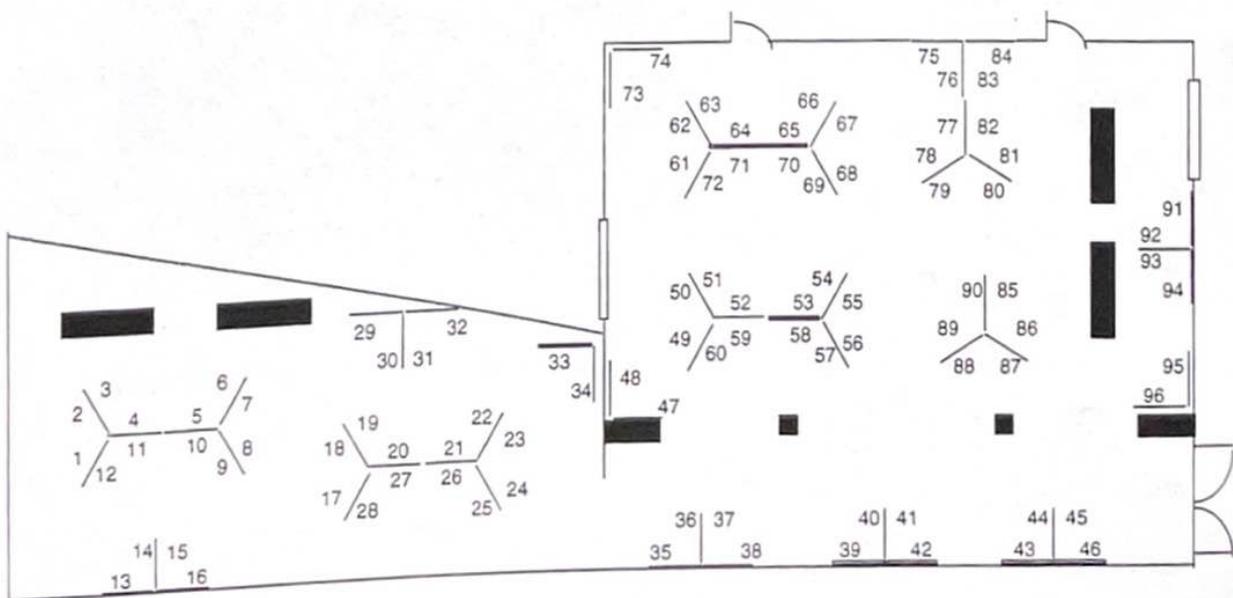
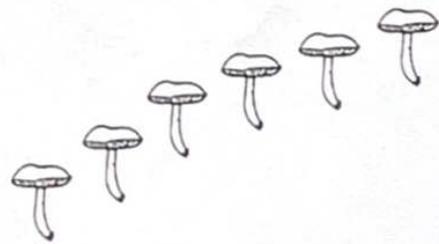
(includes Assembly Room,
Recreation Room and Stern Hall)



Poster Session Room Maps



RR: Recreation Room - Building 5 Level 2



SH: Stern Hall Dining Room

ABSTRACTS

Arranged Alphabetically by First Author

poster session 8 WF AR 47

ABE, JUN-ICHI P.¹ & K. KATSUYA¹. ¹Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan. - Zonation of vesicular-arbuscular mycorrhizal fungi along a saline gradient on three coastal sand-dunes in Japan.

Salinity is one of the environmental factors which has a strong influence on the zonal distribution of plants in coastal sand-dunes. The distribution of vesicular-arbuscular mycorrhizal fungi (VAMF) were examined from shoreline to inland along a saline gradient. Two study sites along the coast of the Pacific Ocean and one on the Sea of Japan differing in vegetation were chosen. Sand samples were collected from 4-6 sampling points at each line transects. Sampling was carried out from uncovered sand-dunes below the edge of the vegetation up to 190 m far from the shoreline. Three genera and 9-10 species of VAMF were isolated and identified at the three study sites. Many common species (up to 89%) were observed between the study sites. The frequency of occurrence of VAMF spores in sand samples were low on uncovered seaward sides and increased towards the landward sides. However the total number of spores per samples were higher in covered sand dunes near the vegetation limits. *Scutellospora gregaria* and *Acaulospora* sp. 1 were predominantly isolated and their frequency of occurrence increased in samples collected further inland. *Glomus* sp. 2 was predominantly present on covered sand-dunes near the shoreline and *Glomus* sp. 1 and *G. aggregatum* were predominantly isolated from the landward sides. These results showed evidence for zonation of *Glomus* spp. on coastal sand dunes, which appeared to be dependent on the saline gradient rather than on the zonation of sand-dune plants.

poster session 3 ST SH 49

ABOU-GAZIA, HOSNEY, AHMED ELSETTAWY, TALAT OMRAN & KHAIRIA ABOUELKHAIR. Dept. of Forestry & Wood Tech., Faculty of Agriculture, Alexandria University, Alexandria, Egypt. - VA-mycorrhizae associated with different plant species irrigated with sewage water.

A study was carried out to identify Vesicular-arbuscular mycorrhizae spores associated with different plants grown in the reclaimed soils of New Burg El-Arab city. One year old trees were used for planting in January 1994. The pH of soil was 8.1 with E.C. 3.41 and 9.38 dS/m. The salinity of soil increased with using the sewage water. The Na content increased until 52.75 meq./L and Cl content was 59.32 meq./L after one year from planting. The pH of sewage water was 7.7, with E.C 2.29 to 3.25 dS/m.

The survey of VAM spores showed that nine species were associated with the different plant species. The identified species were *Acaulospora foveata*, *A. laevis*,

A. scrobiculata, *A. spinosa*, *A. tuberculata*, *Glomus claroideum*, *G. convolutum*, *G. mosseae* and *G. tortuosum*.

poster session 3 ST SH 50

ADDY, HEATHER¹, MURRAY MILLER² & LARRY PETERSON³. ¹Dept. of Horticulture, The Pennsylvania State University, University Park, PA 16802 USA. ²Dept. of Land Resource Science and ³Dept. of Botany, University of Guelph, Guelph, Ont., Canada N1G 2W1. - Infectivity of the propagules associated with extraradical mycelia of two VAM fungi following winter freezing

VAM fungi have been thought to survive adverse environmental conditions primarily as spores. However, evidence from previous research in our laboratory suggested that infective extraradical mycelia survived in frozen soil over winter. We conducted two experiments to investigate the infectivity of mycelia of two *Glomus* species after prolonged periods in frozen soil. Extraradical mycelia were produced in pouches made of fine nylon mesh, which excluded roots but not hyphae. Mycelia in these pouches were exposed to freezing conditions both in the field and in a controlled-temperature chamber. Following freezing, soil in half of the pouches was disturbed by sieving. In the second experiment, an additional treatment was included such that a third set of pouches contained only isolated spores. The relative infectivity of mycelia and spores following freezing was determined by growing bioassay plants directly in the pouches. Spores were not an effective inoculum in any of the bioassays, regardless of freezing. Soil disturbance following freezing reduced subsequent colonisation of bioassay plants, indicating that mycelia were responsible for mycorrhizal formation following freezing. These results are consistent with the hypothesis that mycelia remain infective in frozen soil over winter. The persistence of mycelia in frozen soil was not dependent on connection to root systems nor on the presence of root pieces. The presence of the hyphal network in soil in spring has important implications for the early phosphorous nutrition of seedlings.

poster session 13 WF SH 86

ADHOLEYA, ALOK & VIJAY GADKAR. Tata Energy Research Institute, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi 110 003, India. - A few new structures in *Gigaspora margarita* and *Gigaspora gigintia* under in vitro conditions

While cultivating two *Gigaspora* species under in vitro on carrot roots (*Daucus carota*) it was found a few regular structures are formed apart from those generally known as auxilliary cells and spores out side the roots during symbiosis. When roots were cleared and stained, found no differences in colonization pattern by these fungi. Developmental pattern of these structure was studied. The similar structures were absent in another species cultivated in vitro (*Glomus intraradices*) on carrot roots.

poster session 14 WF RR 01

AGERER, REINHARD, GERHARD RAMBOLD. Section Mykologie, Institut für Systematische Botanik, Universität München, Menzingerstr.67, D-80638 München, Germany. - DEEMY, a DELTA-based information system for DEtermination and characterization of EctoMYcorrhizae

Determination of ectomycorrhizae is until now exclusively based on dichotomous keys with alternatives and ranks selected by the constructors of the keys. DEEMY provides an interactive key in a hypertext version with the possibility to start the determination process with any feature. As an aid, the system ranks the characteristics regarding their distinguishing capacity. To date, features useful for characterization of ectomycorrhizae are discussed and sometimes depicted in different publications. DEEMY, however, compiles all useful features in a character-list with one up to several states, gives explanations, provides informative illustrations, and therefore helps to prepare detailed descriptions of ectomycorrhizae. The determination procedure and the multiple evaluation possibilities are explained on the poster. DEEMY system is designated for mycorrhizasts, mycologists, and botanists working in soil ecology, taxonomy and nature conservation. It supports current and future floristic, ecological and taxonomic projects on ectomycorrhizae and fungi. DEEMY will be firstly issued, presented and demonstrated at ICOM 1. Actually, only a selected number of ectomycorrhizal species are included, but each second year an updated version will be available.

poster session 5 ST AR 01

AGUILERA, L.², F.T. DAVIES, JR.¹, V. OLALDE - PORTUGAL², S.A. DURAY¹ & L. PHAVAPHUTANON¹. ¹Dept. of Horticultural Sciences, Texas A&M University, College Station, TX 77843, and ²CINVESTAV Plant Biology Inst., Irapuato, Mexico. - Effect of phosphorus stress on gas exchange, plant growth and mycorrhizal development of *Capsicum annuum* cv. San Luis (chile ancho pepper).

Seedlings of *Capsicum annuum* L. cv. San Luis were grown in pots containing a pasteurized mixture of sand and sandy loam soil inoculated or noninoculated with the endomycorrhizal (VAM) fungus *Glomus intraradices*. Long Ashton nutrient solution (LANS) was modified to supply phosphorus at 0, 11 or 44 $\mu\text{g P ml}^{-1}$. Diurnal gas exchange measurements were taken 15, 30 and 50 days after the experiment was initiated. Gas exchange and net photosynthesis were enhanced by mycorrhiza and full strength LANS fertilization (44 $\mu\text{g P ml}^{-1}$). Under P stress (0 $\mu\text{g P ml}^{-1}$), VAM plants had greater gas exchange and net photosynthesis compared to Non-VAM plants. At all P levels, the symbiosis increased leaf nutrient content of P, K, Mg, S, Fe, Mn, Zn, Cu, B, Mo, and Al. Mycorrhizal plants had higher shoot dry weights, leaf number, leaf area, and fruit primordia than nonmycorrhizal plants at 0 and 11 $\mu\text{g P ml}^{-1}$ P. Growth of VAM plants at 0 $\mu\text{g P ml}^{-1}$ was similar to Non-VAM plants at 11 $\mu\text{g P ml}^{-1}$ P. Root colonization (arbuscules,

vesicles, internal and extraradical hyphae development) were higher at 0 and 11 $\mu\text{g ml}^{-1}$ P. The quantity of spores recovered in soil was independent of P treatments.

poster session 2 ST SH 21

AGWA, HAMDY & IRIS CHARVAT. Department of Plant Biology, University of Minnesota, 220 Biological Sciences, 1445 Gortner Ave., St. Paul, MN 55108-1095. - Species of arbuscular mycorrhizal fungi from a remnant prairie in Minnesota, USA

Arbuscular mycorrhizal symbiosis is widespread in natural ecosystems. In prairie grasslands, mycorrhizal fungi play an important role in ecosystem maintenance. Plant reliance on mycorrhizae appears to be heavy when inorganic phosphorus levels are low. The objective of this study was to do a survey of the Glomales from a remnant prairie called Crosstown Prairie, located in Minneapolis, Minnesota, USA. A small plot is all that remains of the large prairie that once occupied this region. The descriptions and observations reported in this study are from trap cultures as well as single spore cultures using big bluestem, *Andropogon gerardii* Vitman as the host plant. Big bluestem is native to this prairie. *Glomus occultum* Walker, *G. intraradices* Schenck & Smith, *G. Mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe, *G. Etunicatum* Becker & Gerdemann, *G. Constrictum* Trappe, *Gigaspora gigantea* (Nicolson & Gerdemann) Gerdemann & Trappe, *Scutellospora pellucida* (Nicolson & Schenck) Walker & Sanders, *Entrophospora infrequens* (Hall) Ames & Schneider have been identified.

*Visiting professor from Tanta University, Faculty of Education, Biology Department, Kafr El-Sheikh, Egypt.

poster session 8 WF AR 45

ALARCON, CLARA¹ & GISELA CUENCA². ¹Postgrado de Botánica, UCV - CIEZA, UNEFM, Venezuela. ²Centro de Ecología, Instituto Venezolano de Investigaciones Científicas. Apartado 21827. Caracas 1020-A Venezuela. - Mycorrhizal Status of psamophilous meadows in sand dunes of Venezuela.

Colonization percentage by mycorrhizal of the most abundant herbaceous and arbustives plant species was determined in the psamophilous meadows of the coastal sand dunes in Paraguaná Peninsula, Venezuela. The presence of AM fungi in soils was quantified by measuring mycorrhizal colonizations within fine roots with a light microscope using a clearing and staining procedure. Samples of rootlets were taken in twelve plant species: *Acacia tortuosa*, *Argusia gnaphaloides*, *Croton punctatus*, *C. ramifolium*, *Egletes postrata*, *Fimbristylis cymosa*, *Leptothrium rigidum*, *Melochia crenata*, *Panicum vaginatum*, *Scaveola plumieri*, *Sporobolus virginicum* and *Suriana maritima*. Colonization percentage by mycorrhizal was evaluated by Trouvelot *et al* (1986) method, which considers both arbuscules and vesicles. Pelotons and hyphae were also evaluated. *C. ramifolium* had the highest AM colonization frequency (92.0 %), when compared with *L. rigidum* and *F.*

cymosa which had very low mycorrhizal colonization frequencies. These frequencies varied from 49.3 to 86.0 % for the other species. The psamphilous meadows had 68.8% and 7% of average frequency and intensity of mycorrhizal colonization, respectively. When our results are compared with other tropical ecosystems, we can conclude that the frequency of mycorrhizal colonization in the meadows sand dunes in northern Venezuela is similar to other tropical natural ecosystems like herbaceous savannas, but their colonization intensity by mycorrhizal is typical of perturbed ecosystems.

poster session 4 ST AR 41

ALBRECHT, CATHERINE, RENE GEURTS, KATHARINA PAWLOWSKI, AB VAN KAMMEN, TON BISSELING. Molecular Biology, Agricultural University, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands. - Are early nodulins genes express during endomycorrhizal symbiosis?

Legume mutants have been generated that are unable to form nodules in symbiosis with *rhizobia*, a subset of which are also unable to enter mycorrhizal symbiosis. The frequent coincidence of the Nod⁻ and Myc⁻ phenotype suggests that "general symbiotic genes" allow plants to live with endosymbiotic partners. To further investigate whether development of endomycorrhizal and *Rhizobium* symbiosis share common functions we have examined induction of some early nodulins genes during the establishment of endomycorrhiza. For this purpose, specific primers for the early nodulins genes, ENOD 5 and ENOD 12, have been used in a RT-PCR strategy to detect a gene expression in wild type and mutants pea roots colonized with the endomycorrhizal fungus *Gigaspora margarita*.

poster session 13 WF SH 41

ALLAWAY, WILLIAM G.¹ & ANNE E. ASHFORD². ¹School of Biological Sciences A12, The University of Sydney, NSW 2006, Australia. ²School of Biological Science, The University of New South Wales, Sydney, NSW 2052, Australia. - Choice of technique as an influence on perceptions of apoplasmic pathways, localization of small molecules and fine structure in mycorrhizas.

The influence on physiological interpretation due to microscopical preparation techniques, including chemical fixation, dehydration and embedding, precipitation methods, and cryofixation and freeze-substitution, is discussed. Problems including changes in structure, diffusion from *in vivo* locations, binding to tissue components, and extraction by solvents, are considered. It is concluded that physiological interpretations of images and localizations obtained with microscopic techniques require rigorous experimental investigation of the effects of these techniques.

poster session 8 WF AR 44

ALLEN, MICHAEL F.¹, MATTHIAS C. RILLIG¹, JOHN N. KLIRONOMOS², NONA R. CHIARIELLO³ & CHRISTOPHER B. FIELD⁴. ¹Biology Department, San Diego State University, San Diego, CA 92182, U.S.A. ²Department of Botany, University of Guelph, Guelph, ON, Canada N1G 2W1. ³Department of Biological Sciences, Stanford University, Stanford, CA 94305, U.S.A. ⁴Department of Plant Biology, Carnegie Institution of Washington, Stanford, CA 94305, U.S.A. - Root infection by arbuscular mycorrhizal and other fungi in response to elevated atmospheric carbon dioxide and fertilization in a mediterranean annual grassland.

In a 5 x 2 x 2 factorial experiment, 5 plant species of an annual mediterranean grassland were grown for four months in pots inside open-top chambers at the Jasper Ridge Biological Preserve MECCA facility (San Mateo County, California). The plant species consisted of three annual grasses (*Bromus hordeaceus*, *Vulpia microstachys* var. *pauciflora*, *Avena barbata*) and two herbs (*Linanthus parviflorus*, and *Calycadenia multiglandulosa*). Per plant species, four pots were used for each of the 4 treatment combinations, in which atmospheric carbon dioxide concentration (elevated and ambient) was crossed factorially with NPK fertilization (fertilized and unfertilized). Root infection by arbuscular mycorrhizal and other fungi (e.g., melanized, clamped, regularly septate hyphae) was assessed, and extraradical hyphal lengths were measured.

poster session 8 WF AR 68

ALSHEIKH, ABDULMAGID. Kuwait Institute for Scientific Research, P.O.B. 24885, Safat 13109, Kuwait. - Pure culture mycorrhizal synthesis of Cistaceae with desert truffles in the genera Terfezia and Tirmania

Desert truffles, *Terfezia* and *Tirmania* species, are collected from sites where the annual and perennial *Helianthemum* and other plants in the family Cistaceae are abundant in north Africa and southwest Asia. The mycorrhizal relationship between Cistaceae and *Terfezia* and *Tirmania* spp. has been suggested and realized for some species. In this study two annual Cistaceae, *H. ledifolium* and *H. salicifolium*, and four perennials, *H. kahiricum*, *H. lippii* (from Kuwait desert), *Cistus albidus* and *Fumana procumbens* (from southern Europe) were used. Mycelial cultures from spores of *Terfezia boudieri*, *T. clavaryi*, *Tirmania nivea* and *T. pinoyi* germinated *in vitro* served as inoculum. Although the roots were surrounded by profuse septate hyphae, penetration and colonization of rootlet cells did not always occur. When colonization did occur, hyphae either penetrated cortical cells with intercellular branching and grew randomly within the cells or formed beginnings of a loose mantle as in *F. procumbens*. Colonized rootlets were thicker and darker than nonmycorrhizal ones. This work suggests that desert truffles survive during unfavorable weather conditions by forming mycorrhizae with some perennial hosts. Mycelial cultures of desert truffles can possibly be used as inocula for perennial species in desert habitats to help increase rangeland productivity, shelter belt projects,

and ornamental planting. The success in germinating dried *Terfezia* and *Tirmania* spp. spores suggests experiments with dried specimens of other truffles.

poster session 1 ST RR 41

ALVARENGA, FLÁVIA¹, ROSADO, SEBASTIÃO¹ & SIQUEIRA, JOSÉ². ¹Ciências Florestais and ²Ciência do Solo, Universidade Federal de Lavras, Lavras -MG, 37200-000, Brazil. - Growth and phenotypic stability of ecotypes of *Trema micrantha* Brume (Ulmaceae) as affected by AM fungus inoculation and superphosphate

Growth response and phenotypic stability of the pioneer wild woody specie *T. micrantha* from different seed sources to increased phosphorus rates and inoculation with the arbuscular mycorrhizal fungus *Glomus etunicatum* (Ge) were studied in an 5x2x4 factorial design composed of five P-rates (0, 60, 120, 240 and 480ppm), two mycorrhizal conditions (I-inoculated and NI-non-inoculated) and four seed sources (designated as S1...S4). The study was conducted in a grasshouse using a soil-mix with pH_{water}=7.0 and Mehlich-P=2, 14, 25, 25, 272µg.g-1 soil. Seedlings from all sources grew better when inoculated with Ge, irrespective to P-rates. Root colonization (RC) and mycorrhizal dependency (MD) were maximum when P was not added to soil-mix. In absence of P, RC was high ranging from 66.5 to 93.1% whereas MD was low ranging from 8.8 to 25.2%. There was no correlation between RC and MD. Linear regression coefficients (β) for shoot dry weight as related to environmental index (P-rates) were used to assess seed source stability by the Finlay and Wilkison's method. Stability for non-inoculated and inoculated seedlings differed only for seed source S2 with values of $\beta=0.53$ and 0.33 for NI and I plants, respectively. Therefore, indicating mycorrhizal contribution to increased stability, which means higher adaptability of I plants to varied soil P-availability.

poster session 1 ST RR 03

ALVAREZ, I.F., J. PERA & J. PARLADE. IRTA, Dept. de Patologia Vegetal, Ctra. de Cabrils s/n, 08348 Cabrils (Barcelona), Spain. - Effect of mycorrhizal inoculation, hormone treatment and substrate disinfection on rooting, mycorrhiza development and growth of Douglas-fir cuttings

Douglas-fir cuttings were taken from three-year-old mother plants and inserted in a substrate composed of peat and vermiculite (1:1, v:v) previously disinfected with either steam or methyl bromide. Two hormonal treatments were applied: 24 h dipping in L-exuberone or IBA mixed with talc powder. Inoculation of cuttings with vegetative inoculum of *Laccaria bicolor* S-238 N was established at insertion at the dose 1:20 (v:v, inoculum substrate). Inoculation with spores of *Melanogaster ambiguus* and *Rhizopogon subareolatus* were established two months after insertion at the dose of one million of spores per cutting. A treatment of non inoculated seedlings was also considered. After rooting, the mean percentage of mycorrhizal plants with each fungus for the

whole experiment was 82 % for *L. bicolor*, 30% for *R. subareolatus* and 21% for *M. ambiguus*. Substrate disinfection with methyl bromide decreased significantly the number of rooted cuttings and the percentage of mycorrhizal plants with *R. subareolatus* (for both hormone treatments) and *L. bicolor* (only for exuberone). Inoculation with either *L. bicolor* and *R. subareolatus* significantly increased the percentage of rooted cuttings. No significant effects in growth were observed.

poster session 11 WF RR 37

AMICUCCI, ANTONELLA, ISMAELA ROSSI, LUCIA POTENZA, ALESSANDRA ZAMBONELLI, DEBORAH AGOSTINI, FRANCESCO PALMA and VILBERTO STOCCHI. Istituto di Chimica Biologica "Giorgio Fornaini", Università di Urbino, Via Saffi, 2 - 61029 Urbino, Italy. § Dipartimento di Protezione e Valorizzazione Agroalimentare, Via Filippo Re, 8 - 40126 Università di Bologna, Bologna, Italy. - RFLP analysis of the ITS region for the identification of ectomycorrhizae from Tuber species.

The ectomycorrhizae of two economically important white truffle species, *Tuber magnatum* and *Tuber borchii* have similar morphological features from which is difficult to obtain a correct identification. In an attempt to distinguish these two species during the symbiotic phase we combined the amplification of the internal transcribed spacer (ITS) of the ribosomal DNA with the RFLP analysis. The DNA from fruitbodies of *T. magnatum* and *T. borchii*, from leaves and roots of *Tilia platyphyllos* and from roots of this host plant inoculated with the two ectomycorrhizal species was amplified using the "universal primers" ITS1 and ITS4. The analysis of the ITS amplification products allowed a preliminary discrimination between the two species and showed that the lengths of the two bands from the ectomycorrhizae were the same as those obtained from the two symbionts. In contrast with what we had observed after analyzing the uncut ITS region, the restriction studies with Hinf I, Mbo I and Taq I endonucleases suggested that the mycorrhizae presumed to be of *T. magnatum* belonged neither to the species utilized for the mycorrhization nor to other *Tuber* species studied in our lab. The results of this research demonstrate that ITS amplification combined with RFLP analysis represents a simple, sensitive and reliable strategy to type ectomycorrhizae from the two different species of *Tuber* studied.

poster session 10 WF SH 19

ANDRADE, GALDINO, LINDERMAN, ROBERT G. & BETHLENFALVAY, GABOR J. USDA-ARS, Horticultural Crops Research Laboratory, 3420 NW Orchard Ave, Corvallis OR 97330, USA. - Microbial populations in the mycorrhizosphere of sorghum inoculated with 3 species of *Glomus*.

Bacterial surround arbuscular mycorrhizal (AM) hyphae on water agar using of hyphal exudates. We studied bacteria on sorghum mycorrhizae in pasteurized-soil pot cultures. Plants were inoculated with 2 different isolates of three AM fungal species, or were left uninoculated (7

treatments). Bacterial populations in the rhizosphere soil or soil colonized by AM fungal hyphae were counted on TSA medium (total bacteria) or P1 medium (fluorescent pseudomonads). Twenty-four isolates were selected for taxonomic study. Bacterial proliferation was greater in rhizosphere than in hyphosphere soil. *Bacillus* (5) and *Arthrobacter* (3) species dominated the latter, vs. 1 species of each in the rhizosphere. The rhizosphere had more species of *Pseudomonas* (3) than the hyphosphere (1). The bacterial community (TSA medium) was more diverse in the hyphosphere (16 species) than in the rhizosphere (11), but these numbers varied greatly within AM treatments. The hyphosphere of an isolate of *Glomus intraradices* was associated with 1 bacterium (*Burkholderia cepacia*), while an isolate of *Glomus mosseae* was associated with 5 bacterial species. One bacterium (*B. cepacia*) occurred in the soils of all 6 fungi, but not in the nonAM control) while others, like *Variovax paradoxus*, colonized only an isolate of *G. mosseae*. *Burkholderia pickettii* was found only in nonAM soil. AM fungi selectively stimulated specific bacterial strains in hyphosphere soil. Thus, different fungus-bacterial associations may affect plant growth and health differently.

poster session 15 WF RR 13

ANTA, BERTA¹, MARIO HONRUBIA², CATALINA CARRILLO², ALMUDENA GUTIERREZ² & VICTOR RUBIO¹. ¹Centro Nacional de Biotecnología (CSIC-UAM), E-28049 Madrid, Spain. ²Dpto. Biología Vegetal (Botánica). Facultad de Biología. Universidad de Murcia. Campus de Espinardo 30100, Murcia, Spain. - Characterization by molecular methods (ITS-RFLP) of the different *Suillus* species of the mediterranean region.

The relatedness of 17 isolates of *Suillus*, belonging to six typical species from the mediterranean region (*S. bellini*, *S. collinitus*, *S. granulatus*, *S. luteus*, *S. mediterraneus* and *S. variegatus*) and other isolate of *Suillus granulatus* from Kenya, was determined comparing the ITS region. The amplification of the ITS region with the universal primers ITS 1 and ITS 4 produces a fragment of constant size in all strains. The digestion of these fragments with different restriction enzymes, first with *Alu I* and *Sau 3A*, results in a common pattern for almost every strain; second with the combination of *Taq I*, *Msp I* and *Hinf I* identifies the different species, except of *Suillus mediterraneus*. Intraspecific variation was observed in all the species. Data presented do not support the maintenance of *Suillus mediterraneus* and *Suillus collinitus* as different species. Results indicate that PCR-RFLP can be used as a simple method for ecological studies of the *Suillus* species in this semiarid area in which they are predominant. Finally we present the relationship of these *Suillus* with *Suillus grevillei*, *Suillus spraguei* and *Rhizopogon subcaurelescens* (from the data base) in a phylogenetic tree.

poster session 15 WF RR 14

ARREDONDO, FELIPE¹, VIC CLAASSEN² & BRETT M. TYLER¹. ¹Department of Plant Pathology, and ²Department of Land, Air and Water Resources, University of California, Davis, CA95616. - Diversity of rRNA Spacer Regions among VA Mycorrhizal Fungi

Revegetation of degraded soils is important in a wide variety of agricultural and wildlands management contexts. Establishment of VA mycorrhizae is critical for sustainable revegetation of degraded soils where there are harsh physical conditions and poor nutrient availability. The long term aims of this research are to characterize the genetic structures of vesicular-arbuscular mycorrhizal fungal populations and to identify the strains which are effective for revegetation of degraded soils, through the use of molecular markers. Our goal is to develop molecular markers that can be used to characterize VAM populations by direct extraction of DNA from soil. The internal transcribed spacer (ITS) and extragenic spacer (EGS) of the ribosomal DNA repeats were amplified from DNA extracted from about 100 spores each from multiple accessions of *Glomus intraradices*, a common species in dry areas. We are presently assessing the variability of these regions at the inter-generic level down to the intra-species level in order to determine their usefulness for characterizing DNA molecules obtained directly from soil. In a parallel project we are refining techniques for extracting and amplifying VAM fungal DNA directly from unfractionated soil, and are estimating the limits of detection of this assay in field and greenhouse soils.

poster session 3 ST SH 52

ASBJORNSEN, H¹, A. CAYETANO², M. VALDÉS², S. PALACIOS-MAYORGA³ & K. VOGT¹. ¹Yale University, School of Forestry and Environmental Studies. New Haven, CT 06511. ²National School of Biological Sciences, Polytechnic Institute. 11340 Mexico DF, ³Institute of Geology, National Autonomous University, México D. F. - Mycorrhizal Inoculum Potential as an Indicator of Ecosystem Disturbance in the Mountain Tropics of Southern Mexico.

Among the legacies observed within disturbed oak forest ecosystems in the mountains of the state of Oaxaca, Mexico are the clearings (patches). These clearings were used for cultivating various subsistence crops, and many were abandoned within the last 20-30 years. The dominant trees (oak and pine) are strongly ectomycorrhizal (EM). We hypothesize that the mycorrhizal status of the soils may serve as an indicator for the degree of disturbance. We evaluated the mycorrhizal potential (MIP) of the soil for two ecosystems with different degrees of disturbance. Within each ecosystem, MIP was determined for three microsites (forest, edge and open). The method used was the Most Probable Number technique, and the trap plants were *Alnus acuminata* and *Pinus oaxacana*. The greatest degree of variation in the ecto-MIP was observed in the most disturbed ecosystem (200-2500 propagules/g soil). For the least disturbed ecosystem the lowest ecto-MIP was

observed in open microsites (1000 propagules/g soil) We also observed strong differences in the percentage of EM feeder roots of pine seedlings between the different microsites within each ecosystem. The greatest degree of variation in the number of propagules was observed in the most disturbed ecosystem. MIP of arbuscular propagules was the same in all microsites including the forest sites, but percent of colonization was higher in the forest of the most disturbed ecosystem (73%) compared to the other microsites (30-60%). Only arbuscules were found in the roots from the forest sites and vesicles in addition of arbuscules were observed in roots from the clearings.

talk session 6

ASHFORD, ANNE, GEOFF HYDE & LARA PERASSO. School of Biological Science, The University of New South Wales, Sydney NSW 2052, Australia - Fungal vacuoles, endosomes and transport

Vacuole systems of *Pisolithus tinctorius* labelled with 6-carboxyfluorescein can form pleiomorphic dynamic networks in the hyphal tips. We have been able to visualise these networks unstained by DIC optics, showing that their form and motility are not dependent on loading of fluorochrome. Tubules may also be seen connecting the larger vacuoles in mature hyphae, indicating that the system has potential for transport in both tip and mature regions. The integrity and motility of tubules is dependent on microtubules but not microfilaments. In the presence of anti-microtubule drugs tubules disappear leaving a series of rounded vacuoles, while cytochalasin apparently has no effect. Experiments using these drugs could provide the opportunity to separate transport via vacuoles from cytoplasmic streaming. Various characteristics including motility suggest that the vacuolar networks are equivalent to animal cell endosomal networks which interconnect and transfer material by fusion and fission rather than vesicle transport. Both endosomal networks and vacuolar networks have varicosities that progressively move along tubules. Endosomal varicosities are multivesicular bodies that are sites of molecular sorting and transport within linear, continuous compartments. Multivesicular bodies are very common in electron micrographs of hyphae, and we propose that similar mechanisms may operate in fungi. We are studying this using the styryl dye FM 4-64 which labels vacuolar membranes. Vacuoles of *Pisolithus tinctorius* contain high levels of phosphorus. We have suggested that at least some of this phosphorus is polyphosphate which occurs in a dispersed form rather than as granules. This view is further supported by the very low frequency of granules in live, unlabelled and fluorochrome-labelled vacuole systems.

poster session 6 WF AR 16

ASIF, M & ABDUL G. KHAN. Department of Biological Sciences, The University of Western Sydney - Macarthur, P. O. Box 555, Campbelltown NSW 2560, Australia. - Axenic growth and sporulation of *Glomus intraradices* from a transformed carrot root grown in the

proximity of a pre-colonized sweet potato root from an aeroponic system.

Aeroponically grown sweet potato roots infected with *Glomus intraradices* were surface-disinfected and placed on a minimal medium with or without a non-infected Ri-T DNA transformed carrot root. Extensive mycelium and spores developed on both the plates, although the growth was more profuse on the plate with the transformed root, which also became infected. When subcultured on minimal medium, without any root segment, axenic growth occurred on the plates inoculated for 6 weeks in dark at 25°C. The fungus possessed aseptate mycelium with angular projections typical of VAM fungi. Successful vesicular infection developed in the roots of Sudan grass when inoculated with infected hairy roots or a small agar block with axenic fungal growth only as sources of inoculum. No arbuscules were observed. Inoculated plants produced significantly higher PDW than the controls but compared to plants inoculated with sand culture, PWD were not significantly different. However, when both sources of inoculum were used collectively, a significant and perhaps synergistic effect on PDW was observed.

poster session 12 WF SH 63

AUGÉ, ROBERT¹, XIANGRONG DUAN¹, DAWN NEUMAN², JANET REIBER², CRAIG GREEN¹.

¹Institute of Agriculture, OHL, University of Tennessee, P.O. Box 1071, Knoxville, TN 37901-1071, USA. ²Department of Biological Sciences, University of Nevada, Las Vegas, NV 89154-4004, USA. - Mycorrhizal symbiosis, stomatal sensitivity and chemical signals of soil drying.

AM symbiosis can alter stomatal behavior of host leaves but the mechanism is not always clear. We tested if colonization by *Glomus intraradices* would result in altered stomatal sensitivity to hormonal root-to-shoot signals or altered movement of hormones in xylem as a function of soil water. In whole *Vigna unguiculata* plants, AM symbiosis allowed transpiration (E), stomatal conductance (g_s) and shoot water potential to be maintained to lower soil moisture than in comparative nonmycorrhizal plants. Fluxes and concentrations of ABA in xylem were lower in mycorrhizal than in nonmycorrhizal plants once soil drying was sufficient to begin closing stomates. Stomatal sensitivity of leaves to xylem sap pH or to fluxes or concentrations of ABA or zeatin riboside in xylem was not changed by AM colonization. Transpiration assays with detached leaves of *V. unguiculata* and *Pelargonium hortorum* suggest that there is generally no residual influence of the symbiosis in foliage; we almost always see mycorrhizal influence on g_s only in plants having intact root systems (*Rosa hybrida* was an exception). Involvement of xylem sap pH, calcium or phosphate has been previously implicated in a chemical root-to-shoot signaling process; E of mycorrhizal and nonmycorrhizal leaves of *V. unguiculata* and *P. hortorum* remained similar when pH and these ions varied in the feeding solution. Higher g_s of mycorrhizal plants in dry soil appeared primarily due to a

greater hydraulic efficiency of the mycorrhizal root system.

poster session 5 ST AR 02

AYLING, S.M., S.E. SMITH & F.A. SMITH. Department of Soil Science, Waite Research Institute, University of Adelaide, Adelaide, S.A. 5064, Australia. - The physiology of the plant-mycorrhizal fungus interface: preliminary observations.

The symbiosis between a host plant and mycorrhizal fungus involves bidirectional transfer of nutrients. Numerous workers have demonstrated enhanced uptake of phosphorous from the soil to the plant in the presence of mycorrhizal fungi; and export of carbon metabolites from the plant to the fungal hyphae. The mechanisms controlling these transfers must involve some modification of both plant and fungal membranes.

There is no information about the physiological conditions in the region of contact between the two organisms. Histochemical techniques have demonstrated high ATPase activity in the periarbuscular membrane, while molecular biological methods have identified a phosphate transporter in a VAM fungus. In the absence of detailed knowledge of the membrane potentials and ionic gradients in this zone the functional behaviour of these proteins can only be assumed. In this study we are combining electrophysiological techniques and confocal laser scanning microscopy to characterise the host-fungus interface.

poster session 13 WF SH 42

AZEMAR-LORENTZ, STEPHANIE, MICHEL CHALOT, BERNARD BOTTON & JEAN DEXHEIMER. University Henri Poincaré-Nancy I, Faculty of Sciences, Laboratory of Forest Biology, U.A. INRA 977, BP 239, F-54500 Vandœuvre Cedex, France. - Morphological and physiological evidences for the involvement of the root-cap in ectomycorrhiza formation between *Eucalyptus globulus* and *Pisolithus tinctorius*.

Observations on the root-cap anatomy in ectomycorrhizas, autohistoradiography techniques and capillary zone electrophoresis (CZE) applied to soluble carbohydrate determinations have shown a particular relationship between the host plant (*Eucalyptus globulus*) and the mycorrhizal fungus (*Pisolithus tinctorius*). In the functional part of the mycorrhiza (biotrophic area), the interactions between the plant and the fungus induced morphological changes in both partners of the symbiosis in order to facilitate the mutual exchanges. In contrast, the root-cap behaviour was completely different: morphological changes appeared only in the plant parts whereas the fungus was not affected. However, the fungus benefited of the nutrient supply resulting from a significant degradation of the cell walls (visualised by PATAg Test in M.E.T and detection of galactose in CZE), from depolymerisation of mucilage (PATAg Test and detection of fucose in CZE) and from degradation of phenolic compounds (M.E.T). Autohistoradiography showed a transfer of [3H]glutamate from the fungus to

the host plant with an accumulation in the meristematic zone. These results suggest that the root-cap is not a biotrophic area but rather a saprophytic zone which may be involved in the achievement of the mycorrhizal process.

poster session 2 ST SH 02

BAAR, JACQUELINE. Biological Station of the Wageningen Agricultural University, Kampsweg 27, 9418 PD Wijkster, The Netherlands. Present address: Department of Zoology and Physiology, University of Wyoming, Laramie, Wyoming 82071-3166, U.S.A. - The ectomycorrhizal flora of primary and secondary stands of *Pinus sylvestris* in relation to soil conditions and ectomycorrhizal succession

Ectomycorrhizal species composition and sporocarp abundance in two 15-20-year-old primary stands of *Pinus sylvestris* in the central part of The Netherlands were compared with those in two 16- and 27-year-old secondary stands of *P. sylvestris* in the northeastern part of The Netherlands. The trees of the primary stands were spontaneously seeded in a drift sand area. Only thin (< 4 cm) litter and humus layers were present. The trees of the 16-year-old secondary stand were planted on podsolic sandy soil and those of the 27-year-old stand on non-podsolic sandy soil. In both secondary stands, thick (4-10 cm) litter and humus layers had been developed. The litter and humus partly originated from former stands at the same sites, partly from the present stands. In the secondary stands, the thick litter and humus layers and understory vegetation were removed ("sod-cutting") in order to simulate the thin litter and humus layers in the primary stands. Untreated plots served as controls. Surveys conducted in 1991, 1992 and 1993 indicated that sod-cutting enhanced abundance and diversity of ectomycorrhizal fungi; however, the species richness and diversity in the sod-cut plots did not reach those in the primary stand plots. High species richness and diversity were associated with low concentrations of nitrogen and relatively high pH values in the litter and humus layers and mineral soil. These results suggest that sod-cutting may be beneficial in restoring ectomycorrhizal flora in Scots pine stands.

talk session 2

BAGO, BERTA¹, LUC SIMON² and YVES PICHÉ¹. ¹Centre de recherche en biologie forestière, ²Recherche en sciences de la vie et de la santé, Pavillon C.-E. Marchand, Université Laval, Québec, G1K 7P4 Canada. - In situ PCR: applications and prospects in the study of arbuscular mycorrhizal fungi.

The polymerase chain reaction (PCR) has been successfully applied to the study of arbuscular-mycorrhizal (AM) symbiosis, especially after the design of specific primers targeting ribosomal genes. However, DNA extraction prior to solution-phase PCR makes it impossible to directly correlate nucleic acid analysis with the histological features of the sample. Until recently, in situ hybridization protocols had to be developed to

address this question. In situ PCR, a relatively new technique, allows both more sensitive and more specific detection of the target sequence within specimens fixed on microscope slides, permitting to spatially correlate PCR results with cell morphology. Although it has never been applied before to AM-research, in situ PCR could prove an interesting tool in the study of AM genetics (e.g. to confirm the presence of different alleles in AM fungal (AMF) genes, to show possible different nuclear populations within a single AMF spore), cytology (e.g. nuclei behavior, distribution of different endophytes colonizing the same plant) and physiology (e.g. expression of symbiosis-related genes along the different stages of mycorrhiza formation). We have developed a fluorescent in situ PCR protocol which has allowed us to obtain specific amplification signal in the nuclei of two AMF. The sensitivity and specificity of this technique and its suitability for AM research will be discussed.

poster session 1 ST RR 09

BAGYARAJ, D.J. & CAROLINE MACHADO. Agricultural Microbiology, The University of Agricultural Sciences, GKVK Campus, Bangalore, 560065, India. - Effect of soil solution P levels on VAM symbiosis in mulberry

An investigation was conducted to predict phosphorus concentration established in the soil solution relevant for VAM symbiosis in mulberry. Soil was limed and equilibrated to raise the soil pH to 6.2, which is optimum for VAM symbiosis. Four different levels of P were established by applying varying amounts of KH₂PO₄ to limed soil (0, 2S, 100 and 400 mg/Kg soil) in pots containing 3.5 Kg of unsterile soil; other essential nutrients were supplied at optimum levels for VAM activity and subjected to 3 cycles of wetting and drying which resulted in soil solution P levels of 0.007, 0.022, 0.088 and 0.232 mg P/L respectively. *Glomus fasciculatum* and *Glomus aggregatum* inoculum were added at the rate of 2S g/Kg of soil. Cuttings of mulberry cultivars S-34 and K-2 were planted at the rate of two cuttings per pot and maintained in a glass house. Number, chlorophyll content, fresh and dry weight of leaves; biomass and P content of the plant, and mycorrhizal colonization of the root were determined 60 and 150 days after planting. Mycorrhizal inoculation effect was also calculated. The results brought out that both the varieties of mulberry responded to VAM inoculation as well as P application. Mulberry responded better to inoculation with *G. fasciculatum* compared to *G. aggregatum*. Of the two cultivars of mulberry, S-34 responded better to mycorrhizal inoculation. S-34 cultivar performed better at soil solution P level of 0.022 mg P/L while K-2 cultivar responded to mycorrhizal inoculation better at 0.007 mg P/L. Soil solution P level more than 0.022 mg P/L though enhanced P uptake by mulberry plants did not benefit biomass production. The present study brings out that any additional amount of P fertilizer to soil resulting in more than 0.022 mg soil solution P/L will not benefit either VAM symbiosis or plant growth in mulberry.

morning talk

BAINBRIDGE, BRIAN. Microbiology Group, Division of Life Sciences, King's College, Campden Hill Road, London W8 7AH, UK. - Fungal Biodiversity: origins, estimation and significance.

Interest in biodiversity may be motivated by a desire to conserve rare species, to investigate the effects of fungi on environmental or agricultural productivity or to search for novel compounds of therapeutic value. Biodiversity at the morphological level must be reflected in diversity at the genetic and molecular levels. The origin of diversity is complex and involves mutation/selection as well as random and neutral changes. The processes resulting in change can be classified into genetic, molecular and cytoplasmic effects. Genetic processes involve mutation, mitotic and meiotic recombination, nondisjunction, chromosomal rearrangement and translocation. Molecular processes include gene conversion, transposition, molecular drive and molecular switching. Cytoplasmic factors such as mitochondrial DNA, mycoviruses, retrotransposons and plasmids have all been shown to influence phenotypes. Estimation of fungal biodiversity is difficult for a variety of reasons. Fungi can reproduce asexually and may even be imperfect. This can mean that the concept of the organism or of the species, in the plant or animal sense, is problematical. Consequently it is difficult to estimate richness or abundance by simple counts. Molecular methods show considerable promise and have influenced the analysis of phylogenetic relationships, the detection of organisms in the environment and the structure of fungal populations. However the choice of molecular targets is crucial and can vary from fungus to fungus. For example mitochondrial DNA may show high variability, where recombination between heterologous molecules can occur, but very little where there is uniparental inheritance. Major progress has been made by the application of the polymerase chain reaction which offers the possibilities of detecting and sequencing DNA from unculturable fungi as well as the *in situ* analysis of hyphae in the rhizosphere.

poster session 1 ST RR 08

BAKARR, MOHAMED I.^{1,2}, MARIA Q. TARLUE² & DAVID SUALE³. ¹Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, FL 33124 U.S.A. ²Department of Biological Sciences, Njala University College, PMB, Freetown, Sierra Leone, West Africa. ³Institute of Agricultural Research (IAR/MAFF), PMB, Freetown, Sierra Leone, West Africa. - Arbuscular mycorrhizae affect growth of maize (*Zea mays*) in monocrop and intercrop with cowpea (*Vigna unguiculata*)

We conducted an experiment in fumigated (with granular Dazomet) field microplots (1 X 1 m) to determine the effects of arbuscular mycorrhizae (AM) on growth of maize in monoculture and intercropped with a nodulated legume, cowpea. Monocultures consisted of 25 maize plants/m²; intercrops alternated three five-plant rows of cowpea with two rows of maize for the same total density. We used a 2 X 2 factorial design with

mycorrhizal inoculation and cropping system as treatments. We harvested ten maize plants per plot, and assessed treatment effects on plant height, leaf area, shoot fresh mass, and total mass per plot. We terminated the experiment eight weeks after planting. AM had a significant positive effect on all measures of maize growth. Presence of cowpea had a significant negative effect on maize height and total mass of maize. Lack of significant interaction indicates that intercropping did not change the relative benefit of AM.

poster session 4 ST AR 42

BALAJI, BOOVARAGHAN, ARMAND SEGUIN & YVES PICHE. CRBF, Université Laval, Ste-Foy, Québec G1K 7P4, Canada. - Expression of PAL and CHS mRNAs during appressoria formation in a non-mycorrhizal (Myc⁻) and colonization in a mycorrhizal (Myc⁺) transformed roots of pea (*Pisum sativum* L.)

Arbuscular mycorrhizal (AM) fungi associate with most of the vascular plants for which they provide nutrients and protect against drought and plant pathogens. Though the physiology of the AM symbioses has been extensively studied, little is known of the biochemical and genetic processes involved. Following microbial attack, plants accumulate phytoalexins, phenylalanine ammonia-lyase (PAL) activity and the corresponding PAL or chalcone synthase (CHS) mRNAs. PAL and CHS are regulatory enzymes of flavonoid and isoflavonoid biosynthesis. In our present study, we examined the accumulation of phytoalexins and the expression of PAL and CHS mRNA in the transformed roots of pea (*Pisum sativum* L.) cv. Frisson following inoculation with a AM fungus, *Glomus intraradices* (Schenck & Smith) during different stages of AM colonization, viz; contact, penetration and colonization of AM fungi. We have established transformed roots cultures of pea, either resistant to AM invasion (Myc⁻), or normally AM colonized parental line, Frisson (Myc⁺). Pisatin, a phytoalexin of pea, was analyzed by HPLC and Northern blot hybridization was done to study the expression of PAL and CHS mRNAs. Our model with Ri T-DNA transformed roots of Myc⁻ and Myc⁺ pea would allow one to eliminate other contaminating microbes and provides an ideal system for physiological and genetic studies.

poster session 2 ST SH 03

BALDWIN, QUENTIN and KEITH EGGER. Biology Dept, Memorial University of Newfoundland, St. John's, Nfld. A1B 3X9, Canada. Natural Resources and Environmental Studies, The University of Northern British Columbia, Prince George, BC, V2N 4Z9, Canada. - Diversity of postfire mycorrhizal fungi on *Picea mariana* Mill. within prescribed burn sites along the west coast of Newfoundland.

Three year old Black Spruce (*P. mariana*) seedlings were collected for mycorrhizal diversity assessment as part of a Canadian Forest Service project studying the effects of

prescribed burning. In the fall of 1993, 25-week old seedlings were planted into 4 experimental blocks located in a clear-cut region. Each block consisted of 3 randomly allocated treatments (high intensity burn, low intensity burn, unburned). In the fall of 1995 randomly harvested seedlings were surveyed for mycorrhizal fungi using molecular identification techniques. Root tips were randomly selected from each seedling and frozen for later molecular analysis. Individual root tips were crushed and the fungal DNA amplified via the polymerase chain reaction (PCR) procedure. Fungal specific primers were used to amplify an approximately 1100 base pair segment of the ribosomal DNA. Restriction fragment length polymorphisms (RFLP) for each fungal isolate were obtained using three restriction endonucleases (Alu I, Hinf I, Rsa I). Fragment patterns were analyzed to match isolates and measure biodiversity. Preliminary results show that at least 9% of root tips analyzed harbored more than one fungal species per root tip. It has also been observed that some root tips which show mycorrhizal habitation cannot be successfully amplified. At present the RFLP data for each of the burn treatments is being compared to determine shared and unique fungal species within and between treatments.

poster session 1 ST RR 37

BARNOLA, L.¹, C. HAMEL² and D. SMITH¹.¹Department of Plant Sciences, McGill University, Macdonald Campus. ²Department of Natural Resources Sciences, McGill University, Macdonald Campus, 2111 Lakeshore Road Ste-Anne-de-Bellevue, Quebec, Canada, H9X 3V9. - Evaluation of the efficiency of different AM fungi on sweet corn (*Zea mays*) and pepper (*Capsicum frutescens*) under greenhouse and field conditions.

The objective of this research is the selection of the most efficient arbuscular mycorrhizal fungus on pepper (*Capsicum frutescens* L. cv. North star) and sweet corn (*Zea mays* L. cv. Bicolor) growing under greenhouse and field conditions. Nine different AM strains were produced in pot cultures with marygold plants (*Tagetes patula* L.). Both pepper and sweet corn were inoculated with each one of the nine AM fungi and a non-mycorrhizal control and planted in pasteurized soil (greenhouse) in 4 different locations in the field. Under greenhouse conditions a strain of *Glomus intraradices* showed the highest levels of colonization for both pepper and sweet corn. At the same time, the same inoculum exerted a significant decrease of pepper mass compared with the control. In the other hand, corn plants showed a significant increase of mass when inoculated with *G. microcarpum*. Under field conditions, no significant results were obtained for pepper mass whereas a significant decrease of mass was observed for sweet corn inoculated with the same strain of *G. intraradices*. No differences were observed in both sweet corn and pepper when comparing the effect of the same mycorrhizal species, *G. aggregatum*, but from different sources (undisturbed habitat and a field sample).

poster session 8 WF AR 32

BARROW, JERRY¹, BOBBY MCCASLIN², CURTIS MONGER², INEZ FEDER². ¹USDA-ARS, Jornada Experimental Range, Las Cruces, NM 88003. ²Department of Agronomy and Horticulture, New Mexico State University, Las Cruces NM 88003. - Mycorrhizal similarities of septate fungal root endophytes in native plant species of arid ecosystems.

Levels of fungal colonization in the roots of fourwing saltbush *Atriplex canescens* and other dominant native grass and shrub species from an arid ecosystem have been studied. It was shown that colonization by septate fungal colonization was 2.7 times greater in fourwing saltbush than colonization by VAM fungi. Historically studies have focused on the role of VAM fungi and how they affect the plant. The septate fungi bear several similarities to other types of mycorrhizae. They formed extensive non-destructive interfaces, primarily by vegetative hyphae, within the root cortex. They enhance nutrient uptake at low concentrations and restrict uptake at high concentrations. They also protect against high salt concentrations. Some are competent decomposers, and appear to enhance seedling vigor and establishment by supplying nutrients from external organic matter. They modify soil by binding sand particles to the root surface. We propose that septate fungal endophytes have a major function in ecosystem processes.

poster session 3 ST SH 53

BAXTER, JAMES W.¹, STEWARD T. A. PICKETT², MARGARET M. CARREIRO³ & JOHN DIGHTON¹. ¹Rutgers University, Piscataway, NJ 08855-1059 USA, ²Institute of Ecosystem Studies, Millbrook, NY 12545-0129 USA, ³Fordham University, Louis Calder Center, Armonk, NY 10504 USA. - Ectomycorrhizae and oak seedling performance in human altered forest ecosystems.

We examine ectomycorrhizal (EM) status, oak seedling performance, and soil nutrient availability in similar mature oak-dominated forests in urban Bronx Co., NY, and rural Litchfield Co., CT, U.S.A. Consistent with their increased exposure to pollution, the urban forest soils contain elevated levels of heavy metals and inorganic N (primarily as nitrate). Red oak (*Quercus rubra*) seedlings were grown for 16 weeks in the greenhouse in soil cores collected from 5 urban and 5 rural forest stands. EM infection and morphotype composition was quantified on half the seedlings (n = 50), while plant height, leaf area, leaf number, root and shoot biomass, and total N and P status were measured on the other half (n = 50). Soil cores were also collected from the field sites to quantify EM status. Soil N and P availability was assessed by measuring NH_4 and PO_4 uptake rates into mature red oak roots collected from the field. Total EM infection was higher in urban seedlings. However, EM richness was higher in rural seedlings and EM composition differed between the treatments. Similar differences in EM status were observed on oak roots collected from the field. Seedlings grown in the rural forest soils had higher leaf area and total N content with

trends toward increased foliar biomass and P content. Uptake rates of NH_4 and PO_4 in urban roots were 75% higher for NH_4 and 3 times higher for PO_4 , suggesting that both of these nutrients are relatively limiting in the urban forest soils. Whether it is altered EM composition and abundance or changes in N and P availability that are responsible for decreased oak seedling performance in the urban soils is a question that is currently being investigated.

poster session 8 WF AR 39

BEARDEN, BIRGITTE NEERGAARD. The Royal Veterinary and Agricultural University, Department of Chemistry, 40, Thorvaldsensvej, DK-1871 Frederiksberg C, Denmark. - Influence of arbuscular mycorrhiza on aggregate stability of Vertisols.

Low infiltration, leading to runoff and loss of sparse precipitation, is a problem in many semiarid tropical areas. Where the soil contains swelling clay minerals, as in semiarid India where this work was conducted, water stable aggregates are essential for infiltration and hence availability of water for plants. The influence of arbuscular mycorrhiza (AM) on aggregate stability of vertisols was studied in a pot experiment in which sorghum was grown in sterilized soil inoculated with mycorrhizal inoculum as well as in sterilized and unsterilized control soils. Part of the soil in the pots was separated from the roots by a nylon mesh bag. Aggregate stability of the soil inside and outside the mesh bags was studied at harvest, after 10 weeks of plant growth. Water stability of aggregates was determined by wet sieving and turbidimetric measurements, and AM was quantified as colonized root length and hyphal length.

Wet sieving of soil from outside the mesh bags showed greater "mean diameter weight" (MDW) in the soil inoculated with mycorrhiza than in non-inoculated soil. However, unsterilized soil showed equally high MDW despite the fact that the sterilization process itself had been proven to increase the MDW before the experiment. Inside the mesh bags there was no significant effect of the inoculation, but the unsterilized soil showed a higher MDW. The turbidimetric measurements of the bulk soil showed the highest aggregate stability in the sterilized and inoculated soil. Although the unsterilized soil had the lowest stability, it showed the largest increase in stability. These results show that mycorrhiza affects the aggregate stability and that both increased root growth and mycorrhizal hypha is involved.

talk session 3

BECKMANN, SUSANNE & INGRID KOTTKE. Eberhard-Karls-Universität, Tübingen, Botanisches Institut, Spezielle Botanik und Mykologie, Auf der Morgenstelle 1, 72076 Tübingen, Germany. - Deposition of nitrogen in ectomycorrhizae of Norway spruce (*Picea abies* (L.) Karst.) on NH_4^+ -fertilized sites in the Black Forest, Germany

A nitrogen-fertilization experiment has been carried out on two plots of the ARINUS-Projekt, Schluchsee and

Villingen and the Nitrogen-deposition in ectomycorrhizae of Norway spruce was studied. The survey intended to prove whether accumulation of nitrogen containing granules takes place in field material as was shown as in vitro. Different mycorrhizal types and observation sites were compared. Proof and relative quantification of N-accumulation was aided by EELS, Electron Energy Loss Spectroscopy. Compared to the vacuole, the N-content was up to 53 times increased in the granules. All of the investigated types of mycorrhizae were able to form the granules, though to a different extent. The number of granules per mm² of hyphal mantle increased after fertilization. The increase depended on the mycorrhizal type and more marked on the Schluchsee plot. *Xerocomus badius* mycorrhizae and *Cenococcum geophilum* showed already high amounts of granules on the control plot. The N-content of the granules of *Xerocomus badius* was always high. In contrast, *Tylospora fibrillosa* contained a higher number of granules and higher N-contents of the granules only after the fertilization. Only *Tylospora fibrillosa* showed clear differences among the soil horizons. In conclusion mycorrhizal types reacted differently. Apart from mediating the N-nutrition of the host trees, ectomycorrhizae are shown to play a role in the regulation of the nitrogen household of forest soils because of their capacity of N-accumulation.

talk session 7

BEGUIRISTAIN, THIERRY, JIANGUO HUANG & FREDERIC LAPEYRIE. Equipe de Microbiologie Forestière, I.N.R.A., Centre de Nancy, 54280 Champenoux, France. - Host plant stimulates the tryptophan betaine, hypaphorine, accumulation in *Pisolithus tinctorius* hyphae during ectomycorrhizal infection. Fungal hypaphorine controls K⁺ uptake, H⁺ extrusion and root hair development.

Hypaphorine, the betaine of tryptophan, is the major indolic compound which accumulates in *Pisolithus tinctorius* hyphae. Hypaphorine is over-accumulated in *P. tinctorius* tissues as soon as hyphae are in contact with host plant root surface. This phenomenon observed, 24 hours after inoculation was recorded as well in several month old ectomycorrhizae. Such over-accumulation is controlled by specific root diffusible molecules, it could be induced through a membrane, but non-host plants are inactive.

Hypaphorine solutions (0.1-10 µM) applied to fungal colonies stimulated net K⁺ uptake and net H⁺ extrusion. Hypaphorine and IAA activities were in the same range of magnitude, while tryptophane, a precursor of IAA and hypaphorine in *P. tinctorius*, was inactive. *P. tinctorius*, a hypaphorine producer, was much less responsive to exogenous micromolar hypaphorine solutions, than *Paxillus involutus*, a non-hypaphorine producer. Hypaphorine might be in some way contributing to ion fluxes regulation through plasma membrane, in hyphal tip for cell elongation and in mycorrhizae for nutrient exchange between hyphae and root cortical cells.

Fungal hypaphorine had no IAA like activity on *Eucalyptus* root elongation and ramification and therefore

could not be considered as an auxin analogue, instead a strong reduction of root hairs elongation was recorded (10-1000 µM)

poster session 5 ST AR 04

BEGUIRISTAIN, THIERRY & FREDERIC LAPEYRIE. Equipe de Microbiologie Forestière, I.N.R.A., Centre de Nancy, 54280 Champenoux, France. - The tryptophan betaine, hypaphorine, produced by *Pisolithus tinctorius* during ectomycorrhizal infection is readily absorbed and metabolised by *Eucalyptus globulus*, the host plant.

Hypaphorine the betaine of tryptophan, is over-accumulated in *Pisolithus tinctorius* tissues as soon as hyphae are in contact with host plant, *Eucalyptus globulus*, root surface. This phenomenon observed, 24 hours after inoculation was recorded as well in several month old ectomycorrhizae. *P. tinctorius* synthesise hypaphorine from externally applied ¹⁴C-tryptophan and ¹⁴C-hypaphorine was purified by HPLC.

Eucalyptus globulus seedlings incubated in fungal hypaphorine were taking up hypaphorine at a slightly lower rate than tryptophan. Consequently, after 9 hours, *E. globulus* over-accumulated in roots a yet non-identified compound RAC-11 (Root Aromatic Compound, 11 min. retention time). RAC-11 is synthesised by *E. globulus* from ¹⁴C-hypaphorine (15% of absorbed hypaphorine), but not from ¹⁴C-tryptophan. Apart from RAC-11, absorbed ¹⁴C-hypaphorine was recovered in roots mainly as hypaphorine (20%) and tryptophan (10%).

talk session 2

BÉLAND, MARC, PETER MOUTOGLIS¹, PAUL WIDDEN & J. ANDRÉ FORTIN¹. Biology Department, Concordia University, 1455 de Maisonneuve Blvd. W., Montreal, Québec, Canada H3G 1M8. ¹Institut en Biologie végétale, Université de Montréal, 4101 est rue Sherbrooke, Montréal, Québec, Canada H1X 2B2. - rDNA characterization of VAM fungi colonizing *Medeola virginiana* in three Québec forests.

It is generally assumed that there is a lack of host-specificity in vesicular arbuscular mycorrhizal fungi (VAMf). Experiments using pot-cultured VAMf under controlled conditions show that a single plant species can be colonized by a wide range of taxa. Host-specificity under field conditions is difficult to investigate due to our inability to identify VAMf *in plantae*. The development of fungal specific primers for the amplification of rDNA genes using the Polymerase Chain Reaction (PCR) provides a powerful tool to address the question of host-specificity in the field. We have developed a method to extract DNA from the roots of colonized *Medeola virginiana* plants from the field and to amplify rDNA genes (Internal Transcribed Spacer regions and the 5.8s gene) of the VAMf, using PCR. Restriction analysis, combined with sequencing of the genes was used to characterize the VAM genotype in three natural forest populations of this plant in Québec.

poster session 13 WF SH 68

BELGRAVE, CAROL, LOUIS E. CHINNERY & SUSAN B. PERSAD-CHINNERY. Department of Biology, The University of the West Indies, P.O.B. 64, Bridgetown, Barbados. - Mycorrhizal induced changes in root morphology, nuclear diameters and mitotic indices of corn and leek.

Corn (*Zea mays*) and leek (*Allium porrum*) separately colonised by two species of VA mycorrhizal fungi, *Gigaspora decipiens* and *Scutellospora gregaria*, showed decreased numbers of lateral roots and reduced lengths of primary roots but there were no significant differences in the mean numbers of these primary roots when compared to uncolonised controls. At distances 0 - 1, 1 - 2 and 2 - 3 cm behind the root meristems mean nuclear diameters were greater in cortical cells from colonised than from uncolonised corn roots. The size difference was more pronounced further from the root tip. Increased nuclear diameters were also recorded within 1 cm of the root tip of colonised leek. Necrotic meristematic apices were prevalent in both corn and leek roots colonised by both species of VA mycorrhizal fungi. The mitotic indices in healthy root meristems of mycorrhizal corn and leek were lower than those recorded for uncolonised plants.

poster session 5 ST AR 05

BERBARA, RICARDO¹, HENRIQUE FONSECA², MELVIN DAFT³. ¹Universidade Federal Rural do Rio de Janeiro, Departamento de Solos, Seropedica, Itaguaí, RJ, CEP 23851-970, Brazil. ²Universidade de Aveiro, Departamento de Biologia, 3800 Aveiro, Portugal. ³Biological Sciences Department, University of Dundee, Dundee, DD1 4HN, Scotland, UK. - The role of calcium in the germination and germinative hyphal growth of *Gigaspora rosea* Nicolson and Schenck.

The role of lanthanum (calcium blocker), EGTA (calcium chelator) and TFP (calmodulin antagonist) on the spore germination; hyphal growth, branching and gravitropism of *Gigaspora rosea* Nicolson and Schenck were studied in media with and without calcium. Spore germination was influenced by EGTA and La, although in the former the effect was only significant at the highest level (32mM). Hyphal growth decreased with increasing levels of La, EGTA and TFP. Conversely, higher number of hyphal tips were observed at higher levels of these reagents. Increasing concentrations of La, EGTA and TFP caused mycelia to branch and lose gravitropism. It is proposed that calcium mobilisation within the fungi affect the morphogenesis *G. rosea*.

poster session 1 ST RR 11

BERMAN, JENNIFER, CAROLINE BLEDSOE. Soils and Biogeochemistry, Dept. of L.A.W.R., The University of California, Davis, CA 95616. - The effect of forest soil inoculum on mycorrhizal root development and growth of valley oak seedlings

A field experiment was carried out to assess the effects of riparian forest soil on ectomycorrhizal root development

and seedling growth of valley oak (*Quercus lobata* Née.). Soil collected from a mature riparian valley oak forest containing abundant potential fungal inoculum was put in acorn planting holes in an agricultural field. Two additional treatments were set up as controls: agricultural field soil and steam sterilized forest soil. When seedlings were harvested ten months later mycorrhizal roots were found in all three treatments. The greatest percent mycorrhizal infection and fine root biomass were found for seedlings grown in forest soil. Shoot biomass was greatest in the forest soil treatment and least in the agricultural soil, while biomass was intermediate for the sterilized forest soil. Our data suggest that mycorrhizal activity in the forest soil contributed to the increased seedling growth of the forest soil grown seedlings. The sterilized forest soil also contributed to growth through unknown mechanisms.

talk session 5

BERTA, GRAZIELLA¹, GUIDO LINGUA¹, ANNA FUSCONI², ANTONIO TROTTA². ¹Dipartimento di Scienze e Tecnologie Avanzate, corso Borsalino 54, 15100, Alessandria, Italy. ²Dipartimento di Biologia Vegetale, Viale Mattioli 25, 10125, Torino, Italy. - Quantitative and structural variations in host cell nuclei of arbuscular mycorrhizal and/or pathogenic systems.

Some evidence exists that arbuscular mycorrhizas (AM) increase tolerance of host plants to soil-born fungal pathogens. A strong protective effect has been shown in tomato (*Lycopersicon esculentum*) against *Phytophthora nicotianae* v. *parasitica*, resulting in a significant reduction of plant growth inhibition and of necrotic root zones. We investigated quantitative and structural nuclear variations induced in tomato roots by *Glomus mosseae*, by *P. nicotianae* v. *parasitica*, and by their interactions, using a combination of transmission electron microscopy (TEM), flow cytometry and cytochemical techniques. Nuclei extracted from tomato roots of mycorrhizal and control plants (forty-five days old), infected and uninfected by the pathogen, were analysed by flow cytometry after staining with saturating DAPI concentration (DNA quantity), or with under-saturating DAPI or saturating propidium iodide (PI) (chromatin structure). Saturating DAPI staining caused higher fluorescence in control and mycorrhizal root nuclei compared to those extracted from *P. n. v. parasitica* inoculated roots, suggesting DNA loss in the latter. Undersaturating DAPI and saturating PI staining of non-mycorrhizal *P. n. v. parasitica* inoculated plants gave lowest fluorescence, while mycorrhizal plants, both with and without *P. n. v. parasitica*, had the highest ones. TEM analyses confirmed this pathogen effect on chromatin condensation, which was attenuated by AM. Finally, both saturating DAPI and PI staining gave a peak corresponding to a 4C nucleus population, and the ratio of number of 4C nuclei to 2C nuclei was always significantly higher in non-mycorrhizal plants. These last results are particularly intriguing and suggest that in tomato, AM fungi could interfere with DNA endoreduplication processes.

poster session 1 ST RR 13

BETHLENFALVAY, GABOR, PAUL SCHREINER & KEIKO MIHARA. USDA Agricultural Research Service, Horticultural Crops Research Laboratory, Corvallis, OR 97330, USA. - Mycorrhizal fungi enhance weed control and crop growth in a soybean-cocklebur association treated with herbicides.

We grew soybean (*Glycine max*) and cocklebur (*Xanthium strumarium*) plants in pots with or without a mixed inoculum of *Glomus*. Labeled N (1 mL, 100 mM $^{15}\text{NH}_4\text{NO}_3$, 98 atom % ^{15}N) was applied to cocklebur leaves 4 d before spraying all plants with the herbicide bentazon at rates of 0, 1/3, 2/3, 3/3, & 4/3 of the field-recommended (FR, 1.12 kg a.i./ha) rate. Plants were assayed for growth parameters 2 weeks after spraying; soybean leaves were also tested for ^{15}N to determine its transfer from cocklebur. The AM fungi did not modify the herbicide effect in soybean or cocklebur at 0 and 4/3 FR. At the intermediate FR levels, growth and nutrient contents of +AM soybean were significantly enhanced, while cocklebur shoot dry mass was inhibited. Labeled N in +AM or -AM soybean leaves did not differ at 0 and 4/3 FR, but at the intermediate rates ^{15}N abundance was significantly higher in +AM plants. Cocklebur, competitive at 0 FR, apparently did not transfer assimilates to soybean, while at 4/3 FR rapid and pervasive necrosis of leaf tissues prevented export. At 2/3 FR, however, conditions were ideal for nutrient transfer. The results indicate that significant shifts in intra- and inter-plant source-sink relations occurred as a result of selective stress imposed on cocklebur by the herbicide, and this shift in competitiveness permitted a mycorrhiza-mediated flux of nutrients from cocklebur to soybean. Foliar herbicides apparently affect weed-crop relations in ways that involve the below-ground components of agrosystems.

poster session 5 ST AR 07

BLAUDEZ, DAMIEN, MICHEL CHALOT, PIERRE DIZENGREMEL & BERNARD BOTTON. University Henri Poincaré-Nancy I, Faculty of Sciences, Laboratory of Forest Biology, U.A. INRA 977, BP 239, F-54500 Vandœuvre Cedex, France. - Uptake and metabolism of [^{14}C]glutamate and [^{14}C]malate by *Betula pendula* / *Paxillus involutus* mycorrhizas.

Modifications of nitrogen assimilation pathways during mycorrhizas formation have been demonstrated by several authors. Labelling experiments with [^{14}C]glutamate and [^{14}C]malate were performed with detached mycorrhizal (MYC) or non mycorrhizal (NM) roots from 6 and 15 day-old and from 3 month-old seedlings. The newly-formed ECM roots (6 and 15 day-old) took up the label more efficiently than the oldest one did. MYC roots took up the label at a 5- to 10-fold higher rate, when compared with NM roots. Most of the label in the MYC or NM root system was recovered in the soluble fraction (amino and organic acids) but the ratio of the radioactivity recovered in the soluble fraction on the radioactivity recovered in the insoluble fraction was higher in MYC roots. Under both [^{14}C]glutamate and

[^{14}C]malate feeding, glutamine was the main labelled amino acid, the remaining radioactivity being recovered in aspartate, glutamate, GABA and alanine in MYC and NM roots. However, in MYC roots the ratio [^{14}C]glutamine/[^{14}C]glutamate remained higher compared with NM roots. The low recovery of label in organic acids suggested that amino acid synthesis is an important sink for TCA cycle intermediates. The distribution of label differs also with the age of the roots. For instance [^{14}C]aspartate and [^{14}C]alanine were detected in 6 day-old MYC roots but not in 15 day-old MYC roots. The implications of such modifications for N and C interactions during the early stages of mycorrhizas formation are discussed.

poster session 4 ST AR 59

BLEE, KRISTOPHER A. & ANNE J. ANDERSON. Department of Biology, Utah State University, Logan, Utah, 84322-5305, USA. - A comparison of responses in bean roots to colonization by *Glomus intraradices* and *Fusarium solani*.

Dark red kidney bean (*Phaseolus vulgaris* L. cv. Moncalm) roots were differentially colonized by pathogenic and beneficial fungi. The pathogenic *F. solani* f. sp. *phaseoli* grew inter- and intracellularly throughout the root cortex and penetrated the vascular cylinder. *G. intraradices* grew intercellularly until reaching certain cortical cells surrounding the endodermis which were penetrated and arbuscules formed within. Invading hyphae of *F. solani* f. sp. *phaseoli* autofluoresced yellow. Arbuscules of *G. intraradices* caused a pink autofluorescence and vesicles yellow. Whether the autofluorescence is due to plant or fungal cell wall components is not known. Accumulation of transcripts for defense-related proteins were detected differentially in roots colonized by the pathogenic and beneficial fungi. *In situ* hybridizations showed the arbusculated cortical cells responded with increased accumulations of transcripts for the defense-related enzymes phenylalanine ammonia-lyase and chitinase. Transcripts for two cell wall-associated proteins peroxidase (PER) and hydroxyproline-rich glycoprotein (HYP) were elevated in cells in contact with the pathogen. HYP but not PER transcripts were increased in arbusculated cells of mycorrhizal roots. PER transcript accumulation in the *F. solani* f. sp. *phaseoli* infected roots was not accompanied with lignification as detected by phloroglucinol-HCl staining.

poster session 8 WF AR 48

BODDINGTON, CLAIRE L.¹ & JOHN C. DODD¹. ¹International Institute for Biotechnology/Dept. of Biosciences, University of Kent, Canterbury, Kent. CT2 7YW. UK. - A study of the hyphal dynamics of tropical species of arbuscular mycorrhizal fungi (AMF) from 4 genera in association with a plant host.

The extraradical mycelium (ERM) of AMF is the interface between the plant root system and the soil matrix. This mycelial network allows direct recycling of

nutrients, e.g. P, and helps stabilise the soil structure. Information is required on the morphology and function of the ERM for effective application of AMF in ecosystem restoration projects. A study of fungal morphogenesis of 4 species of AMF from 4 genera: *Glomus manihotis* (INDO-1), *Gigaspora rosea* (PHIL-INVAM), *Scutellospora heterogama* (BEG-35) and *Acaulospora tuberculata* (BEG-47), has been made. Spores were placed between Millipore membranes in simple laboratory microcosms in the presence of a host root system. Results show clear differences in the hyphal architecture and dynamics between the 4 species following spore germination. The ecological relevance of these data will be discussed. The function of the ERM of 3 of these 4 species of AMF has been studied using inoculated *Desmodium ovalifolium* plants grown under tropical glasshouse conditions in an experimental pot system. A 37µm mesh pouch was inserted into each pot to allow root-free hyphal growth. Hyphae were extracted from these pouches over 3 successive harvests during a 20-week time period. Hyphal lengths were compared for each species of AMF used, metabolic activity was measured using alkaline phosphatase stain (AP). The arbuscular colonisation of roots was quantified and stained for AP. The results of this work will be discussed.

poster session 15 WF RR 08

BONELLO, PIERLUIGI & THOMAS D. BRUNS. Dept. of Environmental Science, Policy, and Management, University of California at Berkeley, 151 Hilgard Hall, Berkeley, CA 94720-3110, USA. - Population structure of *Suillus pungens* in a coastal *Pinus muricata* community of Northern California

The population structure of *Suillus pungens* was investigated in a 40 year old coastal forest of Bishop pine (*Pinus muricata*) in Northern California. Using regular PCR, RAPDs, and AP-PCR (arbitrary primer PCR) of fruitbody tissues, 11 polymorphic loci were selected for single stranded conformational polymorphism (SSCP) analysis. A total of six different genotypes were detected in a 1200 m² study area. Five of the six genotypes were represented by single fruitbodies. The largest genotype, comprising 13 fruitbodies, covers an area of at least 300 m², with maximum measured dimensions of 40 m and 14 m. This genotype is the largest vegetative clone of an ectomycorrhizal fungus ever described, because apomixis could be excluded as a propagative mechanism.

The fruitbody population had an observed average heterozygosity level of 0.423, indicating that outcrossing is a major component of the reproductive behavior of *S. pungens*. This implies that potential secondary homothallism, suggested by the dikaryotic nature of a proportion of the basidiospores, and which would result in selfing, is not ecologically significant.

The combination of rapid growth and the abundant fruiting suggests *S. pungens* has a larger carbon budget than might be expected for a species known to be only a very minor ectomycorrhizal component of this community. Evidence suggests that this ectomycorrhizal

fungus has saprophytic potential: *S. pungens* was able to grow in vitro with peat as the sole organic carbon source.

poster session 6 WF AR 11

BONET, JOSE A., JUAN J. BECERRIL, JORDI ROMA & CARLOS COLINAS. Dep. Proteccion de cultivos, Centro UdL-IRTA, Av. Rovira Roure 177, E-25198 Lleida, Spain. - The relationship between orientation, thinning and forest age on the production of sporocarps of ectomycorrhizal fungi in *Pinus sylvestris* forests of the Central Pyrenees

Harvest of edible sporocarps of ectomycorrhizal fungi in the Pyrenees has increased over the past five years with interest in managing forested lands for mushroom production. This study was designed to examine the influences of silvicultural treatments and forest conditions on sporocarp production over four years. Weekly collections were made from research plots from mid-Sept. through Nov. Results presented here were obtained from 1994 and 1995.

The average fresh weight of sporocarps of ectomycorrhizal fungi was 105 kg/ha (dry weight = 11 kg/ha). Of this total, 61 kg/ha fw. (dw. = 6 kg/ha) were edible sporocarps. The most abundant genus among the edible sporocarps was *Suillus* (33%) with 12 kg/ha fw. of *Suillus luteus*. Next in abundance was *Tricholoma* (30%), with 18 kg/ha fw. of *Tricholoma imbricatum*. Mature plots (65-75 yr.) had high productions of *Suillus variegatus* and *Cortinarius* sp. while plots of 45-55 yr. were dominated by *Inocybe* sp. In young plots (25-35 yr.) the most abundant genus was *Laccaria*. Diversity of taxa collected increased significantly in mature forest plots. Orientation was significant in the phenology of the harvest, with east and south orientations similar. Harvest initiated and endured longer on south-facing plots. The L. delicious harvest began on plots with a southern aspect and continued westward, ultimately to plots with a northern exposure.

With regard to thinning, the only taxonomic group that showed significant increase in production in thinned forests was the genus *Cortinarius* although *Suillus luteus* showed a tendency to increase production with thinning and the genus *Inocybe* showed the opposite tendency.

poster session 4 ST AR 44

BONFANTE, PAOLA¹, A. GENRE¹, R. BERGERO¹, P. PUIGDOMENECH². ¹Dipartimento di Biologia vegetale dell'Università, CSMT-CNR, Viale Mattioli 25, 10125 Torino, Italy. ²Departament de Genetica Molecular. CID-CSIC. Jordi Girona, 18. 08034 Barcelona, Spain. - Activation of a maize α -tubulin promoter and tubulin location in mycorrhizal transgenic tobacco roots

Transformed tobacco plants are good hosts for AM fungi and were used to test the hypothesis that changes in the expression of cytoskeleton genes are involved in the substantial morphological modifications described in root cells upon fungal colonization. Tobacco plants containing constructs where the promoters of either a maize *at1* or an

a3 tubulin gene were fused with the GUS reporter gene showed an intense GUS activity in the meristematic region. When colonized by *Gigaspora margarita*, only plants with the *tuba3* construct showed gene activation in the differentiated root regions. We have detected i) GUS activity in the cortical root cells which contain arbuscules, and ii) accumulation of *a3* tubulin transcripts in maize root cells where arbuscules are formed.

Immunofluorescence experiments revealed an altered pattern of expression of α and β tubulins in the infected cells. Microtubules organization in the apical root meristeme reveals the different mitotic phases, while microtubular bundles in the parenchymatic cortical cells showed reduction both in thickness and in number. The penetration of the symbiotic fungus into these cortical cells caused a change in organization of microtubules. They run along the intracellular hyphae and the arbuscular trunk, and branch around the small arbuscular hyphae as short bundles. In conclusion, on the basis of GUS technology and *in situ* hybridization, we offer evidence that *tuba3* gene changes its expression during establishment of symbiosis with AM fungi in both maize and transgenic tobacco. In addition, immunofluorescence clearly reveals that microtubules represent another cellular compartment that changes its organization upon fungal colonization.

poster session 1 ST RR 14

BOSWELL, EDWARD, ROGER KOIDE & DURLAND SHUMWAY. Department of Horticulture, The Pennsylvania State University, University Park, PA 16802, USA. - The effects of a winter cover crop on VA mycorrhizal inoculum potential

In this study we tested the hypothesis that a winter wheat (*Triticum aestivum* L.) cover crop will increase the vesicular-arbuscular mycorrhizal (VAM) fungal inoculum potential of the soil and that it will do so by increasing amounts of extraradical hyphae. Cover crop and no cover crop plots were created in the autumn. The following spring the VAM fungal inoculum potential of the soil was estimated by an 8 d *in situ* maize (*Zea mays* L.) bioassay. The winter wheat cover crop increased VAM fungal inoculum potential of the soil. Soil disturbance, in combination with the 8 d *in situ* bioassay, was used to estimate the inoculum potential due to extraradical hyphae. The cover crop increased the amount of infective extraradical VAM fungal hyphae in the soil. The results of this study indicate that winter cover crops can be used to manage VAM fungi in an agricultural system.

poster session 3 ST SH 54

BRATEK, ZOLTÁN¹, FERENC LÁNG¹, IBOLYA VÖRÖS², KLÁRA BUJTÁS². ¹Department of Plant Physiology, Eötvös Loránd University, H-1088 Budapest, POB 330, Hungary. ²Research Institute for Sciences, H-1022 Budapest, Hermann O. út 15., Hungary. - Effect of heavy metals on mycorrhizated red clover (*Trifolium pratense* L.): Examination of photosynthetic properties.

The photosynthetic processes of mycorrhizated (AM) red clover were examined after heavy metal treatment. Cd, Zn or Ni were applied in the same concentrations: 90 kg/ha, 270 kg/ha, 810 kg/ha respectively. The control soil was sterilized by γ -radiation. The other control version was made by giving back the bacterial suspension of the original soil. The mycorrhizated plants were taller, their leaf number were higher, and the total dry weight of shoots were larger. The mycorrhiza was not able to prevent the toxic effect of Cd at the highest concentration, but it could protect plants at medium Cd-concentration. The *in vivo* photosynthetic activity of mycorrhizated plants measured by ¹⁴CO₂-fixation and fluorescence induction were significantly higher than those of nonmycorrhizated ones in parallel with a similar increase in the chlorophyll content. However, the total chlorophyll content decreased at the highest Cd concentration. There were no significant differences in the chlorophyll a/b ratios. The chlorophyll fluorescence emission and excitation spectra showed a better organized photosynthetic apparatus in mycorrhizated plants.

poster session 5 ST AR 08

BRAUNBERGER, PETER, DAVID READ, IAN WOODWARD. Department of Animal and Plant Sciences, The University of Sheffield, Sheffield S10 2TN, UK. - The growth of mycorrhizal and non-mycorrhizal ragwort (*Senecio jacobaea*) in ambient and elevated CO₂ environments

Mycorrhizal and non-mycorrhizal ragwort (*Senecio jacobaea*) plants were grown in ambient (350 μ mol mol⁻¹) and elevated (550 μ mol mol⁻¹) CO₂ environments in glasshouses. Plant growth, rate of CO₂ assimilation and mycorrhizal colonization were assessed over a summer growing season. Mycorrhizal colonization in a low phosphorus treatment increased the rate of CO₂ assimilation in ambient and elevated CO₂ environments similarly. Mycorrhizal colonization resulted in only a small increase in plant growth in both ambient and elevated CO₂ environments. The proportion of the root length colonized by hyphae was not different in ambient and elevated CO₂ environments. These preliminary results indicate that the function of mycorrhizas of ragwort is similar in ambient and elevated CO₂ environments.

poster session 2 ST SH 04

BROWN, DARYL¹ & NICK MALAJCZUK². ¹Department of Agricultural Science, University of Tasmania, Hobart, TAS. 7001, Australia. ²Division of Forestry and Forest Products, CSIRO, Wembley, WA.. 6014, Australia. - A survey of mycorrhizal fungi growing in association with hazel (*Corylus avellana*) in Tasmania, Australia.

Hazelnut trees (*Corylus avellana*) inoculated with the French Black Truffle (*Tuber melanosporum*) are being planted in Tasmania, Australia. The factors most likely to influence the production of the French Black Truffles in Tasmania are soil properties and competing fungi. *Tuber*

melanosporum inhabits calcareous soils. Such soils are not common in Tasmania, and consequently, slightly acidic soils have been heavily limed in an attempt to create a suitable environment. Tasmania has a great diversity of indigenous ectomycorrhizal fungi, which have evolved in association with eucalypts and other native flora. Many of these genera were believed to be host specific to their hosts and hence, possibly would not pose a threat to the establishment of a Black Truffle industry in Tasmania. To determine whether this was the case, a fungal fruiting survey was conducted over three years to determine which ectomycorrhizal fungi are forming relationships with uninoculated hazel trees, previously planted in Tasmania. The majority of fungi collected were northern hemisphere species probably introduced to Australia on the roots of these hazels or other northern hemisphere tree species. Australian mycorrhizal fungi including *Descomyces* and *Hydnangium* were found to form mycorrhizal relationships with *Corylus avellana* but were shown to be poor competitors when the pH of the soil was raised with the addition of lime.

poster session 13 WF SH 43

BRUN, ANNICK¹, M. CHALOT¹, J.S. MATHISEN², P.O. OTTERSEN² & B. SÖDERSTRÖM³. ¹University Henri Poincaré-Nancy I, Faculty of Sciences, Laboratory of Forest Biology, U.A. INRA 977, BP 239, F-54500 Vandœuvre Cedex, France. ²Anatomical Institute, University of Oslo, Karl Johansgate 47, N-0162 Oslo 1, Norway. ³Department of Microbial Ecology, University of Lund, S-223 62 Lund, Sweden. - First direct visualization of amino acids in ectomycorrhizal fungi by immunocytochemistry.

The localization of amino acids was investigated in the ectomycorrhizal fungus *Paxillus involutus* (Batsch) Fr. by immunocytochemistry. Antibodies raised against glutamate and glutamine have been produced which allow the utilization of immunogold labelling techniques to explore the cellular and tissular distribution of amino acids. Classical fixation procedures with glutaraldehyde and paraformaldehyde resulted in a loss of amino acids during the incubation step as demonstrated with [¹⁴C] labelled amino acids. Therefore we applied a freeze-substitution technique to fungal cells which allow better conservation of the vacuole content. Immunogold labelling of freeze-substituted cells clearly showed a cytosolic localization of glutamate and glutamine. However, there was a great heterogeneity of labelling distribution between different cells, with cells intensely labelled whereas other cells were poorly labelled. When using a double labeling protocole, with two particle sizes of 10 and 30 nm for glutamate and glutamine, respectively, it appeared that glutamine amount was 3 to 5 fold higher than glutamate amount, which is in good agreement with quantification of amino acids by HPLC. This study represents a first step towards the use of electron microscope immunocytochemistry as a tool to assess the distribution and the absolute concentrations of amino acids in fungal cells and symbiotic tissues.

BRUNDRETT, MARK. CSIRO Forestry and Forest Products, Private Bag, PO, Wembley, Western Australia, 6014, Australia. - The ecology of VAM fungi in undisturbed and disturbed habitats in tropical Australia

The diversity and distribution of Glomalean fungi forming VAM associations were examined in undisturbed and disturbed habitats in the vicinity of Kakadu National Park in tropical Australia. Methods used to examine fungal populations in soils included (a) Spore numbers (b) spore biovolumes (c) identification of fungi by colonisation patterns in bioassay plant roots and (d) isolation of fungi in pot cultures. Topsoil was collected from 32 sites, including eucalypt savanna, rocky hill, wetland, rain forest and minesite rock dump habitats. 15 species of VAM fungi were identified from spores found in these samples and 7 additional fungi were recovered from the same soils using 4 complimentary pot culturing methods. Different methods of detecting fungi produced different answers about which fungi were most important in soils. Undisturbed sites contained between 7 and 12 species of VAM fungi, but disturbed sites had a much lower diversity. Most species were widespread, but some only occurred in disturbed habitats or waterlogged soils. Fungi were associated with patches of vegetation in disturbed habitats and the abundance of their spores and other propagules increased with plant cover, reaching levels well in excess of those found in undisturbed plant communities. Bioassay results for different genera of VAM fungi were well correlated with spore numbers, but not biovolume data. Differences in propagule strategies suggest that fungi had different life history categories, that corresponded with genera or groups within genera. Observations of fungi in pot cultures provided valuable information about spore features, spore size, spore numbers, external hyphae, vesicles, etc.

poster session 5 ST AR 10

BRUNNER, IVANO & BEAT FREY. Swiss Federal Institute for Forest, Snow and Landscape Research, 8903 Birmensdorf, Switzerland. - Effects of media lacking one nutritional element on ectomycorrhizal development and plant growth or 'how to recognize a functioning ectomycorrhiza'

Birch seedlings (*Betula pendula*), inoculated with the ectomycorrhizal fungus *Paxillus involutus*, were grown in Petri dishes on media deficient in K, Na, Ca, or Mg, and with Cl lacking in all treatments. The experiments have shown, that the formation of ectomycorrhizae occurred frequently in the K- and Na-lacking treatments, but only occasionally in the Mg- and Ca-lacking treatment. The lack of either one of these nutritional elements resulted in a discoloration of leaves other than cotyledons. With addition of the lacking nutritional elements as chloride solutions into small aluminum pots accessible only to extramatrical mycelia, however, a recovering of the leaf color was evident. Additionally, CsCl was applied as a tracer solution. In freeze-fractured ectomycorrhizal roots, the detection of elements using energy dispersive X-ray analysis (EDX) lead to the following conclusions:

1) Cs and Cl are useful tracers detectable in various compartments of ectomycorrhizae indicating an uptake by hyphae and transport across the interface; 2) K is not a valuable parameter, since it also can be detected in plant parts grown on media deficient in K (K probably originates from the seeds); 3) Na, Ca, and Mg often are not in a sufficient amount present which prevents a clear interpretation.

In addition, the above mentioned media were supplemented with 525 mM N (as ammoniumnitrate), since earlier studies showed the induction of callose formation in epidermal and cortical cells of birch ectomycorrhizae due to elevated N-concentrations. The potential and limitations of the systems and techniques used to show the 'functioning' of such symbioses are discussed.

poster session 15 WF RR 15

BUCKNER, B., S.K. HARNEY., F.S. EDWARDS & M.F. ALLEN. San Diego State University, Biology Department, San Diego, CA, 92182. - Preliminary identification of selected species of arbuscular mycorrhizal fungi using PCR amplified rDNA.

The obligately symbiotic group of fungi, the arbuscular mycorrhizal (AM) fungi, possess several characteristics (e.g. inability to be grown in pure culture) which make identification, and therefore ecological field studies, difficult. Traditional methods of identifying this group rely on the isolation of asexual spores from the soil. These spores, which are resting structures, do not necessarily reflect what species are actively colonizing the plant root. We have recently utilized molecular methods to develop a protocol for identifying AM fungi. Field collected spores were identified using traditional microscopy, and DNA was extracted for amplification. DNA was extracted using crude lysate, which eliminates the need for a large amount of tissue, and produces a DNA template suitable for PCR amplification. Results indicate that size polymorphisms in the ITS region of rDNA can distinguish among genera, and can be used to screen species. Amplification products of field collected plant roots showed that these fungal endophytes may be identified to species based on size polymorphisms.

poster session 5 ST AR 11

BURKE, RONALD M.¹, & JOHN W. G. CAIRNEY². ¹Biochemistry and Applied Molecular Biology, UMIST, PO Box 88, Manchester, M60 1QD, UK. ²Biological Sciences, University of Western Sydney, Nepean, PO Box 10, Kingswood, NSW 2747, Australia. - A β -1,4-endoxylanase from the ericoid mycorrhizal fungus *Hymenoscyphus ericae*.

There has been much recent emphasis placed on the role of proteases and phosphatases in the ericoid mycorrhizal symbiosis. However, there is increasing evidence that ericoid fungal endophytes also produce enzymes capable of degrading the plant cell wall. Such activities are likely to be significant in a number of ways, namely: in the penetration of the wall of host cortical cells during

establishment of the ericoid mycorrhizal symbiosis; in the exploitation of nutrients sequestered within plant cell walls; and in the degradation of moribund plant material during saprotrophic growth or symbiosis. Here we report the purification to electrophoretic homogeneity of a β -1,4-endoxylanase (E.i.3.2.1.8) from *H. ericae* using isoelectric focusing, ion exchange and gel permeation chromatography. Physicochemical characterization of the purified enzyme showed an isoelectric point of 4.85-5.20 and a molecular weight of 31.6kD. The enzyme has an apparent $S_{0.5}$ of 3.75 mg.ml⁻¹ for soluble birchwood arabinoxylan and a V_{max} of 468.0 nkatal.(mg protein)⁻¹. The pH optimum for activity is 4.5 and that for stability is 3.5-4.0. These values are discussed in the context of the ecology of *H. ericae*.

talk session 5

BURLEIGH, STEPHEN & MARIA HARRISON. Noble Foundation, Plant Biology Division, Ardmore, OK 73401 USA. - The cloning of two genes involved in the *Medicago truncatula*/*Glomus versiforme* mycorrhizal symbiosis.

A differential screening procedure was used to identify plant and fungal genes involved in the *M. truncatula*/*G. versiforme* mycorrhizal symbiosis. Total RNA from mycorrhizal and non-mycorrhizal roots of the *M. sativa* mutant MNN-1008 was isolated, reverse transcribed to cDNA, labeled and used to screen a *M. truncatula*/*G. versiforme* cDNA library. Since the infection of MNN-1008 by mycorrhizal fungus is limited to the early stages of the infection process, cDNA clones identified by this strategy may represent early symbiotic genes. Screening 17,000 plaques yielded several dozen clones of interest, two of which were closely studied. Clone 4 is a *M. truncatula* cDNA that has homology to transcripts expressed only in non-mycorrhizal roots of *M. truncatula* and *M. sativa* and clone 54 is a fungal cDNA that has homology to transcripts expressed only in mycorrhizal roots. The influence of another VAM fungus, infection with *Rhizobium*, and infection with a root pathogen on the expression of transcripts homologous to these two cDNAs in *Medicago*, as well as the localization of gene expression by *in situ* hybridization, will be presented.

poster session 12 WF SH 35

CADE-MENUN, BARBARA & SHANNON BERCH. Department of Soil Science, The University of British Columbia, 139-2357 Main Mall, Vancouver, BC V6T 1Z4. BC Ministry of Forests, Glyn Road Research Station, 1320 Glyn Road, Victoria, BC V8W 3E7 Canada. - Can western red cedar trees use P from organic sources?

In some BC forests, western red cedar grows well in soils containing P mainly in organic form, and shows less response to P fertilization than other conifers. It was hypothesized that VA mycorrhizae allow cedar to mineralize organic P. In a year-long greenhouse study, 2-year-old cedar trees in pots with Turface were fed every 9 days with one of 11 P treatments (in Long Ashton

solution): No P; high phytic acid; low phytic acid (NOTE: for all treatments, high is 50 mg P/L; low is 10 mg P/L); high ATP; low ATP; high glycerophosphate; low glycerophosphate; high pyrophosphate; low pyrophosphate; high orthophosphate; low orthophosphate. Ten mycorrhizal trees were used per treatment; in half, colonization was reduced with the fungicide benomyl. Growth was good, relative to the No P and orthophosphate controls, with the organic compounds glycerophosphate and ATP and with the inorganic pyrophosphate, especially at the higher rates. The trees grew very poorly with phytic acid, which may have complexed Ca, Zn and Cu from the nutrient solution, inducing Ca deficiencies. The P source influenced the activities of acid and alkaline phosphatase of roots, but had no significant effect on phosphodiesterase or pyrophosphatase in the soil or the plant roots. It was impossible to distinguish enzyme activity of the plants and mycorrhizae from that of rhizosphere microorganisms. Colonization improved foliar P content, but other mycorrhizal effects may have been masked by N enrichment from the benomyl.

poster session 5 ST AR 12

CALDERON, FRANCISCO, D. HARRIS, & E. A. PAUL. Department of Crop and Soil Sciences and Center for Microbial Ecology, Michigan State University, East Lansing, MI 48824-1325, USA - The role of lipids in the C cycle of mycorrhizae.

Lipids are the dominant component of the cytoplasm of the arbuscular fungi and have been proposed as one of the main carbon storehouses of the endophyte. The carbon turnover of the lipids of mycorrhizal roots has never been directly measured. This is important information since arbuscular mycorrhizae are known to impact the C budget of the host plant. We carried out a pulse-labeling experiment to study the length of the carbon cycle in the mycorrhizal lipids of the *Sorghum-Glomerus clarum* association. This involved the exposure of mycorrhizal and control *Sorghum* to $^{14}\text{CO}_2$ for a 3 hr period, followed by five sequential harvests during 24 days after the labeling. After each harvest, the plants were analyzed for the specific activity and biomass of shoot and roots. The total lipids of the fine roots were further extracted and analyzed for the incorporation of ^{14}CO . Additionally, the below-ground respiration of selected plants was measured for 60 hours after the pulse labeling. Our results show an increase on root respiration and shoot ^{14}CO incorporation of inoculated plants. This replicates the results of previous studies by other groups. Other measurements are ongoing and we will present preliminary data and discussion regarding the C cycle of mycorrhizal lipids.

poster session 12 WF SH 56

CALDWELL, BRUCE A., JIM TRAPPE & ARI JUMPPONEN. Department of Forest Science, Oregon State University, Corvallis, Oregon 97331-7501, USA. - Physiological characters of dark-septate root endophytes.

Pioneer plant communities, forming as discrete islands of vegetation, frequently trap and retain a significant fraction of the community nutrient pool as detritus. This suggests a major role for mycorrhizal associations capable of processing the various detrital organic nitrogen and phosphorus compounds into forms that can be translocated back to the host plant(s). Ten isolates of putatively mycorrhizal dark septate root endophytes (*Phialocephala fortinii*) from various plants colonizing the forefront of a retreating glacier in Washington State were screened for ability to access major detrital sources of carbon, nitrogen and phosphorus. All isolates were able to hydrolyze a(1,4)-, b(1,3)- and b(1,4)- glucans (starch, laminarin and cellulose), as well as fatty acid esters for carbon; protein for nitrogen; ribonucleic acids for phosphorus. Although production of phenoloxidasas has been previously reported for this fungus, our assays using polymeric dye decoloration gave no evidence of significant lignolytic capacity.

poster session 3 ST SH 55

CASTELAO, ANA¹, MARIA J. SAINZ¹ & ANTON VILARIÑO². ¹Departamento de Ingenieria Agroforestal y Produccion Vegetal, Universidad de Santiago de Compostela, E-27002 Lugo, Spain. ²Instituto de Investigaciones Agrobiologicas de Galicia (C.S.I.i.), Apdo. 122, E-15080 Santiago de Compostela, Spain. - Evolution of mycorrhizal propagules in a burnt soil after fertilization and revegetation treatments.

The surface of a hill soil covered by shrubs was burnt for about one hour. Representative samples of the burnt soil were taken at 2-5 cm depth to evaluate mycorrhizal propagules left in soil and be compared with the corresponding samples in the unburnt soil. After the fire, a revegetation experiment was designed to control soil erosion, using two seed mixtures with the following species: i) *Lolium perenne*, *Dactylis glomerata*, *Trifolium repens* and *Trifolium pratense*, ii) *Lolium multiflorum*, *D. glomerata*, *T. repens* and *T. pratense*. Each mixture was sown in autumn under two fertilization treatments: a) an unfertilized control, b) liming and addition of a NPK (8:24:16) fertilizer. There were four replicates of 12 m² per treatment. In every replicate, samples of soil were taken in July and November to study the evolution of mycorrhizal propagules; samples of roots were also taken to determine the mycorrhizal colonization of the dominant plant species established. The introduced plant species failed to compete with the indigenous flora (mainly *Pteridium aquilinum* and *Arrhenatherum* sp. with high percentages of root mycorrhization), which covered most of the soil surface in all treatments. Only a small proportion of *Lolium multiflorum* and *Dactylis glomerata*, both also extensively mycorrhized, could grow in the fertilized treatments. The number of propagules was strongly reduced after the fire, remaining low both in the fertilized and unfertilized treatments for about six months. This fact might help to partly explain the failure of the introduced plant species to get established in the burnt soil.

poster session 1 ST RR 01

CAVALLAZZI, JOSE R. P. & MARGARIDA MENDONCA. Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, Florianopolis, Santa Catarina, CEP 88040-900, Brazil. - Infectivity and effectiveness of arbuscular mycorrhizal fungi populations of apple orchards in Santa Catarina - South Brazil.

This work is part of a project that aims to produce virus-free micropropagated and mycorrhizal apple rootstocks for transplantation into nurseries located in regions of low pH soils. The objective of this work was to select arbuscular mycorrhizal fungi (AMF) infective and efficient to apple rootstocks plantlets in greenhouse (acclimatization) and nursery conditions. To estimate the populations infectivity, bioassays were established using six soil samples collected from orchards in two experimental stations and from a commercial apple plantation in the state of Santa Catarina (Brazil). Sites from where soil samples were collected differed in management practices, particularly fertilization and pesticide use. The AMF populations were compared for the ability to colonize corn roots. Estimates of root colonization in four periods showed that soil samples from the experimental stations contained mycorrhizal fungal populations more infective than the ones from the commercial orchards. To determine the effectiveness of the AMF population, growth promotion of corn plants was assessed when soil of these sites was used as the growth substratum. The experiments on efficiency are being carried out. In general, infectivity was higher in soils collected in experimental stations than in commercial orchards. Our results suggest that the use of heavy fertilization and pesticide applications may have reduced infective potential of mycorrhizal fungi present in soils from commercial plantations. Research funded by Financiadora de Estudos e Projetos (FINEP) and CNPq/RHAE - Brazil.

poster session 10 WF SH 15

CHAKRAVARTY, P. and M. KEAN. MikroTek Labs, 36 Emerald Street, P.O. Box 2120, Timmins, Ontario, CANADA P4N 7X8. - Transfer of symbiotically fixed nitrogen from alder to white spruce via ectomycorrhizal hyphae

Transfer of labeled nitrogen from speckled alder [*Alnus incana* (L.) Moench] inoculated with a nitrogen-fixing actinomycete, *Frankia*, and an ectomycorrhizal fungus, *Paxillus involutus* (Batsch:Fr.) Fr., to white spruce [*Picea glauca* (Moench) Voss] was studied. *Alnus incana* that were exposed to a ^{15}N -enriched atmosphere fixed more ^{15}N than did *A. incana* maintained in air at natural ^{15}N abundance. The total ^{15}N excess content of *A. incana* was similar whether or not inoculated with *P. involutus*, but *A. incana* had significantly higher ^{15}N excess content when inoculated with *Frankia*. The total ^{15}N excess content was higher in *P. glauca* seedlings when grown together with *A. incana* inoculated with *Frankia* and *P. involutus*. The presence of a common mycorrhizal fungus on *P. glauca* and *A. incana* increased

the transfer of symbiotically fixed nitrogen from *A. incana* to *P. glauca* seedlings.

talk session 1

CHALOT, MICHAEL¹, ANNICK BRUN¹, BERNARD BOTTON¹ & BENGT SÖDERSTRÖM². ¹University Henri Poincaré-Nancy I, Faculty of Sciences, Laboratory of Forest Biology, U.A. INRA 977, BP 239, F-54500 Vandœuvre Cedex, France. ²Department of Microbial Ecology, University of Lund, S-223 62 Lund, Sweden. - Kinetics, energetics and specificity of a general amino acid transporter from the ectomycorrhizal fungus *Paxillus involutus*.

The kinetics, energetics and specificity of a general amino acid transporter were studied in the ectomycorrhizal fungus *Paxillus involutus*. The uptake of amino acids showed characteristic features of active transport. After correction for a non-mediated component, the kinetics of glutamate, glutamine, alanine and aspartate uptake measured over a wide concentration range followed the simple Michaelis-Menten saturation curves. The apparent K_m derived from the Eadie-Hofstee plots ranged from 7 μM for alanine to 27 μM for glutamate. Dinitrophenol, carbonyl-cyanide-*m*-chlorophenylhydrazone and NaN_3 strongly inhibited amino acid uptake whereas dicyclohexyl-carbodiimide, vanadate and the ionophores monensin and nonactin were without effect on the uptake. Both pH dependence and inhibition by protonophores are consistent with a proton symport mechanism for amino acid uptake by *P. involutus*. Competition studies indicate a broad substrate recognition by the uptake system, which resembles general amino acid permease of yeast. Dixon plots of the inhibition of glutamate uptake by alanine, lysine and methionine sulfoximine showed that inhibitions were competitive. The physiological importance of this transporter for the exchange of nitrogenous compounds between fungal and host plant cells in ectomycorrhizal associations is discussed. Further experiments using modern molecular techniques will be directed to elucidate nitrogen transport mechanisms at the biotrophic interface during the early stages of mycorrhiza formation. They will lead to a better understanding of many aspects of ammonium, amino acid and peptide transport that are not well defined at present.

poster session 8 WF AR 36

CHAN, W.K. Department of Applied Biology & Chemical Technology, Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong. - The seasonal effects on VA mycorrhization in *Machilus chinensis* Hemsl. and *Acacia confusa* Merr.

Machilus chinensis and *Acacia confusa* are two very important tree species in Hong Kong. *M. chinensis* (Lauraceae) is a native species of Hong Kong and South China and is one of the most dominant species in the natural secondary forests in Hong Kong. *A. confusa* (Mimosaceae) is native of Taiwan and the Philippines and grows well in Hong Kong, it has been widely grown as road-side plants and in plantations. Both species are

evergreen and found to be infected by VA mycorrhizal fungi. One of the studied site is a natural succession secondary forest of *M. chinensis*, the other is a plantation of *A. confusa*; the two sites were in close proximity. The soil conditions of both sites are typically sandy, nutrient poor and acidic (pH3.5-4.9). The survey have been carried out for 12 months from June 1994 to May 1995. Four trees of the same species were sampled at each site; soil and root samples were taken from the top 10cm of the rhizosphere at four different directions. Various parameters including number of VA spores in soil, infection rate of roots, hyphal length in soil, pH and relative humidity of the rhizosphere were monitored and studied. The average number of spores were higher in the *A. confusa* site through out the 12 months period, however both the percentage of infection in roots and the hyphal length in soil were higher in the *M. chinensis* site. This could perhaps explain the phenomenon that *Machilus* spp. can establish successfully only through natural succession but not in plantations using nursery grown seedlings.

poster session 1 ST RR 15

CHANG, DORIS C.N.¹, S.W. LEU¹ & S.R. CHANG JAN². ¹Department of Horticulture, National Taiwan University, Taipei, Taiwan, ROC (10617) & ²Crop Improvement Department, Tao Yuang Agricultural Improvement Station, Tao Yuang, Taiwan, ROC. - VAM Symbiosis in Plug System May Benefit Vegetable Production.

The inoculation of VAM fungi (*Glomus* spp. or *Gigaspora* spp.) in plug system for the growing of vegetable seedlings showed great potential for practical use. Such as asparagus, cucumber, eggplant, tomato and watermelon. The beneficial effects which the mycorrhizal vegetable seedlings resulted were as following:

1. increased plant height, leaf area and plant weight.
2. Increased transplanting survival rate.
3. Earlier flowering and fruit production.
4. Decreased the fruit drop.
5. Increased total fruit production.
6. Prolonged the productive period for the crop.
7. Decreased the application of phosphate fertilizer.

The VAMF inoculation should be done during seed sowing in the plug. The growing of mycorrhizal tomato seedlings was recommended as following: In a 128 plug, use BVB#4 and vermiculite #2 as the growth medium, inoculate about 40-50 spores in the middle of each plug under the seed, then cover it. Apply Johnson solution once a week, but contained only 1/4 (less than 20 ppm) of phosphate fertilizer. Seedlings grow faster than tomato (e.g. cucumber, watermelon) may choose bigger size of plug and inoculate more spores. In Taiwan, each mycorrhizal watermelon seedling could sell 1 dollar more than each non-mycorrhizal control. The mycorrhizal seedlings had resulted in beneficial effect for vegetable production.



poster session 1 ST RR 17

CHAREST, CHRISTIANE¹ & YOLANDE DALPÉ². ¹Department of Biology, University of Ottawa, PO Box 450 STN A, Ottawa, ON, K1N 6N5 Canada. ²Eastern Cereal Oilseed Research Centre, Agriculture and Agrifood Canada, Ottawa, ON, K1A 0C6 Canada. - Use of endomycorrhizae as a non-chemical method for the production of agrostid lawn grass.

In order to evaluate the effectiveness of mycorrhizal inoculation as an alternative for chemical fertilizers in the production of quality lawn grasses, the growth of the agrostid grass (*Agrostis palustris* Huds.), inoculated with *Glomus versiforme* or *G. etunicatum*, was assessed in a greenhouse experiment. The agrostid grass production was studied on parcels grown with (M+) or without (M-) mycorrhizae, subjected or not to herbicide treatment and to four fertilizer concentrations achieved by varying P & N contents. Biomass, leaf water, chlorophyll, and protein contents have been measured. Results showed that root colonization % was inversely proportional to the concentration of fertilizer added. While leaf water content remained unchanged under all treatments, protein levels significantly increased in M+ plants, especially with *G. etunicatum*. The chlorophyll content remained constant in M+ plants but significantly decreased in M- plants with low fertilizer level. Herbicide treatment did not affect the chlorophyll content of M+ plants but tended to decrease the chlorophyll level of M- plants. Herbicide application combined with low P treatment caused a slight decrease in shoot biomass but this impact on M+ was lower than on M- plants. This study suggests that beneficial effects may be expected from mycorrhizal inoculation in relation to a reduced input of chemicals employed in lawn production.

poster session 2 ST SH 51

CHARVAT, IRIS, ALYCIA KLUENENBERG & JUDI TAMASI. Department of Plant Biology, University of Minnesota, 220 Biological Sciences, 1445 Gortner Ave., St. Paul, MN 55108-1095. - Comparison of AMF spores at two wetland ecosystems at different stages of succession

The restoration of native wetland/prairie plant communities has received considerable attention in recent years. Roadside projects need to be carried out with more information about the status of the edaphic and the arbuscular mycorrhizal fungal (AMF) parameters. This study determined several edaphic physical characteristics and the seasonal fluctuation of the AMF spores at two sites: a recently created wetland at an early stage of succession and an undisturbed wetland at a later stage of succession. Both of these wetlands are adjacent to roadways near Cambridge, Minnesota. The soil characteristics analyzed included nutrient composition, amount of organic matter, percent water, density and pH. The amount of total carbon, the percent organic matter, and the percent water by volume of the soil was greater at the undisturbed wetland. Soil samples were cored at five time periods during the year and the spores were isolated from the dried soil and quantified. The undisturbed

wetland had a considerably greater fluctuation of spores per gram of dried soil than the newly created wetland had. Fall was the peak time for spore numbers at the undisturbed wetland, but no significant difference between time periods was present at the newly created one. The biological and physical tests employed in this study will be used in the future to evaluate the condition of the recently created wetland at progressive stages of succession.

poster session 8 WF AR 62

CHINNERY, LOUIS E. & LYNDON F. ROBERTSON. Department of Biology, The University of the West Indies, P.O.B. 64, Bridgetown, Barbados. - Using phytometers to assess the mycorrhizal potential of soils

Soils were sampled from each of the ten soil associations of Barbados. At each site two subsamples were placed, with as little disturbance as possible, into 15cm plant pots and a third subsample bagged for subsequent spore extraction. Three seedlings each of corn (*Zea mays*), pepper (*Capsicum annuum*) and bean (*Phaseolus vulgaris*), germinated in autoclave sterilised growth media (soil:sand:vermiculite), were transplanted into one set of soil samples and 24hr pre-soaked corn achenes planted in the second set. Vesicular-arbuscular mycorrhizal (VAM) fungal colonisation of roots was determined 2, 3 and 4 wk after transplanting. Spore populations were low and varied between 1.02 and 2.63 g⁻¹ soil with the lowest populations being found in the agriculturally most productive soil type. VAM colonisation increased with time and corn plants showed the greatest level of colonisation. Significant differences between VAM fungal colonisation of phytometer roots between soil types was closely related to spore density. This study has not shown that the use of phytometers provides a better assessment of the VAM potential of soils than counting spores.

poster session 8 WF AR 26

CLAPPERTON, JILL, DERRICK KANASHIRO, & BOB BLACKSHAW. Agriculture and Agri-Food Canada, Research Centre, P.O. Box 3000, Main, Lethbridge, Alberta, T1J 4B1 Canada. - A preliminary study on the influence of VAM fungi on the ecology of grassy weeds in agroecosystems

The objective of this study was to compare the inoculum density and colonisation by VAM fungi of the grassy weeds downy brome (*Bromus tectorum*), green foxtail (*Setaria viridis*), foxtail barley (*Hordeum jubatum*), and wild oats (*Avena fatua*) with that of hard red spring wheat (*Triticum aestivum*). Percent of root length colonised by VAM fungi was compared in both grassy weed species and wheat which had been collected at the end of the growing season. Soil was also collected at the field sites from both the weed plots and adjacent wheat plots and used in greenhouse studies to compare the infectivity of the soils using the weed species, wheat, and green onions. Our preliminary results from the field collections showed that the weeds were more heavily colonised by VAM fungi than wheat, and that foxtail barley was the

most colonised, and green foxtail the least. When all the weed species, wheat, and green onions were grown in each soil, the wheat and green foxtail were not infected, the remaining weed species were infected and showed some colonisation in spots along the root, with no preference for a particular soil. The onions were infected in all the soils, with soil from under wheat having the least infectivity (< 10%) and soil from under downy brome, foxtail barley, and green foxtail having the most (70%).

poster session 3 ST SH 48

CLARK, R. B. & S. K. ZETO. Appalachian Soil & Water Conservation Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, P.O. Box 867, Beckley, WV 25802USA. - Mineral acquisition by mycorrhizal maize grown on acid and alkaline soil

Arbuscular mycorrhizal (AM) fungi can enhance plant acquisition of mineral nutrients and function over relatively broad pH ranges. This study was conducted to determine the effect of the AM fungi *Glomus etunicatum* WV579A (Ge), *G. diaphanum* WV579B (Gd), and *G. intraradices* WV894 (Gi) for acquisition of minerals in maize (*Zea mays* L.) grown on acid (pH 4.2 and 4.6) and alkaline (pH 7.8 and 8.0) soils in a greenhouse. Shoot concentrations of P, Ca, Mg, K, Cu and Si were lower and Mn, Fe and Zn were higher while contents of N, P, S, Ca, Mg, K, Na, Cu, B and Si were lower and Mn, Fe and Zn were higher in plants grown on acid compared to alkaline soil. With few exceptions, mycorrhizal (AM) plants had higher mineral concentrations and contents than non-mycorrhizal (nonAM) plants. The AM isolates were generally comparable for enhancing acquisition of elements in plants grown on acid soil, while Gi and Ge plants absorbed more nutrients than Gd plants grown on alkaline soil. Many of the enhanced mineral acquisition effects were greater than could be accounted for by increases in shoot biomass. Reduced acquisition of P by Ge and Al by Gi plants grown on acid soil were noted. These results provide evidence that *Glomus* isolates can be effective in enhancing mineral acquisition over wide ranges of soil pH (± 3.5 pH units), and that AM isolates varied extensively in enhancing or reducing mineral acquisition in maize.

poster session 15 WF RR 16

COLLINS, R.A.¹, M.R. ALDERTON¹, C. MCLEAN² & A.C. LAWRIE¹. ¹Department of Applied Biology & Biotechnology, RMIT, GPO Box 2476V, Melbourne, Vic. 3001, Australia, ²Faculty of Agriculture, Forestry and Horticulture, Burnley College, University of Melbourne, Burnley Gardens, Swan St, Richmond, Vic. 3121, Australia. - Use of antibodies to differentiate among root endophytes of Epacridaceae and Ericaceae

The aim of this study was to develop a serological method for differentiating among hyphae in and on roots of species of the Epacridaceae. Polyclonal antisera were prepared in BALB/c mice and binding to fungi detected

microscopically using fluorescence. A polyclonal antiserum to pelotons from 'typical' ericoid endophytes of *Epacris impressa* showed strong immunofluorescence with both hyphae and pelotons in fresh roots, but not with some external hyphae, uninfected roots or cultures of *Hymenoscyphus ericae* or *Oidiodendron griseum* isolated from species of the Ericaceae. A polyclonal antiserum to *H. ericae* reacted strongly with *H. ericae* cultures, external hyphae and pelotons from 'typical' ericoid mycorrhizal roots, and weakly with *O. griseum*, suggesting some similarity. Neither antiserum gave any reaction with the morphologically different internal hyphae in roots of epacrids in the Grampians, Victoria. This evidence suggests that more than one type of fungus infects these epacrids, that these fungi are different from those tested which infect the Ericaceae and that antibodies can be used to differentiate among root endophytes of the Ericales.

poster session 5 ST AR 13

COLPAERT, JAN V.¹, ANDRÉ VAN LAERE¹ & KATIA K. VAN TICHELEN². ¹Laboratory of Developmental Biology, ²Laboratory of Plant Ecology, Institute of Botany, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Leuven, Belgium. - The use of phytate as a phosphorus source by mycorrhizal and non-mycorrhizal Scots pine (*Pinus sylvestris*)

The use of phytate-P by mycorrhizal and non-mycorrhizal pine seedlings was investigated. Mycorrhizal plants were either inoculated with *Thelephora terrestris* or *Suillus luteus*. Plants were grown for 9 weeks in a semi-hydroponic system with P as the growth-limiting factor. Addition of a relatively high concentration of soluble Ca-phytate resulted in an increased relative growth rate (RGR) in both mycorrhizal and non-mycorrhizal plants. The effect of the mycorrhizal fungi on the use of the phytate-P was small. The addition of an insoluble phytate to the growth substratum did not result in an increase of the RGR and P uptake neither in the non-mycorrhizal, nor in the mycorrhizal pines. Soil phytate, which has a low solubility, is probably not a very useful P source for *Thelephora terrestris* and *Suillus luteus*. Nevertheless the external mycelia of these fungi exhibited a substantial extracellular acid phosphatase activity. The activity was correlated with the fungal biomass in the growth substratum and decreased with an increasing P nutrition. Phytase activity of the external mycelia could not be detected although a low phytase activity could be observed at the root surface. Mycorrhizas showed significantly higher activities than uninfected roots. The orthophosphate, labile and stable P fractions in the substratum were analysed at harvest. In the mycorrhizal treatments the Pi pool decreased significantly whereas the more stable P pool tended to increase.



talk session 1

CONJEAUD, CORINNE, PASCALE SCHEROMM, PASCAL TILLARD, CLAUDE PLASSARD & DANIEL MOUSAIN. Laboratoire de Recherches sur les Symbiotes des Racines, INRA, 2 Place Viala, 34060 Montpellier Cedex 1, France. - Effects of ectomycorrhizal infection with *Hebeloma cylindrosporum* and *Rhizopogon rubescens* on the carbon balance in *Pinus pinaster* seedlings

The study of the effects of ectomycorrhizal infection on the carbon balance of the host-plant was carried out by measuring (i) the biomass production and the net rates of photosynthesis, (ii) the rates of root respiration and (iii) the distribution of carbon compounds (soluble sugars, starch) in the host-plant. The results showed that each ectomycorrhizal association constituted a different model of carbon balance in *Pinus pinaster* seedlings. The association with *Hebeloma cylindrosporum* was characterized by higher rates of photosynthesis, a very strong increase in root respiration and finally caused a growth depression in mycorrhizal plants compared to the control ones. On the contrary, *Rhizopogon rubescens* had no effect on photosynthesis nor root respiration rates and finally increased the growth of mycorrhizal plants. The distribution of carbon compounds as a function of ectomycorrhizal association was then studied by supplying ¹³CO₂ to the plants, whether mycorrhizal or not, for 4 or 10 days. The results obtained showed again that the presence of *H. cylindrosporum* increased significantly the carbon allocation to the roots and to the fungus associated to the roots compared to the figures obtained with *R. rubescens*. These data indicated that the mycelium of *H. cylindrosporum* increased the sink of carbon compounds, which in turn increased the photosynthesis rate (source-sink concept). Finally, application of different light intensities did not change the effects of both fungal species. Application of different N-regimes during the culture suggested that the competition between *H. cylindrosporum* and the host plant for N-compounds might be also responsible for growth decrease of mycorrhizal plants.

poster session 2 ST SH 05

CONN, CHRISTINE & DIGHTON, JOHN. Rutgers Pinelands Field Station, P.O. Box 206, New Lisbon, NJ, 08064. - The influence of litter quality on mycorrhizal communities.

The influence of litter quality on root growth, mycorrhizal communities and decay processes was investigated through a litterbag experiment. Litterbags containing either pine needles, oak leaves or oak/pine mix were placed within the O horizon of a lowland pitch pine (*Pinus rigida*) forest in the New Jersey Pinelands. Upon retrieval, ingrown pine roots were removed and quantified for total length and percent mycorrhizal colonization by morphotype. Ergosterol content (fungal biomass indicator) and phosphatase activity was determined for dominant morphotypes. In addition, litter decay rates, hyphal colonization of litter and N and P litter content were measured. Preliminary data show

mixed litter (oak + pine) had highest total pine root ingrowth. Pine and mixed litters had greater mycorrhizal morphotype diversity than oak. Dominant mycorrhizal morphotype differed in response to litter type. A tuberculate form dominated (35%) in pine litters while distinctly different nontuberculate morphotypes dominated in oak and mixed litters. Mixed litters generally had greater hyphal length colonization than pure litters. Pine needle decay was slower in the presence of oak leaves (mixed litter) than when alone.

talk session 6

CORDIER, CHRISTELLE, VIVIENNE GIANINAZZI-PEARSON & SILVIO GIANINAZZI. Laboratoire de Phytoparasitologie, INRA/CNRS, SGAP, INRA, BV 1540, 21034 Dijon cédex, France. - Resistance mechanisms to *Phytophthora nicotianae* var. *parasitica* in mycorrhizal tomato : pathogen development within root tissues and host cell responses

Arbuscular mycorrhiza are known to decrease root damage caused by fungal pathogens, but the mechanisms involved are not well understood. Our research is aimed at analysing interactions between *Glomus mosseae* and the pathogen *Phytophthora nicotianae* var. *parasitica* in roots of tomato (*Lycopersicon esculentum*) at tissue and cellular levels, in order to better understand the bioprotective effects of arbuscular mycorrhiza. The use of antibodies, in the absence of an appropriate stain, to estimate infection intensity of the pathogen in whole root fragments showed that this was considerably lower in mycorrhizal roots as compared to non-mycorrhizal controls. Infection patterns of *P. n. v. parasitica* in mycorrhizal and non-mycorrhizal root tissues of tomato were analysed by immunocytochemistry to discriminate between the two fungi. *P. n. v. parasitica* colonizes tomato root tissues inter- or intracellularly, and in non-mycorrhizal roots the presence of the pathogen in the root cortex induces necrosis and phenolics accumulation in cells containing or not the fungus. However, this plant reaction does not prevent pathogen spread through the root tissue to the central cylinder. In pathogen-infected mycorrhizal root systems, hyphae of *G. mosseae* and *P. n. v. parasitica* are, in most cases, found in different root regions but they can also develop in the same root tissues. Quantification of *P. n. v. parasitica* hyphae in root tissues showed that pathogen spread was greatly reduced in both mycorrhizal and non-mycorrhizal portions of mycorrhizal root systems. *P. n. v. parasitica* never invaded arbuscule-containing cells in mycorrhizal tissues. Moreover, arbuscule-containing cells surrounded by intercellular *P. n. v. parasitica* hyphae did not necrose and only a weak autofluorescence was associated with the host cells. These observations suggest that bioprotection by *G. mosseae* against *P. n. v. parasitica* may be due both to direct interactions between the fungi in the same root tissue as well as long-distance effects of mycorrhizal development on pathogen infection.

poster session 1 ST RR 18

CRAVEN-GRIFFITHS, AMANDA¹, JOHN C DODD² & PETER JEFFRIES¹. ¹Research School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ UK. ²International Institute of Biotechnology, University of Kent, Canterbury, Kent CT2 7YW UK. - The effects of inoculation with arbuscular mycorrhizal fungi on indigenous plant species from a desertified ecosystem in Southern Spain.

The EU ENVIRONMENT project REDEEM aims to improve the rate of revegetation by native plant species in a desertified ecosystem in Southern Spain. A glasshouse experiment was designed to study the effects of AMF on the growth of 3 indigenous plants: the predominant legume pioneers *Retama sphaerocarpa* L. and *Anthyllis cytisoides* L. Boiss. and *Thymus vulgaris* L. a non-legume. Plants were inoculated with either *Glomus coronatum* (BEG-49; an indigenous isolate) or *G. microaggregatum* (AD-2; an exotic isolate from a similar ecosystem). Deep pots were used to suit the deep rooting nature of desert plants and roots were collected separately from the 0-10cm, 10-20cm and 20-30cm rooting depths at harvest. At 12 weeks most root development had occurred in the upper 10cm rooting zone. There was no effect of AMF on total root fresh weight. However, *G. coronatum* (BEG-49) increased root fresh weight in the upper 10cm rooting zone for the three plants whilst *G. microaggregatum* (AD-2) also altered the root architecture of *R. sphaerocarpa*, increasing the proportion of root tips within the upper 10cm rooting zone. Thus, 12 weeks after inoculation both AMF had induced significant yet different changes in root growth and development. This has implications for the placement of inoculum in a restoration programme. Isozyme analysis has been used to monitor the development of AMF colonising roots of *R. sphaerocarpa* and *A. cytisoides* and this will be discussed further.

talk session 3

CRIPPS, CATHY L. Dept. of Plant Pathology, Montana State University, Bozeman, MT 59717. - Ectomycorrhizal communities with aspen on smelter-impacted sites: how ecosystems recover from airborne pollution

Airborne pollution from copper smelters denuded vast acreages in western Montana in the early 1900's, and left a legacy of acidified soil laced with heavy metals. Aspen are colonizing these areas and provide an opportunity to examine the role of ectomycorrhizal fungi in the natural recovery of smelter-impacted sites. This study examined the species of ectomycorrhizal fungi which can survive in, and are important to, these stressed systems. The long range goal is to incorporate this information into reclamation strategies to accelerate successional processes with appropriate fungal inoculum for a specific set of conditions.

Aspen stands in smelter-impacted areas near Butte and Anaconda hosted a subset of the ectomycorrhizal fungi which occur with aspen in Montana. Some stands were dominated by a few tolerant species such as *Inocybe*

lacera, *Paxillus vernalis*, and *Laccaria proxima*. Other aspen-covered areas displayed a greater diversity of ectomycorrhizal fungi which are discussed. Over 14 species of *Inocybe* were associated with aspen on these stressed sites.

Ectomycorrhizae were examined in one stressed aspen stand to determine if sporocarps are a reliable measure of the (inferred) importance of fungal species in stressed systems where fruiting may be limited. Only half of the fungal species in the aspen stand fruited in 4 years. Only one fruiting body of *Paxillus vernalis* appeared in 4 years, yet 25% of the ectomycorrhizae sampled were this type. In addition, a majority of the ectomycorrhizae occurred deep in the soil of the aspen stand, from 16 to 48 cm.

talk session 8

CUENCA, GISELA, ZITA DE ANDRADE & GLADYS ESCALANTE. Instituto Venezolano de Investigaciones Científicas (IVIC), Centro de Ecología, Apartado 21827, Caracas 1020-A, Venezuela. - Arbuscular mycorrhizae in the rehabilitation of tropical fragile degraded lands

The aim of this work was to study the effect of arbuscular mycorrhizae (AM) in the rehabilitation of areas that have been severely degraded. A field experiment was carried out in La Gran Sabana, Venezuela, in an area that was disturbed five years ago and in which no spontaneous recolonization by plant species has occurred. Five treatments were performed in which and introduced grass, *Brachiaria decumbens*, was sowed (30 kg/ha). The treatments were: NI= non inoculated control; I= inoculated with AM fungi at 1650 kg/ha; P= fertilized with triple superphosphate, 100 kg/ha; I+P= inoculated with AM fungi and fertilized with triple superphosphate; and S= previously sterilized AM inoculum. A soil microorganism inoculum free of mycorrhizae, was added to all the treatments. Five months after sowing the grass, above and below ground biomass, % AM colonization, root length and nutrient uptake were measured. The re-entry of plant species native to the neighboring undisturbed savanna was also evaluated. The results showed a noticeable increase in plant cover, biomass and uptake of nutrients in the I+P treatment in comparison with all the other treatments. The reintroduction of mycorrhizae (I) produced a slight increase in the growth of *B. decumbens*. The P uptake of this treatment increased tenfold in relation to the control even in the absence of exchangeable P in the soil. Of native species re-entering the different treatments, 81% were mycorrhizal. The results obtained indicate that the presence of mycorrhizae seems to be a required condition to achieve the rehabilitation of these lands.

poster session 2 ST SH 06

CULLINGS, K.W., W.W. STOLL, & V.T. PARKER. San Francisco State University, Department of Biology, 1600 Holloway Ave., San Francisco, CA. 94132. - Changes in distribution and relative frequency of *Pinus contorta* mycorrhizae through succession.

The Greater Yellowstone Ecosystem is fire driven, and forest succession proceeds from pure seedling *Pinus contorta*, to adult *P. contorta*, to mixed *P. contorta*, *Picea engelmannii*, and *Abies lasiocarpa*. In this study, we determined the location and relative frequency of active mycorrhizae of each tree species in the system through succession. In each forest type, mycorrhizae were sampled by soil core (10 cm- diameter) and separated into three, 10 cm. deep sections. Three cores were taken from 2 locations in 3 different stands (total of 18 cores) cores were sifted, and mycorrhizae were divided by morphotype. In pure *P. contorta* stands, mycorrhizae in each core were counted. In mixed stands, tree species forming ectomycorrhizae were identified using PCR; DNA from 5-10 mycorrhizae of each morphotype was amplified using the plant specific primer combination 28KJ/28C, and tree species forming individual mycorrhizae identified by comparing RFLP patterns of DNA amplified from root tips to reference DNA amplified from needles. Results indicate that mycorrhizae of *P. contorta* occupy the upper 10 cm layer in pure stands, but once *P. engelmannii* and *A. concolor* are established, mycorrhizae of *P. contorta* become restricted to the bottom 10 cm of the soil column, while the upper layers are dominated by mycorrhizae of the late successional species. Furthermore, *P. contorta* mycorrhizae do not dominate even in the lowest soil layer, even though individuals of this species are always larger and more numerous in the mixed species stands. This result suggests that mycorrhizae of late successional species are displacing mycorrhizae of *P. contorta* for space, and that *P. contorta* may therefore be at increasing nutritional disadvantage as stands develop.

poster session 7 WF AR 12

DANELL, ERIC¹, FRANCISCO J. CAMACHO². ¹Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, P.O. Box 7026, S-750 07 Uppsala, Sweden. ²Department of Botany & Plant Pathology, Oregon State University, 2082 Cordly Hall, Corvallis, OR 97331-29024, USA. - Fruitbody of *Cantharellus cibarius* forming in the greenhouse

Our greenhouse experiments with the edible ectomycorrhizal mushroom *Cantharellus cibarius* have produced a fruitbody. This result is an important step towards a greater understanding of the biology and life cycle of this fungus species. We are examining competition of *C. cibarius* with other ectomycorrhizal fungi and the bacterial associations with the fruitbody. This discovery allows for many other possibilities of study such as physiology of fruitbody formation, physiology of long lived ectomycorrhizal species, genetics and systematical studies, and methods for reestablishing endangered ectomycorrhizal species. Since the *C. cibarius* complex is a group of economically important edible mushroom species worldwide, these techniques may have future commercial applications.

At this time we are investigating the possibility of protecting techniques under the intellectual property law.

Further information can therefore not be published until an oral presentation at ICOM1.

talk session 2

DAVIES, DEBORAH¹ & JONATHAN GRAVES¹.
¹Department of Biology, PO BOX 373, University of York, York YO1 5YW. - Interaction modification in the Lolium perenne-Rhinanthus minor host-parasite association: the role of mycorrhizas

Arbuscular mycorrhizal (AM) fungi are ubiquitous components of the natural grasslands that the annual hemiparasitic angiosperm *Rhinanthus minor* colonizes. As such, there is a high probability of simultaneous infection of a host by these two symbionts. Given that both angiosperm parasites and mycorrhizas act as sinks for host carbon, dual infection could result in competition for photosynthate and hence the occurrence of higher order interactions. This paper describes the results of a glasshouse experiment which investigated the outcome of simultaneous infection of *Lolium perenne* by *R. minor* and AM, at two phosphorus levels (0.13mM and 0.65mM P). Host biomass accumulation was markedly reduced by *R. minor*; a response which appeared to be related to not only sequestration of carbon by the parasite, but also morphological changes in the host. AM colonization did not affect host performance either in the presence or absence of the parasite. The most startling findings were the occurrence of very strong and conflicting parasite responses to AM and phosphorus. Parasite growth, fecundity and attachment rates were significantly higher in the presence of AM, yet were markedly reduced by increasing phosphorus supply. Given that angiosperm parasites are known to be non-mycorrhizal, the AM response must have been mediated via the host.

poster session 3 ST SH 61

DE ANDRADE, ZITA, GLADYS ESCALANTE, ERASMO MENESES & RAFAEL HERRERA. Centro de Ecología, Instituto Venezolano de Investigaciones Científicas, IVIC, Apartado 21827, Caracas 1020-A, Venezuela. - Soil mycorrhizal potential in a seasonally flooded forest in Venezuela

The objective of this work was to assess the soil mycorrhizal potential and the AMF spores types communities at different locations along a gradient in a seasonally flooded forest at Mapire river: the studied field plot is localized near Mapire, Anzoátegui State, South-East of Venezuela. Soil sampling was carried out during February and May, 1992, in three zones along a gradient perpendicular to the course of the river. Flooding reaches a maximum of up to 12 m and last up to 8 months (MAX) near the river's normal border; approximately 100 m away from the river's normal border an intermediate zone occurs where the flooding reaches a maximum of up to 5 m and lasts for up to 5 months (MED); and there is a third zone which is the zone where flooding reaches a maximum of 1 m for 1 or 2 months (MIN). Soil samples (0-20 cm) were taken from each site. Spores were

isolated from soil by wet-sieving and decanting and centrifuged with sucrose solution (750 g/L). The soil mycorrhizal potential was estimated using the Porter's most probable number (MPN) method. Soil mycorrhizal potential was found to be the lowest in MAX site both for February and for May. At MIN site, the number of colonizing propagules was found intermediate and the highest values were those found in MED for both sampling times. The values reported here are in general lower than those for other tropical ecosystems. Many species of AM fungi were found particularly those belonging to *Scutellospora* spp. and *Glomus* spp.

poster session 12 WF SH 28

DECKER, KELLY L.M, SHERRI J. MORRIS, and RALPH E.J. BOERNER. Department of Plant Biology, Ohio State University, Columbus, OH 43210 U.S.A. - Ca:Al ratio effects on competition between arbuscular mycorrhizal and ectomycorrhizal forest trees.

In the deciduous forest of eastern North America, the abundance of species of trees dependent on ectomycorrhizae, such as oaks (*Quercus* spp.) and hickories (*Carya* spp.) has declined over the last two decades while the abundance of species dependent on arbuscular mycorrhizae, such as maples (*Acer* spp.) and yellow-poplar (*Liriodendron tulipifera*) has increased. We hypothesize that this shift in dominance is the consequence of the effects of heavy N deposition and decreasing soil Ca:Al ratio on both plants and fungi. In this study, we examine the effect of lowering the Ca:Al ratio from 100 to 4 under high N conditions on growth and competitive interactions between second year seedlings of ectomycorrhizal red oak (*Quercus rubra*) and arbuscular mycorrhizal yellow-poplar (*Liriodendron tulipifera*). Overall, the relative growth rate of yellow-poplar seedlings exceeded that of red oak seedlings, although the difference was greater at the higher Ca:Al ratio. When in interspecific competition, yellow-poplars outgrew red oaks at both Ca:Al ratios. The relative growth rate of red oak seedlings was relatively insensitive to competition, whereas yellow-poplar plants grew faster when in inter- than intraspecific competition. Under these high N conditions, yellow-poplars were competitively superior to red oaks regardless of Ca:Al ratio. We will also discuss the impact of Ca:Al ratio on N uptake, C and N allocation to leaves, stems, and roots and the effects of Al on the fine structure of ectomycorrhizal roots.

poster session 1 ST RR 20

DEKKERS, DORINE, PETER VAN DER WERFF & PETRA FRISSEN. Ecological Agriculture, Wageningen Agricultural University, Wageningen, 6709 RZ, The Netherlands. - AMF colonization, phosphate uptake and nitrogen management in spring barley as affected by different precrops

The influence of sugarbeet, flax and fieldbean as a precrop was examined on the indigenous AMF colonization, phosphate management and nitrogen management in spring barley. For this purpose a field experiment was conducted on an organic farm in

Flevoland, The Netherlands, which had a silty clay loam soil with pH=8.0 and organic matter=2.8%. At the beginning of the growing season soil samples were taken, plant and root samples were taken during the growing season. The precrops did not influence the direct phosphate availability of the soil. In all experimental plots the $P_{water}=6.4$ mg P_{2O_5}/l soil and the $P_{total}=540$ mg/kg dry soil. However, the precrops did affect the amount of mineral nitrogen in the soil. The initial amount of mineral nitrogen in the soil was higher after flax and fieldbean, compared with sugarbeet. The organic material originated from the sugarbeets residues mineralized later in the growing season. The crop density of spring barley was 275 plant/m² for all mentioned precrops. The tillering of spring barley was positively correlated to the initial amount of mineral nitrogen, and was higher in flax and fieldbean. The AMF colonization reached its highest point just before flowering. At this time the colonization after flax (65%) and fieldbean (71%) were significantly higher than after sugarbeet (51%). The good tillering and AMF colonization after flax and fieldbean were positively correlated with a higher phosphate uptake before flowering. Between flowering and ripening nitrogen originated from the organic material of the sugarbeets mineralised. In this period this resulted in a significantly higher phosphate uptake rate after sugarbeet than after flax or fieldbean. Finally the crop yield was equal for sugarbeet, flax and fieldbean. From this we conclude that precrops do not only influence the AMF colonization but have also an important impact on the timing of nitrogen mineralization.

morning talk

DELL, BERNIE¹ & NICHOLAS MALAJCZUK².

¹CSIRO, Division of Forestry, Perth, Western Australia, 6014. ²School of Biological Sciences, Murdoch University, Perth, Western Australia, 6150. - A role for Australian ectomycorrhizal fungi in the rehabilitation of denuded landscapes in Asia

Fast growing trees are being used to revegetate denuded landscapes in many parts of eastern Asia. These areas include grasslands in the Philippines that once supported rainforests dominated by dipterocarps, and lowland subtropical and upland temperate regions of southern China. Soils of these denuded landscapes are typically acidic, highly leached, low in P, N and some micronutrients and, in some places, are high in Ni and Cr (ultramafic soils). There is very low species diversity of ectomycorrhizal fungi in these disturbed sites compared to the isolated pockets of remnant vegetation remaining in some areas. The reclamation of these lands with trees is successful where there are tangible short-term financial benefits to the local people. Consequently, Australian trees are favoured in initial rotations because of their potential high growth rates and wood quality. Our experience is that establishment and growth of eucalypts in disturbed sites is enhanced by inoculation with isolates of ectomycorrhizal fungi that are matched to site type and host tree. Inoculation programs are desirable because where eucalypts are planted, the few indigenous ectomycorrhizal fungi are mostly ineffective in promoting

the growth of eucalypts. Examples of inoculation trials in China and the Philippines are given to illustrate these principles. Once the disturbed sites have been stabilised through the establishment of a tree cover, options then arise for the reintroduction of indigenous commercial species such as dipterocarps. The development of inoculum for mixed tree plantations and multipurpose trees are challenges for research. Considerable effort will be required to understand the behaviour of potential inoculum fungi in the field before their commercial release.

poster session 4 ST AR 46

DELP, GABRIELE¹, SALLY E. SMITH², SUSAN. J. BARKER¹. ¹Dept. of Plant Science and ²Soil Science, Waite Campus, University of Adelaide, Glen Osmond, S.A. 5064, Australia. - Two differential display products from the VA fungus *G. intraradices*: one contains a homeobox, the other shows homology to a human thyroid receptor-interacting protein.

In our molecular study of the mycorrhizal symbiosis between barley and *Glomus intraradices* we are using the differential display technique to identify genes that are differentially expressed in mycorrhizal roots. Roots with uniform infection stages were produced using the nurse pot method. This inoculation method, which allows a rapid synchronised infection of the roots by transplantation of seedlings into pots with an established inoculum, has revealed that establishment of the mycorrhiza involves a uniform, reproducibly timed series of developmental events. We used mRNA from an early infection stage prior to the development of arbuscules as the template for differential display, compared to non-mycorrhizal root mRNA at the same stage after transplantation. Using this material, several PCR products were consistently differentially amplified in the differential display reaction. Sequence analysis of two of the fragments obtained from infected roots revealed homologies to regulatory genes from higher eucaryotes: one (dd11) contains a homeobox, which indicates that it binds DNA and may act as a transcriptional regulator; the other (dd5) shows homology to trp 15, a human protein that interacts in a hormone-dependant manner with the thyroid receptor. PCR with primers specific for dd5 and dd11 using *G. intraradices* and barley DNA as templates showed that the differential display products are of fungal origin. Using the same specific primers, fragments of the expected size can be amplified from total and mRNA of infected but not uninfected barley roots for both dd5 and dd11, which indicates that the corresponding genes are expressed during intraradical growth of *G. intraradices*. Our results show that differential display is a suitable technique to obtain cDNA sequences from fungal genes that are expressed during VA mycorrhizal interaction. These products will be used to isolate the corresponding genes to allow a more detailed study of their expression pattern and function.

poster session 12 WF SH 74

DENISOVA, NINA. V.L.Komarov Botanical Institute of Russian Academy of Sciences, prof. Popov str. 2, St.Petersburg, 197376, Russia. - Can we account the proteolytic peculiarity of ectomycorrhizal basidiomycetes as an adequate characteristic of their ecological feature?

The correlation between the level of proteolytic activity (especially fibrinolytic, FA, and trombolytic, TA) and ecological belonging of ectomycorrhizal basidiomycetes was revealed on the base of extensive experimental researches of basidiomycete cultures (more than 700 strains from the Botanical Institute Collection) and fruit bodies (about 400 species). The most strongly pronounced synthesis of the proteinases with FA is typical for saprotrophic basidiomycetes (especially for species connected to utilisation of ligno-cellulose complexes). We propose that proteinases with FA and TA can take part in the ecologo-trophic function for such mushrooms. The macromycetes with symbiotrophic (or parasitic) type of life have not marked level of these enzymes activity. Some species which traditionally were considered as the obligate symbiotrophs were the exception from the rule and had high FA and TA levels. There were, for example, some species from genera *Tricholoma*, *Boletus*, *Russula*, *Lactarius*. We think that there examples could provide evidence of the ability of some strictly mycorrhizal species to switch from obligate into facultative type of symbiotrophism. Apparently, symbiotrophs dwelling in well-being forests do not need to have a high level of proteolytic activity. However it can not be excluded that symbiotrophs can have pronounced biosynthesis of mentioned proteinases as an additional mechanism of regulation of ecologo-trophic functioning in the ecosystems where symbiotic relations between myco- and phitobionts are broken. It is possible that the obligate character of symbiotic relations for some species of macromycetes should be corrected.

morning talk

DHILLION, SHIVCHARN. Department of Biology and Nature Conservation, Agricultural University of Norway (NLH), PB 5014, Aas, N-1432, Norway. - Mycorrhizal relationships with plant diversity and elevated CO₂ conditions

There is a increasing interest in the importance of diversity in the functioning of microbial and plant communities. Recent studies show that microbially-mediated soil processes (e.g., enzyme activities, microbial substrate utilization and nutrient cycling) are linked to plant diversity, and inturn, plant diversity may be linked to the mycorrhizal species within the system because plant species vary in their mycotrophy. Furthermore, microbially-mediated soil processes may be modified by elevated CO₂ conditions. Studies manipulating plant functional groups (e.g. grasses, legumes, composites, annuals and perennials) show that functional groups influence microbially mediated processes, especially nutrient cycling, and thus emphasize the role of mycorrhizas. This paper addresses the following questions: Can differences in the the overall

mycotrophic nature of communities influence processes associated with plant diversity? Do changes in CO₂ levels influence species responses within plant communities? It highlights the inter specific role of plant species, and groups of plants, which may determine the impact of mycorrhizas in plant communities, and stresses the need for evaluating microbially-mediated soil processes parallel to those of mycorrhizae and to those associated with plant diversity.

talk session 8

DI BATTISTA, CÉLINE, DANIEL BOUCHARD, *BENOIT GÉNÈRE, FRANCIS MARTIN, FRANÇOIS LE TACON. Equipe de Microbiologie Forestière-INRA Nancy, France 54280 Champenoux, *CEMAGREF 45290 Nogent/Vernisson, France. - Monitoring the persistence of the ectomycorrhizal basidiomycete *Laccaria bicolor* S238N on Douglas-fir and Norway Spruce in nursery and forest sites by PCR

The growth of Douglas-fir (*Pseudotsuga menziesii*) and Norway spruce cuttings (*Picea abies*) is improved in nursery by artificial inoculation with the ectomycorrhizal strain *Laccaria bicolor* S238N. To evaluate its competitiveness and its persistence after transplantation in forest, the mycorrhizal infection of Douglas fir and Norway spruce cuttings were determined morphologically and by ribotyping. The polymorphism of the internal transcribed spacer (ITS) and the intergenic spacer (IGS1) of the ribosomal DNA was assessed by RFLP of PCR amplified DNA. Eighty to 90% of mycorrhizal tips of the 1 or 2-year-old Douglas fir and Norway spruce cuttings, grown in nursery, were colonized by *Laccaria bicolor* S238N. The inoculated Norway spruce cuttings were outplanted on 2 forest sites (St Germain l'Herm, 1000m of altitude ; Vidaillat, 560m of altitude) where the competition with indigenous strains was higher than in nursery. In Vidaillat, 1 year after outplanting, only 15% of mycorrhizae on inoculated spruce cuttings were formed by *L. bicolor* S238N. Other mycorrhizal tips (85%) belonged to indigenous species. In St Germain l'Herm, 2 years after outplanting, only 18 % of the total mycorrhizal tips of the inoculated Norway spruce cuttings were colonized by *L. bicolor* S238N. Four years after outplanting, only 3% of the mycorrhizal tips of *L. bicolor* S238N on inoculated spruce cuttings were still associated with the roots. In these sites, the introduced strain was rapidly replaced by local strain. However in other investigated forest sites, *L. bicolor* S238N associated with Douglas-fir persisted at least 10 years after transplantation. Persistence of the inoculated strain therefore depends on the host plant, the presence of competitive local strains, and/or edaphic and climatic conditions.

poster session 3 ST SH 74

DIAZ, GISELA¹ & Mario Honrubia¹. Departamento de Biología Vegetal (Botánica). Facultad de Biología. Universidad de Murcia. Campus de Espinardo 30100, Murcia. Spain. - Effect of simulated acid rain on

mycorrhizae of Aleppo pine (*Pinus halepensis* Miller) in calcareous soil

Mycorrhiza formation and plant growth, in particular root development, of *Pinus halepensis* were studied in relation to the influence of pH from simulated rain in pot cultures. Four treatments of water (7.5, 6.0, 4.5 and 3.0) were established by adding a mixture of sulphuric and nitric acids (2:1, v/v) or 10% NaOH to distilled water. Three experiments, that differed in the inoculation treatment, type of substrate and age of seedlings, were carried out: 1) Three-month-old seedlings growing in untreated calcareous forest soil; 2) two-year-old naturally mycorrhizal seedlings, transplanted into vermiculite in order to differentiate old and new-formed roots; and 3) seedlings growing in peat-vermiculite, inoculated with mycelial inoculum of *Suillus collinitus*. Although no visible effects on the aerial part were observed, a reduction of root length in the most acidic treatment was noted. The dominant morphotype observed in experiments 1 and 2 was of the *Suillus* kind. Enhancement of ectomycorrhizae formation was also recorded in this treatment in the three experiments. In conclusion, in substrata of neutro-basic pH, short-term exposures to acid rain positively affected ectomycorrhizal fungi, in particular, *Suillus* species.

poster session 5 ST AR 14

DICKSON, SANDY¹, SALLY SMITH¹ & ANDREW SMITH². ¹Department of Soil Science, Waite Campus, University of Adelaide, S. Australia 5064, ²Department of Botany, University of Adelaide, S. Australia 5005. - P fluxes from two VA mycorrhizal fungi to leeks under two soil phosphate levels

An experiment was carried out to demonstrate the efficiency of transfer of P by two VA mycorrhizal fungi. Two soil P levels were used (no added P (P0) and 0.4 mmol P per kg soil (P1)), with two fungi (*Glomus* sp. 'City Beach' WUM 16 and *Scutellospora calospora*) on the host plant *Allium porrum*. Harvests were taken at 2,3,4 and 6 weeks and fungal efficiency was measured as the flux (transfer of P to the plant per unit area of symbiotic interface). Vital staining to show metabolically active fungal structures was carried out on sections, in order to quantify intercellular hyphae, arbuscules and vesicles within the root. The percent of root colonised was also measured using non-vital staining of root segments. Although the results using the two stains were not significantly different, there was a trend for sections with vital staining to show slightly lower levels of (viable) colonisation than those stained with a non-vital stain. Flux values have been obtained using the percent colonisation shown by vital staining as the measure of active arbuscules and hyphae. Fluxes across both the intercellular and arbuscular interfaces, or across the arbuscular interface alone, vary with the fungal species and with the addition of P in the soil. At P0 fluxes were low with both fungi (0.81 - 6.57 nmol m² s⁻¹). In contrast, fluxes across both interfaces were greatly increased at P1, but to differing degrees depending on the

fungus. It appears that *S. calospora* is more tolerant of additional P in the soil than is *Glomus* sp. 'City Beach'.

poster session 11 WF RR 42

DÍEZ, JESUS¹, FRANCIS MARTIN² & JOSE L. MANJÓN¹. ¹Biología Vegetal, Universidad de Alcalá de Henares, 28871 Alcalá de Henares, Spain. ²Microbiologie Forestière, I.N.R.A., Centre de Nancy, 54280 Champenoux, France. - Biodiversity of the desert truffles associated with *Tuberaria guttata* in the Mediterranean Spanish vegetation.

A "dehesa-like" vegetation exists in the Extremadura Region of Spain. This one comprises a grass land with some trees, mainly holm oaks (*Quercus rotundifolia*) and/or cork trees (*Quercus suber*). During the spring season, the local inhabitants collect desert truffles near the annual plant *Tuberaria guttata*. The desert truffles are known to form mycorrhizas with the Cistaceae. However, no genetic diversity studies have been reported for these fungi. The main objective of the present study is to assess the genetic biodiversity of the desert truffles in this region. Three different mycorrhizal fungal species were identified by studying morphological features of the fruiting bodies, by optical and scanner microscopy. They were *Tuber* sp., *Terfezia arenaria* and *T. leptoderma*, which form mycorrhizal communities with *Tuberaria guttata* in siliceous acid-sandy soils. The internal transcribed spacer (ITS) of the nuclear ribosomal DNA (rDNA) from fruiting bodies and isolates have been amplified by PCR. RAPD and polymorphism of microsatellite rich DNA were also carried out. PCR-RFLP was very homogeneous within species. In contrast, RAPD and polymorphism of microsatellite rich regions were much larger. These data suggest that RAPD and polymorphism analysis of microsatellite rich DNA have a higher power of resolution for assaying the variability of these mycorrhizal communities.

poster session 1 ST RR 23

DÍEZ, JESUS & JOSE L. MANJÓN. Biología Vegetal, Universidad de Alcalá de Henares, 28871 Alcalá de Henares, Spain. - In vitro mycorrhization of micropropagated plantlets of *Tuberaria guttata* with desert truffles, and mycorrhizal persistence after acclimation monitored by PCR.

Tuberaria guttata belongs to the Cistaceae and establishes mycorrhizal association with desert truffles. This symbiosis is common in the Western Mediterranean Basin. The mycorrhizal synthesis has been carried out in axenic and gnotoxenic conditions. However, no mycorrhization of micropropagated plantlets of *T. guttata* has been reported. The main objectives of the present study were to develop the micropropagation and in vitro mycorrhization of *T. guttata*, and to design tools for monitoring the mycorrhizal status of plantlets. Mycelial cultures of desert truffles were obtained from fruit-body tissues in the Fontana and BAF medium. Micropropagation of *T. guttata* was carried out by microcutting of shoot tips and nodal segments in MS

medium with a low cytokinin concentration. Microcuttings rooted spontaneously, but indole-3-butyric acid or macronutrient reduction increased rooting percentages. Mycorrhization of micropropagated plantlets was carried out on the Sommer medium with some modifications, and then acclimatized to the greenhouse conditions. Mycorrhizal status was confirmed by PCR/RFLP of the internal transcribed spacer (ITS) of the nuclear rDNA. The present study is the first report of the mycorrhizal inoculation of *T. guttata* microcuttings.

talk session 9

DODD, J.C.¹, B. TISSERANT², N. REQUENA³, V. BRENAC¹, J.M. BAREA³ & P. JEFFRIES².

¹International Institute for Biotechnology, ²Dept. of Biosciences, University of Kent, Canterbury, Kent CT2 7YW, UK. ³Estación Experimental del Zaidín, C.S.I.I., Profesor Albareda 1, E-18008 Granada, Spain. - The detection of arbuscular mycorrhizal fungi (AMf) in root systems of different plants using fungal/mycorrhizal-specific isozymes as molecular markers.

Isozyme analysis is a simple and relatively inexpensive technique which can discriminate closely-related organisms. The use of FSI/MSIs as molecular markers has practical use in the detection and identification of AMf in plant roots. Our aim has been, therefore, to use non-denaturing polyacrylamide gel electrophoresis to separate isozymes and to track well-characterised AMf in young root systems. In a project funded by the EU called REDEEM (Restoration of Environmental Diversity by Effective Ecosymbiont Monitoring) we have used this technique to identify individual AMf in the roots of *Anthyllis cytisoides* (a desert shrub legume) and other test plants. The results from a screening of various *Glomus* spp. in laboratory microcosms using inert growth media (Terragreen, durite sand), in soil from the study site in Almeria (S. Spain), and ultimately in the field, have shown that a cocktail of enzyme stains can be used to identify individual AMf colonising roots at different stages of the symbiosis. The use of an alkaline phosphatase (ALP) stain showed that the non-indigenous symbiont, *Glomus microaggregatum* (BEG 56), used as the fungal inoculant of *A. cytisoides* seedlings could be detected 3 months after outplanting into the field. The presence of the ALP marker bands also implied that the fungus was probably still active in the roots, with respect to P transfer, at a time when the seasonal rains had finished. Staining of the roots for ALP activity showed that most occurred in the arbuscules. The results will be discussed with respect to the use of the technique to study the functional ecology of AMf in natural ecosystems.

poster session 10 WF SH 18

DOMINGUEZ de TOLEDO, L.¹, E. NOUHRA¹, R. MOLINA² & A. BECERRA³. - ¹Instituto Multidisciplinario de Biología Vegetal, Casilla de Correo 495. 5000, Córdoba, Argentina. ²USDA Pacific Northwest Research Station, Forestry Science Laboratory, Corvallis, OR. 97331. ³Fac. Cs. Ex. Fis. y Nat. U.N.i. Av. V.Sarsfield 299. 5000, Córdoba, Argentina. - Development, function and specificity of

actinorhizae and mycorrhizae on *Alnus jorullensis* H.B.K from Argentina.

Alnus species are important components in many native forests. *Alnus* forms both N-fixing nodules (actinorhizae) as well as vesicular-arbuscular mycorrhizae and ectomycorrhizae. Such tripartite or tetrapartite symbioses contribute to successful colonization of *Alnus* in nutrient poor or disturbed sites, with subsequent benefits to site quality. *Alnus jorullensis* occurs in the Andes of South America in wet montane habitats above 1300 m. In three related studies we examined occurrence and function of root symbiosis of *A. jorullensis* in its native forests and following inoculation with forest soil, *Frankia*, and the ectomycorrhizal fungus *Alpova diplophloeus* native to *A. rubra* habitats in Oregon, (USA): (1) Roots of *A. jorullensis* were collected from forests in Catamarca and Tucuman Provinces (Argentina). We describe two ectomycorrhiza types, presence of nodules and colonization by dark septate endophytes; (2) Seedlings of *A. jorullensis* were greenhouse grown in sterile soil and inoculated with Oregon cultures of *Frankia* and spores of *A. diplophloeus*. N-fixing nodules and *A. diplophloeus* ectomycorrhizae became well developed; (3) Seedlings of *A. jorullensis* and *A. rubra* were grown in soil taken from beneath *A. rubra* and harvested at three times. Both *Alnus* species developed nodules and shared compatibility for three distinct ectomycorrhiza types.

poster session 12 WF SH 36

DOUDS, DAVID, JERRY NAGAHASHI, & GLORIA ABNEY. USDA-ARS ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038-8038 USA. - Phosphorus amendment inhibits hyphal branching of the AM fungus *Gigaspora margarita* directly and indirectly through its effect on root exudation.

The direct or indirect nature of the inhibition of mycorrhizal development by phosphorus is still debated, but the exact effect upon the mycorrhizal fungus in the soil is not understood. To this end, the effect of solution P concentration upon growth of pregerminated spores of the arbuscular mycorrhizal fungus *Gigaspora margarita* was examined in vitro. One mM P significantly inhibited branching of the primary germ tube. The number of branches and the total hyphal length were both significantly inhibited at 10 mM P. In addition, germinated spores exposed to exudates produced by Ri T-DNA transformed roots of *Daucus carota* grown in the presence of P showed significantly less hyphal branching than those exposed to exudates produced by P stressed roots. These phenomena could contribute to the observed inhibition of mycorrhiza formation by high P.

poster session 12 WF SH 37

DUGASSA-GOBENA, DEREJE & F. SCHÖNBECK. Institut für Pflanzenkrankheiten und Pflanzenschutz, Universität Hannover, Herrenhäuser Strasse 2, D-30419 Hannover, Deutschland. - Physiology and assimilate allocation in triplicate system, host plant, arbuscular mycorrhiza (AM) and biotrophic pathogen.

Plant health and growth are greatly influenced by physiological state and assimilate allocation in plants which may alter also by AM and biotrophic pathogens, due to their sink activities. Such interactions has been investigated in *Linum usitatissimum*, *Glomus intraradices* and *Oidium lini*. The pathogen sink activity is expressed as sporulation rate and it is higher on AM plants than on non-AM plants. In spite of higher pathogen activity AM plants show less damage than non-AM plants as expressed in CO₂ assimilation in mildewed plant parts and content of sucrose in non mildewed shoot apex. These and other physiological parameters will be discussed, with emphasis on mechanisms of improved plant health by AM development.

poster session 4 ST AR 49

DUMAS-GAUDOT, ELIANE¹, BARBARA DASSI¹, SOPHIE SLEZACK¹, ASSEM SAMRA¹, MARIA JOSE POZO² & SILVIO GIANINAZZI¹. ¹Laboratoire de Phytoparasitologie INRA/CNRS, SGAP, INRA, BV 1540, 21034 Dijon Cedex, France. ²Departamento de Microbiologia, CSIC, Estacion Experimental del Zaidin, 18008 Granada, Espagna. - Researches on specific plant protein expression in arbuscular mycorrhizal symbioses.

Targeted and untargeted approaches have been used in several plant species (tomato, pea and tobacco) to look for changes in plant protein expression linked to arbuscular mycorrhizal (AM) symbioses, pathogenic infections and pathogen-mycorrhizal root interactions.

The targeted approach, selected to study modifications in plant hydrolytic enzymes following arbuscular symbiosis, pathogenic infections, or chemical elicitation, allowed us to demonstrate, as a general phenomenon, the existence of mycorrhiza-induced specific chitinase isoforms, probably of host origin. The expression of several groups of Pathogenesis Related (PR) proteins was investigated by immunological detection (Western blotting) in plant roots inoculated with AM and fungal root pathogens or during bioprotection in roots pre-colonized with an AM fungus and post-infected with a root pathogen. While the AM symbiosis more often leads to weak expression of PR proteins compared to that in pathogenic root interactions, a different situation occurs during bioprotection.

An untargeted approach, using 2D-PAGE to analyze protein content of mycorrhizal roots, has permitted to demonstrate the existence of endomycorrhizins. This result has been further supported by *in vivo* and *in vitro* protein synthesis analyses. Studies by 2D-PAGE are in progress for identifying polypeptides specifically expressed during bioprotection in pathogen-challenged mycorrhizal roots.

poster session 10 WF SH 20

DUNSTAN, WILLIAM A.¹, NICHOLAS MALAJCZUK² & BERNIE DELL¹. ¹School of Biological and Environmental Sciences, Murdoch University, Perth, W.A. 6150, Australia. ²CSIRO Forestry & Forest Products, CSIRO Centre for Mediterranean Agricultural Research, Private Bag P.O.

Wembley, W.A. 6014, Australia. - Effects of selected bacteria on mycorrhizal development and growth of container grown Eucalyptus diversicolor seedlings.

Eucalyptus diversicolor seedlings were co-inoculated with one of three isolates of two Laccaria spp. and an isolate of known Mycorrhization Helper Bacteria (MHB) or a bacterium isolated from Laccaria spp. sporocarps, or Eucalyptus marginata - L. fraterna ectomycorrhizas. No mycorrhizas were formed by L. laccata S238, a conifer mycorrhizal isolate. One MHB isolate, Bacillus subtilis Bbc6, (obtained from INRA, Champenoux, France), significantly reduced mycorrhization by L. laccata E766 but significantly increased mycorrhization by L. fraterna E710. One bacterium (Elf29), isolated from E. marginata - L. fraterna ectomycorrhizas, significantly increased mycorrhization by L. fraterna E710. In treatments with bacteria alone, one isolate significantly increased shoot dry weight. A significant increase in shoot dry weight was also observed in three bacterial treatments co-inoculated with L. laccata S238. Bacterial isolates that appeared to stimulate shoot growth did not increase mycorrhiza development.

poster session 2 ST SH 07

DURALL, DANIEL¹, MELANIE JONES¹, ELAINE WRIGHT², & DAVID COATES². ¹Biology Department, Okanagan University College, Kelowna, B.C. V1V 1V7, Canada. ²British Columbia Ministry of Forests Research Section, Prince Rupert Forest Region, Bag 5000, Smithers, B.C. V0J 2N0, Canada. - The effect of opening size on ectomycorrhizal diversity and fruitbody production in the coast-interior transition forests of northwestern British Columbia.

Public concern and changing professional perspective has resulted in a new forest policy in B.C. that calls for all silvicultural systems to be considered prior to harvesting. This has prompted the B.C. Forest Service to initiate several studies whose objectives are to investigate not only the economic feasibility of alternative (to clearcutting) silvicultural systems, but also to determine how flora (including fungi) and fauna respond to these treatments. Roots were sampled from *Tsuga heterophylla* and *Pinus contorta* seedlings, from a range of opening sizes (49 m² to 20 ha), 3 years after outplanting. Two hundred mycorrhizae from each seedling were carefully examined under 400 X and/or 1000X magnification and the mycorrhizae placed into categories based on morphology. The diversity of ectomycorrhizal types found on *P. contorta* seedlings dropped significantly between seedlings planted 2 m versus 20 m from the intact forest canopy ($r^2=0.57$, $P=0.003$; $r^2=0.50$, $P=0.001$; $r^2=0.52$, $P=0.0007$; for richness, Shannon's and Simpson's diversities, respectively), but data between 20 m and 120 m was much more variable and no significant relationships were detected over 2 to 120 m. The number of ectomycorrhizal types associated with *T. heterophylla* decreased significantly between seedlings planted 2 m versus 120 m from the canopy ($r^2=0.2$; $P=0.04$). Fruitbodies of ectomycorrhizal fungi were sampled in August and September 1995 along 300 m

transects in the 20 ha clearcuts and in 20 ha uncut blocks, and along the longest N-S transect (6 m to 76 m) in smaller openings. Species richness per m of transect was quite variable (2.5 - 10) in openings smaller than 250 m², but was uniformly low (<1.2) in openings larger than 900 m².

poster session 12 WF SH 38

EATON, GREGORY K.¹ and ORSON K. MILLER, JR.² ¹Department of Biology, Dartmouth College, Hanover, NH 03755-3576, USA, and ²Department of Biology, Virginia Tech, Blacksburg, VA 24061-0406, USA. - Nutrition and growth of a calcifuge plant in a model calcareous system: effects of mycorrhization and rhizosphere oxalic acid.

Rhizosphere production of oxalic acid may enhance localized nutrient cycling and drive soil chemical reactions that markedly change the environment experienced by plants. We evaluated the influence of oxalic acid produced in ectomycorrhizal symbiosis on rhizosphere nutrients and on the nutrition and growth of a calcifuge plant grown in calcareous soil. *Eucalyptus diversicolor* seedlings were grown with or without the ectomycorrhizal fungus symbiont *Hebeloma westraliense* in calcareous and non-calcareous media in synthesis tubes in a growth chamber. The calcareous system had increased solution pH and Ca and decreased solution P. Seedlings without mycorrhizae had decreased shoot P, reduced height and mass and macroscopic symptoms of severe nutrient deficiency. The effects of mycorrhization on calcareous solution included increased pH, decreased Ca and P and substantially increased rhizosphere oxalates. Mycorrhizal seedlings had increased Ca and P in shoots, greater height and mass and no symptoms of nutrient deficiency. Oxalate binds Ca in calcareous soil to form Ca oxalate crystals. This allows increased P in the rhizosphere solution and increased plant uptake of this nutrient. Oxalic acid production in mycorrhizal symbiosis may ameliorate the intolerance of *Eucalyptus* spp. to calcareous soil.

poster session 8 WF AR 64

EBERHARDT, URSULA & INGRID KOTTKE. Spezielle Botanik und Mykologie, Universität Tübingen, Auf der Morgenstelle 1, D 72076 Tübingen, Germany. - Effectiveness of different mycorrhizal fungi inoculating spruce seedlings (*Picea abies* (L.) Karst.) in the forest environment

The study was carried out to investigate whether among fungi mycorrhizal with mature spruce trees differences can be found in respect to their ability to inoculate spruce seedlings. In a mature forest stand near Bad Waldsee (Baden-Württemberg, Germany) of 120 x 120 cm of the forest floor were divided into 64 squares and the organic layer and the mineralic topsoil were sampled including spruce seedlings of the year before. The mycorrhizae of the seedlings and of the roots of mature trees contained in the samples were identified morphologically. From mature trees 10 different mycorrhizal types could be

distinguished 7 of which could also be found on seedlings. Assuming that the inoculation of seedling roots is carried out by emanating hyphal elements of adjoining mycorrhizae, the mycorrhizae of the roots of mature trees in each square were considered responsible for the inoculation of seedlings rooting in the same square. The proportion of seedlings mycorrhizal with a given fungus of the total of seedlings coming from squares containing mycorrhizae of the same type on roots of mature trees was calculated for 7 fungi. This proportion was considered a measure for the effectiveness of a fungal species to inoculate seedlings. Though *Tylospora fibrillosa* had inoculated the greatest number of seedlings, *Thelephora terrestris* was found to be most effective in inoculating seedlings among the encountered mycorrhizal fungi.

poster session 8 WF AR 50

EBERHART, J.L., D.L. LUOMA and M.P. AMARANTHUS. Department of Forest Science, Oregon State University, Corvallis, OR 97331 USA. - Dynamics of Ectomycorrhizal Fungi - The link between ectomycorrhizae and sporocarp production.

Experimental plots have been installed in the Siskiyou National Forest that will compare long-term ecosystem productivity under different silvicultural manipulations. We have collected baseline data (prior to treatment) on ectomycorrhizal fungus diversity. Collections include 43 species of ectomycorrhizal truffles from plots totaling 0.75 hectares, and about 100 species of ectomycorrhizal mushrooms from plots totaling 4.5 hectares. From 189 soil cores totaling a soil surface area of only 0.45 m², 192 ectomycorrhizal morphotypes were discerned. For most types the identity of the fungus remains unknown. Frequency of occurrence of each morphotype was used to determine relative abundance of ectomycorrhiza types within a particular core. Total number of types and mean relative frequency of types were assessed in pretreatment comparisons. The mean number of types (12) per 350 cc soil core was stable among 14 of the 15 stands. The development of a relative frequency index for ectomycorrhiza morphotypes has provided an efficient way to assess fungal populations so that, with adequate pretreatment sampling, treatment effects can be measured. Our results show that we cannot assume uniformity of fungal populations in these stands and demonstrates the necessity and utility of pretreatment sampling. This poster focuses on six of the more common ectomycorrhiza types that can be identified to species. Our results demonstrate for the first time that, for some species, variation in mycorrhizae abundance is matched by variation in sporocarp production at a landscape level. Results also showed a fairly constant presence of these mycorrhiza types across seasons in contrast to the strong seasonality of sporocarp production.



ECKERT, GREGORY¹, R. MICHAEL MILLER² & CHARLES RHOADES¹. ¹Institute of Ecology, University of Georgia, Athens, Georgia 30602-2202 U.S.A. ²Argonne National Laboratory, ER-203 Argonne, Illinois 60439-4843 U.S.A. - Arbuscular mycorrhizae dynamics in Andean andisols

Long term study plots of mycorrhizae and other soil ecosystem components have been established in a mid-elevation montane zone of western Ecuador. The zone is characterized by allophanic soils, steep slopes and 3,000 mm of precipitation over 10 months of the year. Land in the study area is managed for pasture and sugarcane leaving a fragmented landscape. Study plots are along a vegetation gradient of 1. planted pasture; 2. unplanted, unmanaged pasture; 3. Small trees and shrubs; 4. young forest and 5. mature forest. Mycorrhizal infection levels of field roots collected during the 2 - 3 month dry season were very low. Assays of mycorrhizal inoculum potential (MIP) were indirectly related to levels of plant-available phosphorus ($r^2=0.68$). Plant-available phosphorus (P) concentration was determined by sequential extraction of samples with anion-exchange resins and sodium bicarbonate. P levels were high in forest soils (62 ug/g soil) where MIP was lowest (14% of 1 cm root lengths with infection). Shrub patches showed depleted P (41 ug/g) with a related increase in MIP (61%). Future work at this site will include wet season studies of mycorrhizae and experiments to evaluate the role of mycorrhizae in managed ecosystems.

EGERTON-WARBURTON, LOUISE M., & EDITH B. ALLEN. Botany and Plant Sciences, University of California, Riverside, CA 92521-0124, USA. - Variations in density and diversity of arbuscular mycorrhizal fungi in coastal sage scrub communities in response to soil nitrogen eutrophication

Arbuscular mycorrhizal fungi populations were assessed for *Artemisia californica*, *Encelia farinosa* and *Eriogonum fasciculatum*, at the Box Springs Mountain Reserve, an area with elevated N eutrophication (30 kg N ha⁻¹ year⁻¹), and Lake Skinner Reserve, an intact coastal sage scrub community with low N eutrophication (5 kg N ha⁻¹ year⁻¹). In coastal sage, propagule production was highest in August/September which coincides with completion of the plant growing season, and lowest during December/January (winter). While there was no significant difference in propagule number between sites from April until August, for the remainder of the year (and overall) a significantly higher density of propagules was recovered from Lake Skinner than Box Springs. This difference was also reflected in both species diversity and richness measures. The genera *Acaulospora*, *Scutellospora*, *Gigaspora*, *Sclerocystis*, *Glomus* were identified, with both sites being dominated by *Glomus* species, particularly Box Springs (14 species identified, 12 *Glomus* spp), while *Sclerocystis* and *Gigaspora* species were only recovered from Lake Skinner (19 species identified, 14 *Glomus* spp). Host plant x site interactions

were absent. However, a greater number of arbuscular mycorrhizal species were associated with *Encelia farinosa*, while *Gigaspora* was not associated with *Eriogonum fasciculatum*. These data indicate that soil eutrophication may be directly associated with decreases in both propagule numbers and diversity, particularly of larger spored genera, and indirectly associated with the host plant species.

EGGER, KEITH. Natural Resources and Environmental Studies, The University of Northern British Columbia, Prince George, BC, V2N 4Z9, Canada. - Molecular Biodiversity: What are we measuring?

Interest in the use of molecular techniques to estimate mycorrhizal biodiversity is intensifying. As a result, it is becoming increasingly important to consider the correspondence between morphologically-based methods of estimating biodiversity and molecular assessments. Molecular estimates are ideally suited for differentiating mycorrhizal types from single root tips, and should be better at differentiating complexes of convergent morphotypes. However, molecular techniques are unique in that they measure both species diversity and population genetic diversity. As a result, molecular measures will tend to give a higher estimate of diversity, because a component of the estimate will be population-level variation, and multiple colonization of single root tips is more likely to be detected. On the other hand, molecular diversity will not be sensitive to morphotype overestimates that are due to host or environmental variations. In this talk I will discuss several aspects of molecular biodiversity, including: 1) sources of interspecific and intraspecific molecular variation, 2) differences in intraspecific variability among species and the factors responsible for generating population-level variation, 3) how different diversity indices respond to differing levels of intraspecific variation, and 4) the level of sampling required to capture a significant proportion of the variation at the molecular level.

EGLI, SIMON & FRANCOIS AYER. Swiss Federal Institute for Forest, Snow and Landscape Research, 8903 Birmensdorf, Switzerland. - Changes in macromycete diversity - results of 20 years experimental study on man-made influences on fungal flora, with special emphasis on mycorrhizal species.

In 1975, a long-term mycoecological research project was started to establish a scientific basis knowledge on the decrease of fungal diversity, namely on the role of fungi-harvesting as well as on the influence of forest management, microclimate and environmental changes. Special emphasis was laid on the long-term dynamics of the different ecophysiological groups of fungi (mycorrhizal, saprobe, parasitic, acidophilous, basophilous, nitriphilous species) and on the representativity of fruiting body patterns for underground mycorrhizal diversity. It is hoped that results will also

contribute to the discussion concerning fungi conservation in Switzerland.

Some changes recorded in species diversity can be attributed to silvicultural operations and to changes in site conditions. Fungi harvesting seems not to influence fungal productivity significantly, but concomitant trampling of the forest floor has clearly negative effects. The ratio between mycorrhizal and saprobe fungi has changed in the last 20 years, most probably due to atmospheric deposition of nitrogen.

Beside the presentation of results the various methodical difficulties in recording macromycetes and assessing their diversity will be discussed.

poster session 3 ST SH 65

EOM, AHN-HEUM, DAVID HARTNETT, GAIL WILSON, & DEBORAH FIGGE. Division of Biology, Kansas State University, Manhattan, KS, 66506, USA. - The effect of fire, mowing and fertilizer amendment on mycorrhizas in tallgrass prairie

The effects of annual mowing, burning and N and P fertilization regimes on arbuscular mycorrhizal (AM) symbiosis in tallgrass prairie were studied ten years after treatment initiation. Spring burning of native prairie field plots significantly reduced AM fungal species diversity and evenness, while increasing spore abundance. This increase in abundance (total spore number) was due to an overall response of most of the 17 fungal species present. In general, the various management treatments had larger effects on the AM species richness component than on the evenness of fungal species diversity. Burning and mowing had no significant effects on AM fungal colonization of roots or extramatrical hyphal (EMH) development. However, nitrogen fertilization significantly increased root colonization and EMH, and P amendment decreased EMH development. There was no significant effect of fertilizer amendment on AM spore abundance, species diversity or richness, but N and P fertilization decreased fungal species evenness. Effects of management practices on AM fungi may be mediated through changes in soil resources or microclimate, or through changes in their host plants. These effects on AMF symbiosis and community structure are important because AMF strongly influence the growth, demography, competitive relationships, relative abundances, and diversity of plants in grassland communities.

poster session 5 ST AR 16

EZAWA, TATSUHIRO¹, MASANORI SAITO², MASAHIRO HAYATSU² & TOMIO YOSIDA³. ¹University Farm, School of Agriculture, Nagoya University, Togo-cho, Aichi, 47001, Japan. ²National Grassland Research Institute, Nishinasuno, Tochigi, 32927, Japan. ³Faculty of Horticulture, Chiba University, Matsudo, Chiba, 271, Japan. - Characterization and localization of phosphatases in the plant and fungal symbionts of arbuscular mycorrhiza.

The enzymatic property and localization of phosphatases specific to arbuscular mycorrhizal

symbiosis were investigated to consider the role of these enzymes in the symbiotic phosphate metabolism. Marigold was inoculated with *Glomus etunicatum* and the soluble enzyme was extracted from the roots. The infection-specific phosphatase (ISPase) was detected by electrophoresis followed by phosphatase staining. The ISPase activity increased as the mycorrhizal colonization increased, but decreased at the stationary phase of the host growth although the colonization level was still high. The activity of ISPase was correlated with the growth of host plant. The ISPase was purified and characterized, then it was identified as non-specific acid phosphatase (E.i.3.1.3.2). The electrophoretic mobility of the ISPase was almost the same among different mycorrhizal associations. The ISPase was not detected in the spore extract. These findings and the N-terminal amino acid sequence of the enzyme suggested that it may be of host plant origin.

The localization and characteristics of phosphatases in the intraradical hyphae isolated from enzymatically digested roots were also examined.

poster session 8 WF AR 38

FARIAS, JAVIER, LUIS BOJALIL, ANTONIO FLORES, SALVADOR GUZMAN & ARNOLDO MICHEL. Facultad de Ciencias Biológicas y Agropecuarias. Universidad de Colima. Apartado postal 36. 28100 Tecoman, Colima, Mexico. - Influence of a Host Plants on Arbuscular Mycorrhizal Infection of Purple Nutsedge (*Cyperus rotundus* L.).

Members of the Cyperaceae are reported to be non-mycorrhizal. In this study the influence of bean (*Phaseolus vulgaris* L.) on the VAM colonization and nutrient status of purple nutsedge roots was determined in pot culture experiments. Five treatments were evaluated: 1) soil alone, 2) purple nutsedge, 3) bean, 4) purple nutsedge + bean (sown and grown simultaneously) and 5) purple nutsedge + bean (this was sown fifteen days after). These were arranged in a completely randomized design with four replications. *Glomus intraradices* Shenck & Smith was utilized as inoculum. Plants were harvested at 15, 30 and 45 days after inoculation. *C. rotundus* plants grown with bean together developed VA infection and VA colonization in bean roots was not decreased by the presence of the "non-host". In contrast no infection was observed in purple nutsedge when these plants were grown alone. High (65.6 and 61.8%) infection (cortical mycelium, vesicles and chlamidospores, but no arbuscules) developed when bean host plant was present. Although plant tissue nitrogen, phosphorus and potassium level were not affected by association of bean and purple nutsedge or mycorrhizal status. These results suggest that barriers to mycorrhizal infection in "non-host" are intrinsic and more probably related to characteristics of the root cortex or epidermis than to any infection-inhibition factors that might be released in root exudates.

poster session 10 WF SH 03

FILION, MARTIN, MARC ST-ARNAUD & J. ANDRÉ FORTIN. Institut de recherche en biologie végétale, Université de Montréal and Jardin botanique de Montréal, 4101 est, rue Sherbrooke, Montréal, Québec, Canada, H1X 2B2. - Negative effect of soluble substances released by the VAM fungus *Glomus intraradices* on the *Fusarium oxysporum* f. sp. *chrysanthemi* conidial germination in an in vitro system.

VAM fungi can reduce the incidence and importance of plant diseases caused by soilborne phytopathogenic fungi. Some hypothesis were put forward, trying to explain the mechanisms inherent to this situation. We are here testing the hypothesis according to which VAM fungi could interact directly with soilborne pathogens in the mycorrhizosphere of mycorrhizal plants via substances released in the environment by the mycelium or spores. A two compartment in vitro system (modified from Mycorrhiza 5:431-438) was used to isolate potential exudates or intra-fungal active substances released by the mycelium or spores of the VAM fungus *Glomus intraradices*. An extraction technique was designed to extract all soluble compounds released in the compartment containing the fungus only and these substances were tested for their potential effects on the conidial germination of *F. o. chrysanthemi*. The results indicated that the VAM fungus released substances that have an important negative effect on the *F. o. chrysanthemi* temporal conidial germination. Concentrations of the substances were carry out and were correlated to the enhancement of the effect. Moreover, pH was evaluated as a potential agent causing the diminution of germination but no correlation was obtained between this factor and the results. This experience support the hypothesis of direct interactions between VAM fungi and pathogens, therefore contributing to reinforce the possibility of using VAM fungi as potential biocontrol agents.

poster session 7 WF AR 10

FISCHER, CHRISTINE¹ & CARLOS COLINAS².

¹Depto. de Investigacin Forestal de Valonsadero, Apdo. 175, E-42080 Soria, Spain. ²Dep. Produccion Vegetal i Ciencia Forestal, Universitat de Lleida, Av. Rovira Roure 177, E-25198 Lleida, Spain. - Methodology for certification of *Quercus ilex* seedlings inoculated with *Tuber melanosporum* for commercial application

The decrease in edible truffle (*Tuber* sp.) production over the last 30 years from naturally occurring sites in France, Italy and Spain has promoted a great interest in cultivation. Commercial inoculation methods have been employed in France and Italy since 1979, with certification of seedling quality managed by regulatory bodies of the appropriate country. In the past five years interest in trufficulture has increased in calcareous regions of Spain and a practical, economical method for certification of commercially inoculated seedlings of Spanish nursery production is needed. The objectives of this method are to characterize the ectomycorrhizae of *Quercus ilex* with *T. melanosporum* as well as important

competitors of the same genera (*T. brumale*, *T. indicum*) and commonly occurring greenhouse ectomycorrhizae, such as *Sphaerospora brunnea*, which can potentially displace the desired *Tuber*. Based on these descriptions, a synoptic key has been developed for use in the random examination of a statistically tested minimum number of root apices and plants within a homogenous lot to ensure 95% compliance with seedling quality and colonization level. A laboratory assistant will be trained in the methodology.

Further objectives for certification include the development of European Community standards as well as followup studies of competing ectomycorrhizae to examine their outplanting performance under different field conditions with respect to their potential capacity for displacement of *Tuber* species.

poster session 2 ST SH 09

FOMINA, ELIZAVETA. Laboratory of Systematics and Geography of Fungi, Komarov Botanical Institute of the Russian Academy of Sciences, 2 Prof. Popov St., St.Petersburg, 197376, Russia. - The species diversity and spatial distribution of ectomycorrhizal fungi in Scotch pine forests of Nizhnesvirskii natural reserve

The species diversity and spatial distribution of ectomycorrhizal (ECM) fungi in Scotch pine forests were investigated in Nizhnesvirskii natural reserve (middle taiga, Leningrad region, Russia). There were revealed 185 species of ECM fungi belonging to Basidiomycota. The most species of ECM fungi had single ectomycorrhizal partner. The pine and the birch demonstrated the highest number of mycobionts. The results of distribution study and comparative floristic analysis confirmed the point of view that ECM fungi diversity as well as their fruitification are influenced mainly by the plant symbionts and are almost independent of plant species composition of phytocoenosis. The research of spatial distribution of ECM fungi in sample plots was carried out. Statistically significant correlation was shown between findings of some species of ECM fungi. The results obtained depended of plot size.

poster session 2 ST SH 10

FRANKE-SNYDER, MARLISE¹, DAVID DOUDS¹, LARISA GALVEZ¹, LAURIE DRINKWATER². ¹USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038. ²Rodale Institute Research Center, 611 Siegfriedale Rd., Kutztown, PA 19530 - Diversity of Glomales in conventional and low-input agricultural settings at the RODALE Institute farms in Pennsylvania

Low-input agriculture is becoming more widespread as farmers seek an alternative to chemical-based agriculture. We are studying the diversity of fungi established in a conventional (CV) and two low-input farming systems: animal (LI-A) and cash grain (LI-CG). Corn rhizosphere soil samples were taken in October, 1995. Preliminary data indicate that CV and LI-A share the same basic species composition, but LI-A had twice as many spores.

They were: *G. gigantea*, *G. mosseae*, *G. etunicatum*, *G. occultum*, *G. microaggregatum*, *G. geosporum*, and 7 other undescribed species. CV was dominated by spores of *G. etunicatum*, a *G. occultum*-like species and *G. mosseae*, while LI-A was dominated by spores of 2 undescribed *Glomus* species, *G. mosseae*, and *G. microaggregatum*. LI-CG had the fewest number of spores and a smaller range of species present. As in previous work, more spores of *G. gigantea* were found in LI than in CV. Fewer species were recovered from trap culture samples of the different treatments after 5 months. Consecutive trapping will be performed to try to maximize the number of species recovered, including some that may not sporulate under field conditions. Statistical measures of these communities, such as species richness, dominance and biovolume, will be presented as part of an effort to understand how farming systems affect Glomalean fungi. We also have isolated some of these fungal species, including four of the undescribed ones, for further studies and identification.

talk session 5

FRANKEN, PHILIPP¹, LAURENCE LAPOPIN¹, BRITTA RESSIN¹ & VIVIENNE GIANINAZZI-PEARSON². ¹Max-Planck-Institut fuer terrestrische Mikrobiologie, Karl-von-Frisch-Strasse, 35043 Marburg, Germany. ²Laboratoire de Phytoparasitologie, INRA-SGAP, BV 1540, 21034 Dijon, Cedex, France. - RNA accumulation and gene expression in arbuscular mycorrhizal fungal spores

The molecular analysis of the arbuscular mycorrhizal (AM) fungi is very difficult, since they are obligate symbionts and cannot be grown in pure culture. Genetic and biochemical information about these organisms are therefore very poor. Spores are the only source of pure fungal material from which DNA can be extracted for isolation and characterization of certain genes or for the establishment of genomic libraries. We have also detected RNA accumulation in AM fungal under certain conditions. In *Gigaspora rosea*, this accumulation showed a time course variation after isolation of spores from soil. RNA was extracted from *Gig. rosea* and *Glomus mosseae* spores and used for RT-PCR amplification of transcripts to detect expression of genes encoding glycerol aldehyde phosphate dehydrogenase (GAPDH), α -tubulin and different ATPases. In the case of the GAPDH gene from *Gig. rosea*, sequencing revealed highest similarity to that of nematodes, but Northern blot analysis showed that this was not due to contamination of spores. The PCR fragments are currently used to clone the corresponding genes and for analysis their expression during mycorrhiza development.

poster session 13 WF SH 67

FREY, BEAT¹, IVANO BRUNNER¹, PAUL WALTHER², CHRISTOPH SCHEIDEGGER¹ & KARL ZIEROLD³. ¹Swiss Federal Institute for Forest, Snow and Landscape Research, 8903 Birmensdorf, Switzerland. ²Laboratory of Electron Microscopy, Federal Institute of Technology, Schmelzbergstr. 7, 8092

Zürich, Switzerland. ³Max-Planck-Institute for molecular physiology, Rheinlanddamm 201, 44139 Dortmund, Germany. - Element localization in ultrathin cryosections of high-pressure frozen ectomycorrhizal spruce roots

Quantitative energy dispersive X-ray analysis (EDX) was performed on freeze-dried cryosections of high-pressure frozen ectomycorrhizal roots of *Picea abies* - *Hebeloma crustuliniforme*. In a first part, *Picea abies* was grown under two different nitrogen regimes to determine the modification in the element composition due to nitrogen fertilization, in a second part, cationic tracer such as Cs and Sr were applied to the fungal hyphae in order to assess its distribution in cryosections, and, in a third part, toxic elements such as Al and Zn were localized.

Cryosectioning resulted in thin fragments rather than sections. Good ultrastructural preservation of the major root subcellular compartments and of Hartig net structures was observed in the scanning-transmission electron microscope (STEM). X-ray microanalysis to localize and to quantify characteristic elements in the cryosections could be performed separately on root and fungal cell compartments.

poster session 5 ST AR 17

GABROVSEK, KARIN, NADA GOGALA, MARJANA REGVAR. Department of Biology, BF, University of Ljubljana, Vecna pot 111, 1000 Ljubljana, Slovenia. - Ergosterol content in the ectomycorrhizal fungal mycelium is affected by growth substances.

Analysis of ergosterol by HPLC is an acknowledged method of determining fungal biomass in mycorrhized roots. Various growth regulators are synthesized in the roots, among them cytokinins and jasmonates. Because of the assumption that these substances can influence the structure of biomembranes, we analyzed ergosterol in the ectomycorrhizal fungi *Pisolithus tinctorius* and *Laccaria laccata* treated with various concentrations of zeatin riboside or jasmonic acid.

In both fungi jasmonic acid increased ergosterol. In *Pisolithus tinctorius* zeatin riboside in concentrations of 0.1 or 10 μ M caused a significant decrease of ergosterol. Thus, the level of ergosterol in mycorrhized roots is also dependent on the physiologic state of the plant, not only on the fungal species and degree of mycorrhization.

poster session 1 ST RR 25

GAGNON, JEAN. Direction de la recherche forestière, Ministère des ressources naturelles (MRN), 2700, rue Einstein, Sainte-Foy, Québec, Canada, G1P 3W8. - Growth stimulation of containerized jack pine 2+0 seedlings inoculated with fungus *Laccaria bicolor* and with bacteria *Pseudomonas fluorescens*.

This study was carried out to determine the best treatment for the growth and ectomycorrhizae formation of container-grown jack pine 2+0 (*Pinus banksiana* Lamb.) seedlings. At the time of sowing (May), mycelial suspensions of two fungal strains (MRN 92.4 and MRN 92.9) of *Laccaria bicolor* (Maire) Orton were mixed with the potting substrate whereas for the bacterial suspension

of *Pseudomonas fluorescens* L-26.1, it was injected in the cavities of the Styroblocks 45 containers (45 cavities of 340 cm³). The six treatments were seedlings inoculated with *L. bicolor* 92.4, *L. bicolor* 92.9, *P. fluorescens* (B), *L. bicolor* 92.4 + B, *L. bicolor* 92.9 + B, and uninoculated seedlings. Thirteen months after sowing (June), 48 seedlings per treatment were harvested to evaluate their mycorrhizal formation and to measure their growth parameters. Jack pine 2+0 inoculated with *L. bicolor* 92.4, 92.9, 92.4 + B, and 92.9 + B had respectively 22%, 23%, 22%, and 37% of their short roots ectomycorrhizal. Shoot and total dry weights of seedlings inoculated with *L. bicolor* 92.4, 92.9, and 92.4 + B were significantly greater than *L. bicolor* 92.9 + B, *P. fluorescens* (B) and control seedlings. Compared to the controls, the inoculation of seedlings with *L. bicolor* 92.4 + B, 92.9 and 92.4 increased significantly the seedling biomass by 19%, 20%, and 21%, respectively. The seedling inoculation with only the bacteria *P. fluorescens* (B) led to a significant reduction of the ectomycorrhizae formation by naturally occurring fungi (*Thelephora* sp.), with a colonization rate of 16% compared to 32% for control seedlings.

morning talk

GARBAYE, JEAN. Laboratoire de Microbiologie Forestière, INRA, F54280 Champenoux. - New results and hypotheses about mycorrhiza helper fluorescent pseudomonads.

Some bacteria associated with mycorrhizal fungi in the soil and in the rhizosphere have been shown to promote the establishment of the symbiosis. These so-called mycorrhiza helper bacteria (MHB) can be found with ecto- or endomycorrhizas and belong to various taxonomic groups; however, to date, most available data concern fluorescent pseudomonads. This communication presents recent results obtained with the Douglas fir - *Laccaria laccata* ectomycorrhizal system and an helper strain of *Pseudomonas fluorescens*. Experiments were performed *in vitro* as well as in the glasshouse or in the nursery, using a population approach: phenotypic and genotypic typing of fluorescent *P.* in the soil and the rhizosphere, population kinetics of a rifampin-resistant mutant of the introduced helper strain and monitoring mycelial development and mycorrhiza formation. The results provide new insights into interactive mechanisms underlying the MHB effect. A new set of hypotheses is proposed and discussed as a starting point for further research. For instance, the helper *P. fluorescens* studied belongs to a rare genotype of biovar I selectively associated with the mycorrhizosphere of *L. laccata* and preferentially using trehalose as growth substrate, while *L. laccata* mycelium produces high amounts of this fungus-specific sugar. In addition, BBc6 is not rhizospheric but adheres to the hyphae, promotes the growth of *L. laccata* and benefits from the presence of the fungus for its own survival in the soil. This suggests that an explanation of the helper effect of this particular isolate of *P. fluorescens* might be the close association and mutually-beneficial trophic interdependence of the two microorganisms, leading to faster colonization of the soil

by the fungus and higher probability of root-fungus encounter.

poster session 2 ST SH 11

GARCIA, GREGORIO, MARIO HONRUBIA & GISELA DIAZ. Departamento de Biología Vegetal (Botánica). Facultad de Biología. Universidad de Murcia. 30100 Murcia. Spain. - Ectomycorrhizal inoculum potential (ECMIP) in *Pinus halepensis* Miller forests of the Sistema Ibérico Mountains

Up to date, very little is known about ecological and biological aspects of the ectomycorrhizal fungi from Mediterranean forest ecosystems in Europe. Our study was designed to determine the variation of the number of propagules of these fungi along a seasonal cycle in natural soils. Greenhouse bioassays were established by mixing natural soils, which provided the inoculum, with the same sterilised soil at dilutions of 1/1, 1/10, 1/102, 1/103, 1/104 and 1/105. Soils from *Pinus halepensis* stands in Castellón and Teruel provinces (Spain) were sampled every 3 months from winter 1993 up to winter 1994. Six months after germination, seedling root systems were examined for the percentage of mycorrhizas formed. The Most Probable Number (MPN) test was used to determine the potentiality of the propagules. The most frequent ectomycorrhizal morphotypes were also described. The estimated number of propagules ranged from 0,61 to 5,40 propagules per cm³ with a maximum in the winter season and a minimum in the fall season. The relationship between the number of propagules and environmental parameters, such as temperature and precipitation, were also studied.

poster session 13 WF SH 80

GARCÍA-MONTERO, L.G.¹, G. MASSIMO² & J.L. MANJÓN¹. ¹Unidad de Investigación y Producción Vegetal, Facultad de Ciencias, Universidad de Alcalá, 28871 Alcalá de Henares (Madrid), Spain. ²Dipartimento di Biologia vegetale dell'Università degli studi di Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy. - Synthesis and description of *Tuber malençonii* Don., Riouss. & Chev. ectomycorrhiza

The University of Alcalá develops different ectomycorrhizal synthesis of fungi, mainly based on truffle culture, with several procedures of inoculation and spore activation (MANJÓN & *al.*, 1991; MANJÓN & *al.*, 1994). For the morphological ectomycorrhizal descriptions, we follow the terminology and recommendations by AGERER (1987-1991); BENCIVENGA & *al.* (1995); DONNINI & BENCIVENGA (1994, 1995) and GRANETTI (1995). The cultivations were carried out with truffles, seeds and substrates from Spanish origin. It has been identified by synthesis the mycorrhiza of *Tuber malençonii* Don., Riouss. & Chev. in plants of *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. (MANJÓN & GARCÍA-MONTERO, in press). According to the bibliography that has been consulted, this mycorrhiza is undescribed until

the date, but it has also been synthesized in the University of Perugia (personal communication of G. Massimo).

poster session 8 WF AR 07

GAUR, ATIMANAV¹, K.G. MUKERJI² & ALOK ADHOLEYA¹. ¹Tata Energy Research Institute, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi 110 003, India. ²Applied Mycology Lab. Department of Botany, University of Delhi, India. - Formation of primary entry points in *Sorghum vulgare* by six isolates of VA mycorrhizal fungi in high pH soils under tropical conditions

Six VA mycorrhizal fungi (at equivalent spore density of 20 spores per gram soil) were compared in a pot experiment for their ability to form primary entry points (PEPs) on roots of sorghum vulgare in native, alkaline soils using a image analysis system, quantimet500 plus (Leica, Cambridge). Conditions were optimised for achieving maximum root interception by fungal propagules present in the soil inoculum and simultaneously avoiding formation of secondary entry points. At 12 days harvest, response of PEP formation to changes in inoculation density was similar for mixed indigenous culture, *Glomus caledonius* (indigenous), *G. mosseae* (INVAM isolate, FL 156B) and *G. mosseae* (INVAM isolate, 336) and reached a plateau level in PEP formation at higher concentration levels. Nevertheless, mixed indigenous cultures produced more number of PEPs (2216.82) than *G. caledonius*, *G. mosseae* (FL 156B) and *G. mosseae* (336) (2183.29, 1809.10 and 1215.67 respectively). *G. intraradices* (Lavel Univ., Canada) and mixed exotic inocula (a combination of *G. intraradices* and *G. mosseae*) formed minimum number of PEPs (1040.25 and 852.01 respectively). Inoculum density did not seem to effect the root length at 12 day harvest. Differences among isolation of VA mycorrhizal fungi in forming PEPs may be important when selecting fungi for infectivity, compatibility with a host and colonizing ability for a given soil conditions.

poster session 1 ST RR 26

GÉNÉRÉ, BENOIT. Cemagref, Domaine des Barres, Nogent sur Vermisson, 45290, France. - Survival of late outplanted Norway Spruce cuttings depends on their mycorrhizal status

In mountains, Norway spruce is often planted late in spring (in April or May) due to climatic conditions. Under similar conditions, a field trial was established in 1992 on a classic forest site. Rooted cuttings grown in fumigated nursery beds for 2.5 years were used as planting stock. Different effects were studied : mainly, the 2 nursery sites (Les Barres and Peyrat-le-Château) and inoculation with *Laccaria bicolor* S 238 N (presence versus absence). Prior to planting, the planting stock was analyzed as regards morphology, oven-dry weight, mycorrhizal status and nutrition (foliar analysis). Two years after planting, the survival rate was markedly lower on inoculated cuttings (65 to 70% instead of 86 to 97%),

whatever the nursery site. Their mycorrhizal infection rate with pure *Laccaria bicolor* S 238 N was very high at the time of planting (50 to 70%), and about 20% two years later. The poor performance of inoculated cuttings proved to be connected to late outplanting, when budbreak occurs. This finding is based on the results of a second experiment in 1995 with two crossed factors : inoculation (with *Laccaria bicolor* S 238 N or no inoculation) and planting date (March 10 or May 10). Thus, being planted in March, the inoculated cuttings displayed a very high survival rate, one year after planting (99% instead of 77% for May).

poster session 2 ST SH 12

GIACHINI, ADMIR JOSE & VETURIA LOPES DE OLIVEIRA. Depto. de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, C.P. 146, 88010-970, Florianopolis, SC, Brazil. - Ectomycorrhizal fungi in *Eucalyptus* and *Pinus* plantations in Santa Catarina (Southern Brazil).

The occurrence and diversity of ECM fungi was assessed in *Eucalyptus dunnii* Maiden and *Pinus taeda* L. plantations in two seasons in Santa Catarina, Brazil. The plantations were 1, 7, and 9 years-old for *E. dunnii* and 1, 9, and 18 years-old for *P. taeda*. The fungi were identified by macroscopic and microscopic characteristics of fruitbodies and the Relative Importance (RI) was determined for each fungus based on the spatial frequency and dry weight of fruitbodies. In each plantation 18 species were harvested during spring 1995 (Sep-Dec). In the summer (Dec-Mar) 15 species were obtained for *E. dunnii* and 16 for *P. taeda*. The following species occurred under *E. dunnii* in these two seasons: *Chondrogaster* sp., *Descomyces albus*, *Laccaria altaica*, *L. amethystea*, *L. amethystina*, *L. montana*, *Laccaria* spp. (2), *Scleroderma areolatum*, *S. citrinum*, *Scleroderma* spp. (10), and 4 unidentified genera. Under *P. taeda*, *Laccaria altaica*, *L. amethystea*, *L. montana*, *Laccaria* spp. (2) *Lactarius rubrilacteus*, *Lactarius* sp., *Rhizopogon* sp., *Scleroderma citrinum*, *Scleroderma* spp. (11), *Suillus cothurnatus*, and 8 unidentified genera were detected. *S. areolatum* and *S. citrinum* were those presenting the highest RI values in both plantations, regardless the season. The results show that richness of ECM fungi in Brazil is higher than previously described. The importance of this study in relation to ecological and applied aspects is discussed.

morning talk

GIANINAZZI-PEARSON, VIVIENNE. Laboratoire de Phytoparasitologie INRA/CNRS, SGAP, INRA, BV 1540, 21034 Dijon Cedex, France. - Current challenges in mycorrhizal research: getting to the cellular, molecular and genetic roots of the symbioses

The generalized phenomenon of reciprocal functional compatibility between plants and mycorrhizal fungi must be regulated by dominant mechanisms that have been maintained through evolution. Investigations of systems modulating mycorrhiza development and function have been largely descriptive in the past, but new perspectives

have recently been opened by the merger of biochemistry, cytology, molecular biology and genetics in studies of root-fungus interactions. The coordinated changes in cell and tissue differentiation imposed on both mycorrhizal partners, and culminating in the symbiotic interface, require continual recognition events and signal exchange. Alterations in gene expression are a common feature of mycorrhizas and some are analogous to those governing other plant-microbe associations. Events like the low and limited spatio-temporal expression of plant defence responses in mycorrhizas raise the more general question of genetic and molecular strategies impeding incompatible plant-microbe interactions (plant susceptibility/fungal compatibility factors), whilst similarities in nodule and mycorrhiza symbioses open a challenging area of targeting evolutionary common determinants. Analysis of genetic programmes specific to mycorrhiza development and symbiotic function represents one of the main research developments for the next few years. Knowledge of the fungal genome and identification of plant genetic determinants will have major impacts in characterizing molecular markers for monitoring mycorrhiza activities and in genetic tagging of responsive genotypes for plant breeding.

poster session 10 WF SH 09

GOEDE, R.G.M. de, F.W. de VRIES & TH.W. KUYPER. Biological Station, Wageningen Agricultural University, Kampsweeg 27, 9418 PD Wijkster, the Netherlands. - Does grazing by fungivorous soil fauna really affect ectomycorrhizal symbioses?

The sheath and extramatrical mycelium of ectomycorrhizal fungi seem a good potential food source for fungivorous soil animals. The fungivorous nematode *Aphelenchoides* sp., a common inhabitant of Scots pine (*Pinus sylvestris* L.) plantations in Drenthe, the Netherlands, fed and reproduced when grazing the ectomycorrhizal fungi *Suillus bovinus*, *Laccaria bicolor*, and *Rhizopogon luteolus*, grown on MMN-agar in the absence of tree roots. Nematode populations grew from 10 to 176,000 (*Suillus*), 34,000 (*Laccaria*) and 33,000 (*Rhizopogon*) individuals in 9 cm diameter Petri dishes after 40 days. However, when grazing on *Suillus bovinus* and *Laccaria bicolor* growing in symbiosis with Scots pine seedlings in microcosms in a peat/perlite mixture, nematode numbers did not increase significantly, despite a proliferation of extramatrical mycelium and ectomycorrhizas. This discrepancy in development of nematode populations grazing on the mycobiont with and without the phytobiont could either be due to unfavourable conditions within the microcosms or be the result of the presence of the plant. In a subsequent experiment the extramatrical mycelium was allowed to grow out on MMN-agar. Under these conditions the nematode populations increased only slightly (from 10 to 19-336 individuals per Petri dish). We therefore conclude that plant-induced changes in the ectomycorrhizal fungus reduce the population development of the fungivorous grazer.

poster session 14 WF RR 12

GOLLDACK, JUDITH¹, BABETTE MÜNZENBERGER², REINHARD AGERER³ & R. F. HÜTTL¹. ¹Brandenburg Technical University of Cottbus, Chair of Soil Protection and Recultivation, P.O. 101344, 03013 Cottbus, Germany. ²ZALF, Institute of Microbial Ecology and Soil Biology, Dr.-Zinn-Weg 18, 16225 Eberswalde, Germany. ³University of Munich, Institute for Systematic Botany, Section Mykologie, Menzinger Str. 67, 80638 München, Germany. - "Pinirhiza granulosa" and "Pinirhiza stellaria", two ectomycorrhizae of *Pinus sylvestris* L. on a reclamation site in the Lusation lignite mining region

Two unidentified mycorrhizal types "Pinirhiza granulosa" and "Pinirhiza stellaria", collected from a 34 years old *Pinus sylvestris* stand on a reclamation site of the Lusation lignite mining region, are described morphologically and anatomically. The yellowish "Pinirhiza granulosa" ectomycorrhizae have a grainy surface due to heaps of thick-walled cells, which are star-like linked by irregularly inflated cells. The outer surface of the mantle is loosely plectenchymatous. The deeper layers of the hyphal mantle are plectenchymatous to pseudoparenchymatous while the hyphae of the inner mantle surface are ring-like arranged. Emanating hyphae are infrequently present. "Pinirhiza stellaria" ectomycorrhizae are dark brown with a silvery appearance. The thin hyphae of the mantle are ring- to star-like arranged in the outer and the middle layers. The hyphal pattern of the inner mantle layers is star-like. The silvery brownish rhizomorphs are undifferentiated. Their hyphae have thick cell walls, the cell lumen is partially not discernable. The corresponding and different features of "Pinirhiza granulosa" and "Pinirhiza stellaria" and of the respective similar ectomycorrhizae are discussed.

talk session 6

GOLLOTTE, ARMELLE¹, MARK MUNRO¹, CHRISTELLE CORDIER², LUCY HARRIER¹, DIEDERIK VAN TUINEN², VIVIENNE GIANINAZZI-PEARSON², CHRISTA GOEBEL³, ALEXANDER HAHN³, SILVIO GIANINAZZI² & JOHN HOOKER¹. ¹Soil Biology Unit, Land Resources Department, SAC, Craibstone Estate, Aberdeen AB2 9TQ, UK. ²Laboratoire de Phytoparasitologie, INRA/CNRS, SGAP, INRA, BV 1540, 21034 Dijon Cedex, France. ³Lehrstuhl für Botanik, Technische Universität München, 85350 Freising, Germany. - Immunological and molecular biological studies of interactions between *Glomus* and *Gigaspora* in roots of leek and tomato

Little is known about interactions between different species of AMF in soil and roots. Such studies have been impeded in the past due to technical limitations for differentiating between AMF *in situ*. In order to solve these problems, we have developed immunological and molecular biology techniques for their identification and we are studying interactions between *Glomus mosseae* (BEG 12) or *Glomus coronatum* (BEG 22) and *Gigaspora rosea* (BEG 9) in roots of leek and tomato.

Immunocytochemical techniques were used to study interactions between different species of AMF in extraradical or intraradical hyphae using genus specific antibodies. The results were compared to those obtained using molecular biology techniques with AMF-specific primers (Simon et al., 1993). Moreover, in order to better understand how AMF interact with each other and how this affects plant metabolism, differences in gene expression of both plant and fungus in the different interactions were analysed by differential display reverse transcription polymerase chain reaction (DDRT-PCR).

[Simon L. et al., 1993. Appl. Environ. Microbiol. 59: 4211-4215]

poster session 2 ST SH 13

GOODMAN, DOUG, & J.A. (TONY) TROFYMOW. Pacific Forestry Centre, Canadian Forest Service, 506 Burnside Rd. W., Victoria, British Columbia, Canada, V8Z 1M5. - Distribution of ectomycorrhizal types among habitats in mature Douglas-fir stands

Diversity of ectomycorrhizal communities may be valuable for forest health. To provide clues to the individual roles and possible interactions of ectomycorrhizal fungi, ectomycorrhizal types were distinguished and root tips counted in several soil habitats. Habitats examined were logs, stumps, the forest floor over rock or gravel, the forest floor elsewhere, and the mineral soil. Nutrient levels of soil cores were determined. Of the 18 most frequent fungi, four showed a distinct habitat preference. Distinct patterns of ectomycorrhizal occurrence in mineral soil compared to decayed wood and the forest floor were not surprising considering the latter habitats contained 5-10X more carbon, nitrogen, and mineralizable nitrogen. Most of the common types were found abundantly in all habitats. Only weak correlations were found between ectomycorrhizal abundance and levels of carbon, nitrogen, phosphorous or moisture. A more detailed understanding of ectomycorrhizal distribution likely requires consideration of micro-habitats within a soil core, and the extent and location of emanating elements.

poster session 6 WF AR 21

GOULART, B.L., KATHLEEN DEMCHAK and WEI Q. YANG. Department of Horticulture, The Pennsylvania State University, University Park, PA 16802 USA. - Interactive effects of cultural practices on mycorrhizal infection intensity level in field grown 'Bluecrop' highbush blueberry

In Spring of 1992, a field planting of 'Bluecrop' highbush blueberry was established to evaluate the effects of various cultural practices on plant growth and mycorrhizal level. Treatments included mulch or no mulch, pre-plant amendment or no pre-plant amendment, and 4 levels of nitrogen fertilization (from 0 to 100 g/plant in year 2, and from 0-120 g/plant in year 3) arranged in a complete factorial experiment. After two years, interactions among the treatments characterized the plant's responses. When no mulch was employed, increasing nitrogen level resulted in decreased

mycorrhizal concentration in the roots. When plants were mulched, effects were inconsistent. It was also found that the intensity of mycorrhizal infection level varied with location in the rhizosphere, and was dependent on whether or not mulch was employed, as well as on the mulch composition. Mulch and/or amendment increased plant growth and vigor. For plants with no mulch and no amendment, canopy volume and mycorrhizal concentration level decreased with increasing nitrogen.

poster session 12 WF SH 39

GRAHAM, JAMES & DAVID EISSENSTAT. University of Florida, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850. - Field demonstration of carbon cost of mycorrhizae on young Valencia orange trees at high P supply.

We sought to limit mycorrhizal colonization of 2-year-old Valencia orange trees on 4 rootstocks after orchard establishment in a low P fertility soil under high and low P supply. We predicted that control of mycorrhizal fungi with the fungicide, benomyl, would reduce colonization rate and P uptake by the rootstocks in low P soil resulting in P limitation of young tree growth. By contrast, at high P supply, control of mycorrhizae would reduce carbon cost of fungal colonization in the carbon-limited host, citrus, and result in positive growth response to fungicide treatment. Repeated soil drenches (approx. every 45 da) of benomyl reduced total incidence of colonization in roots of all rootstocks for the duration of two growing seasons. No other known citrus root pathogens controlled by benomyl were present in the orchard. Leaf P concentration in the spring growth of trees on all rootstocks was significantly reduced by fungicide treatment at both low and high fertilization. At high P supply, leaf P concentration remained within the sufficiency range for optimum growth. In low P soil, trees treated with fungicide had lower leaf P concentrations than nontreated trees in the second and third seasons, but leaf P was not in the range that would limit tree growth. Leaf nitrogen, potassium, and copper status of trees was not affected by benomyl treatments. At both low and high P supply, benomyl treatments increased the rate of growth in stem caliper for trees on all four rootstocks. The benomyl effect on tree growth rate increased for the duration of the first two seasons but did not increase further in the third season.

poster session 2 ST SH 08

GROGAN, PAUL¹, F.S. III. CHAPIN² & T.D. BRUNS³. ¹111 Koshland Hall, Dept of Plant Biology, U.i. Berkeley, CA 94720. ²Dept. of Integrative Biology, U.i. Berkeley, CA 94720. ³Dept. of ESPM., U.i. Berkeley, CA 94720. - Mycorrhizal community composition in Alaskan tussock tundra in response to climate and nutrient additions

Betula nana is the major ectomycorrhizal host in Alaskan tussock tundra. This vegetation occurs along an 825 km latitudinal transect from Fairbanks north across the Brooks mountain range into the arctic coastal plain.

The influence of climate on ectomycorrhizal community composition was examined by sub-sampling mycorrhizal tips from 10 replicate plants at four similar tussock tundra sites along the transect. Morphological differences between types were subsequently confirmed using extracted DNA from the tips for restriction fragment length polymorphism analysis. A similar approach was used to investigate the effects of annual nutrient additions on ectomycorrhizal community composition in long-term fertilised tussock tundra plots at Toolik Lake Research Station in N. Alaska.

The data clearly demonstrates the importance of climate in determining ectomycorrhizal community composition in tussock tundra. The distribution ranges of two thirds of the 32 morphotypes observed were grouped into distinct categories on the basis of latitude and elevation. The remaining types occurred throughout the transect. *Betula nana* has previously been shown to increase in abundance under elevated air temperatures in Alaskan tussock tundra. This study strongly suggests that greenhouse warming will not only increase the abundance of associated mycorrhizae but also change their species composition.

The strongest effect of nutrient treatment (0, N, P, NP) was on morphotype richness which was significantly lowered in the plots where only N was added. These data suggest that fungal community diversity associated with *Betula nana* in tussock tundra is dependent upon ectomycorrhizal species interactions with the cycling of nitrogen rather than phosphorus.

poster session 8 WF AR 75

GROGAN, PAUL¹ & F.S. III. CHAPIN². ¹111 Koshland Hall, Dept of Plant Biology, U.i. Berkeley, CA 94720. ²Dept. of Integrative Biology, U.i. Berkeley, CA 94720. - The effects of benomyl fungicide and nutrient additions on the productivity of a Californian annual grassland

Vesicular-arbuscular mycorrhizal (VAM) functioning under natural conditions has proved extremely difficult to demonstrate. This study contributes to the small body of literature which attempts to elucidate VAM functioning by investigating the effects of benomyl fungicide and nutrient fertilisation on plant community production in a Californian annual grassland. This system was chosen because production is primarily limited by nitrogen supply. Translocation of nitrogen by VAM has been observed in greenhouse studies but has yet to be demonstrated in the field. A full factorial design (benomyl x nitrogen x phosphorus) of 10 replicate plots was established in an ungrazed field during the 1994/95 growing season. Benomyl was applied at relatively high concentrations every 4 weeks from January through to the harvest in April which was just prior to flowering.

Nitrogen additions resulted in a significant increase in shoot biomass demonstrating that plant productivity in these soils was N-limited. Fungicide application had no effect on shoot N concentration suggesting that VAM association did not enhance plant access to nitrogen. By contrast, in plots that were not fertilised with P, shoot P concentrations were significantly reduced (by 26%) in

plots where benomyl was also added. In plots that were P-fertilised, shoot P concentrations were unaltered by the fungicide addition but the presence of benomyl significantly increased shoot biomass. These data demonstrate that a mutualistic relationship between VAM and plants can exist under natural field conditions during the growth phase leading up to flowering. Plants receive a net benefit from mycorrhizal association in terms of exchanging photosynthate for phosphorus, even in N-limited soils.

poster session 15 WF RR 17

GRUBISHA, LISA¹, JAMES M. TRAPPE², RANDY MOLINA³, MICHAEL CASTELLANO³, & JOSEPH W. SPATAFORA¹. ¹Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331, USA. ²Department of Forest Science, Oregon State University, Corvallis, Oregon 97331, USA. ³U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 Jefferson Way, Corvallis, Oregon 97331, USA. - Re-examination of species concepts in Rhizopogon section Villosuli using molecular characters.

Rhizopogon is a genus with more than 125 species of sequestrate ectomycorrhizal fungi which are host specific to members of the Pinaceae. Rhizopogon section Villosuli exhibits host specificity with *Pseudotsuga*, a genus of ecologically and economically important trees in the Pacific Northwest. Twenty-one Rhizopogon species are recognized within the section Villosuli, however, due to morphological similarities and a gradient of ontogenetic characters it is often difficult to make distinctions at the species level. We are re-examining species concepts of the genus Rhizopogon with special consideration of the section Villosuli through phylogenetic analysis of nucleotide data determined from the internal transcribed spacer (ITS) and the intergenic spacer (IGS) regions of the nuclear-encoded ribosomal DNA. Smith and Zeller's (1966) work on North American taxa is serving as a working hypothesis for testing species concepts in this study. Preliminary data will be discussed pertaining to sectional relationships within Rhizopogon and the feasibility of developing section-specific molecular markers.

poster session 1 ST RR 29

GRUHN, CHRISTINE M. Department of Biology, Nazareth College of Rochester, Rochester, New York 14618. - The arbuscular mycorrhizal status of vegetable crops grown on an organic farm.

This three year study evaluated the percentage of the root system colonized by arbuscular mycorrhizal fungi and determined the mycorrhizal inoculum potential of the soil (MIP) for a variety of vegetable crops collected on an organic farm in western New York. The farm has used only organic methods of fertilization and pest control for the past fifteen years. The MIP was obtained using a corn/mung bean greenhouse bioassay. Results show that crops from the organic farm had higher levels of

colonization than crops of the same species sampled from farms using chemical pesticides and fertilizers. The MIP was determined by the previous year's crop, but did not correlate with root colonization levels of the present crop, demonstrating that soil MIP is not a good predictor of crop mycorrhizal status. The abundance of natural areas and hedgerows on this small farm likely provide a constant source of mycorrhizal inoculum, so that the field is not completely dependent on the existing crop to maintain the diversity of mycorrhizal species. Some crops (e.g. garlic) achieved a uniform percentage of colonized roots regardless of field and previous cropping history, while the mycorrhizal status of others (e.g. lettuce) appear to be more sensitive to the mycorrhizal status of the previous crop. This second group may benefit from greenhouse inoculation with endomycorrhizal fungi prior to transplanting in the field. These results demonstrate that although mycorrhizae are present in relatively high levels in organically grown vegetable crops, recommendations for cultural practices to enhance mycorrhization will likely need to be highly specific.

poster session 5 ST AR 18

GRYNDLER, MILAN, HANA HRSELOVÁ. Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídenská 1083, Prague 4, 142 20, Czech Republic. - Proliferation of intraradical hyphae of arbuscular mycorrhizal fungi in vitro.

The technique of observation of proliferation of intraradical hyphae of arbuscular mycorrhizal fungi and quantification of their growth was improved. Surface decontaminated, mycorrhizal (*Glomus fistulosum*, *G. fasciculatum*) root segments are incubated in a liquid medium, pH 6.3, and the length of hyphae is measured using grid-line intersect method. Using this technique, we studied the effects of glucose, root exudates, vitamins, mannitol, trehalose, glycin, FeNaEDTA, inositol, macrobiogenic and trace elements and selected plant growth regulators. No significant effects of root exudates (0 - 1.4 g/L) and glucose (0 - 5 g/L) on the growth of hyphae were observed. The optimum concentrations of vitamins are (mg/L): thiamin, 10; biotin, 0.01; riboflavin 0.01 - 0.1, pyridoxin, 1; panthothenic acid, 1; nicotinic acid, 1 - 10; B₁₂, 1; and folic acid, 1. Complete inhibition of proliferation by riboflavin at a concentration of 1 mg/L was observed. A similar inhibition was also observed for indole-3-acetic acid (IAA) at 5µM/L. No significant effects of zeatin and gibberellin GA₇ were obtained. The study enabled us to design an incubation medium containing compounds ordinarily used in plant cultivation media at concentrations non-suppressive to the arbuscular mycorrhizal fungi. The technique used is also suitable for the collection of basic data concerning the effects of various soil parameters on the growth of mycelium of arbuscular mycorrhizal fungi.



poster session 11 WF RR 40

GUERIN-LAGUETTE, ALEXIS, SYLVIE MONTEBAULT, BRIGITTE BRUNEL & DANIEL MOUSAIN. Laboratoire de Recherches sur les Symbiotes des Racines, INRA, 2 Place Viala, 34060 Montpellier Cedex 1, France. - Study of the diversity of the ribosomal DNA of ectomycorrhizal *Lactarius* species associated with pines, using PCR-RFLP analysis

Eighty-five isolates of *Lactarius* species of the *Dapetes* Section were collected in South of France and maintained in pure culture. After DNA extraction from the dikaryotic mycelia, 3 sections of the nuclear rDNA were submitted to PCR amplification and endonuclease restriction digest: the ITS and IGS spacers, and the 18S gene. IGS amplification with the universal primers 5SA and CLN12 gave no reproducible products and could not be used for enzymatic restriction. PCR-RFLP analysis of the ITS section of 82 isolates of *Lactarius deliciosus* and *L. sanguifluus*, revealed sequence polymorphism at both specific and intraspecific levels, whereas no variation was observed on the 18S gene. Within the whole population and among the 10 restriction endonucleases tested, *Hinf*I, *Cfo*I and *Msp*I gave numerous different patterns characterizing 17 different genotypes at the ITS locus. An UPGMA cluster dendrogram was inferred from these data. Sequencing of the ITS amplification products was performed on isolates of both *Lactarius deliciosus* and *L. sanguifluus* species and confirmed the existence of two heterologous dikaryotic populations of rDNAs within some of the strains nuclei. PCR-RFLP analysis of the ITS of ectomycorrhizae sampled from the field, including *Lactarius*-like, orange-coloured ectomycorrhizae, was also completed. For the latter, it gave restriction patterns similar to the main genotypes obtained from the mycelia collection. From a genetic point of view, the high sequence polymorphism level of the ITS section within *L. deliciosus* and *L. sanguifluus* species was focused. The intraspecific ITS polymorphism provides a powerful tool for further applied studies on the persistence of introduced *Lactarius* strains in the field.

poster session 1 ST RR 27

GUISSOU, T.¹, A. M. BA¹, J. M. OUADBA¹ and S. GUINKO². ¹Laboratoire de Microbiologie Forestiere, Institut de Recherche en Biologie et Ecologie Tropicale, Centre National de la Recherche Scientifique et Technologique, BP. 7047, 03 Ouagadougou, Burkina Faso, Phone: 226 33 40 98, Fax: 226 31 49 38. ²Departement de Biologie et d' Ecologie Vegetale, Faculte des Sciences et Techniques, BP. 7021, Universite de Ouagadougou, Burkina Faso, Phone: 226 33 20 41, Fax: 226 30 72 42. - Dependency of three multipurpose fruit trees (*Parkia biglobosa* (Jacq.) Benth. *Tamarindus indica* L. and *Zizyphus mauritiana* Lam.) on arbuscular mycorrhizal fungi.

Growth and mineral nutrition responses of three multipurpose fruit trees, *Parkia biglobosa* (Jacq.) Benth, *Tamarindus indica* L. and *Zizyphus mauritiana* Lam., to the inoculation of five arbuscular mycorrhizal fungi (AMF) *Acaulospora spinosa* Walker & Trappe, *Glomus*

mosseae (Nicol. & Gerd.) Gerd. & Trappe, *Glomus intraradices* Schenck & Smith, *Glomus aggregatum* Schenck & Smith emend. Koske or *Glomus manihotis*. However, Sieverding & Schenck, were compared in a phosphorus deficient sandy soil (Bray I-P: 3.8 ppm). Analysis of variance showed that the interactive effects between AM fungi and fruit trees were statistically significant ($p < 0.05$) for all variables measured. Percent fungal root colonization by AMF was high in fruit trees reaching at least 80%, except with *G. mosseae* which presented a relative low infectiveness. Growth responses of *P. biglobosa* and *T. indica* to the inoculation with AM fungi did not differ significantly. However, *Z. mauritiana* responded to inoculation better than other fruit trees irrespective of the AMF. Moreover, the *Z. mauritiana* seedlings inoculated by *A. spinosa* were the most responsive of the fruit tree species in terms of seedling volume (486%), growth response (373%), mycorrhizal dependency (78%) and mineral absorption of P, N and K (1389%, 252% and 531%, respectively). Thus, the Ca and Mg uptake in shoots of *Z. mauritiana* inoculated by *A. spinosa* increased five and four times higher than those of controls, respectively, whereas the respective values were less than one time in shoots of *P. biglobosa* and *T. indica*. The data clearly differentiate the degree of mycorrhizal dependency of these three fruit trees and, thus, show the appropriate mycorrhizal fungus must be selected during their first phase development.

poster session 5 ST AR 19

GUREVICH, LUYDMYLA & NADEZHDA PSURTSEVA. Komarov Botanical Institute of the Russian Academy of Sciences, Prof. Popov str. 2, 197376 St. Petersburg, Russia. - Influence of some chemical substances on the growth of ectomycorrhizal basidiomycete *Amanita rubescens* in culture.

Amanita rubescens (Pers.: Fr.) S.F.Gray is a widespread species forming ectomycorrhiza with a wide range of host plants in temperate latitude. It is known that plants metabolites play an important role in establishing of symbiotic relationships and have essential influence on the growth of mycorrhizal fungi (Piche, Vierheilig, 1994; et al.). Growth of *A. rubescens* strain 0756 LE(BIN) was studied on the synthetic nutrient medium with supplement of chemical substances (5×10^{-6} - 10^{-3} M) of different structure. There were tested 25 substances: aminoacids, vitamins, phenolic compounds, flavonoids, pyrimidine derivatives, etc. It was noticed a good correlation between the concentrations of the certain added compounds (ferulic, cinnamic, p-coumaric, sinapic acids, quercetin, morin, methionine) and strain growth rates. The best results were obtained with adding to the nutrient medium of methionine (10^{-5} - 5×10^{-6} M), 1,3,4,5-tetrahydroxy-cyclohexane-carboxylic (5×10^{-3} M) and shikimic (10^{-5} - 5×10^{-6} M) acids; slightly lower growth stimulation was observed with ferulic acid (10^{-3} M); very slight stimulation was noticed with chlorogenic (10^{-3} M), sinapic (10^{-3} - 10^{-4} M), coumaric (10^{-3} M), glutamic (10^{-3} M) acids, thymine (10^{-3} M), morin (10^{-4} - 10^{-3} M), a-catechin (10^{-4} M) and thiamine (vitamin B1, 5000, μ l). At the same time the addition of cinnamic (10^{-3} - 10^{-4} M) and

usnic ($0,1$ - $0,01$ g/l) acids, pyrocatechol (10^{-3} - 10^{-4} M), morin (10^{-3} M) and quercetin (10^{-3} - 10^{-5} M) resulted in a strong growth inhibition. Thus, tested substances, typical for the higher plants, may have an appreciable effect on the growth of ectomycorrhizal macromycetes.

talk session 6

GUTTENBERGER, MARTIN, & MARIA HARRISON. The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73402, USA. - Carbon exchange between mycorrhizal symbionts: Are carbohydrates the whole story?

Although the chemical nature of the carbon-transport metabolite(s) exchanged between the mycorrhizal symbionts is unknown, there is a striking consent that sucrose, the general transport metabolite *in planta*, and the products of its breakdown (glucose and fructose) are the most likely candidates. Dicarboxylates on the other hand, a common and abundant component of plant root exudates, have been suggested repeatedly (e.g. Smith *et al.* (1994) *Plant Soil* 159, 103-113) but nevertheless widely neglected in mycorrhizal research. Biological considerations suggesting an important role for dicarboxylates as additional or even exclusive carbon source for mycorrhizal fungi will be presented together with first histochemical data consistent with this hypothesis. A novel activity stain for arbuscules indicates that the periarbuscular space is a highly acidic compartment. This adds to the similarities with the nitrogen-fixing root nodules of legumes which harbor the bacterial symbiont in an acidic compartment, the peribacteroid space (Blumwald *et al.* (1985) *Plant Physiol.* 78, 665-672). In root nodules dicarboxylates are the essential carbon source for the functional symbiosis (Ronson *et al.* (1981) *Proc. Natl. Acad. Sci. USA* 78, 4284-4288). This comparison provides a starting point for a new "dicarboxylate hypothesis". It integrates theoretical implications of transport processes between the symbionts as well as evolutionary aspects and observed physiological, hormonal, and cultural characteristics of VAM associates. Attempts to clone the genes encoding the respective transport proteins are under way.

poster session 12 WF SH 62

GUTTENBERGER, MARTIN, & MARIA HARRISON. The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73402, USA. - VA-mycorrhizal fungi inhabit an acidic compartment: A new activity stain for arbuscules

Most transport processes in plants and fungi are coupled to pH-gradients across membranes which are established by membrane-bound H^+ -ATPases. The activity of H^+ -ATPases has been localized histochemically to plant and fungal plasma membranes of the arbuscules (Gianinazzi-Pearson *et al.* (1991) 117, 61-74) which are generally considered to be the primary sites of nutrient exchange. My current work tries to elucidate the actual pH of the periarbuscular space. Exploratory experiments were

performed with mycorrhizal roots from pot cultures (*Glomus versiforme*-*Allium porrum*). Excised roots were stained with neutral red, a dye accumulating in acidic compartments by the ion-trap mechanism. Arbuscules were brightly stained within hours. The specificity of the staining reaction was checked by variation of the staining conditions. Stained arbuscules were completely destained after membrane disruption (mechanically or by freezing and thawing) and by addition of the protonophore CCCP. The pH of the periarbuscular space is estimated to be in the range between 2 and 5. The results were confirmed with mycorrhizas raised in sterile culture (*G. versiforme*-*Medicago truncatula*) and collected in the field (*Trifolium incarnatum*). Further work on the mechanism of the acidification process is under way. The results suggest an additional similarity to root nodules of legumes and will be discussed with respect to their importance for the nutrient exchange in mycorrhizas.

poster session 4 ST AR 50

GUZMAN, SALVADOR¹, FEDERICO SANCHEZ², GABRIEL GUILLEN², JAVIER FARIAS¹ & ARNOLDO MICHEL¹. ¹Facultad de Ciencias Biológicas y Agropecuarias, Universidad de Colima, Apdo. postal 36. Tecoman, Colima, 28100, Mexico. ²Instituto de Biotecnología, U.N.A.M. Cuernavaca, Morelos, 62271, Mexico. - Differential display of messenger RNA of bean (*Phaseolus vulgaris* L.) roots colonized by *Glomus fasciculatum*.

The molecular basis of fungus-plant interactions in mycorrhizal vesicular-arbuscular (MVA) associations during its establishment is still poorly understood, however there is some evidence for the production of new specific polypeptides in response to MVA colonization. The aim of this work was to identify and isolate genes that are differentially expressed in bean roots colonized by *Glomus fasciculatum*. Control plants and plants inoculated with *Glomus fasciculatum* were grown in a growth chamber under the same conditions. DNA-free RNAs isolated from endomycorrhizal and nonmycorrhizal roots were analyzed by reverse-transcription, followed by polymerase chain reaction (PCR) using a set of degenerated anchored oligo-dT primer and an arbitrary decamer. PCR products were resolved on a DNA sequencing gel and differentially displayed cDNA bands were excised and reamplified. Twelve different cDNA bands were identified and isolated, with length between 200 to 600 bp. Nine of them appeared in endomycorrhizal roots and others three appeared in nonmycorrhizal roots. Only a 450 bp clone specific for endomycorrhizal roots has been cloned in pBluescript II SK- and partially sequenced. These findings suggest that differential display technique can be a useful tool for the characterization of the MVA fungus-plant interaction at different stages of root development.



poster session 8 WF AR 67

HABTE, MITIKU. Dept. of Agronomy and Soil Science, University of Hawaii, Honolulu, HI 96822, U.S.A - Effectiveness of benomyl for obtaining nonmycorrhizal conditions in greenhouse studies.

Three successive greenhouse experiments were carried out to determine the effectiveness of an initial application of benomyl for obtaining nonmycorrhizal conditions. An oxisol was amended with P to obtain target concentrations for mycorrhizal host growth or near-sufficient for mycorrhiza-free growth. The soil was not inoculated or inoculated with the vesicular-arbuscular mycorrhizal fungus *Glomus aggregatum* and amended with benomyl to obtain concentrations of 0-100 mg kg⁻¹. At the former soil P concentration, benomyl completely suppressed mycorrhizal formation and effectiveness indicated by pinnule P content and shoot yield of *Leucaena leucocephala* in the initial experiment. The fungicide did not have significant effect on pinnule P content or shoot yield at the latter soil P concentration. In the second experiment, the inhibitory effect of the fungicide persisted through the first 30 days after which time low levels of VAM activity became evident. In the third experiment, mycorrhizal activity in the benomyl-treated and untreated low P soils were similar if they were inoculated with *G. aggregatum*. Mycorrhizal development and function were significantly suppressed by benomyl in the uninoculated soil, although to a lesser extent than in the second experiment. Our data suggest that benomyl could be used to obtain nonmycorrhizal conditions at concentrations as low as 25 mg kg⁻¹ for at least 90 days.

poster session 8 WF AR 33

HADI, SOETRISNO, ERDY SANTOSO & MAMAN NURZAMAN. Fakultas Kehutanan Institut Pertanian Bogor, Bogor 16001, Indonesia. Pusat Penelitian dan Pengembangan Hutan dan Konservasi Alam, Bogor 16610, Indonesia. - The Association of *Pisolithus tinctorius* with *Pinus merkussi* roots in the natural forest in Aceh, Sumatera.

Pisolithus tinctorius sporocarps which were never reported before to be found elsewhere in Indonesia, were observed in association with *Pinus merkussi* roots in the natural forest in Aceh, North Sumatera. Under glasshouse conditions, the fungal spores extracted from the fruiting bodies proved to be able to form ectomycorrhiza not only on *P. merkusii* but also on exotic *Eucalyptus pellita* and *E. urophylla* seedlings. The mycorrhizal fungus also promoted the growth of the latter two tree species. Study needs to be undertaken to determine whether the Aceh P. strain is as effective as the strains found elsewhere in promoting the growth of different indigenous forest tree species.

talk session 8

HALL, IAN R. New Zealand Institute for Crop & Food Research Limited, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand. - The New Zealand Périgord black truffle programme - the first decade

Many ectomycorrhizal fungi have edible fruiting bodies and their collection and sale is a major industry in the Northern Hemisphere. However, because fruiting is seasonal, few preserve well, and generally they do not occur outside of the Northern Hemisphere, for most of the year they are unavailable. There is, therefore, the opportunity to introduce these fungi into Southern Hemisphere countries and produce their fruiting bodies out-of-season for Northern Hemisphere markets.

Research in New Zealand began in 1985 with *Tuber melanosporum* Vitt. (Périgord black truffle) and by 1987 the first *T. melanosporum*-infected *Quercus robur* L. (English oak) and *Corylus avellana* L. (hazelnut) were available for planting. Since then 32 privately owned truffières (truffle plantations) have been established throughout New Zealand. Information will be presented on the techniques that have been employed, selection of suitable sites, problems that have been encountered and the management of the fledgling industry.

poster session 1 ST RR 30

HAMEL, CHANTAL¹, YOLANDE DALPÉ², SUSAN PARENT³ & VALENTIN FURLAN⁴. ¹Natural Resource Science, Macdonald College of McGill University, H9X 3V9 Canada. ²ECORC Agriculture and Agri-food Canada, K1A 0C6 Canada, ³Premier Tech C.P. 2600, G5R 4C9 Canada. ⁴Agriculture and Agri-food Canada, G1V 2J3 Canada. - The indigenous arbuscular mycorrhizal fungal (AMF) community is a major determinant of plant response to inoculation of leek.

Plant response to AMF inoculation is unpredictable. Data collected from 60 agricultural soils in 1993 and from 21 agricultural soils in 1994 were used to determine the soil characteristics most influential on leek response to inoculation with *Glomus intraradices* and *G. versiforme*. At each site leek plants colonized with *G. intraradices*, *G. versiforme*, or non-mycorrhizal plants were transplanted in three rows, in the spring. Differences in stalk diameter of pre-mycorrhized and non-inoculated plants at harvest were considered in relation with soil texture, bulk density, particule density, porosity, organic matter content, pH, available soil P, K, Ca, Mg, Fe, Zn, Cu, Mn, stability of soil aggregates of different size classes, soil mycorrhizal potential, previous crop mycorrhizal potential and spore abundance of indigenous mycorrhizal fungal species in fall, using Principal Component analysis and Regression analysis. Because of interactions between available soil P and other variables, the data from soil having more than 200 µg PO₄-P and less than this quantity of P were considered separately. Soil mycorrhizal potential and species composition appeared important factors influencing leek response to inoculation.

poster session 15 WF RR 18

HARNEY, S., C. DOLJANIN and M. ALLEN. San Diego State University, Biology Department, San Diego CA. 92182. - Characterization of native and non-native isolates of *Pisolithus tinctorius* (Pt)

Pisolithus tinctorius (Pt), a cosmopolitan ectomycorrhizal fungus with a broad host range, is an early successional symbiont, generally occurring on early invaders. Pt is commonly found on native California plant species, i.e. oaks and pines, and had recently been observed in pure *Adenostoma* stands. Pt also occurs on Australian Eucalyptus, which have been widely imported into southern California. Recently, Pt isolates originally associated with the non-native Eucalypts are thought to be expanding their range onto native species. This paper reports on the molecular characterization of native and non-native Pt isolates, from a number of hosts, using PCR amplified rDNA.

poster session 1 ST RR 32

HARNIMAN, MICHELLE, DANIEL DURALL, MELANIE JONES. Biology Department, Okanagan University College, Kelowna, B.C. V1V 1V7, Canada. - The potential of woody angiosperms and naturally regenerated conifers to act as refuge for ectomycorrhizal inoculum following logging in a dry belt Douglas-fir forest.

It has been shown that large clear-cuts have caused a reduction in diversity of ectomycorrhizal fungi (ECM). One source of ectomycorrhizal inoculum for newly planted seedlings on clear-cuts can be the remaining woody angiosperms and naturally regenerated conifers. In July 1995, ectomycorrhizal roots of 14 woody angiosperm species and 2 conifer species (*Juniperus communis* and *Pseudotsuga menziesii*) were collected from a dry belt Douglas-fir forest and described using morphological characters. Twenty eight ectomycorrhizal types were distinguished from ten of the 16 woody plant species.

Six of the fifteen woody angiosperms had greater than 25% of their fine roots colonized with ECM fungi. Six woody angiosperms were not colonized with ECM fungi. From these data, it was determined that six of the woody angiosperm species and the naturally regenerating *Pseudotsuga menziesii* (NR) had a high potential to provide ectomycorrhizal inoculum to out-planted seedlings: *Betula papyrifera*, *Pseudotsuga menziesii*, *Populus tremuloides*, *Alnus viridis*, *Arctostaphylos uvaursi*, *Salix commutata*, *Amelanchier alnifolia*.

poster session 4 ST AR 52

HARRISON, MARIA J. & MARIANNE L. VAN BUUREN. The Samuel Roberts Noble Foundation, Plant Biology Division P.O. Box 2180, Ardmore, Oklahoma 73402, USA. - Molecular cloning of genes induced during a VA mycorrhizal symbiosis: Identification of a cDNA encoding a phosphate transporter.

We are using *M. truncatula*, a model legume, in combination with the VA mycorrhizal fungus *G. versiforme*, as a model system for genetic and molecular studies of the VA mycorrhizal symbiosis. In order to isolate genes that are involved in the formation and functioning of the symbiosis we have prepared cDNA libraries from mycorrhizal roots of *M. truncatula* colonised with *G. versiforme*. The library has been

screened using both specific probes and also by differential screening to isolate genes whose expression is induced during the symbiosis.

The yeast *PHO84* gene which encodes a high affinity phosphate transporter, was used as a probe to identify potential phosphate transporters clones from the mycorrhizal cDNA library. A full length clone (GvPT) sharing 47.9% sequence identity with *PHO84* was selected for further analysis. GvPT is 1932bp in length and encodes a protein of 521 amino acids. The encoded protein is extremely hydrophobic and is predicted to be an integral membrane protein with 12 membrane spanning domains. The function of the protein encoded by GvPT was confirmed by complementation of a yeast phosphate transport mutant. Southern blotting, PCR amplification and sequencing were used to demonstrate that GvPT represents a gene from the fungal genome and not from the plant. Expression of GvPT was localised to the external hyphae, which is the initial site of phosphate uptake from the soil.

morning talk

HARTNETT, DAVID. Division of Biology, Kansas State University, Manhattan, KS 66506, USA. - Mycorrhizal mediation of plant competition, demography, and species diversity in grasslands

Arbuscular mycorrhizal fungi (AMF) are ubiquitous and abundant in tallgrass prairie and other grasslands. Tallgrass prairie soils are low in phosphorus, and plant species vary considerably in their growth responses to mycorrhizal infection and their dependency on the symbiosis. Greenhouse studies and field mycorrhizal suppression experiments in Kansas tallgrass prairie have shown that AMF influence plant competitive relationships and plant-grazer interactions. The competitive dominance of the warm-season C₄ tallgrasses is highly dependent upon their mycorrhizal associations, and host plant benefits from the symbiosis are density dependent. Experiments in native tallgrass prairie show that effects of mycorrhizae on the population dynamics of co-occurring plant species vary significantly among species and among different life history stages (e.g. seedling establishment, tillering, flowering) within species. A long-term (5-year) field experiment and greenhouse prairie microcosm study further show that AMF influence plant community structure. In these grasslands, mycorrhizal suppression decreases dominance by the obligately mycotrophic grasses, increases the relative abundances of C₃ graminoids and forbs, and increases the evenness of species abundances and plant species diversity. Mycorrhizal mediation of plant population dynamics and species diversity differ among grasslands depending upon the relative mycorrhizal dependency and growth responses of the community dominants and the competitively subordinate plant species.



poster session 2 ST SH 57

HASHIMOTO, YASUSHI & MITSURO HYAKUMACHI. Laboratory of Plant Disease Science, The United Graduate School of Agricultural Science, Gifu University, 1-1 Yanagido, Gifu, 501-11, Japan. - Vertical distribution of mycorrhizae and mycorrhizal inoculum potential in birch forest soil.

The vertical distribution of mycorrhizae and mycorrhizal inoculum potential were determined in 40-year-old birch forest, *Betula platyphylla* var. *japonica*, in central Japan. Soil samples were taken from soil depth of 45 cm every 5 cm distance from soil surface. The amount of ectomycorrhizae and the number of arbuscular mycorrhizal spores were determined using stereomicroscope in each soil core sample. The inoculum potentials of ectomycorrhizal and arbuscular mycorrhizal fungi were evaluated by counting the number of ectomycorrhizal and arbuscular mycorrhizal formation in roots of birch seedlings planted in each soil sample using a bioassay system. The ectomycorrhizae was mainly distributed (>50%) in the top soil (0-5 cm) of organic horizons. The percentage of ectomycorrhizae in total fine roots gradually declined with the depth of soil. Ectomycorrhizal formation in birch seedling roots in the bioassay system was high in soil depth 0-5 cm and 10-15 cm while the amount was lower in the soil depth 25-45 cm. The arbuscular mycorrhizae produced more spores in soil depths 0-5 cm and 10-15 cm while the amount was lower in soil depth 20-25 cm until 45 cm. The arbuscular mycorrhizal formation was high in the soil depth of 0-5 cm, but was lower in soil depth of 10-45 cm. Therefore, in soil depth 0-15 cm, a different tendency exists between the distribution of both ectomycorrhizae and arbuscular mycorrhizal spores and their inoculum potentials.

talk session 1

HEBE, GERHARD, PETER SALZER, ACHIM HAGER. Botanisches Institut, Allgemeine Botanik und Pflanzenphysiologie, Universitaet Tuebingen, 72076 Tuebingen, Auf der Morgenstelle 1, Germany. - Signal transduction in spruce cells triggered by chitin elicitors released from ectomycorrhizal fungi.

Ectomycorrhizal fungi release elicitors from cell walls without being influenced by their host. These elicitors induce several rapid reactions in suspension-cultured cells of their mycorrhizal partner *Picea abies*, including extracellular alkalinisation, efflux of K⁺ and Cl⁻, influx of Ca²⁺ and synthesis of H₂O₂ (Salzer et al. (1996) *Planta* 198: 118-126). Treatment of elicitors with chitinase purified from spruce cells by affinity and ion exchange chromatography inhibits the ability of this elicitors to induce these rapid reactions in spruce cells. We suggest elicitor-active chitin fragments of higher degree of polymerisation are inactivated by spruce chitinase by cleaving them to small inactive N-acetylglucosamine oligomers. Moreover, chitin oligomers with four or more residues induce the whole set of rapid reactions. The chitin elicitor-induced phosphorylation of a 63 kD protein seems to play a pivotal role in mediating extracellular alkalinisation and H₂O₂ synthesis. As

revealed by pulse-labeling experiments with [³²P]phosphate the phosphorylation of the 63 kD protein starts as soon as 1.5 to 3 min after elicitor application and is directly followed by H₂O₂ synthesis and the alkalisation response. The kinase inhibitor staurosporine prevents phosphorylation, H₂O₂ synthesis and extracellular alkalisation. The finding that the protein phosphatase inhibitor Cantharidin induces the phosphorylation of the 63 kD protein, alkalisation and H₂O₂ synthesis indicates that elicitor action is based on kinase activity and suppression of a competing protein phosphatase activity.

talk session 9

HEIJDEN, ELISABETH W. van der and THOMAS W. KUYPER. Biological Station of the Wageningen Agricultural University, Kampsweg 27, 9418 PD Wijkster, The Netherlands. - Dual mycorrhizal infection, a matter of priority or selection?

Most plants form only one type of mycorrhiza. A very few plants, however, form both VAM and ectomycorrhiza (ECM). *Salix repens* is one of them and is widespread in a great variety of plant communities, in particular in the coastal dunes of the Wadden Isles of western Europe. In some of these communities many species and sporocarps of ECM symbionts are present, while in others they are almost lacking. In order to investigate the functional significance of different mycorrhizal symbionts of *Salix repens* in relation to survival, growth, biomass allocation and nutrient uptake of the host, *Salix repens* cuttings were inoculated with two vesicular arbuscular mycorrhizal fungi, *Glomus mosseae* and *Acaulospora laevis* and three ectomycorrhizal fungi, *Paxillus involutus*, *Hebeloma leucosarx* and *Inocybe vulpinella*. Stimulation of growth and phosphorus uptake was higher by *Glomus mosseae* than by ECM fungi. *Acaulospora laevis* depressed growth and phosphorus uptake compared to the control. Even with a very low VAM colonization (< 20%) as compared to the ECM colonization (60-100%). In addition the ECM plants have been inoculated with VAM and VAM plants with ECM fungi. Colonization percentages show that ECM prevent roots from VAM infections, more unexpected VAM also inhibits ECM formation. When we inoculate cuttings with VAM and ECM at the same time ECM are the better competitors. The significance of these results as they relate to the below ground field observations will be discussed.

poster session 2 ST SH 14

HEIJDEN, MARCEL van der, IAN R. SANDERS, THOMAS BOLLER & ANDRES WIEMKEN. Botanical Institute, University of Basle, Hebelstrasse 1, 4056 Basle, Switzerland. - Diversity of arbuscular mycorrhizal fungi as a potential determinant of plant community structure

In species-rich grasslands, plants are colonized by many different arbuscular mycorrhizal fungi (AMF). If AMF species differ in their effects on the growth of each of the plant species then the species composition of the AMF

community could be important for determining plant community structure. To test this, three plant species were inoculated with 4 different AMF species, all of which originated from the same species-rich grassland. The plant species responded differently to each of the AMF species. Furthermore, by using multivariate statistical techniques, it is shown that the degree to which the investigated plant species responded differently to each of the AMF isolates varied among the plant species. Multivariate techniques were used because they can summarize overall plant responses to AMF instead of plant responses to single variables. This is important in situations such as these where AMF affect several different variables in different plants. The results suggest that in some plant species the fitness may be much more dependent on which AMF species colonizes their roots than in other plant species and it also indicates that plant species coexistence may strongly depend on which AMF species colonizes plant roots. AMF diversity may, therefore, strongly influence plant community structure.

poster session 2 ST SH 15

HELDRETH, KELLY & JOSEPH MORTON. 401 Brooks Hall, West Virginia University, Morgantown, WV 26506-6057. - Threshold levels of mycorrhizal colonization are needed before sporulation is initiated in species of all genera in Glomales.

It is not uncommon in field situations for sporulation to be absent in arbuscular mycorrhizal plant communities, especially in arid habitats where colonization often is less than 25%. Moreover, trap cultures often produce sporulation by fungal species not detected in the field. This study was undertaken to determine if a minimum threshold level of mycorrhizal development is needed to induce sporulation by an isolate of *A. morrowiae*, *Gl. etunicatum*, *Gl. intraradices*, *Gi. gigantea*, and *S. heterogama*. Intact mycorrhizal sudangrass plants with roots encased by nylon mesh were transplanted into the centers of 15-cm diameter pots containing soil at three phosphorus levels and seeded with red clover. Clover plants were harvested at four sampling dates; percentage mycorrhizal colonization and spore numbers were measured. Data fit a Gompertz regression curve with R² values that ranged from 0.49 (*G. etunicatum*) to 0.96 (*S. heterogama*). Threshold levels of mycorrhizal development varied considerably with species, with a minimum of 10% (*G. etunicatum*) to a maximum of 30% (*Gi. gigantea*, *S. heterogama*). Other researchers have observed a similar threshold in subterranean clover colonized by an undescribed *Acaulospora* (WUM18). These results indicate that niche occupation by arbuscular fungi can be substantial in roots before they are detected by sporulation.

talk session 9

HELM, D.J.¹, E.B. ALLEN², & J.M. TRAPPE³. ¹University of Alaska Fairbanks, 533 E. Fireweed Ave., Palmer, AK 99645. ²Department of Botany and Plant Sciences, University of California, Riverside, Riverside, CA 92521-0124. ³Department of Botany and Plant

Pathology, Oregon State University, 3200 SW Jefferson Way, Corvallis, OR 97331-4401. - Mycorrhizal and plant community development on recently deglaciated terrain, Exit Glacier, Alaska.

Mycorrhizal types changed as vegetation developed over the chronosequence from barren to forest communities on the forefront of a retreating glacier in Alaska. The earliest plant colonizers included *Populus balsamifera* ssp. *trichocarpa*, which may be ectomycorrhizal (EM) or arbuscular mycorrhizal (AM), and is a major component of communities developing for over 100 yr after deglaciation and resultant flooding. EM developed on some *P. balsamifera* seedlings a few weeks after germination on mineral forefront soils although over 1 yr was needed for others. EM in the early successional stages tended to have simpler morphologies, thinner mantles, and fewer hyphal strands than those in nearby Spruce-Hemlock forests. Simpson's diversity index using EM type formation increased with site age across the chronosequence mostly from an increase in evenness, rather than richness, of EM types. To assess the mycorrhizal benefits of a nurse plant to a seedling in establishing patches, we transplanted indoor-grown seedlings of *Epilobium latifolium*, *P. balsamifera*, *Alnus sinuata*, and *Picea sitchensis* in nurse-seedling pairs on the barren floodplain. Seedlings that were planted next to nurse plants inoculated with propagule-bearing soil had greater EM formation than seedlings next to uninoculated nurse plants. Small nurse plants, typical of those found on the outwash plain, were effective only in providing inoculum, not in ameliorating the environment or catching seeds.

poster session 3 ST SH 66

HERNANDEZ-SEBASTIA, CINTA, GUY SAMSON, YVES PICHE¹, YVES DESJARDINS. Centre de Recherche en Horticulture, Environtron. ¹Centre de Recherche en Biologie Forestière, Université Laval, Québec, G1K 7P4 Canada. - Effect of PEG-induced water stress on *in vitro* mycorrhizal strawberry plantlets.

Previous findings indicate that water status (relative water content) of microcultured strawberry plantlets grown under optimal conditions (4000 ppm CO₂ and 100% RH) is increased after tripartite culture with *Glomus intraradices* (1). In the present study, the effect of PEG-induced water stress on plant-water relations of *in vitro* mycorrhizal plants was assessed. Tissue-cultured plantlets (*Fragaria x ananassa*, cv. Kent) were inoculated during 4 weeks following the tripartite culture (2), then removed and subjected to a series of water deficit conditions. The plantlets were placed in PEG-8,000 solutions during 4, 8 and 12 hours and gas exchange, chlorophyll fluorescence parameters and plant water status were measured. Leaf conductance, photosynthetic efficiency, water and osmotic potential were affected by the PEG-treatment but not by the presence of the AM fungi. Our results suggest that the intraradical phase of AM colonized plantlets contribute little to the global water status regulation of the plant.

(1) Hernandez-Sebastià, C. *et al.*, (1995). Effect of Vesicular-Arbuscular Mycorrhizal Fungi on water relations of micropropagated strawberry plantlets during rooting phase. In: Mycorrhizae in Integrated Systems. From Genes to Plant Development. C. Azcon-Aguilar and J. Barea Ed. In press. (2) Elmeskaoui *et al.*, (1995). A tripartite culture system for endomycorrhizal inoculation of micropropagated strawberry plantlets *in vitro*. Mycorrhiza, 5 : 313-319.

talk session 7

HERRMANN, SYLVIE¹, FRANÇOIS BUSCOT¹ & JEAN CHARLES MUNCH². ¹Soil Biology, Federal Center for Agriculture Research, Bundesallee 50, D-38116 Braunschweig, Germany. ²Soil Ecology, GSF-Research Center for Environment & Health, Postfach 11 29, D-85758 Oberschleißheim, Germany - Differential effects of mycobionts with different ecological strategies on oak morphology

The presented work is a unique approach on influence of mycobionts with different ecological strategies on morphology of the aerial and subterranean parts of plants. Micropropagated oaks were mycorrhized in a semi sterile system in which roots grow two dimensionally in a sterile recipient. Non destructive morphological observations were performed with a digitized image analysis system during the culture and completed by destructive analyses at the end of the assays. The morphological effects differed extremely according to the mycobiont species. Certain fungi influenced plant development only quantitatively whereas others modified internal correlations concerning leaf formation and production of the different root categories (main roots, mother roots of 1st and 2nd orders, fine rootlets). With certain mycobionts, the morphological effects started before mycorrhization. For example, *Piloderma croceum* stimulated both leaf and root production before a symbiotic relationship was detectable. This stimulating effect was not reflected by weight increase, suggesting that the fungus links photoassimilates before it forms mycorrhizae. The experimental models we established allow exciting comparative studies on recognition prior to formation of ectomycorrhizas. They are also useful to interpret influence of mycorrhization on dynamics in individual plants and in plant communities.

talk session 2

HOBBIE, ERIK, STEPHEN MACKO, & HERMAN SHUGART. Department of Environmental Sciences, University of Virginia, Charlottesville, VA 22903 USA. - Stable isotopes in mycorrhizal and saprotrophic fungi: insights into carbon and nitrogen cycling.

The successful use of natural abundances of carbon and nitrogen isotopes in the study of ecosystem dynamics suggests that isotopic analysis could give new insights into fungal functioning in nitrogen and carbon cycling. Along a 230-year-old successional sequence in Glacier Bay National Park, Alaska, mycorrhizal fungi, saprotrophic fungi, vegetation, and soils were collected in

young, deciduous-dominated sites and older, coniferous-dominated sites for $k^{13}C$ and $k^{15}N$ signatures. $k^{13}C$ of mycorrhizal fungi averaged -26, whereas saprotrophic fungi averaged -21. Plant foliage $k^{13}C$ ranged from -25 to -32, with soils averaging -28. Nitrogen signatures were more variable for both types of fungi, with values ranging from -7 to +1 for older sites and -1 to +11 for younger sites. *Cortinarius* sp. were particularly high, averaging +9. In contrast, no plant species averaged greater than +1 for $k^{15}N$, with most averaging from -6 to -1. The wider range of fungal $k^{15}N$ signatures suggests that fungal strategies for obtaining nitrogen are more specific and more varied than those of plants. Because only some of the mycorrhizal fungi have $k^{15}N$ signatures that resemble the putative host plants, comparison of these signatures may allow determination of which mycorrhizal species are responsible for supplying nitrogen to host plants. Carbon signatures indicate that saprotrophic fungi may be drawing on fractionated organic matter relative to the bulk soil.

talk session 9

HORTON, THOMAS R., TOM BRUNS & IGNACIO CHAPELA. University of California, Department of ESPM, 108 Hilgard Hall, Berkeley, CA 94720. - Ectomycorrhizal community structure in Andean Monterey pine plantations, Ecuador.

Hedger (1986) reported an annual production of *Suillus luteus* in Ecuadorian Monterey pine (*Pinus radiata*) plantations of 78,000 to 156,000 fruit bodies per hectare, or about 1 metric ton dry weight per hectare. The trees are introduced into a VAM associated bunch grass community native to this region of the Andes. We hypothesize that the high fruit body production occurs because of a simplified mycorrhizal community resulting in low competition for tree derived carbon. We sampled in two Monterey pine plantations at about 3800 meters near Salinas, Ecuador. *Suillus luteus* fruiting was high in a 5-7 year old stand and low in a 10 year old stand. Conversely, *Thelephora terrestris* fruiting was low in the 5-7 year old stand and high in the 10 year old stand. We are now assessing the below ground diversity of fungi on root tips utilizing PCR-based methods. In contrast to the fruiting pattern, there was no difference between *S. luteus* and *T. terrestris* mycorrhizal biomass in either stand. We also dug through the deep organic soil layer (1.5 meters) at one location in the 10 year old stand and observed *S. luteus* mycorrhizal root tips at 120 - 140 cm. Finally, one year old nursery seedlings used to stock current plantations were primarily colonized by a *Rhizopogon* sp. and to a lesser extent by *Laccaria tortilis*; *S. luteus* and *T. terrestris* were not detected on the seedlings.

talk session 7

HOSNY, MICHEL^{1,2}, MOHAMED HIJRI¹ & HUBERT DULIEU^{1,2}. ¹Laboratoire de Phytoparasitologie INRA/CNRS, SGAP, INRA, BV 1540, 21034 Dijon Cedex, France. ²Laboratoire de Génétique, Université de Bourgogne, 6 Boulevard Gabriel, 21000 Dijon, France. -

Molecular genetics as a unique tool for identification of biodiversity and evaluating genetic exchanges within endomycorrhizal species.

We have developed different approaches to design molecular tools useful in genome studies and in molecular marking of species or genotypes of glomalean fungi. Genome sizes were studied by flow cytometry. The nuclear DNA contents ranged from 0.14pg to 1.12pg among 12 species. These results indicate higher sizes than those of known primitive fungi (Zygomycetes and Oomycetes). Experiments on DNA denaturation-renaturation suggest a high proportion of repeated DNA (more than 50%) in *Scutellospora castanea*. Genomic clones of a pUC18 library were hybridized with the total genomic DNA as a probe and gave similar results. Analyses were performed on the ribosomal-RNA specifying genes: the number of repeats was evaluated to be 50 to 70 copies. In spite of this low copy number, there are clear indications that several families exist in the bulk DNA of *S. castanea*. We confirmed this by cloning PCR products of the ITS region and classifying them into at least six very divergent families by both RFLP and sequencing. Moreover, PCR performed on single spores gave further evidence that genetic heterogeneity has persisted among nuclei of single spores. Family-specific primers designed from the sequences allowed us to demonstrate the multikaryotic status of spores; this is in favour of the possibility of nucleus transfer by interhyphal conjugation.

talk session 8

HUA, XIAOMEI¹, GUOLONG LIU², XIAOLIN ZHANG², LAIYOU ZHENG¹. ¹Centre for Mycorrhizal Research and Development, Chinese Academy of Forestry (CAF), Wan Shou Shan, Beijing, 100091, China. ²The Experimental Centre of Subtropical Forestry, CAF, Feiyi County, Jiangxi Province, 336600, China. - A technique for mycorrhizal inoculation: the effect and field performance

Based on the principle that the exudation from root can stimulate the germination and growth of the mycorrhizal fungus, the method of growing pine seedlings by cutting their apexes off and inoculating with mycorrhizal fungus is proposed and compared with traditional method or other mycorrhizal inoculation techniques to raise the pine seedlings. The results show that this technique can promote the development and growth of root system and ectomycorrhizae formation, increases the biomass of seedling and improve the quality and yield of plantable seedlings, enhance the survival and growth of outplantings significantly. The benefit analysis confirms that the use of this method has significant economic and social benefits, with a countable value of ¥44662.5 yuan/hm² in nursery alone. The mechanisms of this technique are discussed further.

poster session 11 WF RR 38

HUREK, THOMAS^{1,2}, PICHE, YVES¹. ¹Centre de Recherche en Biologie Forestiere, Faculte de Foresterie et de Geomatique, Pavillon C.E. Marchand, Universite Laval, Quebec, PQ, Canada G1K 7P4. ²Max-Planck-Institut fuer terrestrische Mikrobiologie, Karl von Frisch Str., D-35043 Marburg, Deutschland. - Sequence heterogeneities among 18S ribosomal RNA genes in monoxenic cultures of *Glomus intraradices* DAOM 197198

We studied small ribosomal subunit RNA (SSU rRNA) in spores of in vitro-grown *Glomus intraradices* in order to identify intraspecies-specific polymorphisms. Fourteen terminal secondary spores emerging from a single hypha of in vitro cultivated *G. intraradices* DAOM 197198 were used for initiation of monoxenic mass production of spores in root organ cultures with carrot. From 100000 of these newly produced spores a genomic library was constructed which is free of any bacterial or fungal contamination. The library was screened with an universal rDNA-targeted oligonucleotide probe. 28 clones were obtained and several SSU rRNA genes sequenced. Sequences of SSU rRNA genes did not match with the published sequence on SSU rDNA of *G. intraradices* DAOM 197198 (EMBL X58725) obtained from direct sequencing of pooled PCR products. Cloning of single SSU rRNA genes resulted in sequences that differed from that of others or that of the mean sequence by several mutations. Results suggest, that for identification of unknown AM-fungi a mean sequence or a sequence of a single nonidentified SSU rRNA gene should be avoided. To address the question whether polymorphic SSU rRNA genes occur in the same or in different nuclei, in situ hybridisations with specific rDNA-targeted oligonucleotide probes are carried out. Possible mechanisms creating this diversity are discussed.

poster session 4 ST AR 54

ITZHAKI, HANAN*, YEDIDYA GAFNI, GAD GALILI* & YORAM KAPULNIK. Field and Garden Crops Institute, ARO, The Volcani Center, Bet Dagan 50250, Israel and *Department of Plant Genetics, The Weizmann Institute of Science, Rehovot 76100, Israel. - Differential expression of tobacco genes in response to VA mycorrhizal fungi colonization

The initiation and development of the symbiotic interaction between vesicular arbuscular (VA) mycorrhizal fungi and plant roots has been previously described by others. So far, little is known of genetic mechanisms involved in the development and function of the symbiosis. In order to elucidate host plant genes that are specifically expressed during the development of the symbiosis we have utilized the Differential Display technique (1) to identify changes in expression of tobacco (*Nicotiana glauca* cv. Xanthi-nc) genes following early stages of *Glomus intraradices* colonization. Total RNA from both mycorrhizal and non-mycorrhizal roots was extracted and cDNA was made and amplified by PCR. Thirty bands were found in mycorrhizal roots but not in non-mycorrhizal roots, and about 40 bands were

found to be lighter or absent in the mycorrhizal roots as compared with non-mycorrhizal roots. This findings demonstrate the feasibility of utilizing this technique to isolated transcripts which were differentially expressed following VA mycorrhizal fungi colonization and some of these differentially expressed transcripts may support the activity and maintenance of the symbiosis. Of the 14 bands that were so-far cloned and sequenced, one clone showed high homology to the 3' end of the basic chitinase from *N. tabacum*. Reduction in this clones' transcript was found in mycorrhizal roots and was correlated with a decrease in chitinase enzymatic activity as compared to non-mycorrhizal controls. Studies of other clones, their appearance and their possible role in the plant-VA mycorrhizal interactions is in progress and will be presented.

(1) Liang and Pardee (1992). Science 257:967-971.

poster session 8 WF AR 35

IVORY, MIKE¹, MARIO HONRUBIA², LINUS MWANGI³ & KAMAU MBURU². ¹Field Laboratory, Oxford Forestry Institute, University of Oxford, Wytham, Oxford, OX2 8QT, Englan. ²Departamento de Biologia Vegetal, Universidad de Murcia, 30100 Murcia, Spain. ³Kenya Forestry Research Institute, P.O. Box 20412, Nairobi Kenya. - Ectomycorrhizal associations between *Scleroderma* spp. and forest trees in Kenya.

The small outlier forests of "Miombo-like-vegetation" which still remain in the Coast Province of Kenya contain 4 species in 4 genera of the subfamily Caesalpinioideae which are known to be ectomycorrhizal elsewhere; namely, *Azelia quanzensis*, *Brachystegia spiciformis*, *Julbernardia magnistipulata* and *Paramacrolobium coeruleum*. Plantations of exotic pines and eucalypts have also been established in Kenya since 1900, particularly in inland high altitude regions, but also to a small extent in the Coast Province. The aims of the present project are firstly, to identify and characterize the fungal symbionts in Kenyan forests; secondly, to synthesize and characterize the ectomycorrhiza which they form; and thirdly, to use PCR-based techniques to typify the symbiotic fungi and to facilitate the identification of the fungi and derived ectomycorrhiza. Since May 1993 four species of *Scleroderma* have been collected from lowland forests in the Coast Province of Kenya. Ectomycorrhiza have been synthesized with all of these on a variety of host tree species at Oxford Forestry Institute and their anatomy and morphology have been studied in detail at the University of Murcia.

poster session 5 ST AR 20

IWASE, KOJI & YURIKO IKEDA. Biological Environment Institute, Kansai Environmental Engineering Center Co., Ltd., 8-4 Ujimatafuri, Uji 611, Japan. - Differences in the utilization of sucrose, glucose and fructose among mycorrhizal and saprophytic fungi.

Utilization of sucrose, glucose and fructose in a synthetic medium (MMN) by several kinds of mycorrhizal and saprophytic fungi was examined. Mycelia were grown in

liquid medium containing sucrose as a carbon source (MMN-S), and changes in soluble sugar composition (sucrose, glucose and fructose) in the medium during mycelial growth were determined with HPLC. Most of mycorrhizal fungi could not grow well and utilize sucrose in MMN-S, suggesting that they lack the extra-hyphal invertase. This characteristic seems to be necessary for stable mycorrhizal association, because fungal extra-hyphal invertase could cause the imbalance of soluble sugar composition in mycorrhiza. However, some mycorrhizal fungi such as *Lyophyllum shimeji* and *L. fumosum* grew well in MMN-S and showed high extra-hyphal invertase activity. These fungi could be facultative mycorrhizal species. Some wood decay fungi could grow well in MMN-S, and accumulation of considerable amount of fructose was observed during mycelial growth, suggesting that those fungi prefer glucose to fructose as a carbon source. The extent of fructose accumulation was variable among fungi tested. When mycelia were grown in liquid medium containing glucose and fructose (MMN-GF), some mycorrhizal fungi rather preferred fructose than saprophytic fungi. It is supposed that preferability of fructose is a suitable characteristic for mycorrhizal fungi because plants utilize glucose for growth and the remaining fructose could be available for fungi.

talk session 1

JAKOBSEN, IVER. Plant Nutrition, Environmental Science and Technology Department, Risø National Laboratory, DK-4000 Roskilde, Denmark. - Competition for soil P between roots and hyphae of arbuscular mycorrhizas

Experiments in microcosms and in the field have shown that hyphae of efficient arbuscular mycorrhizal fungi (AMF) may take up as much as or even more P than roots and AMF in combination. This led to the suggestion that a high P inflow from the AMF hyphae may reduce the direct root P uptake by feed-back regulation. But recent work points to a more simple explanation: roots and hyphae compete for the available soil P which, however, may be depleted to the same degree by the hyphae alone. The root-hyphae competition for P was investigated by measuring the transport of ^{32}P to *Trifolium subterraneum* by *Glomus caledonium* hyphae growing into a mesh bag containing ^{32}P labelled soil. Inside the mesh bag subclover was grown at different plant densities (0, 1, 2 or 4 plants). The hyphal transport of ^{32}P out of the mesh bags during the 5 wk growth period was markedly decreased in the presence of roots and decreased with increasing root density to less than 30% of the ^{32}P transport in the absence of roots in the mesh bag. A high degree of overlapping between P depletion zones of individual roots will occur at high root densities and the ability of the AMF hyphae to bypass the depletion zones will then be of limited value to the host. This direct evidence for competition for soil P between AMF hyphae and roots may explain why mycorrhizal growth responses are low at high root density. The P uptake by an AMF from a root-free soil volume should simply be

considered as a measure of the potential contribution to P uptake by that fungus.

poster session 14 WF RR 06

JAKUCS, ERZSÉBET¹, ZOLTÁN BRATEK². Eötvös Loránd University, H-1088 Budapest, Puskin u. 11-13 Hungary. ¹Department of Plant Anatomy. ²Department of Plant Physiology. - Mycorrhizae of some rare hypogeous fungi from Hungary

The mycorrhiza of three hypogeous fungus species from Hungary are demonstrated. *Rhizopogon vulgaris* var. *intermedius* Svrcek (Gasteromycetes): Coralloid, or dichotomical ramification. Colour white with pink or rose marks, like fruitbody. Mantle outer surface: smooth, without cystidia, plectenchymatic. Inner surface: transition from plectenchymatic to pseudoparenchymatic. Rhizomorphs very abundant, composed from flattened hyphae, colour white, glittering. This mycorrhiza is similar to that of *Rhizopogon luteolus*, but the tips are not yellow, and anilin does not give brown reaction. *Genea verrucosa* Berk et Br. (Tuberales): Monopodial, pyramidal ramification with thick emanating hyphae. Mantle surface smooth, dark brown, without rhizomorphs. Outer layer pseudoparenchymatic, with thick-walled angular cells. Walls of emanating hyphae thick at the base. Intercalar hyphae can be seen often, like in *Genea hispidula*. Inner layer of the mantle plectenchymatic. In this genus only *Genea hispidula* has been characterized up to now, being very similar. The only difference is the absence of brown reaction with AgNO_3 and the basal cells of emanating hyphae being more onion-like. *Terfezia terfezioides* (Matt.) Trappe (Tuberales): The fruitbody of this white truffle has been reported in almost all cases under black-locust trees from Hungary. The mycorrhiza can be considered as being endo type since both mantle and Hartig-net are absent. Septate hyphae colonize the cortex cells of the root forming finger-like branches. Neither vesicula nor arbuscula were formed.

poster session 1 ST RR 35

JANERETTE, CAROL A. P.O. Box 693, Glenside, Pennsylvania 19038, U.S.A. - Janerette Mycorrhizal Fungus Inoculum

The Janerette inoculum is produced from cultures of ectomycorrhizal fungi. It has a shelf life of at least one year, is sterile, handles easily, infects both woody and herbaceous plants, and imparts the known beneficial effects of mycorrhizal associations to the plants that it infects. This invention details the production of inocula from 15 genera of ectomycorrhizae-forming fungi. Inoculum prepared from each of these fungi formed ectomycorrhizae with pine seedlings in sterile culture, and also infected herbaceous plants indicating that these fungi are less specific in host choice than is currently thought. Asparagus, onion, wheat and corn plants quickly outgrew the limited amount of nutrients used to synthesize mycorrhizae, and additional studies were done using a dwarf variety of maize. In preliminary tests,

important roles in the aggregation process at more than one hierarchical level. Roots and the hyphae of mycorrhizal fungi are believed to function as a "sticky string bag", enmeshing primary particles and microaggregates together to form macroaggregates and enabling further stabilization to occur as biological activity within the macroaggregate results in the deposition of plant and microbially derived polysaccharides and mucigels. Using a path analysis approach with data from a chronosequence of prairie restorations, we found the relative contributions of roots and external hyphae to macroaggregate formation were greater than the contributions of microbial biomass, hot-water soluble carbohydrates, and soil organic carbon, especially for larger macroaggregates. Other studies of soils from the same site have identified a glycoproteinaceous substance exuded by mycorrhizal hyphae that appears to cement soil particles and smaller aggregates together and may be a major contributor to the "sticky" mechanism of aggregate stabilization. In addition, the hydrophobic nature of this substance may indirectly contribute to aggregate stability by dampening the disruptive forces of rapid water movement into the pores between and within aggregates. At the microaggregate scale, evidence that partially decomposed hyphal fragments encrusted with clays can serve as nucleating sites for microaggregates has been obtained from soil micromorphology studies.

poster session 2 ST SH 31

JAYASUNDARA, FIONA, LYN ABBOTT & DAVID JASPER. Soil Science and Plant Nutrition, Faculty of Agriculture, The University of Western Australia, Nedlands, W.A., 6907, Australia. - Influence of host plant on infectivity and morphological characteristics of arbuscular mycorrhizal symbionts

The potential exploitation of favourable combinations of AM fungi in plant communities depends on distinguishing them among different host plants. AM fungi from a forest (*Glomus* sp. (WUM44); *Acaulospora* sp. (WUM46)) and a pasture (*Glomus* sp. (WUM51); *Scutellospora* sp. (WUM53)) from south-west of Western Australia were each inoculated onto host plants from their natural habitats. Forest fungi were inoculated onto *Acacia pulchella*, *Eucalyptus calophylla* and *Trifolium subterraneum* and pasture fungi were inoculated onto *Lolium rigidum* and *Trifolium subterraneum* to investigate their infectivity and infection morphologies.

Each host-fungus combination was assessed over a period of 3-12 weeks in the glasshouse. The key features measured were the diameter of hyphae at entry points and within the inner cortex, the dimensions of vesicles and their number and position in relation to entry points, dimensions and the number of arbuscules and the number of entry points. Although for each fungus there were differences between some of these characteristics in different hosts, the key features of each fungus were consistent for all hosts. This rigorous examination of AM fungal morphologies suggests that it is possible to distinguish between some fungi in different host plants.

This provides a valuable ecological tool for quantifying at least some components of the communities of AM fungi at field sites.

talk session 6

JOLICOEUR, MARIO¹, SOPHIE GERMETTE², MARTIN GAUDETTE¹, MICHEL PERRIER¹, GUILLAUME BÉCARD². ¹École Polytechnique de Montréal, Chemical Engineering Dept., 2900 Édouard-Montpetit, Montréal, Québec, Canada, C.P. 6079, Succ. Centre-Ville, H3C 3A7. ²Laboratoire de Mycologie Végétale, Bât. 4R1, Université Paul-Sabatier, 118 route de Narbonne, 31062 Toulouse, France. - Measurement of endomycorrhizal fungi intracellular pH

Intracellular pH of *Gigaspora margarita* germ tubes and *Glomus intraradices* extraradical hyphae and germ tubes were determined using BCECF-AM (Molecular Probes) dye under ratiometric measurements at 450 and 490 nm excitation. The method was developed to perform real time analysis of cytosolic pH of AM fungal cells submitted to various experimental conditions. The use of nigericin led to an in vivo calibration curve.

Delicate parameters such as minimum loading time and concentration of the dye, signal to noise optimization were determined so that valid pH measurement could be made for a steady-state period on viable cells. A characteristic pH profile pattern was observed along hyphae of the AM fungi. For *G. margarita*, the pH of the apex (0-6µm) was typically of 6.8 rapidly raising up to 7.1 behind this region (10 µm) and then slowly decreasing the next 300 µm to reach a constant value of 6.7. For *G. intraradices* hyphae, a similar pattern was obtained. The pH profile of *G. margarita* germ tubes was higher when cultured in the presence of *Daucus carota* hairy root (non-mycorrhized). For extraradical hyphae of *G. intraradices*, the presence of root exudates also raised pH of hyphal cytosol. Preliminary results indicate that the method of cytosolic pH measurement could be useful for studying signal perception and/or H⁺/co-transport of nutrient by AM hyphae.

poster session 3 ST SH 30

JONER, ERIK¹ & CORINNE LEYVAL². ¹Dept. of Biotechnological Sciences, Agricultural University of Norway, P.O.Box 5040, N-1432 Aas, Norway. ²Centre de Pedologie Biologique, CNRS, Laboratoire Associe a l'Universite de Nancy I, 17, rue N.D. des Pauvres, BP 5, 54501 Vandoeuvre-les-Nancy Cedex, France. - Uptake of ¹⁰⁹Cd by roots and hyphae of a *Glomus mosseae*/*Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium.

Subterranean clover in symbiosis with *Glomus mosseae* was grown in compartmented pots allowing access to radiolabelled cadmium at three levels of cold Cd by either roots or arbuscular mycorrhizal hyphae. All treatments were replicated with non-mycorrhizal plants. After a growth period of 52 days roots and shoots were analysed for ¹⁰⁹Cd, and lengths of roots and hyphae in respective labelling compartments determined. Uptake by roots was

soybeans and lima beans showed enhanced yields when treated with ectomycorrhizae inoculum. In these experiments, inoculum produced from *Cenococcum geophilum* infected and enhanced the development of all of the herbaceous plants tested.

These results indicate that *Cenococcum geophilum* would make an excellent candidate for a commercial inoculant for both woody and herbaceous plants

talk session 9

JANOS, DAVID P.¹ & GARY S. HARTSHORN².

¹Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, FL 33124 U.S.A. ²Organization for Tropical Studies, Duke University, P.O. Box 90630, Durham, NC 27708 U.S.A. - Arbuscular mycorrhiza limitation of tropical rainforest host community diversity

In 1973, at the La Selva Biological Station in northeastern Costa Rica, we fumigated seven 10 x 20 m field plots with 510 kg/ha of methyl bromide and 2% chloropicrin gas. These plots were contiguous to paired cleared "control" plots treated similarly but not fumigated. Plot pairs were randomly located throughout a 120 X 120 m cleared area surrounded by forest. Fumigation killed soil biota including seeds, and reduced the availability of arbuscular mycorrhizal fungus (AMF) inocula.

We allowed secondary succession to proceed naturally for twenty years, and then enumerated, identified and measured all woody stems greater than 5 cm dbh. We found that fewer tree species recolonized fumigated than control plots (9.4 vs. 13.0 species, respectively). Fumigated plots had lower plant densities (15.7 vs. 25.3 individuals/200 m²) and total basal areas (1,746 vs. 2,910 cm²/200 m²) than controls, but proportional size distributions of stems on fumigated and control plots did not differ.

The one-time reduction of AMF apparently diminished the probability of establishment of mycorrhiza-dependent tree seedlings, thereby limiting opportunities for diverse species to establish. We propose that recovery of tree diversity during tropical secondary succession depends upon seedling chance encounter with surviving or re-introduced AMF.

poster session 12 WF SH 26

JANSA, JAN¹, GRYNDLER, MILAN², SCHÜEPP, HANNES³. ¹Charles University in Prague, Faculty of Sciences, Dpt. of Botany, Benátská 2, 120 00 Praha 2, CR, ²Institute of Microbiology, Academy of Sciences of the Czech Republic, Václavská 1083, 142 20 Praha 4 - Krc, CR, ³Swiss Federal Research Station, 8820 - Wädenswil, CH. - The influence of chitin and its degradation products on mycorrhizal infection, mycelial growth and sporulation of AM fungi.

In a series of pot experiments there was observed an influence of polysaccharides (laminarine, cellulose, chitin) added to cultivation substrate on infection, mycelium growth and sporulation of AM fungi.

Combination of symbionts used was *Zea mays* and *Glomus fistulosum* BEG 23 (CR) or *Glomus intraradices*

F1 (CH). Because of the positive effect of chitin on some attributes of mycorrhizal infection further work was focused on influence of chitin and its degradation products on mycorrhizal fungi. Other experiments were designed to reveal the effects of carbon and nitrogen part of chitin molecule on arbuscular mycorrhiza. Influence of products of degradation of chitin on mycelial growth was observed in *in vitro* system designed for proliferation of AMF mycelium. There was no significance stimulation by the compounds used and that is why we suppose the positive effect of addition of chitin on mycorrhiza to be realized through other microbial populations in the soil. There were run some experiments also for describing the microbial shift in substrate due to enrichment with chitin. Both changes in number of general bacterial population and chitinolytic bacterias were highly significant.

morning talk

JASPER, DAVID. Centre for Land Rehabilitation, Faculty of Agriculture, The University of Western Australia, W.A., 6907, Australia. - AM fungi in land restoration - we can apply the principles, what about a product?

The effect of soil disturbance and storage during mining on AM fungi is now relatively well understood. In Australian natural ecosystems, there is almost inevitably some loss of infectivity with soil disturbance. The severity of this effect depends on propagule number which in turn reflects the proportion of AM hosts and their productivity. Stockpiling of soil has a more severe effect, with substantial losses of infectivity below about 1 m depth. In most cases the fungal population in topsoil recovers within approximately 5 years. As a result of this research mining companies are now able to apply the principles that have been established and manage soils with an understanding of the consequences for beneficial soil micro-organisms.

In mine wastes and severely-degraded soils containing little or no AM fungi, inoculation appeals as an approach for rapidly restoring a functional population. Yet a product that is practical for use over large areas has not been developed. Further, the benefit of inoculation (and of topsoil management) has still to be demonstrated in the field. Inoculum development has progressed in our research program to the point where large scale field trials are planned. One of the first uses of this inoculation technology will be to attempt to answer the question of whether it is required.

morning talk

JASTROW, J.D. Environmental Research Division, Argonne National Laboratory, Argonne, IL 60439, USA. - Contributions of mycorrhizae to the development of soil aggregate hierarchy

In soils where organic matter is the major binding agent, the formation of aggregates appears to be hierarchical in that primary particles and clay microstructures are bound into microaggregates (up to 250 µm in diameter), which, in turn, are bound into macroaggregates (up to several millimeters in diameter). Mycorrhizae play several

important roles in the aggregation process at more than one hierarchical level. Roots and the hyphae of mycorrhizal fungi are believed to function as a "sticky string bag", enmeshing primary particles and microaggregates together to form macroaggregates and enabling further stabilization to occur as biological activity within the macroaggregate results in the deposition of plant and microbially derived polysaccharides and mucigels. Using a path analysis approach with data from a chronosequence of prairie restorations, we found the relative contributions of roots and external hyphae to macroaggregate formation were greater than the contributions of microbial biomass, hot-water soluble carbohydrates, and soil organic carbon, especially for larger macroaggregates. Other studies of soils from the same site have identified a glycoproteinaceous substance exuded by mycorrhizal hyphae that appears to cement soil particles and smaller aggregates together and may be a major contributor to the "sticky" mechanism of aggregate stabilization. In addition, the hydrophobic nature of this substance may indirectly contribute to aggregate stability by dampening the disruptive forces of rapid water movement into the pores between and within aggregates. At the microaggregate scale, evidence that partially decomposed hyphal fragments encrusted with clays can serve as nucleating sites for microaggregates has been obtained from soil micromorphology studies.

poster session 2 ST SH 31

JAYASUNDARA, FIONA, LYN ABBOTT & DAVID JASPER. Soil Science and Plant Nutrition, Faculty of Agriculture, The University of Western Australia, Nedlands, W.A., 6907, Australia. - Influence of host plant on infectivity and morphological characteristics of arbuscular mycorrhizal symbionts

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talk session 6

JOLICOEUR, MARIO¹, SOPHIE GERMETTE², MARTIN GAUDETTE¹, MICHEL PERRIER¹, GUILLAUME BÉCARD². ¹École Polytechnique de Montréal, Chemical Engineering Dept., 2900 Édouard-Montpetit, Montréal, Québec, Canada, C.P. 6079, Succ. Centre-Ville, H3C 3A7. ²Laboratoire de Mycologie Végétale, Bât. 4R1, Université Paul-Sabatier, 118 route de Narbonne, 31062 Toulouse, France. - Measurement of endomycorrhizal fungi intracellular pH

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poster session 3 ST SH 30

JONER, ERIK¹ & CORINNE LEYVAL². ¹Dept. of Biotechnological Sciences, Agricultural University of Norway, P.O.Box 5040, N-1432 Aas, Norway. ²Centre de Pedologie Biologique, CNRS, Laboratoire Associe a l'Universite de Nancy I, 17, rue N.D. des Pauvres, BP 5, 54501 Vandoeuvre-les-Nancy Cedex, France. - Uptake of ¹⁰⁹Cd by roots and hyphae of a *Glomus mosseae*/Trifolium subterraneum mycorrhiza from soil amended with high and low concentrations of cadmium.

Subterranean clover in symbiosis with *Glomus mosseae* was grown in compartmented pots allowing access to radiolabelled cadmium at three levels of cold Cd by either roots or arbuscular mycorrhizal hyphae. All treatments were replicated with non-mycorrhizal plants. After a growth period of 52 days roots and shoots were analysed for ¹⁰⁹Cd, and lengths of roots and hyphae in respective labelling compartments determined. Uptake by roots was

not significantly influenced by the mycorrhizal status of the plant. Uptake from hyphal compartments was higher in mycorrhizal than in non-mycorrhizal plants, but the increased tracer activity was sequestered in the roots. It is concluded that extraradical hyphae of AM fungi may transport Cd from soil to plants, but that transfer from fungus to plant is restricted due to fungal immobilization. No restriction of hyphal growth into soil with up to 20 mg available Cd per kg was observed. The implications for plant uptake of Cd from polluted soil is discussed.

talk session 4

JONES, MELANIE¹ & DANIEL DURALL¹. ¹Biology Department, Okanagan University College, Kelowna, B.C. V1V 1V7, Canada. - Silvicultural practises and ectomycorrhizal diversity in British Columbia.

Many silvicultural practises have the potential to affect the diversity of ectomycorrhizal fungi in the stand which develops following logging. This presentation will summarize work from several studies which have shown that site preparation treatments, cut-block size, and the diversity of species planted can each influence the diversity of ectomycorrhizal types observed on seedlings during the first several years following outplanting. The first study was established on a dry, grassy slope in the Interior Douglas-fir zone of B.C. Mechanical (removal of the forest floor) and chemical (glyphosate) methods of reducing competition from grasses were applied to 1.5 X 1.5 M areas around *Pinus contorta* seedlings at the time of outplanting. The diversity of ectomycorrhizal types, based on detailed morphological examination, was higher in seedlings growing in chemically-treated plots than in mechanically-treated ones at 16 and 28 months after outplanting. A study conducted in the Interior Cedar Hemlock zone (ICH) of B.C. showed that *Tsuga heterophylla* and *P. contorta* seedlings formed a marginally (but significantly) higher diversity of ectomycorrhizal associations when planted in the center of smaller openings than in the center of larger ones. A second study in the ICH zone examined the effect of planting *Pseudotsuga menziesii* and *Betula papyrifera* in single-species and mixed plots. The ectomycorrhizae associated with *P. menziesii* were more diverse when *P. menziesii* was planted together with *B. papyrifera* than when planted in single-species plots. The trend was the same for *B. papyrifera*, but the differences were not statistically significant. In all cases, the effect on diversity was subtle, with the evenness component of diversity usually affected more than the richness component. In general, the frequency of sub-dominants, including the E-strain fungi and *Mycelium radialis atrovirens*, increased. It is important to learn more about the physiology and ecology of these fungal groups in order to determine the biological significance of results such as those described above.

talk session 4

JONSSON, LENA¹, ANDERS DAHLBERG¹, MARIE-CHARLOTTE NILSSON² & OLLE ZACKRISSON². ¹ Department of Forest Mycology & Pathology, Swedish University of Agricultural Sciences, Box 7026, S-750 07 Uppsala, Sweden. ² Department of Forest Vegetation Ecology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden. - Ectomycorrhizal succession during establishment of *Pinus sylvestris* seedlings in virgin forest

Ectomycorrhizal succession was studied over a 10 year period in newly established *Pinus sylvestris* plants growing in three old-growth *Pinus sylvestris* forests in northern Sweden. All sites represent late post fire succession of *Empetrum - Cladina* forest type. At each site 50 seeds were sown at each site in 10 replicate plots each spring between 1986 and 1995. The surviving plants (406) were harvested in September 1995. Where possible two seedlings were sampled from each cohort (168 plants in total). Mycorrhizal root tips were morphologically characterised and counted, and samples of each type were taken for PCR/RFLP analysis. Five soil cores per site were also taken for comparisons between roots of experimentally established seedlings and old trees. In total 19 morphologically distinct types were distinguished. Thirteen of these were common to both seedlings and old trees. Two types were unique to older trees and four types were found only on the roots of the seedlings. One dominant type occurred on 74% of the young plants. Comparative PCR/RFLP data will be presented in the poster and the results will be discussed in terms of succession and possible difference in mycorrhizal colonisation of seedlings and mature, old-growth forest trees.

poster session 12 WF SH 40

JONSSON, TINA¹, MELANIE JONES², SHAHID MAHMOOD¹, ANDERS TUNLID¹, SUSANNE ERLAND¹, & 3. ¹Department of Microbial Ecology, University of Lund, S-223 62 Lund, Sweden. ²Biology Department, Okanagan University College, Kelowna, B.C. V1V 1V7, Canada. ³Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, S-750 07 Uppsala, Sweden. - Growth on organic N and protease production by fungi from two sites with different levels of nitrogen deposition in southern Sweden.

Possible effects of N deposition on forests include both changes in ectomycorrhizal community structure and shifts in patterns of N utilisation and assimilation. Ectomycorrhizal fungi were cultured from fruitbodies and isolated from surface-sterilized mycorrhiza collected from two similar forest sites in southern Sweden. The two sites, Skrylle and Vedby, have similar soil types and were both planted with *Picea abies* in 1934/5 but the annual canopy throughfall of N at Skrylle (24-28 kg/ha) is double that at Vedby (14-15 kg/ha). Fungal isolates were grown on liquid MMN medium containing either nitrate, ammonium, bovine serum albumin (BSA) or

gliadin as the sole N source. Although there were major intra-specific differences between sites there was no overall difference in the ability of the two fungal communities to grow on the two proteins relative to their growth on inorganic N. There is thus no evidence that higher inorganic to organic N ratios at Skrylle have selected against mycorrhizal fungi which can grow better on protein N than on inorganic N. Protease activity in the media was quantified by measuring the hydrolysis of Azocoll, an azo-linked collagen, at pH 3 and pH 7. The relative protease activity at pH 7 appeared to increase with time. The activity at pH 3 was inhibited by pepstatin and EDTA, suggesting aspartate- and metalloproteases. At pH 7 protease activity was inhibited by PMSF, but not pepstatin, suggesting a serine protease. Protease activity in the *Piloderma croceum* isolate, at both pH 3 and 7, was inhibited by E-64, suggesting that it was due to a cysteine protease.

poster session 2 ST SH 16

KABIR, Z.¹ & C. HAMEL¹. ¹Department of Natural Resource Sciences, McGill University, Macdonald Campus, 21111 Lakeshore Rd. Ste-Anne-de-Bellevue, Quebec, Canada, H9X 3V9. ²Department of Land Resource Sciences, University of Guelph, Guelph, Ontario, Canada, N1G 2W1. - Seasonal changes in arbuscular mycorrhizal development as affected by tillage practices and fertilizations.

Tillage practices and manuring may affect the growth and development of arbuscular mycorrhizal fungi (AMF). In the present study, we examined AMF hyphal density, root colonization and P uptake by corn under different tillage intensities and fertilizations. The experiment was conducted in eastern Canada in sandyloam and clay field soils in 1992 and 1993. Samples were collected in spring, at 10 leaf stage, silking stage and in the fall of each year. The AMF hyphal length was not affected by dairy manure or inorganic fertilization in the sandyloam soil. The length and proportion of total and metabolically active hyphae was highest under no-till (NT), lowest under conventional tillage (CT) and intermediate under reduced tillage (RT) system. The percentage of root colonization and P uptake was also lower under CT than under RT and NT. Arbuscular mycorrhizal fungal development pattern followed that of corn plant.

morning talk

KÅRÉN, OLA, ANDERS DAHLBERG, ROGER FINLAY, LENA JONSSON, MARIA JONSSON & JAN-ERIK NYLUND. Dept. of Forest Mycology and Pathology, Sw. Univ. of Agricultural Sciences, Box 7026, 750 07 Uppsala, Sweden. - Diversity of ectomycorrhizal fungi - managed vs unmanaged forests.

The diversity of ectomycorrhizal fungi was monitored over a season in 11 *P. sylvestris* plots in central Sweden. Eight of the plots were 35-40 yrs old, four of these were regenerated from planting following clearcutting ("P"), and four regenerated under shelterwood trees that were successively removed ("S"). The remaining 3 plots

("Old") were 150-200 yrs old, and had not been managed. Fruitbodies were counted on 7 occasions and fine roots were sampled once in the autumn using soil cores, from which mycorrhizal root-tips were removed. To identify the fungal species in individual root tips, the internal transcribed spacer (ITS) region of the rDNA was amplified by the universal primers ITS1 and ITS4, using the polymerase chain reaction (PCR) and subjected to RFLP-analysis with three endonucleases (HinfI, MboI and TaqI). The restriction patterns of mycorrhizal roots were compared with fruitbody reference species that had been analysed in the same way. The fruitbody inventory indicated that clear-cut, planted forest (P plots) had the lowest diversity and species richness, despite the fact that they contained the highest numbers of fruitbodies. The S and Old plots were similar to each other in terms of diversity. The analysis of mycorrhiza revealed that the majority of root tips were formed by species that cannot form large epigeous fruitbodies. In contrast to the fruitbodies, the diversity of mycorrhizal roots was less affected by the forestry management. Comparisons are made with other studies of ectomycorrhizal communities with special emphasis on molecular methods.

poster session 1 ST RR 33

KASUYA, MARIA CATARINA MEGUMI, LUCIANA DA SILVA RODRIGUES & ARNALDO CHAER BORGES. Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil, CEP 36571-000 - Viability of fragmented mycelia of ectomycorrhizal fungi entrapped in a calcium alginate gels.

The viability of fragmented mycelia of *Pisolithus tinctorius* and *Paxillus involutus* entrapped in calcium alginate gels was studied as part of a project on production of ectomycorrhizal fungi inocula. The fungi were grown in 50 mL of MMN solution, in 125 mL Erlenmeyer flasks, at 28°C, before entrapment. Different ratios of (alginate + mycelium):(0.7M CaCl₂) were tested. The best ratio was 8:10 with 98% viability for *P. tinctorius* and 74% for *P. involutus*. The lowest result found was 1:10 for both fungi. The viability in calcium alginate beads was dependent on the age of mycelium when entrapped, and was different for each fungus. The viability of *P. tinctorius* and *P. involutus* increased with the age of the mycelia until 30 and 40 days, respectively. Viability decreased in older mycelia. The effects of temperature (6°C or 25°C) and the storage conditions (water, filter paper, and 0,07 CaCl₂) were studied at 5 days intervals for 100 days. The viability *P. involutus* was higher than *Pisolithus tinctorius*. Detailed results will be shown and the feasibility of this technique for the production of ectomycorrhizal fungi inocula in our conditions will be discussed.

poster session 8 WF AR 37

KENNEDY ELLIOTT, LINDA, JEAN C. STUTZ & RONALD L. TILLER. Department of Botany, Arizona State University, Tempe, AZ 85287 U.S.A. - Seasonal dynamics of mycorrhizal colonization of *Sporobolus wrightii* in the riparian zone of the San Pedro River, Arizona.

Giant sacaton, *Sporobolus wrightii*, was once a dominant species on floodplain terraces along rivers of southwestern North America. Many restoration projects of riparian ecosystems include reintroduction of this perennial bunchgrass, however, little is known of the ecological requirements necessary to establish and maintain this species. As part of our study of the mycorrhizal ecology of *Sporobolus wrightii*, levels of colonization by arbuscular mycorrhizal fungi were determined at different stages in the annual growth cycle. Roots were collected from two habitats in the riparian zone of the San Pedro River; lower floodplain terraces, where giant sacaton grows as isolated plants; and upper floodplain terraces, where remnants of sacaton grassland still exist. Higher levels of colonization were detected in roots collected from lower floodplains than in roots from the more xeric upper floodplains. Host plants in both environments exhibit high levels of colonization during periods of active vegetative growth.

poster session 9 WF SH 01

KERNAGHAN, GAVIN & R.S. CURRAH. Department of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada. - Russulaceous mycorrhizae of subalpine fir at tree line.

A three year survey of the ectomycorrhizal fungi found at elevational tree line in the Front Ranges of the Canadian Rockies has shown that species of *Russula* and *Lactarius* are conspicuous mycobionts of erect and krummholz forms of *Abies lasiocarpa*. Initial identifications of ectomycorrhizae were based on anatomical features and then confirmed by comparing PCR amplified ribosomal DNA (ITS region) from ectomycorrhizae with that of sporocarp tissue from 12 associated species of Russulaceae. Mantles formed by *Lactarii* (*L. alnicola*, *L. caespitosus* and *L. deliciosus*) exhibit characteristic laticifers and pigments analogous to the associated sporocarp. Those formed by two species of *Russula* (subsection *Emeticinae*) bear distinctive cystidia or sulfovanillin reactive cells. There is little variation between RFLP data from *L. caespitosus* and *L. deliciosus* using *AluI*, *HhaI*, *HinfI* and *RsaI* and no variation between the two species of *Russula*.

poster session 3 ST SH 67

KHAN, ABDUL G., TARIQ M. CHAUDHRY. Department of Biological Sciences, the University of Western Sydney - Macarthur, N.S.W. 2560, Australia. - Effects of metalliferous mine pollution on the vegetation and their mycorrhizal associations at the Sunny Corner - a silver town of 1880's.

Sunny corner, located 200km west of Sydney, was the first place in Australia to smelt silver successfully in the 1880's but now it is reduced to an eroded hillside. Numerous spoil heaps around the complex of mine workings (site 1) were studied for vegetation and their mycorrhizal associations and a comparison was made with an area 5km downstream from the collapsed adit (site 2) and a representative of the original bushland vegetation (site 3). The plant species richness was highest at site 3, intermediate at site 2 and least at site 1. *Baeckea utilis* (Mountain *Baeckea*), was most common at heavy metal polluted spoil heaps and often grew in clumps almost to the water level of the Daylight-Williwa Creek System, which drains the area. Its roots were heavily colonized by VA mycorrhizal infection. Roots of *Eucalyptus dives* and *E.dalrympleana* possessed both VA and EC mycorrhizae. *Acacia dealbata* roots were heavily VA mycorrhizal as well as studded with nitrogen-fixing rhizobial nodules. Among the VAM fungi, *Gigaspora* spp were abundant in the rhizospheres of plants growing at site 1 and 2, whereas rhizospheres of plants growing on site 3 harboured *Glomus* spp.

poster session 1 ST RR 38

KHASA, P.D.¹, W. CHIPCHAR², A. JAHN¹, N. BARKER² & B.P. DANCIC¹. ¹Department of Renewable Resources, University of Alberta, Edmonton, Canada T6G 2H1. ²Pine Ridge Forest Nursery, Smoky Lake, P.O. Box 750, Alberta, Canada T0A 3C0. - Large Scale Screening of Ectomycorrhizal Strains for Commercial Conifers in Alberta

It has long been demonstrated that ectomycorrhizae play an important role in the development and enhancement of plant growth and control of fungal root pathogens. A Microfungus collection of about 40 strains is being assessed for plant growth and/or biological control of fungal root pathogens on five conifers (Lodgepole Pine, Jack Pine, White Spruce, Black Spruce and Engelmann Spruce). The project is set up at Pine Ridge Forest Nursery in Alberta, Canada, according to a robust experimental design. PCR-based markers for ectomycorrhizal DNA fingerprinting are being developed. These markers will then be used for monitoring the persistence of introduced selected beneficial ectomycorrhizal strains by inoculation in forest nurseries. Commercial development of ectomycorrhizal inoculants with the best strains for large scale production and inoculation is also planned.

talk session 2

KJØLLER, RASMUS & ROSENDAHL, SØREN. Department of Mycology, Botanical Institute, University of Copenhagen, Oster Farimagsgade 2D, 1353 Copenhagen K, Denmark. - Arbuscular mycorrhizal fungi in roots from a danish pea field determined by polyacrylamide gel electrophoresis of specific fungal enzymes.

Strains of arbuscular mycorrhizal fungi may differ in their effect on the growth of their host plant, but it is difficult

to identify the fungi occurring in plant roots from fields or natural ecosystems. This identification is important to predict the potential effects of cultural practices and other soil treatments on the mycorrhizal flora. Polyacrylamide gel electrophoresis (PAGE) followed by staining of specific enzymes has proven successful to identify specific arbuscular mycorrhizal fungi in root extracts and to quantify the activity of these fungi. The presence and activity of specific arbuscular mycorrhizal fungi in a Danish pea field were recorded from diagnostic fungal bands in root extracts after electrophoresis. The diagnostic fungal enzymes were identified in the root extracts by comparing with extracts of spores isolated from the same field. The results indicate that the mycorrhizal flora detected in the roots was dominated by a single species *Glomus geosporum*. It was further shown that the mycorrhizal flora did not change during the growing season. A number of other species were also found in the trap cultures and directly in the soil, but none of these could be detected in the roots by the electrophoretic method.

poster session 10 WF SH 22

KLETT, PASCALE¹, JEAN-CLAUDE PIERRAT² & JEAN GARBAYE¹. ¹Laboratoire de Microbiologie Forestière, INRA, F54280 Champenoux. ²Laboratoire de Sciences Forestières, ENGREF, 14 rue Girardet, F54000 Nancy. - Very low inoculum doses of a mycorrhizal helper *Pseudomonas fluorescens* stimulate mycorrhiza formation of *Laccaria laccata* with Douglas fir in a forest nursery

A rifampin-resistant mutant of the mycorrhizal helper bacterium (MHB) *Pseudomonas fluorescens* strain BBc6 was inoculated at four doses (0, 8×10^5 , 8×10^7 and 8×10^9 CFU m⁻²) in a fumigated Douglas fir nursery bench at sowing, combined with two inoculum doses of the ectomycorrhizal fungus *Laccaria laccata* S238N (0.05 and 0.1 g m⁻² DW mycelium entrapped in an alginate beads at the constant dose of 1 liter m⁻²). Five months later, the introduced MHB was still detected in the soil by enrichment and selective plating on rifampin medium only in the plots with the highest bacterial inoculation dose; another experiment under similar conditions even showed that the bacterial population was under the detection level (10² CFU g⁻¹ DW soil) as soon as two weeks after inoculation. At five months, the % short roots mycorrhizal with *L. laccata* was significantly improved by the bacterial inoculation at both low and high mycelium doses (44 to 70 and 64 to 76%, respectively) and the lowest bacterial dose was as effective as the highest. These results validate in the field previous ones obtained in the glasshouse by the same authors: the MHB BBc6 acts at an early stage during the course of mycorrhizal establishment (at a time when the first mycorrhizas are not yet formed, suggesting that it stimulates mycelial growth) and at a low population density. They also indicate that co-inoculating an helper bacterium and an ectomycorrhizal fungus is an effective way of optimizing controlled mycorrhization techniques by reducing the quantity of the fungal inoculum needed:

more mycorrhizas (70%) were obtained with 0.05 g m⁻² DW mycelium plus the MHB than with twice this fungal dose and no bacterium (64%).

poster session 5 ST AR 22

KLING, MONICA¹, VIVIENNE GIANINAZZI-PEARSON², JEANINE LHERMINIER³ & IVER JAKOBSEN⁴. ¹University of Agricultural Sciences, Department of Microbiology, S-750 07 Uppsala, Sweden. ²Laboratoire de Phytoparasitologie and ³Electron Microscopy Service, INRA, F-21034 Dijon, France. ⁴Environmental Science and Technology Department, Risoe National Laboratory, DK-4000 Roskilde, Denmark. - The development and functioning of mycorrhizas in pea mutants

Performance of mycorrhizas was studied in two mutants and the wild type of *Pisum sativum* cv. Finale (Engvild, 1987). An early mutant (RisNod9) blocked mycorrhiza formation at the appressorium stage, while in a late mutant (RisNod24) the fungus colonized the root cortex, but formed no true arbuscules. The aim was to study if nutrient exchange between the symbionts occurred despite the lack of arbuscules. The three pea genotypes were grown in association with *Glomus intraradices* and root colonization, fungal SDH activity, growth of external hyphae and potential fungal ³²P transport were measured. The ³²P was applied to a soil compartment which could be accessed by hyphae but not roots. Root colonization was 0, 18 and 90 % in the early mutant, late mutant and wild type, respectively. Fungal SDH activity was absent in the cortex of the late mutant, but present in 83% of the colonized root length of the wild type. Neither external fungal mycelium nor fungal ³²P transport could be detected in the two mutants which accordingly contained less total phosphorus than the wild type. This work indicates that despite the fungal ability to colonize the late mutant roots there was an insufficient host-fungus C transport to support the growth of an external mycelium. These results together with the observed growth patterns of the two mutants suggest that the late mutant is suitable as a control treatment in experiments on arbuscular mycorrhizas.

talk session 2

KLIRONOMOS, JOHN¹ & MICHAEL F. ALLEN². ¹Department of Botany, University of Guelph, Guelph, ON, Canada N1G 2W1. ²Department of Biology, San Diego State University, San Diego, CA 92182, U.S.A. - Arbuscular mycorrhizal fungi within food-webs and their responses to a globally changing environment

Soil microbes are fed primarily by root-derived substrates, fulfill functions such as mineralization, immobilization, decomposition, pathogenicity, and improvement of plant nutrition, and form the base of the below-ground food web. Below-ground processes have generally been monitored using a black-box approach, thereby ignoring effects of global change at a finer level of resolution. In a series of experiments using a variety of

host plants from different ecosystems we have found that some perturbations, such as elevated atmospheric CO₂, shift below-ground carbon flow to a mutualistic-closed, mycorrhizal dominated system, whereas others, such as nutrient fertilization and UV-B radiation, shunt it towards a more opportunistic-open, saprobe/pathogen dominated one. This indicates that such perturbations may lead to far less predictable feedback patterns than previously thought, and that qualitative shifts in the soil community may be far more important than mere changes in total C sink strength.

poster session 2 ST SH 29

KLOPATEK, CAROLE COE¹, G. M. LOVETT², P. DELLA-BETTA³, K. JOHNSON-EDMONDS³, J. SCHMIDT³, E. ZADDY⁴, & M. SHACHAK⁴. ¹USDA Forest Service, Dept. of Microbiology, ASU, Tempe, AZ 85287-2701. ²Inst. of Ecosystem Studies, Box AB, Milbrook, NY 12545. ³Dept. of Microbiology, ASU, Tempe, AZ 85287. ⁴Mitrani Center for Desert Ecology, Ben Gurion Univ of the Negev, Sede Boqer, Israel 84993. - Mycorrhizae and Soil Resource Distribution in the Negev Desert, Israel

This study is examining the role of vegetation patchiness in controlling ecosystem function in the arid lands of the Negev Desert, Israel. We are examining soil resources, including mycorrhizae, bacteria, fungi, N and C along an environmental rainfall gradient in the Negev, ranging from 50 mm to 300 mm per y. Due to the patchy distribution of the vegetation of this area, the capture and redistribution of resources such as water, N, C and mycorrhizae differ significantly between canopy and openspaces (interspaces). We have found that over 80% of all plant species are mycorrhizal along the gradient, but distribution of mycorrhizae between patch type differs by over 50%. In contrast, during prolonged drought, there does not seem to be a large difference in other microbes between individual patch types. It appears that the primary mechanisms for distribution of mycorrhizae are wind and animals. N deposition to the soil is enhanced approximately six-fold under shrub patches relative to the interspace areas. Presumably, most of this enhancement is due to recycling of N within the patch type. This is partially confirmed by the greater microbial populations under the canopies compared to the interspaces.

poster session 13 WF SH 44

KOBAYASHI, HISAYASU¹, KYOKO HATANO² & NAOHIKO SAGARA¹. ¹Life web, Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606-01, Japan. ²Faculty of Integrated Human Studies, Kyoto University, Kyoto 606-01, Japan. - Ultrastructure of the mycorrhizas formed between *Entoloma clypeatum* f. *hybridum* and *Rosa multiflora*

Mycorrhizas formed between *Entoloma clypeatum* f. *hybridum* and *Rosa multiflora* in the field of Japan were studied by light and electron microscopy. The mycorrhizas with fungal sheath were cylindrical to clavate, up to 4 mm in length and 1-2 mm in diameter.

The root cap, the apical meristem and the apical region of the cortex appeared to be destroyed by the invading hyphae, which branched finger-like and reached the endodermis. The cortex was undestroyed at the basal region of the mycorrhiza. The hyphae surrounding the tip of the stele collapsed. The hyphae reaching the endodermis contained many mitochondria, cisternae of endoplasmic reticulum and transitional vesicles. An electron-dense matrix was observed in the interface between the tips of the invading hyphae and the endodermal cells. Also, a thin layer of more electron dense material than the endodermal cell wall was observed on the outer surface of the endodermis. These observations may suggest a mycorrhizal relationship in which hyphal invasion is defended at endodermis.

poster session 14 WF RR 02

KÕLJALG, URMAS. Inst. of Zoology and Botany, 181 Riia St., EE2400 Tartu, Estonia. - On the systematics and phylogeny of *Tomentella* (Thelephorales, Basidiomycota) and related genera.

The number of taxa described depends very much on the concepts and criteria which have been used for delimitation of taxa. In this study a typological species concept, based on morphological criteria has been used to develop a practical species standard. Practical species standard is a model which includes instructions and methods to be used for distinguishing species. The application of this standard has been found to be very useful. In tomentelloid fungi 60 species were recognized instead of more than one hundred. It is possible that the application of other species standards based on biological or genetical species criteria may change the number of species presented in this study. A practical genus standard was developed to distinguish natural groups of tomentelloid fungi. Like the species standard, it includes certain methods and instructions. Phylogenetic (cladistic) method was used in a search for monophyletic groups (genera) inside the tomentelloid fungi and only monophyletic genera, supported at least by one analysis, are recognized. As expected, the monotypic genera based on one very distinct (autapomorphic) character, fell into other genera and the largest genus, with over 40 species, was divided into two monophyletic groups. The use of clear species and genus practical standards seems to be very important in taxonomic studies because: 1) all taxa will be treated by a taxonomist equally; 2) a taxonomist has to work more deeply with methodology; 3) stabilized standards approved by most taxonomists will make classifications more stable and communication between the researches more fruitful.

poster session 13 WF SH 87

KOTTKE, INGRID. Eberhard-Karls-Universität Tübingen, Botanisches Institut, Spezielle Botanik, Mykologie und Botanischer Garten, Auf der Morgenstelle 1, D-72076 Tübingen, Germany. - Fungal adhesion pad formation and penetration of root cuticle in early stage *Picea abies*-*Laccaria amethystea* mycorrhizas.

Primary events during establishment of fungus-root symbiosis in ectomycorrhizas are still little understood. No attention has been paid so far to the meaning of the adhesion of hyphae to the root cuticle and the penetration of this barrier, although the importance of the cuticle has been shown for pathogen-plant interactions. Early developmental stages of *in vitro* mycorrhization of *Laccaria amethystea* on *Picea abies* obtained after short time incubation in growth chambers at elevated concentration of CO₂ were studied by light and transmission electron microscopy. No structural changes in mycorrhization related to elevated CO₂ were found. Prominent adhesion pad formation at the hyphal tips in contact with the root cuticle was observed. The adhesion pad was connected to the outer cell wall layer of the hypha and was stained by the Swift-reaction for cysteine rich proteins. The root cuticle was dissolved and penetrated by fungal tips of the fingerlike branching mycelium attached to the root surface. The possibility of local restriction of the dissolution to the part of the cuticle covering cell junctions is discussed. Results are compared with well documented pathogenic fungus-plant interactions at the cuticle and are discussed in respect to previously described early events in ectomycorrhizas.

poster session 2 ST SH 17

KOVALENKO, ALEXANDER. Laboratory of Systematics and Geography of Fungi, Komarov Botanical Institute of the Russian Academy of Sciences, 2 Prof. Popov St., St. Petersburg, 197376, Russia. - Coenology of ectomycorrhizal fungi in relation to strategy of their conservation

Near half of all species diversity of ectomycorrhizal (ECM) fungi is included in Red Data Lists (RDL) published in different countries. It is the highest share in comparison to any other functional group of fungi. ECM fungi indeed need a special attention because of their important role both in ecosystem dynamics and in economics (harvesting of edible mushrooms, etc.). Besides, a coenological peculiarities of ECM fungi must be taken into account as well. Being strongly connected to their phytobionts and following ecosystem dynamics, ECM fungi form in every forest zone a particular species composition. An essential part of this potential species diversity can be expressed by their fruitification only in sites of natural virgin forests that are in last stages of succession (near climax). The species of climax communities usually are considered as the first of all candidates for RDL, but after restoration of zonal vegetation on the territory their fruitification comes to norm. There are two outcomes may be applied for conservation aim: (1) protection of the most rare and threatened ECM fungi included in RDL is feasible only in the whole fungal groupings, that are an integral component of natural forest ecosystems; (2) several species of ECM fungi (or even some subgenera or genera on the whole) can serve as bioindicators of natural and virgin forests. In the work presented *Hygrophorus* species are suggested as possible candidates for monitoring and estimating biodiversity in the north-western Russia.

poster session 5 ST AR 23

KRAIGHER, HOJKA¹ & DAVID E. HANKE². ¹Slovenian Forestry Institute, Vecna pot 2, 1000 Ljubljana, Slovenia. ²Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge, CB2 3EA, UK. - Cytokinins in needles and exudates of mycorrhizal spruce seedlings.

Spruce seedlings (*Picea abies* (L.) Karst) were grown on a support in synthetic liquid medium with or without the mycelium of *Laccaria bicolor* (Maire) Orton S 004. Purified needle extracts and culture filtrates were analysed by a combined HPLC/ELISA method, adapted for best separation of the isopentenyladenine (iP) -type of cytokinins. Cytokinin contents in needles of spruce seedlings, inoculated with *Laccaria bicolor*, showed an approximately twofold increase in iP-Ribotide, iP-9Glucoside, iP_o and iP_A***. For cytokinins in culture filtrates statistically significant differences were observed as higher levels of cytokinins in dual cultures in comparison to control seedlings (in all four types) and also to control fungi (for iP-Ribotide and iP). Therefore the colonisation of seedlings with mycorrhizal fungi can affect the host plant cytokinin levels. The influences do not correspond directly to the production of cytokinins by ectomycorrhizal fungi in pure cultures, however fungal cytokinin production can be influenced by the presence of the host seedling.

talk session 7

KRETZER, A., Y. LI, T. SZARO & T. BRUNS. University of California at Berkeley; Department of Environmental Science, Policy and Management; 151 Hilgard Hall; Berkeley, CA 94720-3110; USA. - Internal transcribed spacer sequences from 41 recognized species of *Suillus* sensu lato: phylogenetic and taxonomic implications.

Members of the genus *Suillus* sensu lato exhibit a high degree of specificity for ectomycorrhizal hosts mostly in the Pinaceae which makes them particularly suitable to study evolutionary phenomena such as host shifts and/or cospeciation. Phylogenetic studies are also required to clarify the confused state of taxonomy in this ecologically and economically important group of organisms that includes *Suillus* sensu stricto, *Boletinus*, *Fuscoboletinus*, and *Gastrosuillus*. We have sequenced internal transcribed spacers from the nuclear ribosomal repeat of 54 isolates belonging to 41 recognized species of *Suillus* sensu lato and analyzed the sequence data using both parsimony and distance methods. Although deep branches in *Suillus* sensu lato were not strongly resolved, our best phylogenetic estimate suggests that larch association is primitive within *Suillus* sensu lato and that all host switches to other genera such as pines or Douglas-fir have occurred only once. Our results strongly show that neither *Boletinus*, *Fuscoboletinus*, nor *Gastrosuillus* represents a monophyletic group and that all three genera should be collapsed into *Suillus* (sensu lato).

poster session 12 WF SH 57

KYTÖVIITA, MINNA-MAARIT & KRISTINA ARNEBRANT. Microbial Ecology, Ecology Building, Lund University, S- 223 62 Lund, Sweden. - Effect of simulated herbivory and nitrogen and phosphorus availability on carbon and nitrogen translocation in mycorrhizal birch seedlings.

Mycorrhizal associations were synthesised between *Betula pendula* and *Paxillus involutus*. When 8.5 months old the seedlings were transferred into polystyrene observation chambers (25 x 25 x 2.5 cm) filled with peat:sand (2:1) and they were allocated into three different nutrient treatments over a 6 week period (1): 1 mg N and 0.15 mg P, (2) 7 mg N and 0.15 mg P, and (3) 7 mg N and 1 mg P. The N was added as NH_4NO_3 and P as Na_2HPO_4 , all plants received a total of 20 ml of Ingestad nutrient solution in increasing doses during the growth period. Herbivory was simulated by cutting half of each leaf in the end of the growth period. Three days after cutting the plant shoots were enclosed into chambers into which 9.2 μg ^{14}C was released as CO_2 (1.5 MBq) and 1 mg of ^{15}N as $(\text{NH}_4)_2\text{SO}_4$ was added exclusively to the mycelium. The plants were harvested three days after labelling. The relative distribution of ^{14}C and ^{15}N showed that only 5% of ^{14}C remaining (structural C) in the whole plant was allocated into the roots during the chase, cutting the leaves had no effect. In contrast, translocation by the fungal mycelium was reduced by 95% by defoliation in treatments where N was added in excess (trt 2 and 3), however when N was added in balance with other nutrients (trt 1), defoliation did not affect ^{15}N translocation from the fungus to the roots and shoots.

poster session 1 ST RR 40

LAATIKAINEN, TARJA¹, MARJA-LIISA JUNTUNEN² & HELVI HEINONEN-TANSKI¹. ¹Department of Environmental Sciences, University of Kuopio, P.O.Box 1627, 70210 Kuopio, Finland. ²Finnish Forest Research Institute, Suonenjoki Research Station, Juntintie 40, 77600 Suonenjoki, Finland. - The ectomycorrhizal status of container seedlings of Scots pine (*Pinus sylvestris* L.) treated with fungicides.

The ectomycorrhizal status of container-grown Scots pine (*Pinus sylvestris* L.) seedlings treated with fungicides was examined after the first growing season. Seedlings were grown in paper containers filled with low-humified sphagnum peat according to normal forest nursery practice. 12 weeks after sowing seedlings were taken out from greenhouse and the first fungicide application was performed. Fungicide applications were repeated four more times at interval of 2-3 weeks before root samples were taken. The first test group of seedlings was treated with chlorothalonil (Bravo 500) and the second with propiconazole (Tilt 250 EC). The third test group was first treated three times with chlorothalonil and then two times with propiconazole. The amounts of chlorothalonil and propiconazole in every application were 2 000 g/ha (a.i.) and 125 g/ha (a.i.), respectively, which were those recommended to forest nurseries. There was also a

control group which was not treated with fungicides at all. Percent of ectomycorrhizal infection (% of total root tips) was the lowest in non-treated control group (8.1 %) and the highest in propiconazole group (10.7 %), but the difference was not significant. Whereas, the number of total root tips per root dry weight was significantly higher in chlorothalonil group than in control or propiconazole groups due to lower root dry weight in chlorothalonil group.

poster session 4 ST AR 56

LANFRANCO, LUISA¹, M. VAN BUUREN², S. LONGATO¹, L. GARNERO¹, M.J. HARRISON² & P. BONFANTE¹. ¹Dipartimento di Biologia vegetale dell'Università and CSMT-CNR, V.le Mattioli 25, Torino 10125, Italy. ²The Samuel Roberts Noble Foundation, 2510 Highway 199 East, Ardmore, Oklahoma 73402, U.S.A. - Chitin synthase genes in arbuscular mycorrhizal fungi (*Glomus versiforme* and *Gigaspora margarita*)

The molecular mechanisms that control morphogenesis of arbuscular mycorrhizal (AM) fungi and the switch from the non-symbiotic towards the symbiotic phase are largely unknown. Since understanding the mechanisms of chitin synthesis may offer important information on AM morphogenesis, we investigated the occurrence of chitin synthase (*chs*) genes in two AM species: *Glomus versiforme* and *Gigaspora margarita*. Degenerated primers, designed on conserved amino acid sequences, were used in PCR experiments on genomic DNA extracted from spores. Cloning and sequencing of the amplified products led to the identification of two *chs* homologs for both species. In order to analyse their expression, RT-PCR assays were set up using specific primers designed on the nucleotide sequence. Preliminary results performed on the longer PCR clone obtained from *G. versiforme* indicate a low level of gene expression which is found in developing sporocarps and in colonized roots. In order to isolate the whole gene, genomic libraries from both species were constructed in lambda DASH vector (Stratagene). To assess the quality of these libraries, we determined the average size of the inserts and the frequency of ribosomal genes. In conclusion, our data demonstrate the presence of different *chs* genes in the genome of AM fungi, which may suggest specific expression and functions of these genes during different phases of the fungal life cycle.

poster session 10 WF SH 10

LARSEN, JOHN¹, PAUL HENNING KROGH², IVER JAKOBSEN¹. ¹Plant Nutrition, Environmental Science and Technology Department, Risø National Laboratory, DK-4000 Roskilde, Denmark. ²National Environmental Research Institute, Department of Terrestrial Ecology, P.O. Box 314, Vejløvej 25, DK-8600. - Antagonism by arbuscular mycorrhizal fungi towards Collembola

The external mycelium of arbuscular mycorrhizal fungi (AMF) constitutes an important part of the fungal mycelium in soil covered by herbaceous plants, and may

serve as a food source to mycophagous soil animals. We examined the interactions between the external mycelium of AMF and Collembola (Insecta) by means of a compartmented growth system with root-free soil compartments. Interactions between AMF mycelium and Collembola were multiple. The presence of mycelia of *Glomus caledonium* and *Glomus intraradices* markedly reduced Collembola numbers in root-free soil to levels, which were 2-3 orders of magnitude lower than Collembola numbers in the absence of a mycorrhizal mycelium or in the presence of a *Glomus invermaium* mycelium. The high numbers of the Collembola *Folsomia candida* in the presence of *G. invermaium* mycelium coincided with a reduced hyphal length of this fungus and tended to reduce its P transport. The results from this work suggest that the external mycelium of some AMF may antagonize Collembola by unidentified mechanisms.

morning talk

LE TACON, FRANÇOIS. Equipe de Microbiologie Forestière, INRA-Nancy, 54280 Champenoux, France. - Nursery and field response to ectomycorrhizal inoculation of forest trees in western Europe.

It is essential for afforestation or reforestation to produce tree seedlings with efficient mycorrhizal associations enhancing tree growth and survival after transplantation. However, ectomycorrhizal deficiencies are frequent in forest nurseries, as different practices inhibit mycorrhizal development. Techniques of ectomycorrhizal inoculation of bare-root seedlings in classical nurseries after soil fumigation, or for the production of inoculated containerized seedlings on artificial substrate are now available. Sixty field experiments are presently available in western Europe. In almost all of them, several years after outplanting, there is an effect, positive or negative, of the mycorrhizal status inherited from the nursery phase. To improve field responses quantitatively and to enlarge the number of sites where a positive response can be obtained, efforts should be concentrated in three areas: the competitive ability, the efficiency and the stability of the artificially introduced fungi. The differences in effectiveness and competitiveness between fungal species and strains is the basis of practical application. A major problem is the delineation and management of stable strains, and the recognition of these strains in field situations.

Even without sexual reproduction, ectomycorrhizal strains often present variations which could be of epigenetic or genetic origin. Moreover, most of the time, introduced and natural strains produce meiotic spores. The consequence is that introgression of the introduced genes in the local population is possible.

Competition and stability studies are now possible in long term experiments with new methods such as RFLP and PCR, gene sequencing and DNA probes.

poster session 2 ST SH 18

LEACOCK, PATRICK R. & DAVID J. MCLAUGHLIN. Department of Plant Biology, University of Minnesota, St. Paul, MN 55108, USA. - The diversity of ectomycorrhizal fungi in mature and old-growth stands of two Minnesota forest types.

To obtain baseline data on the diversity of fungi of native, undisturbed forests in Minnesota, studies were begun in 1993 in young and old-growth stands of red pine and northern hardwood-conifer forest. Epigeous members of genera and families containing potential ectotrophic mycorrhizal fungi are the focus of this study because of project constraints and because dramatic declines of these fungi have been reported in parts of Europe. Quantitative sampling, using dispersed circular sampling areas along permanent transects, is documenting high fungal species richness for most stands, with a total of 235 species in red pine, 110 in northern hardwood-conifer. Mushroom density ranged from an estimated 200 to 34,900 fruitbodies per hectare. Cortinariaceae and Russulaceae are the most diverse groups for the red pine stands while in northern hardwood-conifer the Entolomataceae and Hygrophoraceae have greater species diversity. There is little overlap in species composition among the two forest types and differences exist between old-growth and younger stands of the same forest type. About 65% of the more than 300 species will be new state records including several undescribed species. Contributions will be made to knowledge of the region's flora, baseline data for monitoring, and demonstration of the importance of old-growth forest conservation.

morning talk

LEAKE, JONATHAN DAVID READ & SHEENA MCKENDRICK. Department of Animal & Plant Sciences, University of Sheffield, Sheffield S10 2TN U.K. - Myco-heterotrophy as a way of life.

The extreme mycorrhizal dependency of myco-heterotrophic plants has entailed the evolution of some remarkable modifications in plant form and function. The most important of these is the production of minute seeds with undifferentiated embryo which dictates the requirement for carbon to be supplied by a fungal partner from the earliest stages of germination. Extreme microspermy appears to be synonymous with myco-heterotrophic germination. The production of vast numbers of seeds, their characteristic shape, size and structure in myco-heterotrophs presents one of the most striking examples of convergent evolution between distantly related families in both dicotyledons and monocotyledons. By studying *in situ* germination of seeds of myco-heterotrophs in their natural environments we are observing, often for the first time, the sequence of events preceding germination and the subsequent cryptic development of these plants. These studies are indicating the duration of dormancy, the processes of germination and infection and providing clues as to the specificity and identity of the fungi involved. We report the nature of the largely undescribed mycorrhizal infection and root anatomy of a number of Neotropical myco-heterotrophs

including members of the Gentianaceae, Triuridaceae and Burmanniaceae. These studies are helping us to understand myco-heterotrophy as a way of life.

poster session 2 ST SH 19

LEBEL, T.¹ and M.CASTELLANO². ¹Botany and Plant Pathology Dept., Oregon State University, Corvallis OR, 97331 USA. ²USDA Forest Service, PNW Research Station, 3200 Jefferson Way, Corvallis, OR, 97331 USA. - The Diversity and Abundance of Sequestrate Russulaceae of Australia and New Zealand.

Until recently the sequestrate fungi of Australia and New Zealand have received little attention. Currently only 7 species of sequestrate Russulaceae have been described from this region. Recent forays and taxonomic work show this number to be an under representation of the sequestrate Russulaceae flora. Sequestrate Russulaceae from Australia and New Zealand collected from 1989-1995, were critically examined and new taxa differentiated. The majority of these fungi are specific to either Eucalyptus or Nothofagus, with no overlap in species. Most New Zealand taxa are endemic Nothofagus associates [*N.fusca*, *N.menziesii*, *N.solandrii*]. Distinct differences between eastern and western Australian species are also apparent.

poster session 2 ST SH 20

LEBEL, TERESA¹, M.CASTELLANO² & J.TRAPPE². ¹Botany and Plant Pathology Dept., Oregon State University, Corvallis OR, 97331 USA. ²USDA Forest Service, PNW Research Station, 3200 Jefferson Way, Corvallis, OR, 97331 USA. - The diversity and distribution of sequestrate fungi from Australasia.

Until recently the sequestrate fungi of Australasia have received little attention. From 1989-1995 we extensively collected sequestrate fungi from diverse habitats throughout Australasia, described new taxa, revised existing genera, and thereby expanded considerably our knowledge of this diverse group of fungi. We recognize 12 families and 44 genera of sequestrate Basidiomycetes and 18 genera of sequestrate Ascomycetes from Australia and New Zealand. The majority of these fungi fruit with either Eucalyptus or Nothofagus, with no overlap in mycorrhizal host association. Distinct differences in mycota between eastern and western Australia reflects differences in species of Eucalyptus and the absence of Nothofagus from Western Australia.

poster session 13 WF SH 88

LEFEVRE, CHARLES K¹, RANDY MOLINA², & LEWIS H. MELVILLE³. ¹Department of Forest Science, Oregon State University, Corvallis, OR, USA 97331. ²USDA Forest Service PNW Research Station, Corvallis, OR, USA 97331. ³Department of Botany, University of Guelph, Guelph, Ontario, Canada N1G 2W1. - Morphology of *Tricholoma magnevelare* ectomycorrhizae on several host genera.

Tricholoma magnevelare (Peck) Redhead, the 'American matsutake', is an important commercially harvested edible mushroom in regions throughout western North America. Unlike its relative, *Tricholoma matsutake* (S. Ito et Imai) Singer of Japan, little is known about its mycorrhizal ecology. To facilitate study of *T. magnevelare* in the field and to explore the functional nature of its symbioses, we have begun a morphological characterization study of its ectomycorrhizae. Macroscopic similarities with the ectomycorrhizae of *T. matsutake* are evident upon initial examination. Studies of *T. matsutake* and related species have shown morphological characteristics atypical of ectomycorrhizae including some that suggest a degree of parasitism. Macro- and microscopic characters of *T. magnevelare* ectomycorrhizae will be compared on several genera of hosts. These results will be part of a larger program to investigate the ecological functions and management requirements of *T. magnevelare*.

poster session 3 ST SH 69

LILLESKOV, ERIK A. & TIMOTHY J. FAHEY. Department of Natural Resources, Cornell University, Ithaca, N.Y., 14850, U.S.A. - Patterns of ectomycorrhizal diversity over an atmospheric nitrogen deposition gradient near Kenai, Alaska.

Nitrogen (N) availability may be an important factor influencing the abundance, diversity and function of ectomycorrhizal fungi (ECMF). Understanding ECMF responses to N is critical not only because of natural variation in N availability, but also because anthropogenic N deposition has altered N availability in forest soils. Recent evidence suggests that declining production and diversity of ECMF fruitbodies in Europe is correlated with multiple air pollutants, including atmospheric N deposition. The objective of this study was to determine the effect of N deposition on ECMF of white spruce (*Picea glauca*) over a short, steep N deposition gradient in Alaska. Forests there have been receiving high N inputs from an NH₃ production facility for 28 years, but nearby background levels are quite low (~2 kg ha⁻¹ yr⁻¹) We expected lower ECMF abundance and diversity near the NH₃ source. Sampling focused on ECMF species abundance, tree nutrition and growth, plant community composition, precipitation chemistry and soil chemistry. High precipitation N inputs near the NH₃ source were associated with high levels of soil mineral N availability, foliar N:P and N:cation ratios, and grass cover; and lower pH and moss cover. ECMF species diversity was lower at the high N sites. Four common ECMF species showed no change or an increase at the high N sites: *Laccaria laccata*, *Laccaria bicolor*, *Lactarius theiogalus*, and *Paxillus involutus*. Abundance of fruitbodies was negatively correlated with organic horizon mineral N for the following genera: *Cortinarius* ($r^2=0.87$), *Russula* ($r^2=.75$), *Tricholoma* ($r^2=.72$) and *Hebeloma* ($r^2=.61$). The two most species rich genera, *Cortinarius* and *Russula*, declined dramatically in both abundance and diversity with increasing N deposition. These results are consistent with those of N fertilization experiments and with studies of regional patterns of decline in Europe.

poster session 12 WF SH 25

LINDERMAN, R. G. & E. A. DAVIS. USDA-ARS, Horticultural Crops Research Laboratory, 3420 NW Orchard Ave., Corvallis, OR 97330, USA. - Effects of organic substances or extracts on VA mycorrhizae.

The influence of soil organic materials on VA mycorrhizal (VAM) fungi has not been explored. Organic soil amendments, especially humic-rich substances, often stimulate plant growth, possibly due to stimulation of VAM fungi. On the other hand, growth media with high peat moss content have been reported to inhibit VAM formation. We investigated the interactions between VAM fungi and humic-rich composted organic materials or different peat mosses on VAM formation and function on onions grown in P-limiting soil. Solid or water extracts of humic amendments greatly stimulated growth compared to the unamended controls. Addition of VAM fungi in combination with humic extracts caused further growth enhancement but without increasing the level of VAM colonization. Pre-heating the soil to various degrees (50, 80, or 100 C) to eliminate microbes had no effect, suggesting that the additive growth enhancement was due to improved nutrition (presumably P uptake). Addition of 5-30% by volume of sphagnum peat to soil suppressed colonization levels by some VAM fungi but not others. A comparison of different peats showed that some had a suppressive effect, while others did not. Suppressed colonization levels, however, did not always cause reduced plant growth enhancement due to VAM. These studies suggest that VAM fungi can be influenced significantly by organic components of soil or growth media, but the response is both substance and fungus specific.

talk session 8

LINDERMAN, R. G., J. L. MARLOW, & E. A. DAVIS. USDA-ARS, Horticultural Crops Research Laboratory, 3420 NW Orchard Ave., Corvallis, OR 97330, USA. - Contribution of microbial associates of VA mycorrhizae to mycorrhiza effects on plant growth and health.

A number of soil microorganisms can affect the growth and health of plants. Some of them have been shown to positively interact with VA mycorrhizae (VAM) when dual inoculation of plants is made. These findings suggest that growth enhancement of plants by VAM fungi could be the result of combined effects of the VAM and some of the microbial associates. We investigated this hypothesis by isolating antagonistic bacteria (shown in other studies to inhibit several fungal root pathogens and stimulate seedling plant growth) from the mycorrhizosphere soil of onions. Pot cultures were established with onions inoculated with the VAM fungus *Glomus intraradices* (GI) alone, bacterial antagonists alone, GI + bacterial antagonists, and no organisms (control). Onion growth in the pot cultures was enhanced, compared to the control, by GI and bacterial antagonists alone, but most by the combination of GI + bacterial antagonists. A microbial slurry of rhizosphere soil from each pot culture was used to inoculate potting

medium for growing snapdragon seedlings prior to inoculation with the root rot pathogen *Pythium irregulare*. Root rot disease was suppressed by the slurry from pot cultures inoculated with VAM alone and bacterial antagonists alone, but most from that inoculated with GI + bacterial antagonists. These results support the hypothesis that microbial associates of VAM function in tandem to enhance the growth and health of plants.

poster session 4 ST AR 58

LIU, HENRY and MARIA, HARRISON. Samuel Roberts Noble Foundation, Plant Biology Division, Ardmore, OK 73402, U.S.A. - Nutrient transporters in *Medicago truncatula*-*Glomus versiforme* mycorrhizal interaction.

A sugar transporter from *Medicago truncatula* (Harrison, M.J. 1996 *Plant J.* 9:491-503) and a phosphate transporter from *Glomus versiforme* (Harrison, M.J., and van Buuren, M.L. 1995. *Nature* 378:626-629) have recently been cloned. We are now taking an immunological approach to determine the subcellular localization and distribution of these transporters in the mycorrhizal root. Fusion proteins of β -galactosidase and the loop regions between transmembrane helices 6 and 7 of both transporters have been expressed in *Escherichia coli*. These fusion proteins have been purified by affinity column chromatography and used to raise polyclonal antibodies. The immune serum has been purified by affinity chromatography and for both transporters, antibodies specific to the loop region have been obtained. We are currently testing the antibodies against strains of *Saccharomyces cerevisiae* and methylotrophic yeast *Pichia pastoris* expressing the transporters. This will enable us to determine whether the antibodies recognize the whole transporter proteins prior to immunolocalization experiments.

talk session 7

LOBUGLIO, KATHERINE F. & JOHN W. TAYLOR. University of California, Berkeley CA 94720. - Phylogenetic origins of the asexual mycorrhizal symbiont *Cenococcum geophilum* Fr. and other mycorrhizal fungi among the ascomycetes.

Nucleotide sequence data of the 18S rRNA gene for five *C. geophilum* isolates, three *Elaphomyces* species, and 44 additional genera of ascomycetes were included in the phylogenetic analyses. Percent similarity among the 18S rDNA sequences of the *C. geophilum* isolates examined was 99.8% to 100% indicating that *C. geophilum* is monophyletic. Percent similarity of nucleotide sequence among the three *Elaphomyces* species was also high and ranged from 99.4% to 99.5%. DNA parsimony and distance analysis of the sequence data separated these two genera on distant clades when sequence from 44 additional genera of ascomycetes was included. Parsimony and distance analyses positioned *C. geophilum* as a basal, intermediate lineage between the two Loculoascomycete orders, the Pleosporales and the Dothidiales, and strongly supported *Elaphomyces* to be of Plectomycete origin. Among the sexual Ascomycetes

examined, which included representative taxa from four classes of filamentous Ascomycetes (Plectomycetes, Pyrenomycetes, Discomycetes, and Loculoascomycetes), no close sexual relative to *C. geophilum* was identified. At least four independent lineages of mycorrhizal fungi were identified among the ascomycetes examined.

poster session 4 ST AR 62

LORILLOU, SOPHIE^{1,2}, FRANCIS MARTIN² & ROGER FINLAY^{1,3}. ¹Department of Microbial Ecology, University of Lund, Ecology Building, S-223 62 Lund, Sweden. Equipe de Microbiologie Forestière-INRA, Nancy, 54280 Champenoux, France. ³Department of Forest Mycology & Pathology, Swedish University of Agricultural Sciences, Box 7026, S-750 07 Uppsala, Sweden. - Effect of inorganic and organic N sources on the transcript levels of NADP-GDH in different *Laccaria* strains.

Root systems of forest trees are often associated with numerous ectomycorrhizal fungi possessing different abilities to use the various forms of N (NH_4^+ , NO_3^- , amino acids, proteins) present in soils. It is tempting to speculate that this assemblage of ectomycorrhizal associates optimises the simultaneous acquisition of different soil N sources by the host tree. NADP-GDH is a key enzyme in the primary assimilation of NH_4^+ but little is known about its regulation in ectomycorrhiza, especially in situations where organic N is the primary source of nitrogen. The regulation of NADP-GDH was studied in *Laccaria* strains grown on different N sources. A full length cDNA encoding NADP-GDH was isolated by screening a λ gt11D library of the ectomycorrhizal basidiomycete *L. bicolor* S238N and was used to estimate the levels of NADP-GDH transcripts in strains of *Laccaria* grown on different N sources. NADP-GDH transcripts were identified in all *L. bicolor* and *L. laccata* strains tested but the transcript levels were strikingly different between strains, suggesting large intraspecific variation in the use of this metabolic pathway. In two strains of *L. bicolor* with poor (S238N) and intermediate (CRBF 569) proteolytic activity NADP-GDH expression is regulated by the N source present in the medium. In *L. bicolor* S238N transfer of mycelium from a NH_4^+ medium to either a NO_3^- medium or N-depleted medium induced an increase of the transcript levels of the NADP-GDH. When the mycelium of *L. bicolor* CRBF 569 was grown in medium containing BSA or gliadin as the sole N source the transcript level of NADP-GDH was similar to that obtained in NH_4^+ medium, suggesting that the enzyme is involved in the re-assimilation of NH_4^+ produced during protein degradation.

poster session 1 ST RR 42

LOVATO, PAULO EMILIO, SADI CASSOL & LUCIR LOCATELLI. Departamento de Engenharia Rural, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, CEP 88040-900, Brazil. - Effects of arbuscular mycorrhiza on survival and growth

of two micropropagated apple rootstock clones for transplantation into regions of acid soils.

This study is part of a project which intends to investigate the viability of using the association of micropropagation and mycorrhizal fungi inoculation in the production of vigorous apple, virus-free rootstocks for transplantation into nurseries with soils of low pH. The aim of this study was to evaluate the effects of mycorrhization on the survival and growth of micropropagated plantlets during the acclimatization period using a substratum with low pH. Microcuttings of apple rootstock clones were transplanted to a mix of soil, calcinated rice hulls and sand (4:2:3.v/v/v) under greenhouse conditions. Apple rootstocks (M9 and Marubakaido) were grown in this substratum at two pH levels (4.8 and 5.8), uninoculated and inoculated with *Glomus etunicatum* (NPI, USA). Plant survival, shoot growth, root length, and mycorrhizal colonization were evaluated after six weeks. The Marubakaido rootstocks presented higher survival rates than the M9. Inoculated plantlets of Marubakaido presented higher shoot growth than uninoculated ones, and at pH 5.8 inoculated plantlets had lower root length values than uninoculated ones. Mycorrhizal colonization was higher at pH 5.8 (26%) than at pH 4.8 (4%). The Marubakaido clone associated with *Glomus etunicatum* showed better potential for transplantation into nurseries of low pH than uninoculated plants or the M9 clone. The association of these clones with isolates of other arbuscular mycorrhizal fungal isolates from regions of low pH is under evaluation.

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talk session 3

LUIZÃO, REGINA¹, KEVIN INGLEBY² & JULIA WILSON². ¹Departamento de Ecologia, Instituto Nacional de Pesquisas da Amazonia, Cx. Postal 478, 69011-970 Manaus, Am, Brasil. ²Institute of Terrestrial Ecology, Midlothian, EH26 OQB, U.K. - Effects of selective logging on the spore populations and infectivity rate of AM fungi in the Amazonian lowland evergreen rain forest

The potential for timber production is high and logging is an expanding activity in Amazonia. Projeto BIONTE has been set up to examine biomass production and nutrient cycling in the context of sustainable wood production for central Amazonian forests. Selective logging removed 50% of the commercially valuable trees > 50 cm DBH (about 8 trees ha^{-1} or 35 m^3 of wood ha^{-1}). A mycorrhizal study forms part of the project. A field bioassay on logged and control plots was used to examine effects of logging on inoculum potential of soil for AM infection resulting from logging disturbance and to evaluate possible effects on tree seedling regeneration. Seedlings of *Clitoria racemosa* and *Inga edulis* (Leguminosae), pregerminated in sterile soil, were both planted in the forest at different times after logging at random locations, and harvested after 8, 14, 38, 68 and 96 weeks. Soil samples were taken at the same time for spore analysis. There was a

strong seasonal effect on spore populations in all treatments, and logging reduced both the number of spores and rate of mycorrhizal infection over the short term. However, both species of baiting plants behaved like pioneer species and grew better on logged plots where there was increased incidence of light and accumulation of debris. Number of live spores were low, suggesting that root and hyphal fragments were more important than spores as sources of inoculum.

talk session 9

LUOMA, DAN. Department of Forest Science, Oregon State University, Corvallis, OR 97331 USA. - Fifteen years amongst the Snow Plants

The northernmost known population of the mycotrophic plant *Sarcodes sanguinea* (Snow Plant) was discovered at Limpy Rock Research Natural Area, Oregon in 1982. *Sarcodes* occurs in a community type dominated by *Pseudotsuga menziesii*, *Pinus lambertiana*, and *Abies grandis* in the overstory. *Berberis nervosa* and *Linnaea borealis* comprise most of the shrub layer.

The achlorophyllous, mycotrophic nature of this plant has long intrigued botanists. The nutrient transfer process from fungus to plant in the Monotropoideae has been studied by Duddridge and Read and Robertson and Robertson. Mainly because the root epidermal cell wall is not breached but invaginates around the intruding hypha, the hyphal intrusion into the cell is not considered a true haustorium. This type of mycorrhizae has been designated as a separate class *f* monotropoid. Members of the Monotropoideae such as *Sarcodes* can be considered parasites of their mycorrhizal fungi. Since the term "parasite" has negative connotations, the terms "obligate mycotroph", "mycotrophic", or "mycoheterotrophic" may be preferred.

The Limpy Rock population has been monitored annually since its discovery in 1982. Plant locations have been mapped and number of flowering stalks recorded. The results of 15 years of monitoring show that the recurrence of flowering by *Sarcodes* plants is variable and low in frequency. Over 150 plants, occupying an area of about 1 ha, have been located in the study site. The average number of plants flowering per year is 13 with a minimum of 3 and a maximum of 24. Relative number of plants flowering in the year subsequent to first appearance ranged from 0 to 35%. Few flowering recurrences beyond the subsequent year have been recorded. One plant did not flower again until 11 years after its first appearance! The distribution of *Sarcodes* plants suggests a spatial association with specific overstory trees. Future research will explore these patterns in relationship to potential shared mycobionts. Observations are discussed with reference to the implications of mycotrophic dependency to phytogeography in the face of potential climatic change.



poster session 2 ST SH 22

MAHMOOD, SHAHID¹, TINA JONSSON¹, SUSANNE ERLAND¹ & ROGER FINLAY². ¹Department of Microbial Ecology, University of Lund, Ecology Building, S-223 62 Lund, Sweden. ²Department of Forest Mycology & Pathology, Swedish University of Agricultural Sciences, Box 7026, S-750 07 Uppsala, Sweden. - Studies of ectomycorrhizal community structure after biofuel removal from a Swedish spruce forest

Use of biofuel, in particular that derived from the forest industry is an important part of Swedish energy production strategy with regard to climate change. The use of biofuel results in the net emission of only relatively small amounts of carbon dioxide to the atmosphere. Increased biofuel removal may however affect the availability of base cations, phosphorus and nitrogen, as well as the development, community dynamics and function of ectomycorrhiza which in turn may influence nutrient uptake and tree growth. The present study involves screening of ectomycorrhizal species in plots with and without biofuel removal in a spruce forest planted in 1961 in the south of Sweden. We have used PCR to amplify the ITS (internal transcribed spacer) region of ribosomal DNA and subsequent cutting with different restriction enzymes to produce RFLPs specific for different ectomycorrhizal species. The results obtained show a higher percentage of positive PCR amplifications from single mycorrhizal root tips in plots without biofuel removal as compared to those where biofuel was removed. In total 18 different ITS- types were identified, 13 types occurred in each treatment and five were common to both treatments. So far four of the ITS- types have been identified to species by comparison with RFLPs patterns of identified fruitbodies or axenic cultures. *Tylospora fibrillosa* was abundant in plots from which biofuel had been removed and an unidentified ITS-type (1) was common in untreated plots. Further identification of the remaining ITS- types and studies of possible seasonal variation in ectomycorrhizal species composition are in progress.

poster session 1 ST RR 43

MALAJCZUK, N.¹, B.DELL² & T. BURGESS². ¹CSIRO, Division of Forestry, Perth, Western Australia, 6014. ²School of Biological Sciences, Murdoch University, Perth, Western Australia, 6150. - Persistence of Australian *Pisolithus* species in eucalypt plantations in China

The persistence of Australian *Pisolithus* isolates introduced into experimental plantations of eucalypts in southern China was examined by recording the presence of sporocarps one to four years after planting. Electrophoretic patterns of polypeptides extracted from mycelial cultures obtained from *Pisolithus* sporocarps confirmed the successful colonization of eucalypt seedlings in southern China. Although the indigenous Chinese *Pisolithus* was present in the eucalypt plantations, it appears to be a poor coloniser of eucalypt roots and failed to form functionally mature

ectomycorrhizal structures *in vitro*. The pattern of basidiocarp abundance in the field suggests that the Australian *Pisolithus* isolates out-competed the indigenous *Pisolithus* in *Eucalyptus* plantations in China once successful inoculation of seedlings is achieved in the nursery.

poster session 10 WF SH 29

MANSKE, G.G.B.¹, A.B. LÜTTGER², R.K. BEHL³, P.L.G. VLEK². ¹CIMMYT, Apdo. Postal 6-641, 06600, Mexico, D.F. ²IAT, University of Göttingen, Grisebachstr. 6, 37077 Göttingen, Germany. ³Department of Plant Breeding, HAU, 125004 Hissar, India. - Enhancement of mycorrhizal (VAM) infection, nutrient efficiency and plant growth by *Azotobacter chroococcum* in wheat: evidence of varietal effects

Azotobacter seed-inoculation was tested in a field trial in North-India with ten Indian wheat cultivars. The roots of all cultivars were infected by the indigenous VAM fungal population in the soil. Significant main effects of the varieties and seed-inoculation could be observed. The effect of inoculation was more obvious in the root than in the shoot parameters. The total root length and their % VAM infection were improved. However, the response of grain yield to *Azotobacter* was plant genotype dependent and appeared to be related to improved P and N utilization efficiency, stimulated total root length and VAM infection. Phosphate utilization efficiency in grain yield production was more enhanced (average 13%) than N utilization efficiency (5%). Furthermore, N uptake was not qualitatively enough improved by *Azotobacter* inoculation. This supports the hypothesis that *Azotobacter* acts through the production of phytohormones, which stimulate root growth and VAM infection, rather than as an associative dinitrogen fixer.

Azotobacter inoculation was exceptionally effective in improving the grain yield of KAUZ (an important, world wide distributed CIMMYT line). The high yield and adaptation capacity of KAUZ, may be related to its high ratio of total root length / shoot dry weight, and resulting high P and N utilization efficiency as well as to the beneficial effects of rhizospheric associations (VAM, *Azotobacter*).

poster session 15 WF RR 19

MARÍN, MIGUEL^{1,2}, ISABEL PARDO² & SERGI FERRER². ¹COTEVISA, L'Alcúdia, 46250 València, Spain. ²Departament de Microbiologia, Facultat de Biologia, Universitat de València, Burjassot, 46100 València, Spain. - Detection of intraspecific variation of *Lactarius deliciosus* by PCR/RFLP

Restriction site maps have been constructed of the polymorphic Internal Transcribed Spacers (ITS) and the conserved 5.8S of the nuclear ribosomal RNA genes of several strains of *Lactarius deliciosus* and other species of ectomycorrhizal fungi. The samples of this research were taken from different culture collections and from harvested fruitbodies; to have samples of several geographic origins. The RFLP analyses of amplified

regions have been accomplished with twenty restriction enzymes to construct restriction site maps that allow to study the intraspecific variations and the phylogenetic relationships between the strains of *Lactarius deliciosus* and the other species of ectomycorrhizal fungi.

This method allows us to identify ectomycorrhizal fungi by PCR/RFLP analysis. The intraspecific variations found in *Lactarius deliciosus* were low, as in other ectomycorrhizal fungi. The RFLPs patterns are similar between strains of different origins: there is a homogeneity at the level species and the variations are random distributed. Phylogenetic analyses show that is possible to group the genera and species by PCR/RFLP: these genera of different taxons become clustered.

poster session 12 WF SH 51

MARKKOLA ANNA MARI¹, OILI TARVAINEN¹, ULLA AHONEN-JONNARTH², ARI JUMPPONEN³ & RAUNI OHTONEN¹. ¹Department of Biology, University of Oulu, P.O. Box 333, FIN-90571 OULU, Finland. ²Swedish University of Agricultural Sciences, Department of Forest Mycology and Pathology, Box 7026, S-75007 Uppsala, Sweden. ³Oregon State University, Department of Forest Science, Corvallis, OR 97331-7501, U.S.A. - Root peroxidase activity in ectomycorrhizal Scots pine seedlings in polluted environment

Plant biomass, amount of mycorrhiza and peroxidase activity in fine roots (PODroot) were measured in ectomycorrhizal Scots pine (*Pinus sylvestris* L.) seedlings exposed to high N and S concentrations in soil in laboratory conditions. In addition, PODroot and the amount of fungal biomass were monitored during the second growing season of nursery seedlings of Scots pine in an experimental field. Addition of ammonium or potassium sulphate to non-polluted soil did not affect PODroot. However, PODroot was slightly higher in seedlings growing in highly polluted urban forest soils than in those growing in non-polluted soils, and distinctly higher in the autumn harvest compared to summer conditions. On the contrary, in nursery seedlings PODroot showed a distinct decrease during the growing season, while the fungal biomass in roots increased. Elevated peroxidase activity in the roots of seedlings growing in polluted forest soils was hypothesized to be a defence mechanism of Scots pine against either saprophytic or mycorrhizal fungi invading the roots.

poster session 8 WF AR 56

MARLER, MARILYN, CATHERINE ZABINSKI, & RAGAN CALLAWAY. Division of Biological Sciences, University of Montana, Missoula MT 59812. - Effect of spotted knapweed invasion on native mycorrhizae.

We examined the effect of an invasive weed on mycorrhizae of native plants in a Palouse prairie community in western Montana. Roots of *Festuca idahoensis* and *Agropyron spicatum*, both native perennial bunchgrasses, were collected from intact Palouse prairie and from an area being invaded by

Centaurea maculosa (spotted knapweed). Roots of *C. maculosa* were collected from the invaded Palouse and from an area dominated by *C. maculosa*. Roots were sampled 3 times over the summer of 1996. Colonization of the bunchgrass roots by mycorrhizal fungi was not affected by the presence of *C. maculosa*. There was no detectable difference in the phenological development of mycorrhizae in the grass roots in the presence of *C. maculosa*. *Centaurea maculosa* formed more vesicles when growing in the invaded Palouse community compared to the *C. maculosa*-dominated area. Compared to both grasses, *C. maculosa* had a much higher ratio of vesicles to arbuscules over the course of the growing season.

poster session 8 WF AR 41

MARLER, MARILYN, RAGAN CALLAWAY & CATHERINE ZABINSKI. Division of Biological Sciences, University of Montana, Missoula MT 59812. - Mycorrhizal mediation of competition between an exotic forb and a native bunchgrass.

We grew *Centaurea maculosa* and *Festuca idahoensis* in the greenhouse to determine whether competitive balance is influenced by the presence of mycorrhizal fungi. Plants were grown with and without mycorrhizal fungi in inter- and intraspecific combinations, and compared biomass. *Festuca idahoensis* biomass was significantly reduced in the presence of mycorrhizal fungi in both neighbor treatments. The response of *C. maculosa* to mycorrhizae shifted depending on the neighbor. When growing with a conspecific, *C. maculosa* biomass was less with mycorrhizae than without. When growing with *F. idahoensis*, *C. maculosa* biomass was increased with mycorrhizae relative to the nonmycorrhizal treatment. Competition with *C. maculosa* reduced growth of *F. idahoensis* only in the presence of mycorrhizae. These results suggest that mycorrhizae may mediate competition between an exotic forb and a native bunchgrass.

poster session 2 ST SH 59

MARQUES, MARIA ISABEL, READ, DAVID. Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, U.Kingdom. - The relationship between arbuscular mycorrhizal fungi and plants of uncertain mycorrhizal status.

Arbuscular mycorrhizal fungi (AMF) occur in the roots of the majority of plants and their importance in the phosphorus (P) nutrition of their 'hosts' is recognised. However, the extent of colonisation and of the response of plants to the presence of AMF varies in different plant families. In this study, the effect of AMF upon growth of plant species of uncertain mycorrhizal status was investigated in order to determine the nature of the relationship between fungus and plant. The results showed a negative effect of the AM mycelium upon the growth of *Arabis hirsuta* L. and *Sinapidendron gymnocalix* (Lowe) Rustan (Cruciferae), *Echium vulgare* L. (Boraginaceae), *Polygonum persicaria* L. and *Rumex acetosella* L. (Polygonaceae). Usually, these plants are ruderals of disturbed habitats. It is suggested that their

exclusion from undisturbed closed communities of mycorrhizal plants is attributable to their sensitivity to AM fungi and that, as a result, these fungi play a key role in determining the species composition of natural plant communities.

talk session 5

MARTIN-LAURENT, FABRICE¹, Helene ROUSSEL¹, Eliane DUMAS-GAUDOT¹, Diederick VAN TUINEN¹, Vivienne GIANINAZZI-PEARSON¹, Silvio GIANINAZZI¹ and Phillip FRANKEN². ¹Laboratoire de Phytoparasitologie, INRA/CNRS, SGAP, INRA, BV 1540, 21034 Dijon Cedex, France, ²Max-Planck-Institut für Terrestrische Mikrobiologie, Abteilung Biochemie, Karl-von-Frisch-Strabe, 35043 Marburg, Germany, - PSEMYC1, a plant gene induced during early stages of arbuscular mycorrhiza development

Arbuscular mycorrhiza (AM) formation in plant roots is a complex process under genetic control and results from the production and the perception of signals which spatially and temporally regulate the expression of symbiosis related (SR) genes in both partners. Recently, we successfully used differential RNA display to detect modifications in gene expression during early stages of AM development between pea (*Pisum sativum* cv. Frisson) and *Glomus mosseae*. A clone, *psemyc1*, of 1497 bp corresponding to a single plant gene activated during early symbiotic interactions before arbuscule development and possessing an open reading frame of 108 amino-acids with no similarity with known proteins was isolated. Amino-acid sequence analysis predicts a transmembrane hydrophobic helix and a cytoplasmic N-terminus region contains putative phosphorylation sites. A model is proposed which groups the putative *psemyc1* peptide into a class of small proteins with regulatory functions, such as phospholamban which regulates the intracytoplasmic level of Ca²⁺, a potential secondary messenger, in sarcoplasmic cells. In this, the enhanced production of *psemyc1* protein by induced mRNA accumulation during the early stages of AM development could lead to the activation of plant genes via a transduction pathway implicating a secondary messenger.

talk session 5

MARTIN, FRANCIS, UWE NEHLS, THIERRY BEGUIRISTAIN & FREDERIC LAPEYRIE. Equipe de Microbiologie Forestiere, I.N.R.A., Centre de Nancy, 54280 Champenoux, France. - Signaling in the ectomycorrhizal symbiosis. The tryptophan betaine, hypaphorine, produced by *Pisolithus tinctorius* stimulates the expression of an auxin-regulated gene in roots of *Eucalyptus globulus*.

The development of the ectomycorrhizal association between *Eucalyptus globulus* and *Pisolithus tinctorius* induces dramatic changes in gene expression in both symbionts. Amongst the identified symbiosis-regulated genes, a cDNA clone (EgPar) from *E. globulus* shows a high homology with auxin-induced genes from tobacco and *Arabidopsis*. The EgPar gene was expressed at a low

level in roots and shoots of eucalypt seedlings. The steady-state level of EgPar transcripts was drastically up-regulated in roots during the early stages of ectomycorrhiza development. An enhanced level of EgPar transcripts was also induced by incubation of seedlings in the presence of auxins confirming that EgPar belongs to the family of auxin-regulated genes. The up-regulation of the level of EgPar transcripts was also observed in roots incubated in the presence of either *Pisolithus* acellular extracts or the indolic compound hypaphorine, a tryptophan betaine, abundantly excreted by this fungus. The latter data indicates that the fungal hypaphorine is able to trigger gene expression of the host plant and may act as an auxin derivative in eucalypt roots. This is the first report of an alteration of the host plant gene expression by a diffusible signal from an ectomycorrhizal fungus. The putative role of hypaphorine on the root morphology and development will be discussed.

poster session 15 WF RR 20

MARTIN FRANCIS¹, CHRISTINE DELARUELLE¹, MIKE IVORY², ¹Microbiologie Forestiere, I.N.R.A., Centre de Nancy, 54280 Champenoux, France and ²Department of Plant Sciences, University of Oxford, Oxford OX2 8QT, U.K. - Genetic variability of the *Pisolithus* morphotypes associated with pine, eucalyptus and *Azelia* in Lowland Kenyan forests.

Small outlier forests of "Miombo-like" vegetation exist in the Coast Province of Kenya. They comprise mainly legume trees of the Caesalpinioideae, namely, *Azelia quanzensis*, *Brachystegia spiciformis* and *Julbernardia magnistipulata*. Large plantations of *Pinus* spp and *Eucalyptus* spp have also been established in Kenya since 1900, mainly in upland areas. All the above genera are known to form ectomycorrhizal associations elsewhere. However, few epidemiological studies of these associations have previously been conducted in Kenya. The main objective of the present study is to assess the genetic variability of *Pisolithus* morphotypes associated with the above indigenous and exotic trees. The internal transcribed spacer (ITS) and the intergenic spacer (IGS) of the nuclear ribosomal DNA from representative isolates of the three morphotypes of *Pisolithus* identified in the Lowland forests of the Kenyan Coast have been amplified by PCR and sequenced. The ITS of pine-, eucalyptus-, and *Azelia*-types of *Pisolithus* present a high nucleotide sequence variability (34 to 45%). This variability is much larger than the intraspecific ITS sequence variation observed for other fungal species (1 to 10%). In contrast, RFLP and sequences of rDNA spacers, together with RAPDs, were very homogeneous within one morphotype. These data suggest that the different *Pisolithus* types collected in this region of Kenya do not inter-breed.



poster session 3 ST SH 71

MARTIN, CHRIS & JEAN STUTZ. Department of Botany, Arizona State University, Box 871601, Tempe, Arizona 85287-1601, USA. - Soil respiration of sour orange inoculated with mesic or xeric geographic isolates of VAM fungi under two irrigation frequency regimens

Sour orange (*Citrus aurantium* L.) seedlings were inoculated with geographic isolates of *Glomus intraradices* Smith and Schneck from a xeric (New Mexico, USA) or mesic (Florida, USA) climate or not inoculated as controls. Plants were then grown for five months under high (soil water potential >-0.1 MPa, irrigated once every 3 days) or low (>-1.0 MPa, irrigated once every 12 to 15 days) irrigation frequency regimens. Similar leaf P concentrations were achieved in all plants by applying more P fertilizer to non- mycorrhizal plants than mycorrhizal plants. Plants inoculated with the xeric isolate had more arbuscules and fewer vesicles than those inoculated with the mesic isolate. Under high irrigation frequency, soil respiration (R_{soil}) was generally lowest for plants inoculated with the mesic isolate. Under low irrigation frequency, R_{soil} was highest for plants inoculated with the mesic isolate. Results from this study showed that the carbon economy of sour orange plants especially benefitted from inoculation with an isolate from a xeric climate under low irrigation frequency, independent of P nutrition.

poster session 15 WF RR 22

MARTIN, MARIA & JAN-ERIK NYLUND. Department of Forest Mycology & Pathology, Swedish University of Agricultural Sciences, Box 7026, S-750 07 Uppsala, Sweden. - Molecular taxonomy of *Rhizopogon* based on ribosomal DNA polymorphisms.

A fundamental goal in the study of ectomycorrhiza has been the development of an easy method to establish the identity of the particular species or strain(s) of fungus present in a sample of mycorrhizal roots. The main problem with *Rhizopogon* spp. is the different interpretation of the numerous species published by early authors which made classification in Europe exceptionally difficult. A survey of the mycological literature revealed 190 specific names associated with this genus worldwide, most from North America. In contrast the most recent works on European *Rhizopogon* mention only five species:

R. luteolus Fr. & Nordholm, *R. marchii* (Bres.) Zeller & Dodge, *R. vulgaris* (Vitt) M Lange, *R. roseolus* (Corda) Th. M. Fr. and *R. villusulus* Zeller. In our morphological study, after examining 2560 collections, we proposed a new set of characters that allowed us to build a key to identify 21 species in Europe. As an aid in identifying the species of the genus in our area of study, a survey of ribosomal DNA restriction fragment length polymorphisms (RFLPs) from 132 herbarium collections of *Rhizopogon* (32 type specimens) was carried out. The Internal Transcribed Spacer (ITS) region between the 18S and 28S subunits of ribosomal DNA was amplified using the polymerase chain reaction (PCR). Interspecific variation in length and number of restriction sites of the

amplified ITS was observed after cutting the PCR product with six enzymes (*Alu I*, *Cfo I*, *Dde I*, *Hinf I*, *Mbo I* and *Taq I*).

poster session 5 ST-AR 25

MARTINS, ANABELA¹ & M. S. PAIS². ¹Escola Superior Agrária de Bragança, Apt. 173, Bragança, Portugal. ²Faculdade de Ciências de Lisboa, Bloco C2, Campo Grande, 1780 Lisboa, Portugal. - Peroxidase activity in micropropagated *Castanea sativa* Mill. plants mycorrhizal with *Pisolithus tinctorius* and *Hebeloma cylindrosporum*

Micropropagated *Castanea sativa* plants were mycorrhizal *in vitro* with *Pisolithus tinctorius* and four strains of *Hebeloma cylindrosporum*, two of which are IAA overproducer mutants. Peroxidase (PO) activity in the roots, stems and leaves were determined along the mycorrhizal process. Determinations performed ten days after root contact with the fungus show that roots mycorrhizal with *P. tinctorius* (Pt) have lower PO activities than nonmycorrhizal ones these differences being still maintained at 270 days after contact. Roots mycorrhizal with *H. cylindrosporum* (Hc) also present lower PO activities, either mycorrhizal with the wild types of this fungus or with the IAA overproducer mutants. The IAA overproducer mutants, however, present a different pattern of kinetics when compared with the wild type or with Pt. Preliminary results on PO activity obtained in stems and leaves are not conclusive. PO activities in leaves from plants mycorrhizal with Pt and Hc are not significantly different from nonmycorrhizal ones. PO kinetics is different for roots mycorrhizal with Pt and Hc IAA overproducer mutants. These differences seem to be related to IAA production by the strains. The correlation between PO activity, auxin synthesis and root induction capacity of mycorrhizal fungi is discussed. PO activity changes as a response to mycorrhizal establishment is also discussed.

poster session 1 ST RR 45

MASCHKE, MARTIN¹, CHARLES MONCOUSIN², RETO STRASSER¹ & SILVIO GIANINAZZI³, ¹Université de Genève, Laboratoire de bioénergétique végétale, 1254 JUSSY, SWITZERLAND, ²Centre horticole de Lullier, 1254 JUSSY, SWITZERLAND, ³Laboratoire de phytoparasitologie INRA/CNRS, SGAP, BV 1540, 21034 DIJON CEDEX, FRANCE - Endomycorrhization of micropropagated apple rootstock and plant growth regulators.

Endomycorrhizas have a positive influence on growth of young apple trees. We have studied the involvement of plant growth regulators (PGRs) in this mechanism in micropropagated apple rootstock (*Malus domestica* borkh. M26).

Plants were treated with different concentrations (10^{-4} to 10^{-8}) of exogenous PGRs (IAA, ABA, GA₃ and Kinetin) and growth and mycorrhization was assessed. All experiments were performed in growth chamber

conditions on plantlets which were seven weeks old (after weaning and mycorrhization) and lasted five weeks. Plant growth was reduced at higher PGR concentrations whilst mycorrhization was only strongly reduced in plants treated with GA₃: Mycorrhization was 80 % in mycorrhizal control plants and 1.5 % (without any arbuscle) in GA₃ treated mycorrhizal plants.

In another experiment measurement of endogenous PGRs (abscissic acid and cytokinins) showed some variation between mycorrhizal and non mycorrhizal plants which changed during plant development. Implication of these observations in mycorrhizal effects will be discussed.

poster session 8 WF AR 51

MASSICOTTE, HUGUES B.¹, LINDA E. TACKABERRY¹, ELAINE R. INGHAM² and WALTER G. THIES³. ¹Faculty of Natural Resources and Environmental Studies, University of Northern British Columbia, 3333 University Way, Prince George, B.C., Canada V2N 4Z9. ²Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA. ³Forestry Sciences Laboratory, 3200 S.W. Jefferson Way, Corvallis, OR 97331, USA. - Ectomycorrhizae establishment on Douglas-fir seedlings following chloropicrin treatment to control for laminated root rot disease: assessment 4 and 5 years after outplanting.

Laminated root rot, caused by *Phellinus weirii* (Murr.) Gilb., is a serious disease affecting Douglas-fir and other commercially important species of conifers in northwestern North America. Recent work has shown that this fungus is successfully reduced or eliminated by the fungicide chloropicrin. However, the effect of this fungicide on nontarget organisms, including ectomycorrhizae, is uncertain. Following an initial assessment of organisms between 1989 and 1992, a reassessment of the establishment of ectomycorrhizae on Douglas-fir seedlings was undertaken 4.5 and 5.5 years following chloropicrin application. Our findings show that, in areas around stumps treated with 20% and 100% of the labelled dosage and in areas around non-treated stumps, chloropicrin did not adversely affect the formation of ectomycorrhizae on young Douglas-fir seedlings by naturally occurring fungi. No significant effect on the abundance or type of mycorrhizae were detected on this site. Based on these results, chloropicrin may not adversely affect mycorrhizal associations, at least in the first 5 years following application. However, results documented here might not be the same on other sites.

poster session 7 WF AR 22

MASSIMO, G.¹, R. GALÁN², L.G. GARCÍA-MONTERO², J.L. MANJÓN² & J. DíEZ². ¹Dipartimento di Biologia vegetale dell'Università degli studi di Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy. ²Unidad de Investigación y Producción Vegetal, Facultad de Ciencias, Universidad de Alcalá, 28871 Alcalá de Henares (Madrid), Spain ². - New data on the

biology of *Sphaerosporella brunnea* (A. & S. ex Fr.) Svrcek & Kubicka (*Humariaceae, Ascomycotina*) harmful fungi to the truffle culture

Sphaerosporella brunnea (A. & S. ex Fr.) Svrcek & Kubicka has wakened up interest in the European truffle culture in the last years, for the frequency appearance of mycorrhizal plants with *Tuber* Sibthorp ex Fr. in commercial green houses. Their presence has point out in French (CHEVALIER, 1990), Italian (MEOTTO & CARRATURO, 1988) and Spanish green houses (GARCÍA-MONTERO *et al.*, in press; MASSIMO *et al.*, in press), where it have caused important economical damages. New information about the biology of this species is provided.

poster session 2 ST SH 60

MATSUDA, YOSUKE. Laboratory of Forest Protection, School of Agricultural Sciences, Nagoya University, Nagoya 464-01, Japan. - Spatial distribution of ectomycorrhizal fungi on the border of a man-made and a naturally regenerated forest

The spatial distribution of ectomycorrhizal (ECM) fungi was investigated in terms of occurrences of ECM fruitbodies on the forest floor and ECMs on the roots of fir (*Abies firma*) seedlings in adjacent man-made mixed forest of *Cryptomeria japonica* and *Chamaecyparis obtusa*, and a naturally regenerated forest dominated by *A. firma*. The study was carried out between 1993 and 1995 on a sample plot of 10m in size. The sample plot was taken from the boundary between the two stands so as to include samples from both stands. Thirty-nine species from 14 genera of ECM fungi were recorded, the highest occurring species being *Russula* sp.1. Most of the fruitbodies occurred on the side of the *A. firma*, which is ECM associated tree species, stand. *Russula* spp. were mostly found on the side of the *A. firma* stand, whereas the genera *Gomphus* and *Inocybe* fruited mostly on the side of the mixed stand. Eight fir seedlings from the plot were sampled in December 1994, and monthly from May to October 1995. More than 70 % of root tips of the seedlings were ectomycorrhizal in every sampling. Twenty-one ECM types were found. Type 14 was recorded as the highest frequency of occurrences in each sampling. This type of ECM was found on most of the seedlings on the side of the *A. firma* stand. The area of occurrences of Type 14 tended to overlap the fruiting area of *Russula* sp.1.

poster session 14 WF RR 03

MBURU KAMAU¹, MARIO HONRUBIA², MIKE IVORY³, BERTA ANTA⁴, LINUS MWANGI¹ & VICTOR RUBIO⁴. ¹Kenya Forestry Research Institute, P. O. Box 20412, Nairobi, Kenya. ²Departamento de Biología Vegetal, Universidad de Murcia, 30100 Murcia, Spain. ³Field Laboratory, Oxford Forestry Institute, University of Oxford, Wytham, Oxford, OX2 8QT, England. ⁴Departamento de Biotecnología Microbiana, Centro Nacional de Biotecnología, Madrid, Spain. - A new *Pisolithus* taxon from Kenya.

In the small outlier forests of "Miombo-like" vegetation along the coastal belt of Kenya, a *Pisolithus* taxon morphologically very different from the other two *Pisolithus* strains associated with pines and eucalypts appears fructifying beneath *Azelia quanzensis* trees. The macro and micro characteristics of this new taxon are presented. The spores were observed under the scanning electron microscope and compared with those of other *Pisolithus* strains. Slight differences could be appreciated especially their ornamentation. Results of molecular studies of the new taxon comparing them with other *Pisolithus* strains from Kenya, Australia, Spain and Uruguay are also presented. Studies of ITS region using different restriction enzymes (Hinf I, Alu I, Taq I, Msp I) allowed to distinguish the different strains analysed. The phylogenetic trees showing the genetic diversity of these strains are also presented.

poster session 3 ST SH 72

MCGEE, PETER & GREG PATTINSON. School of Biological Sciences, University of Sydney, NSW 2006, Australia. - Lack of decline of infectivity of AM fungi in three cultivated, heavy clay soils over time.

In temperate Australia, long fallow disorder has been associated with a decline in density of AM in roots of many crop plants from 7 months. It is assumed that the low densities are due to a decline in density of AM fungi in soil due to the absence of a suitable host. The hypothesis was examined in 3 heavy clay soils used to grow cotton. Cores of soil were stored dry for up to 18 months, sieved through a 5mm grid and stored, or saturated with deionised water and then dried every three months for 18 months. Density of AM fungi was determined using infection in cotton seedlings sown in cores and harvested weekly for 8 weeks.

Only a small decline in infectivity over 18 months was detected in soils stored dry and soil that had been sieved. After 18 months, wet/dry cycles reduced infectivity by a moderate amount. This result indicates the importance of soil wetting in the absence of plants for the survival of AM fungi in some cultivated soils.

poster session 3 ST SH 75

McGONIGLE, TERENCE, & MURRAY MILLER. Department of Land Resource Science, University of Guelph, Guelph, N1G 2W1, Canada. - Why do plants growing in disturbed soil sometimes - but not always - have reduced arbuscular mycorrhizal colonization?

In our studies plants have invariably had increased P uptake when growing in soil that has been left undisturbed since the shoots of the previous crop were removed, as compared to that in otherwise similar systems where the soil was broken up between growth cycles; however, mycorrhizal colonization is only sometimes reduced for plants in the disturbed soil, while in other experiments the undisturbed and disturbed treatments have similar and relatively high colonization. The interpretation of this puzzling inconsistency has been that the principal action of the disturbance is to destroy the integrity of the extraradical mycorrhizal mycelium left

behind by the previous crop, always reducing P nutrition in the next generation of plants, but only sometimes causing reduced colonization. A satisfactory understanding of the involvement of mycorrhizae in the effect of soil disturbance on plant P nutrition will only be reached when prediction can be made of whether or not colonization will be reduced in the disturbed treatment. This presentation will discuss hypotheses to explain these inconsistencies in experimental outcomes. Hypotheses relate to the frequency and timing of previous disturbance episodes, to the timing of taking root samples in the current growth cycle, to the soil- and plant-P concentrations, and to the inoculum density in soil. Results of a recent experiment showed that the difference between the low colonization of plants in the disturbed treatment and the high colonization of plants in the undisturbed treatment got larger with increasing inoculum potential, suggesting that a high inoculum density does not prevent disturbance from reducing colonization.

talk session 7

McLEAN, C.¹, A.C. LAWRIE², L.E. DE MELIS², & T.W. STEVENSON². ¹Faculty of Agriculture, Forestry and Horticulture, Burnley College, University of Melbourne, Burnley Gardens, Swan St, Richmond, Vic. 3121, Australia, ²Department of Applied Biology & Biotechnology, RMIT, GPO Box 2476V, Melbourne, Vic. 3001, Australia. - Use of PCR to determine relationships among root isolates of Epacridaceae and the endophyte of Ericaceae, *Hymenoscyphus ericae*.

The aim of this study was to use directed oligonucleotide primers specific for *Hymenoscyphus ericae* to determine any relationship between fungi isolated from the roots of Epacridaceae and *H. ericae*. A pair of matched primers based on the ITS (internal transcribed spacer) region of ribosomal DNA specific for *H. ericae* were used in PCR (polymerase chain reaction). Isolates *H. ericae* 100 and 101 both produced a single band that closely matched a known sequence from *H. ericae*. DNA from *Oidiodendron griseum* did not react. Two hundred fungi were isolated from the roots of five plants of *Epacris impressa* from Cranbourne and three plants of *Astroloma pinifolium* from the Grampians. The isolates were divided into 12 groups by morphological characters on quarter-strength PDA. The groups ranged from white through pink to dark olive and all were slow growing. Fifty isolates chosen at random were grown in liquid culture (quarter-strength potato dextrose) for two weeks and the DNA extracted. Over half the isolates produced a band of the predicted size. Isolates that did not produce a band with specific primers were tested by PCR with random primers. They gave reproducible banding patterns that differed from those of *H. ericae* cultures, suggesting that these isolates were different from *H. ericae*.



poster session 3 ST SH 76

MCQUATTIE, CAROLYN¹, KENDALL MARTIN², & PAUL RYGIIEWICZ³. ¹USDA Forest Service, NEFES, Delaware, OH 43015. ²Dynamac Corp., US-EPA, NHEERL-Western Ecology Division., Corvallis, OR 97333. ³US-EPA, NHEERL-Western Ecology Division., Corvallis, OR 97333. - Effects of elevated carbon dioxide and temperature on cytological changes in Douglas fir ectomycorrhizae

Ectomycorrhizal root tips from Douglas fir (*Pseudotsuga menziesii*) seedlings, grown in Soil-Plant- Atmosphere exposure units, were examined by transmission electron microscopy (TEM) to evaluate the effects of elevated atmospheric CO₂ concentration (ambient level and ambient plus 180 ppm) and atmospheric temperature (ambient level and ambient plus 3.5 C) on mycorrhizal ultrastructure. In 1995, the 5-year-old seedlings had been growing under altered climate treatments since spring of 1993. TEM analysis was performed on representatives of the numerically dominant morphotype among ectomycorrhizal root tips harvested from soil cores. A parallel sample was subgrouped by PCR-RFLP "fingerprinting" using four restriction enzymes. This information was used to separate genotypic from phenotypic characteristics. The mycorrhizal mantle appeared to have two distinct layers; i.e., an inner, tightly appressed layer and an outer, loosely-associated layer. Deterioration of the fungal mantle was most severe in seedlings grown at elevated temperature plus ambient CO₂. Mycorrhizal roots from seedlings grown at elevated CO₂ showed increased Hartig net development and accumulations of glycogen granules in fungal hyphae. Intrahyphal hyphae were seen in some roots from each treatment.

poster session 2 ST SH 23

MENDONCA, MARGARIDA, ALEKSANDER W. MUNIZ & ANALIA S. CORDOBA. Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, Florianopolis, Santa Catarina, CEP 88040-900, Brazil. - The diversity of arbuscular mycorrhizal fungi in dunes in three stages of stabilization at the Joaquina Beach, Island of Santa Catarina, Brazil.

The diversity of arbuscular mycorrhizal (AM) fungi was assessed in three different types of dunes in successional stages of stabilization. Data of spore occurrence in the field were supplemented with data from pot culture isolation techniques. The additional use of pot cultures to assess AM diversity is necessary given that many fungi may not sporulate at field conditions, underestimating diversity. Ten soil samples were collected from each type of dune during spring of 1995. Pot cultures were started using soil diluted in sterilized sand (1/1,v/v) as inoculum and *Sorghum bicolor* as the host plant. Bioassays of field collected soils, from these three types of dunes, were established to provide estimates of the capacity of these fungi to form mycorrhizae with a host. A total of twenty species were recovered, three of which were only detected in pot cultures. *Scutellospora* and *Gigaspora* were dominant in the foredune while *Acaulospora*

scrobiculata and *Glomus* spp. were dominant in the intermediate and the stabilized dunes. Infectivity tended to increase with dune stabilization and host root colonization was 14%, 29% and 34% in soils collected from the foredune, intermediate and stabilized dunes, respectively. Our results demonstrate that assessment of biodiversity of AM fungi should involve the use of different procedures of fungal recovery and that AM fungal population composition changes as stabilization proceeds. Research funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Brazil.

talk session 4

MERRYWEATHER, JAMES & A.H. FITTER. Department of Biology, PO Box 373, University of York, York YO1 5YW, U.K. - The diversity of AM fungi associated with *Hyacinthoides non-scripta* (L.) Chouard ex Rothm. in English woodland.

In field experiments we have demonstrated the phosphorus acquisition rôle of arbuscular mycorrhiza in bluebell *Hyacinthoides non-scripta*. At the time we treated the mycorrhiza as a simple dual entity, but, even at our single field site, bluebell roots associate with a range of glomalean fungi and we predict that some fungi may have functions other than P assimilation. The diversity of fungal endophytes in bluebell roots and the spores associated with them are being studied using both traditional and molecular techniques. Roots may contain up to three out of five or more endophytes from the genera *Scutellospora*, *Acaulospora* and *Glomus* as well as the ubiquitous "Fine Endophyte". Spores collected from soil associated with bluebell are even more diverse, and we are finding relationships between spore and root populations and the plant communities within which the bluebells were sampled. We have isolated glomalean fungi from bluebell soil, some of which are not represented by spores in field assemblages. We discuss aspects of morphology, identity and ecology of the many fungi found in the bluebell mycorrhiza and outline experimental work which will assess the performances of pot cultured isolates in order to elucidate the rôle of each in bluebell and an English woodland community.

poster session 3 ST SH 68

MICHEL, ARNOLDO, JAVIER FARIAS, SALVADOR GUZMAN & RAFAEL MARTINEZ. Facultad de Ciencias Biológicas y Agropecuarias, Universidad de Colima, Apartado postal 36. 28100 Tecoman, Colima, Mexico. - Effects of herbicides on vesicular-arbuscular formation in roots of maize (*Zea mays* L.) seedlings.

Herbicides are widely used by producers to obtain maximum crop quality and yield. However, where the chemicals may disrupt symbiosis occurring between plants and arbuscular mycorrhizal fungi. This work reports the effects of different herbicides applied alone and mixture on the mycorrhizal colonization in maize plants roots grown in nursery. Seeds disinfected of maize were sown in pots containing 5 kg of soil sterilized with

methyl bromide and inoculated with 50 g of *Glomus fasciculatum* mixed throughout into soil. Herbicides evaluated were Alachlor, Atrazine, Atrazine+Terbutrine, Atrazine+Paraquat and control, these were applied with manual sprayer to recommended rate at sowing time. Plants were harvested at the 15, 30 and 45 days after sowing. Fresh weight roots, mycorrhizal colonization and intensity of infection were severely affected by different herbicides used. Herbicides significantly reduced mycorrhizal infection in maize roots, however plants treated with Alachlor showed 51% of infection at 45 days after application. Mycorrhizal infection in control plants obtained 84% at 30 days. We suggest that any these herbicides could affect molecular interaction processes which lead to the recognition, binding and penetration of fungus in maize roots. Further investigations would be necessary to elucidate the effect of different rates and other systemic herbicides and to what extend indirect effects on plants growth.

talk session 1

MILLER, STEVEN, TERRY MCCLEAN, & LEA GUTHRIE. Botany Department, University of Wyoming, Laramie, WY 82071, U.S.A. - Differential utilization of cellulose and protein reveals competitive advantage of ectomycorrhizal fungi over saprotrophs for complex organic substrates.

Saprotrophic fungi (SAP) must use carbon they themselves extract to obtain the nitrogen required for metabolic functioning, whereas ectomycorrhizal fungi (EM), with a ready photosynthate supply from their host, can burn as much carbon as needed to obtain their nitrogen. Comparison of basic physiological properties in SAP and EM should reveal differences in organic substrate utilization and the ability to compete for limited resources. To test this hypothesis, utilization of cellulose (carboxymethyl cellulose) and protein (bovine serum albumin) by two EM (*Suillus tomentosus* and *Hebeloma birrum*) and two SAP basidiomycetes (*Agaricus augustus* and *Coprinus quadrifidus*) was compared in vitro by using media containing cellulose only, glucose only and cellulose + glucose at low (25), mid (75) and high (500) C:N with protein as N source. All fungi were able to utilize protein as an N source. All species grew more on the glucose only media and at mid level C:N. At high C:N the EM outgrew the SAP while the reverse was true at low C:N. *Agaricus* used cellulose best at low C:N and best of all species tested if glucose was not present. Both *Agaricus* and *Coprinus* were able to use cellulose at all C:N in the absence of glucose but at a lower level than when glucose was present. *Suillus* was unable to use cellulose at any C:N if glucose was absent and *Hebeloma* used only very little. Both EM and SAP utilized cellulose equally well at all C:N if glucose was present in the medium. *Agaricus*, *Hebeloma* and *Coprinus* used virtually all of the cellulose and *Suillus* used 70% at all C:N when glucose was present. This study supports the idea that EM are at an advantage in the decomposition of complex organic substrates like cellulose and protein in the field because they have a ready supply of glucose from their hosts that would be unavailable to SAP.

Further testing of the above hypotheses in corresponding in vivo experiments is underway.

poster session 1 ST RR 19

MILLER, ROBIN & LOUISE JACKSON. Department of Vegetable Crops, University of California at Davis, Davis, CA 95616. - On Farm Survey of Mycorrhizae in Lettuce Roots

Agricultural practices, including fertilization, pest management, crop rotation and tillage, were documented, and soil nutrient conditions were analyzed, in order to assess the relative contribution and synergistic effects of these factors on VAM colonization of lettuce and soil spore counts in 18 fields in the Salinas Valley of California. Samples were collected two weeks prior to crop harvest at three times during the season. Both colonization levels and spore counts increased as the season progressed. Use of pesticides, inorganic fertilizers and deep tillage practices decreased the amount of infection. Crop rotations predominated by VAM hosts increased the probability of infection. Total soil phosphorous showed a closer relationship to VAM colonization than Olsen available phosphorous. Higher total soil carbon percentages tended to correlate to an increase in root infection levels. The greater incidence of VAM in lettuce in 'organic' fields may indicate that mycorrhizae play a greater role in nutrient acquisition when soil fertility is dependent on the addition of organic matter.

poster session 8 WF AR 49

MILLER, R.M.¹, S.F. WRIGHT², J.D. JASTROW¹ & A. UPADHYAYA². ¹Environmental Research Division, Argonne National Laboratory, Argonne, IL 60439, USA. ²United States Department of Agriculture-Agriculture Research Service, Beltsville, MD 20705, USA. - A hydrophobic glycoproteinaceous substance produced by AM fungi stabilizes soil structure

The aggregated state in soil is important because aggregates not only help to control soil erosion but also facilitate the creation of a soil's nutrient reserve. Without the physical protection afforded within stable aggregates, organic matter and associated nutrients may be lost rapidly through both mineralization and erosion. The stabilization of higher hierarchical orders of soil particles into stable soil aggregates appears to depend on the enmeshing of the particles by living or partially decomposed roots and fungal hyphae. An especially important mechanism is the physical binding of soil particles into stable macroaggregate structures by the hyphae of arbuscular mycorrhizal (AM) fungi. Moreover, the stability of soil aggregates is maintained not only by physical entanglement of the soil particles by roots and hyphae but also by the stabilizing properties of plant and microbially derived polysaccharides and mucigels. We present evidence for the existence of another aggregate binding mechanism associated with the hyphae of AM fungi. The AM fungal hyphae apparently exude a glycoproteinaceous substance, glomalin, that acts as a hydrophobic glue binding smaller soil structural units into

larger rather stable macroaggregates. The deposition of glomalin appears to result in the retardation of water movement into the voids within the aggregate structure and therefore allows the nondisruptive movement of gases from the voids within and between soil aggregates. Hence, the accretion of glomalin may increase the stability of an aggregate directly because of its glue-like properties and indirectly by retarding the movement of water within the aggregate.

talk session 1

MILLER, R.M. & J.D. JASTROW. Environmental Research Division, Argonne National Laboratory, Argonne, IL 60439, USA. - Mycorrhizal effects on carbon gain in facultative and obligate ecotypes of *Andropogon gerardi*

Mycorrhizal and nonmycorrhizal prairie peninsula ecotypes of *A. gerardi* have similar biomasses, even though the mycorrhizal plants have greater tissue concentrations of P. Increases in tissue N concentrations of the host were not found with colonization by mycorrhizal fungi. These results suggest that the relationship between the prairie peninsula ecotype and the mycorrhizal fungus is facultative, contrary to reports on a Konza prairie ecotype of *A. gerardi* demonstrating an obligate relationship. We also found a strong positive relationship between shoot tissue N concentration and net photosynthesis ($A = \text{mmol C m}^{-2} \text{ s}^{-1}$), whereas no relationship was evident between A and tissue P concentration for the prairie peninsula ecotype. In addition, the relationships of tissue P and of tissue N with A were not affected by the presence or absence of mycorrhiza. However, a significant mycorrhizal effect was observed in the relationship between A and the ratio of P to N in shoot tissue (P:N). The values for A were higher for mycorrhizal plants than nonmycorrhizal plants at equivalent shoot tissue P:N ratios. Shoot P:N ratios also have a similar effect on net carbon gain, with mycorrhizal plants having higher carbon gains than nonmycorrhizal plants at equivalent shoot P:N ratios. These results raised the question of how mycorrhizal and nonmycorrhizal plants can produce similar biomasses and yet have very different carbon gains. We are currently determining whether the mycorrhizal fungi are a major component of the host's carbon sink strength and identifying the kinds of carbohydrates that accumulate in mycorrhizal and nonmycorrhizal shoots and roots.

poster session 15 WF RR 23

MILLNER, P., W. MULBRY, S. REYNOLDS, and C. PATTERSON. Soil Microbial Systems Laboratory, USDA-ARS, Bldg. 318, BARC-E, Beltsville, MD 20705. - A taxon specific oligonucleotide probe for temperate zone soil isolates of *Glomus mosseae*

ITS regions of nuclear rDNA from spores of *Glomus mosseae* FL156 and UK118 were amplified by PCR; 95% sequence similarity was found. Major differences with partial sequences from 7 other glomalean taxa occurred. Specificity of 4 oligonucleotides to *G. mosseae*

was assessed by PCR amplification of DNA from spores from 36 glomalean fungi: 13 isolates of *G. mosseae*, 12 other *Glomus* isolates, and 11 other glomalean taxa, as well as the nonmycorrhizal controls, *Endogone pisiformis*, *Pythium ultimum*, and *Zea mays*. GMOS1:GMOS2, reliably primed specific PCR amplification of a sequence (approx. 400 bp) present in *G. mosseae* isolates and 2 *G. monosporum*, and did not prime PCR with templates of other glomalean fungi or the controls. The 24-mer oligonucleotide, GMOS5, hybridized with *G. mosseae* and *G. monosporum* DNA amplified with primer pairs ITS1:ITS4 or GMOS1:GMOS2. Colony-blots assays showed that nearly all target amplification products from FL156 and UK118 DNA contained sequences homologous to GMOS5. *G. mosseae* in sudan grass roots was detected by hybridization of GMOS1:GMOS2 primed PCR product with GMOS5. GMOS1, GMOS2, and GMOS5 show promise for use in PCR-based assays for diagnosis of *G. mosseae* colonization in field collected roots.

poster session 1 ST RR 07

MIZOGUCHI, TAKEO. Nutrient Dynamics Lab., Forestry and Forest Products Research Institute, Matsunosato-1, Kukisaki-cho, Inashiki-gun, Ibaraki 305, Japan. - Growth and nutritional responses of some Japanese conifers for inoculation of arbuscular mycorrhizal fungi and different soil phosphorus regimes

Three species of Japanese conifer seedlings (*Cryptomeria japonica* D. Don, *Chamaecyparis obtusa* Sieb. et Zucc., *Thujopsis dolabrata* Sieb. et Zucc.) those have different growth rate and site adaptability were grown in loam soil under five kinds of phosphorus supply with or without inoculation of arbuscular mycorrhizal fungi (mixtures of *Glomus* spp.). In the first growing season, only *Cryptomeria* was enhanced of its growth by the inoculation. In the end of second growing season, all the inoculated seedlings formed arbuscular mycorrhiza, and growth of *Cryptomeria* and *Chamaecyparis* seedlings were clearly stimulated by inoculation. Growth enhancement was most evident when moderate amount of phosphorus was supplied. Some non-inoculated *Cryptomeria* seedlings grown in most low phosphorus regime were died after showing severe symptom of phosphorus deficiency. Nutrient concentration did not significantly differ between inoculated and non-inoculated seedlings, though phosphorus concentration in *Thujopsis* was somewhat higher in inoculated seedlings.

poster session 11 WF RR 35

MONREAL, MARCIA¹, SHANNON BERCH², MARY BERBEE³, and MONA PIRSEYEDI³. ¹Agriculture and Agri-Food Canada, Brandon Research Centre, P.O. Box 1000 A, Brandon, Manitoba. R7A 5Y3. ²British Columbia Ministry of Forests, Glyn Road Research Station, Victoria, British Columbia. V8W 3E7. ³University of British Columbia, Department of Botany, 6270 University Boulevard, Room 1104, Vancouver, British Columbia, V6T 1Z4. - Identification of ericoid mycorrhizal fungi using molecular methods.

Molecular techniques were used to facilitate the identification of ericoid mycorrhizal fungi associated with an ericaceous plant, salal (*Gaultheria shallon* Purch). The polymerase chain reaction (PCR) was used to amplify the DNA that codes for the entire internal transcribed spacers (ITS1 and ITS2) of the nuclear ribosomal repeat of 28 fungal isolates. Restriction fragment length polymorphism (RFLP) patterns of these isolates were compared and a synoptic key that differentiates these isolates into 14 groups was created. The RFLP synoptic key was used to assess the presence of ericoid mycorrhizal fungi from 3 mm fragments of salal root collected in the field and to identify new isolates obtained from the same root. In order to identify known ericoid mycorrhizal fungi in field roots, we amplified and sequenced the internal transcribed spacer ITS2 region (400 base pairs) of 24 fungal isolates. Sequences data analysis segregated the isolates into three main groups. Accordingly, a set of three different specific primers was designed. Tests performed using the new primers with fungal DNA mixtures of known mycorrhizal fungal isolates, non-mycorrhizal fungal isolates and in mycorrhizal salal plants open to environmental contamination successfully detected only the target fungal DNA.

poster session 2 ST SH1

MONTANEZ, A. & P.J. HARRIS. Soil Science Department, University of Reading, UK. - Small scale comparisons of spatial heterogeneity of arbuscular mycorrhizal fungi.

At present there is not well established strategy available to achieve an understanding of AMF spatial aggregates. Local details on the variation is lost when a great number of samples is bulked and then subsampled. In this study total spore number and AMF infection within the root was assessed in relation to soil depth and root density in three undisturbed systems dominated by clover, grass and barley.

Intact soil cores were removed from plots under continuous grass, clover and barley. Each core was divided into 3 cm sections and each section was divided in four subsamples. Each subsample was analyzed for spore and root colonization.

Total spore number differed both between soil cores and within soil cores. Total spore number decreased with depth. Generally the same range of spore types were present in each core with some variation in composition and number of spores between layers. No significant correlation was found between total root length and total spore number. However the patterns of distribution of the total root length and total spore number are very similar. It suggests that spores are related to root density and distribution and could be related to soil pore structure. The coefficient of total AMF infection (intra and extraradical hyphae) and total spore count number must give a better understanding of AMF dynamics.

This preliminary study confirmed the effect of soil depth on mycorrhizal colonization and root abundance, but the results could not confirm the effect of root density

on spatial distribution of mycorrhizal root infection and spore abundance.

poster session 12 WF SH 58

MORANDI, DOMINIQUE¹, YVAN RAHBÉ² & GÉRARD FEBVAY². ¹Laboratoire de Phyto-parasitologie SGAP, INRA/CNRS, BV 1540 21034 Dijon Cedex, France. ²Laboratoire de Biologie Appliquée 406, UA INRA 23203, 20 Av. A. Einstein, 69621 Villeurbanne Cedex, France. - Influence of *Glomus mosseae* on *Acyrtosiphon pisum* development and pisatin accumulation in leaves of *Pisum sativum*.

The interaction between the pea aphid *Acyrtosiphon pisum* and the mycorrhizal fungus *Glomus mosseae* was studied in pea. In a first experiment, three week old pea plants inoculated (or not) at planting with the mycorrhizal fungus were transferred to transparent plastic cages (one per plant) to be infested (or not) with 50 apterous aphids per plant. Three days later, stem and leaves were collected for pisatin analysis. Pisatin accumulation in pea shoots was induced by aphid infestation only in non mycorrhizal plants where the concentration was significantly higher (75%) than in mycorrhizal ones.

A second experiment was set up using a similar experimental model as above where aphid infestation consisted of 15 aphids nymphs per plant. Twelve days later, aphid adults and nymphs were counted and weighted to evaluate fecundity, survival and biomass production. Biomass production of aphids was higher in mycorrhizal peas and, although these results were not significant, mycorrhizal plants appeared to be better hosts for aphids than non-mycorrhizal ones. Pisatin accumulation was significantly reduced in shoots by mycorrhizal infection, but no significant correlation was found between the level of pisatin in shoots and any of the aphid growth parameters measured.

Toxicity of pisatin against *Acyrtosiphon pisum* was assayed using different bioassays in a synthetic diet: it strongly increased mortality, reduced growth, percentage of settling and was found to be phagodeterrent. The eventual implication of interrelations between aphid development, pisatin accumulation and mycorrhizal infection will be discussed.

poster session 6 WF AR 03

MORTE, ASUNCION & MARIO HONRUBIA. Dpt. Biología Vegetal, Facultad de Biología, Universidad de Murcia, Murcia 30100, Spain. - Nutritional requirements of *Helianthemum* and *Terfezia* for their initial *in vitro* mycorrhizal association.

Micropropagated plantlets of *Helianthemum almeriense* were infected *in vitro* with *Terfezia clavaryi* on a specific agar medium which enhanced initial root colonization *in vitro* respect to that obtained on modified MMN medium. This mycorrhization improved *in vitro* plant survival and growth of mycorrhizal plantlets in comparison to control plantlets. To facilitate the inoculation process and to see the effect of this fungus on rooting, rooted plantlets and unrooted microcuttings were also inoculated with

mycelium. *In vitro* rooting rate of mycorrhizal plantlets was lightly higher than rooting rate of control plantlets. Consequently, this improvement of rooting could be due, not as much to the presence of the mycorrhizal fungus as to the mineral and organic composition of the medium.

poster session 12 WF SH 60

MORTE, ASUNCION¹, NICHOLAS MALAJCZUK², BERNIE DELL³ & MARK BRUNDRETT². ¹Department Biología Vegetal, Facultad de Biología, Universidad de Murcia, Murcia 30100, Spain. ²CSIRO Forestry, Private Bag P.O. Wembley, Australia 6014, ³Murdoch University, School of Biological Sciences, Murdoch, Australia, 6150 - Mycorrhiza and root development of *Eucalyptus grandis* *in vitro*

Fifteen Australian ectomycorrhizal fungi were compared on their ability to stimulate root growth and form mycorrhiza *in vitro*. With the exception of H4325 (*Protuberata*), H4317 (*Setchelliogaster*) and E494 (*Descolea maculata*) all fungi significantly increased the number of lateral root tips at day 27 compared with non-inoculated seedlings. The majority of inoculated seedlings produced from between 20 and 25 tips per seedling compared to (controls?). Seedling inoculated with H2000 (*Scleroderma*), H4403 (*Paxilloogaster*) and E4869 (*Amanita*) produced more than 25 tips. However, not all of these fungi formed mycorrhiza *in vitro*. All fungi decreased the length of the main root of *E. grandis* seedlings at day 14 after inoculation compared with control seedlings with the exception of E766 (*Laccaria laccata*) which stimulated extensive root production. It is possible that this isolate could be used as a tool for tissue culture eucalypts which are difficult to root. Further, the results indicate that this technique is unsuitable for *in vitro* investigations of mycorrhizal development on eucalypt roots for all mycorrhizal isolates.

morning talk

MORTON, JOSEPH. 401 Brooks Hall, West Virginia University, Morgantown, WV 26506-6057. - Measurement and Interpretation of Arbuscular Fungal Species Diversity at Different Scales.

Diversity of arbuscular mycorrhizal fungi species entails many variables, but the two most important are richness and abundance. Richness can be measured directly by identification of spores produced in the rhizosphere. Spatial and temporal heterogeneity dictate seasonal samplings in field situations to capture sporulation by a majority of species, but identification is compromised by predation or decomposition of spores. Sequential trap cultures often induce sporulation and increase richness, especially in situations where overall colonization or infectivity is low. Once trap cultures are established they serve many purposes experimentally. Changes in species richness in experimental microcosms can be used to indirectly measure partitioning effects of host and soil factors on niche occupation. Measurement of alpha and beta species diversity in shared habitats on different continents provides data to separate historical and

contemporary processes, both of which may be equally important in development of a species assemblage. Trap cultures also provide healthy inoculum to establish multispore or single-spore cultures of component species. The former provide the variation to better define species-level boundaries. The latter define intra-organismal variation that can complicate resolution of species boundaries at the molecular level. Abundance (niche occupation) cannot be measured directly by current methods, so that common indices of diversity are almost meaningless. Molecular probes are gradually being developed to solve this problem. They will be powerful tools to strengthen assessment of both richness and abundance, as long as the connections between molecules and organisms are examined and maintained.

poster session 15 WF RR 07

MOUTOGLIS, PETER, LOUISE SAVARD & J. ANDRÉ FORTIN. Institut de recherche en biologie végétale, Université de Montréal, 4101 est rue Sherbrooke, Montréal, Québec, Canada HIX 2B2. - Genotypic and phenotypic variability among two isolates of *Glomus intraradices* aseptically cultured with transformed carrot (*Daucus carota*) roots.

Intraspecific variability was examined among two aseptically cultured isolates of *Glomus intraradices*: one from Pont Rouge, Québec and the other from Kansas, USA. Phenotypic differences were observed *in vitro*, at the level of root colonization, sporulation, spore development, morphology and germination. Genotypic differences were observed using Random Amplified Polymorphic DNA (RAPD) primers on aseptically cultured spores produced in a two-sided Petri system. The results confirm previous studies on intraspecific phenotypic, as well as genotypic variability and allow for the further development of isolate-specific markers.

poster session 2 ST SH 24

MOYERSON, BERNARD¹, ALASTAIR FITTER² & IAN ALEXANDER³. ^{1,2}University of York, Department of Biology, PO Box 373, York YO1 5YW, UK; ^{1,3}University of Aberdeen, Department of Plant and Soil Science, Cruickshank Building, Aberdeen AB9 2UD, Scotland. - P nutrition of ectomycorrhizal and arbuscular mycorrhizal tropical tree seedlings.

It is considered that EcM and AM have different characteristics which confer on them advantages in different habitats, and that these differences are an important factor in the determination of tropical rainforest composition. However, few studies have directly compared EcM and AM with respect to their role in the P nutrition of tropical tree species. In this study, growth parameters, P content and P inflow of EcM (*Tetrahelia moreliana*) and AM (*Oubanguia alata*) seedlings from lowland rainforest in Korup National Park, Cameroon, grown in a greenhouse with different levels of rock phosphate are compared. The effect of different levels of mycorrhizal colonization is evaluated. The results are

discussed in relation to new data on the distribution of EcM and AM in the same forest.

poster session 2 ST SH 58

MRIDHA, M. A. U., RAKHI MAHMUD & ANWAR-UR-RASHID. Department of Botany, University of Chittagong, Chittagong - 4331, Bangladesh. - VA-mycorrhizal fungi of *Hevea brasiliensis* and associated undergrowth.

Preliminary survey on the occurrence of VA-mycorrhizal fungi and root infection of *Hevea brasiliensis* and associated undergrowth was studied in a number of gardens throughout Bangladesh. Roots of *H. brasiliensis* and the selected undergrowth possess high percentage of infection. The wide variation in the intensity of infection is related to the percentage infection. Many different types of mycorrhizal fungi were isolated. Their total number was differed considerably. Different seasons influenced the fluctuation of spore population and types. The variation in VAM spore population and percent root infection in the different rubber gardens depend on indigenous VAM fungi, different edaphic factors, interaction of undergrowth and environmental factors. Large number of VA-mycorrhizal fungi and high percentage of root infection observed in the present study have not been reported earlier from Bangladesh.

poster session 12 WF SH 64

MUKERJI, K.G. & MANJU BANSAL. Applied Mycology Laboratory, Department of Botany, University of Delhi, Delhi - 110 007, INDIA. - Fine root-endomycorrhiza dynamics and their role in nutrient cycling.

Fine root dynamics refer to change of living roots (biomass) into dead (necromass) and ultimate addition to soil fertility. Fine roots and mycorrhiza are key links in below ground ecosystem and are important in nutrient cycling. They are the main site for absorption of nutrients. They exert a significant influence on soil profile development and upon dying contribute substantially to Organic Matter content of soil. They also effect nutrient cycling by altering the microbial makeup of soil in the rhizosphere through root exudation. As pivotal links in below ground ecosystem and nutrient recycling, mycorrhiza and fine roots can be ignored only at a substantial peril of reaching to unreal conclusions about ecosystem processes.

poster session 5 ST AR 26

ÜNZENBERGER, BABETTE¹, TILMAN OTTER², ANDREA POLLE². ¹Centre for Agricultural Landscape and Land Use Research Müncheberg (ZALF) e.V., Institute of Microbial Ecology and Soil Biology, Dr.-Zinn-Weg 18, D-16225 Eberswalde, Germany. ²Albert-Ludwigs-University Freiburg, Institute of Forest Botany and Tree Physiology, Am Flughafen 17, D-79085 Freiburg i.Br., Germany. - Peroxidase and laccase activities in mycorrhizal and non-mycorrhizal fine roots

of Norway spruce (*Picea abies* [L.] Karst.) and larch (*Larix decidua* Mill.)

Peroxidase (EC 1.11.1.7) and laccase (EC 1.10.3.1) activities were determined in mycorrhizal and non-mycorrhizal main and lateral roots of *Picea abies* (L.) Karst. and *Larix decidua* L., and in mycelia of the ectomycorrhizal fungus *Laccaria amethystea* (Bull.) Murr. grown under axenic conditions. Peroxidase isozyme patterns were identified after isoelectric focussing. In both tree species mycorrhizae contained the lowest and laterals of non-inoculated plants the highest peroxidase activities. Pure mycelia of *Laccaria amethystea* contained considerable laccase activity but no peroxidase activity. Laccase activity was barely detected in non-inoculated laterals of spruce, but was present in non-inoculated laterals of larch and in main roots of Norway spruce and larch. Highest laccase activities were found in mycorrhizae of both tree species indicating that most of the activity derived from the fungus. Specific peroxidase isozymes were suppressed in mycorrhizae. To determine localization of this specific isozymes in the cell walls, apoplastic washing fluids and root exudates were analyzed. The significance of the observed changes in isozyme patterns for mycorrhiza formation is discussed.

poster session 3 ST SH 73

NADIAN, HABIB, SALLY SMITH, ANGUS ALSTON & R. MURRAY. Department of Soil Science, Waite Agricultural Research Institute, The University of Adelaide, Glen Osmond, SA 5064, Australia. - The effects of soil compaction on plant growth and P uptake by *Trifolium subterraneum* in the presence of mycorrhizal colonisation.

In a series of experiments, growth and phosphorus (P) uptake by *Trifolium subterraneum* were studied with and without mycorrhizal fungi, in a soil with different bulk densities. Increasing soil bulk density from 1.0 to 1.6 Mg m⁻³ (penetrometer resistance=0.4 to 3.5 MPa at water potential -33 kPa) decreased plant growth and P uptake. Total P uptake and shoot dry weight of the plants colonised by *Glomus intraradices* were greater than those of non-mycorrhizal plants at all levels of soil compaction. However, mycorrhizal colonisation decreased as soil compaction was increased. Soil compaction had no significant effect on the fraction of root length containing arbuscules and vesicles, but compacting the soil to a bulk density of 1.6 Mg m⁻³ dramatically decreased total root length colonised by arbuscules, vesicles or by any combination of arbuscules, vesicles and intra-radical hyphae. Our observations demonstrated that there were significant differences in P uptake and plant growth between mycorrhizal and non-mycorrhizal clover in compacted soil and that soil compaction did not decrease the percentage of root length colonised. This led us to conclude that, under the conditions of this study, decline in benefit of mycorrhizal colonisation in highly compacted soil might be due to inhibition of root growth and not of fungal function.

talk session 7

NAGAHASHI, GERALD, DAVID DOUDS, & GLORIA ABNEY. USDA-ARS ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038-8038 USA. - A rapid micro-injection technique allows for the sensitive detection of root exudate signals which stimulate the branching and growth of germinated VAM fungus spores.

An in vitro method was developed to study the response of VAM fungus spores to root exudates. Pregerminated spores of *Gigaspora gigantea* or *Gigaspora margarita* were transferred to a Petri plate containing M medium. Anytime from 2 through 16 days of growth, small holes near growing hyphal tips were made in the gellan with a disposable sterilized Pasteur pipet. The holes were filled from a tuberculin syringe containing concentrated, filter sterilized, crude exudate. Prolific branching of actively growing hyphal tips of *G. gigantea* occurred within 6 to 12 hrs after injection. The "excited" branching pattern was identical to the pattern observed when a hyphal tip grows near an Ri T-DNA transformed carrot root. *G. margarita* had a slightly different response to the crude exudate but the response was identical to that observed when hyphal tips from this species approached a host root. A dose dependent signal response was observed for both mycorrhizal fungi. This method should lead to the identification of signal molecules in host root exudates which stimulate the growth and differentiation of VAM fungal hyphae.

poster session 4 ST AR 64

NEHLS, UWE, ACHIM WIESE & RÜDIGER HAMPP. University of Tübingen, Botanical Institute, Physiological Ecology of Plants, Auf der Morgenstelle 1, D-72076 Tübingen, Germany. - Identification and first characterization of a Norway spruce mono- saccharide carrier

One of the important features of ectomycorrhizal function is the conversion of plant carbohydrates into amino acids by the fungus and their reimport by the plant. For this purpose photoassimilates are supplied by the host as sucrose. Sucrose however, cannot be utilized directly by ectomycorrhizal fungi investigated so far because they lack both, an uptake system for sucrose and an invertase to hydrolyze sucrose. We thus assume that a plant apoplastic acid invertase (Schaeffer et al., 1995) forms hexoses in the apoplast and that both plant cortical cells and fungal hyphae compete for hexose uptake.

To understand carbohydrate exchange in ectomycorrhiza we started the identification of monosaccharide transporters in *P. abies*/*A. muscaria* mycorrhizas. The strategy we use is the identification of conserved regions of monosaccharide transporters from other organisms, primer design and PCR amplification of mycorrhizal cDNA fragments.

One PCR fragment identified so far by partially cDNA sequencing, alignment of the deduced protein sequence with databases and Northern blot analysis is a Norway spruce monosaccharide transporter. This PCR fragment was used to isolate a full length cDNA clone

from a cDNA library. The identified Norway spruce cDNA clone will be used to complement a yeast mutant defective in monosaccharide import for functional analysis of its transport properties.

Literature: Schaeffer C., Wallenda T., Guttenger M., Hampp R. (1995) Acid invertase in mycorrhizal and non-mycorrhizal roots in Norway spruce seedlings. *New Phytol.* **129**, 417-424

poster session 8 WF AR 42

NERG, ANNE, TOINI HOLOPAINEN & JARMO K. HOLOPAINEN. Department of Ecology and Environmental Science, University of Kuopio, P.O.Box 1627, 70211 Kuopio, Finland. - Does mycorrhizal infection affect the performance of herbivores on Scots pine seedlings?

The performance of various herbivores on differently mycorrhizal Scots pine seedlings was determined in several laboratory experiments and in 2-year field experiment. In laboratory experiments, aseptic petri dish technique was used and part of the seedlings was inoculated with mycorrhizal fungus *Cenococcum graniforme*. Different nitrogen levels in growth medium were also used. In the field experiment, part of the 3-year-old pine seedlings was treated with fungicide in order to reduce mycorrhizal infection level in roots. The performance of aphids, sawflies and tarnished plant bug was determined. Afterwards mycorrhizal infection level in roots was determined and samples were taken from shoots and roots for the analysis of host plant quality. In the laboratory experiment, tarnished plant bug laid more eggs on seedlings with higher nitrogen content, but mycorrhizal infection did not significantly affect the oviposition preference. However, aphids seemed to grow better on mycorrhizal seedlings. In the field experiment, reduced mycorrhizal infection did not affect the growth rates of aphids and tarnished plant bug or the growth rate and oviposition of sawflies. Some changes in the chemical quality of host plant were observed. It appeared, that mycorrhizal infection in petri dish seedlings, compared to totally non-mycorrhizal seedlings, may cause some changes in herbivore performance, but moderately declined mycorrhizal infection in the field does not drastically affect the herbivore performance.

poster session 8 WF AR 28

NICOLAI, LENA S. Department of Biology, University of Michigan, Ann Arbor, MI 48109-1048, USA. - Consumption of hypogeous fungi by rodents and consequent dispersal of mycorrhizal spores.

Many species of mammals consume fungal fruiting bodies such as mushrooms, truffles, and false truffles. Ingestion of sporocarps results in deposition of spores in feces. To determine if small mammals are effective dispersal vectors of fungal spores I studied four species of rodents living in ponderosa pine forests of northern Arizona. At each of three sites where relatively undisturbed forest is adjacent to an open or disturbed area I determined: (1) which species of rodents consume fungi during summer and fall; (2) the genera of fungi

consumed; (3) the relative number of spores of each fungal genus per gram of fecal pellet; (4) the viability of spores contained in feces; and (5) the potential for rodent movement of spores within and between forested and open areas. Each species of rodent consumes fungi to varying degrees. Diversity and number of spores contained in feces are very high. The majority of spores originate in hypogeous fruiting bodies of mycorrhizal fungi. Each rodent species moves spores in either the forest or the open area; only two species transport spores between forest and open areas at two of the study sites. Findings indicate that rodents are potential dispersal vectors for multiple genera of mycorrhizal fungi.

poster session 13 WF SH 89

NIEMIRA, BRENDAN A., LAURA ARRIOLA & GENE R. SAFIR. Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI, 48824. - Arbuscular mycorrhizal colonization and border cell production in the Liliaceae and Amaranthaceae

Different plant species vary in their capacity to produce border cells (BC, aka "sloughed root cap cells"), but production is consistent within families. The propensity of the members of a given family to be colonized by arbuscular mycorrhizal (AM) fungi is potentially related to BC production: families which produce thousands of BC per root tip (e.g. Fabaceae and Cucurbitaceae) tend to be heavily colonized by AM, families which produce hundreds of BC per root tip (e.g. Solanaceae) tend to be less heavily colonized by AM, while families which do not produce BC (e.g. Brassicaceae and Chenopodiaceae) are not colonized by AM. Species (*Asparagus officinalis*, *Allium porrum*, *Al. cepa*, *Al. schoenoprasum*) in the Liliaceae, a family known to be colonized by AM, were assessed for BC production and AM colonization. Preliminary results indicate that species in the Liliaceae produce BC on the order of 250-350 BC per root tip. These results support the hypothesis of a correlation between BC production and AM propensity. BC production and AM colonization of species (*Amaranthus tricolor*, *Am. caudatus*, *Gomphrena globosa*, *Celosia cristata*) in the Amaranthaceae, a family previously shown to produce BC, will be discussed.

talk session 8

NORMAN, JAMES¹, CLARA VIGO¹, JILL POVEY¹, NEIL GOW², MIKE MORRIS², KYRSTEN BLACK¹ & JOHN HOOKER¹. ¹Soil Biology Unit, Land Resources Department, SAC, Craibstone Estate, Aberdeen AB22 9TQ, UK. ²Department of Molecular and Cell Biology, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, UK. - Biocontrol of *Phytophthora* root pathogens by AMF: a study of possible mechanisms

There are many reports in the literature of interactions between arbuscular mycorrhizal fungi (AMF) and *Phytophthora* root pathogens. These reports suggest that AMF can control, increase or have no effect on disease development. Effective management of the symbiont-

pathogen interaction requires an understanding of the mechanisms involved in order to ensure a consistent response. Interactions between *Lycopersicon esculentum*, *Fragariae x ananassa* Duchesne and *Phytophthora* were investigated and up to 60 % disease control was measured in roots of AMF colonised plants. Further studies aimed to identify the mechanisms involved and measured the impact of AMF on root architecture, sporangia formation, zoospore taxis and root tip electrical fields.

poster session 8 WF AR 29

NORTH, MALCOLM¹ & JIM TRAPPE². ¹U.S. Forest Service, Forestry Sciences Lab, Fresno, CA 93710. ²Dept. of Forest Science, Oregon State Univ., Corvallis, OR 97331. - Relative abundance and animal consumption of fungal sporocarps in Pacific Northwest young-, mature- and old-growth forests.

Although fungal fruiting bodies are an important dietary staple for several small mammals, changes in their abundance and consumption with forest succession or disturbance has not been quantified. Epigeous and hypogeous sporocarps were sampled for 46 months in enclosure and open plots in managed-young, natural-mature and old-growth western hemlock (*Tsuga heterophylla*) stands in Washington State. Standing crop of epigeous sporocarps was low in most seasons and then increased 30 fold to an average of 2.28 kg/ha in the fall. Epigeous biomass varied little between stand type, and animal consumption of sporocarps in open plots was not significant. Standing crop of hypogeous sporocarps was 0.78 kg/ha in managed young stands, compared to 4.51 and 4.02 kg/ha in natural-mature and old-growth stands. Animal consumption of hypogeous sporocarps averaged 0.64 kg/ha. In natural-mature and old-growth stands, truffles biomass remained high year round and exceeded the consumption rate in all seasons. In managed-young stands, however, the winter available standing crop of 0.36 kg/ha was below the consumption rate and may indicate hypogeous sporocarps are a limited food source in these stands. The consistent level of animal consumption indicate hypogeous sporocarps may be an important and readily available, year-round food source compared to the ephemeral fruiting of epigeous sporocarps.

poster session 11 WF RR 25

O'DELL, THOM E., GEORGE J. MUELLER, JOE F. AMMIRATI. Department of Botany, Box 355325, University of Washington, Seattle, Washington, 98195-5325, USA. - Restriction fragment length polymorphisms of internally transcribed spacer DNA from dominant ectomycorrhizal fungi of old growth douglas-fir - western hemlock forests.

We are building a database of RFLP patterns for the identification of dominant sporocarp-forming species of ectomycorrhizal fungi in the old growth forests of Olympic National Park, Washington. The database is derived from sporocarps collected during ecological

studies over the past four years with a few older herbarium specimens included for taxonomic breadth. Sporocarps of about fifty species of ectomycorrhizal fungi have been examined, for each taxon three to six samples (collections separated by at least 20m) were used for DNA analysis as follows. DNA was extracted using a CTAB mini prep, amplified by PCR using ITS1f and ITS4 or 4b primers (Gardes and Bruns, 1993), and digested with restriction endonucleases Cfo I and Hpa II. Additional restriction digests were performed to distinguish closely related taxa. In most cases RFLP patterns were identical within species; a few taxa showed variation in the patterns. The database of ITS RFLP patterns being developed is proving useful in identification of species of fungi forming ectomycorrhizae on field collected roots.

talk session 4

O'DELL, THOM E., AMMIRATI, JOE F. Department of Botany, Box 355325, University of Washington, Seattle, Washington, 98195-5325, USA. - Diversity and abundance of ectomycorrhizal fungi: scaling in space and time.

Understanding the community ecology of ectomycorrhizal fungi is hindered by a paucity of data regarding how much area and how long to sample. We present data on variation in species richness and similarity of species detected at several spatial and temporal scales: within stand at the same and different sampling times (both within and between years); between stands; and between different plant associations. We give some examples extrapolating total richness from sample richness. The implications of the results for monitoring target species and selecting species-rich sites for conservation will be discussed. After four years of sampling sporocarps, more species are still being observed in old growth *Pseudotsuga menziesii* - *Tsuga heterophylla* stands in Olympic National Park, Washington. At one site species richness doubled in year four. Average annual precipitation and *P. menziesii* canopy cover are useful predictors of species richness at the landscape scale. Chao's estimate of total diversity based on the proportion of rare taxa suggests that we have so far encountered only about one half of the species present at our study sites.

poster session 9 WF SH 02

OHTONEN, RAUNI¹, TAINA PENNANEN² & HENRY VÄRE¹. ¹Department of Biology, University of Oulu, P.O.Box 333, FIN-90571 Oulu, Finland. ²Finnish Forest Research Institute, FIN 01301 Vantaa, Finland. - Development of the microbial community at a glacier forefront.

Succession of plant communities on primary soils is widely described but the importance and function of mycorrhiza in the process is poorly understood. Studies on microfungus succession are rare, and the function of the microbial community in primary succession, as in all ecosystems is also poorly understood. Plant

establishment and increasing biomass during succession result in carbon accumulation and greater diversity of organic carbon compounds in soil. Decomposing microflora may be substrate specific and thus reflect the structural change in developing plant communities. To detect changes in the microbial community, we analysed the phospholipid fatty acid (PLFA) composition in bare and in the rhizosphere soils collected at a deglaciated chronosequence. Nonmycorrhizal *Saxifraga ferrugineus* and ectomycorrhizal *Salix* spp. were sampled. Different subsets of microorganisms can be characterized by their PLFA patterns. The features of the microbial community can be described in their natural habitat without isolation and culturing the microorganisms. Even though PLFA profile does not give an actual species composition, it gives an overall picture of the community structure.

poster session 6 WF AR 04

OLIVEIRA, A. A. R.¹ & FRANCIS E. SANDERS².
¹Centro Nacional de Pesquisa de Mandioca e Fruticultura/EMBRAPA, 44380-000 Cruz das Almas, BA, Brazil. ²Department of Pure and Applied Biology, University of Leeds, LS2 9JT, UK. - Effects of dazomet treatment and P fertilization on mycorrhizal infection, growth and dry matter partitioning in field-grown *Phaseolus vulgaris*

A field experiment was carried out to study the influence of soil fumigation on the growth and arbuscular mycorrhizal (AM) of common beans. Plants were grown in dazomet-fumigated and non-fumigated soil at five levels of added P (0, 20, 50, 150 and 300 kg P/ha). During the vegetative phase, root length density, leaf surface and dry matter production were affected differently by fumigation under increasing P levels. However, at final harvest there were no significant differences in green pod yield between treatments. Differences observed in plant responses were due to phosphate fertilization rather than soil fumigation. Mycorrhizal infection by indigenous endophytes increased during the growing season in untreated soil and was significantly affected by addition of P fertilizer. There was a decrease in percent colonization as P soil availability increased. The native AM endophytes produced only limited root colonization in fumigated soil and did not differ in soil amended with different rates of phosphate.

poster session 6 WF AR 05

OLSEN, JASON¹, VICTOR GALEA², MAL HUNTER³, DAVID EDWARDS⁴, JAMES SCHAEFER¹ & LEEOLA MULLER¹. ¹Queensland Department of Primary Industries, Bundaberg Research Station, MS108 Ashfield Road, Bundaberg, Qld 4670, Australia. ²Department of Plant Production, University of Queensland, Gatton College, Lawes, Qld 4343, Australia. ³Queensland Department of Primary Industries, Redlands Research Station, PO Box 327, Cleveland, Qld 4163, Australia. ⁴Department of Agriculture, University of Queensland, St Lucia, Qld 4072, Australia. - Greenhouse and field responses of bell

pepper to applied P in association with a network of extraradical mycorrhizal mycelium

Two greenhouse experiments and a field trial were conducted to determine the effect of an established extraradical mycorrhizal mycelium on bell pepper (*Capsicum annuum* L.) grown with a range of P supply. In the greenhouse experiments, a mesh was used to exclude roots from the original sunflower host plants (to which either live [+VAM] or killed [-VAM] mycorrhizal [*Glomus mosseae* and *G. etunicatum*] inoculum was added at sowing), but allowed fungal hyphae to grow into the growth medium contained by the mesh. Within the mesh, the bell pepper seeds were sown in the low P growth medium (5 to 6 mg NaHCO₃- extractable P/ kg) and the P was spot placed at 5 rates ranging from 0 to 248 mg P/ kg oven-dry soil. In the field trial, bell pepper seedlings were transplanted into raised beds of a silt-loam (13.5 mg NaHCO₃-extractable P/ kg) in which the intact roots of a previous sweet corn crop remained in association with a network of *Gigaspora margarita*, *G. etunicatum*, *Scutellospora pellucida*, and *Glomus clarum*. P was applied at 5 rates (0 to 135 kg/ ha) prior to soil fumigation of the -VAM plots. Both the greenhouse and field experiments showed that at the low rates of applied P, +VAM plants had higher P concentrations in index tissues and greater dry weights than -VAM plants. At the higher P rates, dry weights of +VAM plants were lower than those of - VAM plants in the greenhouse experiments, whereas in the field trial, fresh weight of harvested fruit was greatest in +VAM plants. Root starch concentrations of +VAM plants were lower than -VAM plants in the greenhouse experiments, no differences were found in the field trial.

poster session 3 ST SH 77

OMRAN, TALAT, AHMAD ELSETTAWY, HOSNEY ABOU-GAZIA, KHAIRIA ABOUELKHAIR. Dept. of Forestry & Wood Tech., Faculty of Agriculture, Alex. University, Alexandria, Egypt. - Survey of VAM associated with some local and introduced timber trees grown in the newly reclaimed areas.

An experiment was conducted to identify the spores of VAM associated with 17 local and introduced timber trees grown in Abies and Mariout. Trees were eight years old. The soil at Abies had pH 7.72, E.C. 2.16 dS/m and CaCO₃ % 6.35, while the soil of Mariout had 8.81, E.C 11.84 dS/m and 35.4 CaCO₃ %.

Height and diameter of the trees were measured. The species used in this study were *Acacia ampliceps*, *A. karro*, *A. saligna*, *A. sclerosperma*, *A. stenophylla*, *A. victoriae*, two provenances of *Casuarina cunninghamiana* and *C. glauca* from Australia and a local one, *C. obesa*, *Eucalyptus camaldulensis*, *E. gomphocephala*, *E. microtheca* and *E. occidentalis*. The VAM spores associated with the trees were *Acaulospora laevis*, *A. scrobiculata*, *Glomus caledonicum*, *G. constrictum*, *G. convolutum*, *G. deserticola*, *G. leptotichum*, *G. melanosporum*, *G. mosseae*, *G. pulvinatum*, and *G. tortuosum*.

morning talk

OSONUBI, O¹, N. SANGINA² & I.E. OKON¹.
¹Department of Botany and Microbiology, University of Ibadan, Ibadan Nigeria. ²Soil Microbiology, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. - Effects of arbuscular mycorrhiza (AM) and different hedgerow prunings on yield of alley-cropped cassava and intercropped maize in a degraded tropical soil.

The effect of the AM fungus, *Glomus deserticola*, on fresh root yield of three cassava (*Manihot esculent* Crantz) cultivars and intercropped maize (*Zea mays* as influenced by two hedgerow species (*Gliricidia sepium* and *Senna siamea*) and their mixture pruned at two-month intervals were investigated in an alley cropping experiment. Mixing the two (*Gliricidia* and *Senna*) tree species in the same hedgerow, particularly with AM inoculation, significantly improved the cassava root yields compared to either of the tree species used alone as hedgerow. In these alley-cropped plots, AM inoculation increased cassava root yield by 42, 141 and 205% over uninoculated plants for cv TMS 30572, 91934 and 4(2)1425 respectively. Decomposition of the prunings was not affected by AM inoculation. For intercropped maize, there was no significant differences between the grain yield of maize mulched with pure *Gliricidia* and mixture of prunings, inoculated or uninoculated. These grain yields were 24-200% enhanced by AM inoculation than their uninoculated counterparts and controls in this degraded soil.

poster session 10 WF SH 32

PALENZUELA, JAVIER¹, MARCIA TORO² & JOSE MIGUEL BAREA¹. ¹Soil Microbiology and Symbiotic Systems Dept., Estacion Experimental del Zaidin, Profesor Albareda 1, Granada 18008, Spain. ²IVIC, Ecology Center, Postal code 21827, Caracas 10202-A, Venezuela. - Effect of a P-solubilizing bacteria on root colonization and mycorrhization in *Medicago sativa*.

An experiment was carried out to assess if a P-solubilizing bacteria (*Enterobacter* sp.) affects root morphology and arbuscular mycorrhizal colonization of *Medicago sativa*. Bacterial establishment in the rhizosphere of *M. sativa* was also studied. *Enterobacter* was inoculated at the beginning of the experiment, together with an inoculum of *G. deserticola*. The inoculum contained root fragments with 50 % AM colonization and abundant external mycelium. Bacterial inoculation significantly increased the number of mycorrhizal entry points and the percentage of mycorrhizal root length (time-course measurements). Specific root length (cm/g) and plant biomass were also enhanced by the double inoculation after 60 days. *Enterobacter* established itself in the rhizoplane with colonization levels of 10³ cfu/cm root after 60 days. This level of establishment was lowered by mycorrhizal inoculation, indicating a possible competition between the fungus and the bacteria. This same trend was observed not only in the rhizoplane but also in the rhizosphere. The inoculation of this solubilizing bacteria improved the establishment of the mycorrhizal fungus in the root of *M.*

sativa. The double inoculation also improved root length. Our results, therefore support recent descriptions on the so called "Mycorrhiza Helper Bacteria".

poster session 3 ST SH 78

PAWLOWSKA, TERESA & IRIS CHARVAT.
Department of Plant Biology, University of Minnesota, 220 Biological Sciences, 1445 Gortner Ave., St. Paul, MN 55108-1095. - Heavy metal tolerance in arbuscular mycorrhizal fungi from metal contaminated sites

Arbuscular mycorrhizal (AM) fungi colonize roots of most land plants and contribute to their improved mineral nutrition. The role of AM fungi in plant interactions with excess heavy metals remains largely unclear as are the mechanisms of metal tolerance in AM fungi. To investigate mechanisms of heavy metal tolerance in AM fungi three sites were selected as a source of fungal material: a calamine spoil mound originated from medieval lead ore mining in Boleslaw, Poland, an old landfill in St. Paul, MN, with moderately enhanced concentrations of cadmium, copper and lead resulting from a long term disposal of concentrated municipal wastes and a noncontaminated Minnesota native prairie. Individual AM fungal species were recovered from selected sites using pot trap cultures with big bluestem (*Andropogon gerardii*) and corn (*Zea mays*) as host plants. Isolated *Glomus* spp. spores were germinated under increasing concentrations of cadmium, copper, lead and zinc nitrate in order to assess their tolerance to heavy metal stress.

poster session 5 ST AR 28

PEIPP H.¹, W. MAIER¹, J. SCHMIDT¹, V. WRAY² & D. STRACK¹. ¹Institut für Pflanzenbiochemie, Weinberg 3, D-06120 Halle (Saale), Germany. ²Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, D-38124 Braunschweig, Germany. - Arbuscular mycorrhizal fungus-induced changes in the accumulation of secondary compounds in roots of the Poaceae

Cereals and other members of the Poaceae (60 species) were grown in a defined nutritional medium with and without the arbuscular mycorrhizal fungus *Glomus intraradices*. HPLC analysis of methanolic extracts from the roots of mycorrhized and nonmycorrhized plants revealed fungus-induced dramatic changes of some secondary metabolites. These compounds were isolated from the roots of *Hordeum vulgare* (barley) and identified by spectroscopic methods (NMR, MS) to be hydroxycinnamic acid amides, putrescine and agmatine conjugates, exhibiting a transient increase and cyclohexenone derivatives exhibiting a continuous accumulation. The most obvious effect was the induced accumulation of the cyclohexenone derivative 'blumenin' in most mycorrhizas, including barley. Results of an extensive taxonomic study of the occurrence of blumenin accumulation in mycorrhizas of members of the Poaceae are shown. It is suggested that the continuous accumulation of the cyclohexenone derivatives correlates

with the establishment of the mycorrhizas whereas the transient accumulation of the amides in early developmental stages seems to reflect initiation of a defense response.

poster session 14 WF RR 04

PERCUDANI, RICCARDO¹, ZAMBONELLI, ALESSANDRA², OTTONELLO, SIMONE¹. ¹Istituto di Scienze Biochimiche, Universita' degli studi di Parma, Viale delle Scienze, 43100 Parma Italia. ²Dipartimento di Protezione e Valorizzazione Agroalimentare, Universita' degli studi di Bologna, Via Filippo RE, 8, 40126 Bologna Italia - Computer-assisted recognition of truffles

The identification of truffle species has economical as well as basic knowledge implications. An extensive application of morphological characters for truffle recognition, at the level of both fruitbodies and mycorrhizae, is presently limited, among other things, by the lack of suitable determination keys. As an initial step toward an identification method accessible also to non specialists, we have coded a number of available, as well as some novel, character descriptions into a format adhering to the DELTA standard. Based on the above descriptors, we have worked out a computer procedure to assist in taxa identification with the aid of pictures, drawings and selected comments. In its present version, the program allows to correctly distinguish, at the level of fruitbodies, the various genera of hypogeous ascomycetes as well as different species belonging to the genus *Tuber*. Since this approach has proved to be less effective when applied to closely related mycorrhizae, we are taking advantage of currently developed, *Tuber*-specific molecular markers, as additional identification keys that will be entered into the determination program.

talk session 4

PEROTTO, SILVIA¹, C. BANDI², P. YOUNG³, P. BONFANTE¹. ¹Dipartimento di Biologia vegetale dell'Università and CSMT-CNR, V.le Mattioli 25, Torino 10125, Italy; ²Dipartimento di Genetica e Microbiologia, Pavia 27100, Italy; ³Department of Biology, University of York, York YO15DD, UK. - Identification of introns in the nuclear ribosomal genes of ericoid mycorrhizal fungi

Ericoid fungi are a heterogeneous group of soilborne fungi that can associate with the fine roots of Ericaceae to form endomycorrhizae. Only few species of ascomycetes - *Hymenoscyphus ericae* and species of *Oidiodendron* - have been identified taxonomically, as most fungi isolated from mycorrhizal roots grow in culture as sterile mycelia.

We have analysed the nuclear ribosomal genes of several ericoid fungi isolated from distant geographic regions to investigate i) their genetic diversity and ii) the phylogenetic position of sterile mycelia. Regions of the ITS and 18S genes were amplified by PCR and analysed either directly, or by sequencing and restriction digestion. The results suggest that sterile mycelia belong to different taxonomic groups, thus increasing the range of species known to form ericoid mycorrhizae. Moreover, analysis

of the 18S rDNA has revealed in most isolates the presence of intron elements, a rare event in the nuclear rDNA of eukaryotes. One insertion in the small subunit of rDNA of *H. ericae* was already reported by Egger et al (1995). However, we have identified several additional intron elements along the 18S gene of different ericoid species, their position and number depending on the fungal isolate. These introns constitute an additional source of genetic diversity for ericoid fungi. Interestingly, the only other report of a similar situation in the nuclear ribosomal genes concerns a group of polyphyletic fungi forming lichen symbiosis (Gargas et al, 1995), and poses the question of a possible role of these elements in the fungal life cycle.

Egger KN, Osmond G, Goodier JL, 1995. *Biochim Biophys Acta* 1261:275

Gargas A, DePriest PT, Taylor JW, 1995. *Mol Biol Evol* 12:208

talk session 1

PHILIP E. PFEFFER, YAIR SHACHAR-HILL, DAVID DOUDS and GUILLAUME BECARD Eastern Regional Research Center, ARS Wyndmoor, PA 19038 - Metabolism of Glucose, its Uptake and Translocation in Mycorrhizal Leek and Transformed Carrot Roots

Vesicular arbuscular mycorrhizal (VAM) fungi are symbionts for a large variety of crop plants. Although it is known that these fungi can transport phosphate and other minerals to the root cells, it is not established what carbon source they receive from the plant nor is it known where it can be taken up. To trace the course of carbon metabolism we have used NMR spectroscopy with ¹³C glucose labeling *in vivo* and in extracts to examine leek roots colonized by *Glomus etunicatum* (and uncolonized controls) as well as germinating spores. These studies implicate glucose as a likely substrate for VAM fungi in the symbiotic state. NMR time course experiments with simultaneous feeding of 0.6mM ¹³C-1glucose, labeled exclusively the fungal metabolites trehalose and glycogen only when arbuscules were present in the mycorrhiza. By contrast exclusive labeling of sucrose, with twice as much incorporation was observed in uncolonized plants. Despite very minor labeling of mannitol C1,6 in the colonized root tissue there was no detectable scrambling of the label from C1 to C6 position in the glucose moieties of trehalose. Thus the occurrence of mannitol cycling is absent in this VAM fungus in its symbiotic state. In contrast ectomycorrhizal fungi have been shown to produce significant amounts of mannitol and exhibit mannitol cycling to produce NADPH. To differentiate between the uptake of glucose by internal vs. external hyphae under long term labeling conditions we examined the lipid composition of extracts of colonized (*Glomus interadices*) transformed root tissue and its separated, extended hyphae and spores. Labeling of fungal lipids in both root sections and separated fungal tissues were observed only when feeding was initiated through the colonized root. The ratio of specific labeling of fungal lipid to host carbohydrate (sucrose) indicated that direct uptake of glucose by internal hyphae was the favored

pathway. No evidence of glucose uptake by external hyphae or spores was observed.

poster session 12 WF SH 66

PHAVAPHUTANON, L.¹, F.T. DAVIES, JR.¹, T.W. BOUTTON², S.A. DURAY¹ & J.N. EGILLA.¹ Dept. of Horticultural Sciences., and ²Dept. of Rangeland Ecology & Management., Texas A&M University, College Station, TX 77843. - Drought response of mycorrhizal and nonmycorrhizal neem plants of comparable size and tissue nutrition.

Mycorrhizal [*Glomus intraradices* (VAM)] and nonmycorrhizal (Non-VAM) neem plants (*Azadirachta indica*) of comparable size and tissue nutrition were subjected to a slowly developing drought. Only Non-VAM plants received supplemental phosphorus (P). VAM and Non-VAM plants responded to drought similarly. However, mycorrhiza compensated for low P supply, allowing VAM plants to have comparable growth, tissue P, and other physiological parameters as Non-VAM plants – which received higher P supply. Drought decreased growth, transpiration (E), photosynthetic rate (A), stomatal conductance (g_s), and plant water status. Osmotic adjustment did not occur, but the relatively low osmotic potential of this species helped maintain turgor during drought. Plant water relations and A of stressed plants fully recovered in 24 hr after rehydration, while g_s and E partially recovered. Instantaneous water use efficiency (A/E) increased during drought and recovery, except for a decrease at peak stress due to very low A. Carbon isotope discrimination (Δ) values of mature leaves remained constant regardless of mycorrhiza or drought. However, Δ decreased in expanding leaves which developed during a drought period, indicating an increased long-term water use efficiency of these leaves.

poster session 5 ST AR 29

PICHÉ, YVES¹, JEAN-GUY CATFORD¹, JACQUES SIMARD², FERNAND LABRIE² & MARIE-JOSÉE POULIN¹. ¹CRBF, ²CHUL. Université Laval, Québec, Canada, G1K-7P4. - Flavonoids: fundamental growth factors to AM fungi.

Ri T-DNA transformed roots of carrot and non-transformed carrot root seedlings release different flavonoids which are known to be potent signals affecting fungal growth of AM fungi. Among the flavonoids detected in isolated root exudates, quercetin and kaempferol are known to be stimulatory, apigenin is inhibitory, while rutin and luteolin have no significant effect on the hyphal growth of *Gigaspora margarita*. Likewise, fungal growth responses to root flavonoids are not uniform among AM fungi where isoflavones such as biochanin A are known to be stimulatory on hyphal growth of *Glomus intraradices* but inhibitory on *G. margarita*. Yet, the growth response to flavonoids seems highly specific among AM species and appears consistent with the existence of putative target-hyphae reactive sites. According to our results, the specific agonist effects of

quercetin and biochanin A on hyphal growth of *G. margarita* and *G. intraradices* were suppressed efficiently by flavonoid antagonists recently found in our laboratory. Our data suggest that plant flavonoids are involved in a regulatory system which control the fungal growth of AM fungi.

poster session 8 WF AR 30

PICONE, CHRIS. Department of Biology, University of Michigan, Ann Arbor, MI 48109-1048. - Hyphal Networks in Tropical Forests Confound Estimates of AMF Inoculum Availability

Tropical forest trees depend more on mycorrhizal infection than pioneer plants. However, spore counts and Most Probable Number (MPN) estimates of inoculum availability are typically lower in tropical forest than in pasture. Here I propose that forest inoculum consists primarily of hyphal networks rather than spores, and thus soil disturbance required by the MPN method would affect forest inoculum more negatively than pasture inoculum. I tested inoculum availability in forest vs. pasture by recording % root infection over time in undisturbed soil with three host species (*Zea mays*, *Psidium guajava*, and *Swietenia macrophylla*), and in both undisturbed and chopped soil for one host species (*Cedrela odorata*). Root infection was consistently higher in undisturbed forest soil compared to pasture soil for all host species. Chopping the soil reduced forest soil infection to that of sterile, control soil, while chopping had less negative effects on infection in pasture soil. Thus, tropical forest inoculum is far more available than previously suggested, and hyphal networks are relatively more important sources of infection in forest compared to pasture.

poster session 10 WF SH 04

PILLUKAT, ANGELA¹ & GERHARD WANNER². ¹Section Mycology, Institute for Systematic Botany, University of Munich, Menzinger Str. 67, D-80638 Muenchen, Germany. ²Section Electron Microscopy, Botanical Institute, University of Munich, Menzinger Str.67, D-80638 Muenchen, Germany. - The parasitic ascomycete *Hypomyces lateritius* on ectomycorrhizae of the basidiomycete *Lactarius salmonicolor*.

Hypomyces lateritius (Fr.:Fr.) Tul. is a high specialized parasite on fruitbodies which shows a preference for *Lactarius*-species of the *Dapetes* group. In contrast to other *Hypomyces*-species this parasitic ascomycete does not liquify but instead causes hardening of the infected fruitbodies. The popular name 'stone milk cap' originates from this phenomenon. The hardening prolongs the lifespan of the fruitbody and provides the parasitic fungus with sufficient time to develop its own fruitbodies. A *H. lateritius* infection is externally recognizable by a complete suppression of gill development and a white layer of parasitic hyphae on the hymenium, while internally the flesh of the fruitbody is wholly infected and hard in all parts. It can now be demonstrated that infection extends beyond the fruitbody to include the

ectomycorrhizae of the host species. Infected ectomycorrhizae of *Lactarius salmonicolor* Heim & Lecl. develop cone-shaped ends which increase the diameter of the side-branches of the ramification system. Photomicrographs taken with the electron microscope and using Normarski interference contrast light microscopy reveal the parasitic hyphae growing between the host hyphae inside the mantle and the formation of conidia on the surface of the ectomycorrhiza.

poster session 7 WF AR 08

PILZ, DAVID, & RANDY MOLINA. Pacific Northwest Research Station, U.S.D.A. Forest Service, Forestry Sciences Laboratory, 3200 Jefferson Way, Corvallis, Oregon, 97331, USA - Commercially-harvested edible forest mushrooms: Productivity and sustainable harvest research in the Pacific Northwest

The Pacific Northwest Research Station (U.S. Department of Agriculture, Forest Service) has initiated an active research program to address concerns caused by the rapid growth in commercial harvesting of chanterelle, matsutake, and morel mushrooms from forest lands in Oregon and Washington. National forest lands operate under a mandate to manage ecosystems for biological diversity and healthy forests, while simultaneously providing opportunities for the sustainable harvest of commercial products. To predict, regulate, and enhance mushroom harvesting opportunities, forest managers need information on mushroom productivity in diverse habitats and how forest management activities affect productivity over time. Information on the ecology and reproduction of these fungi, and the impacts of collection, is also essential to ensure their sustainable harvest, hence avoiding declines in production such as those noted in Europe.

poster session 6 WF AR 06

POLI, RICHARD, VICTOR GALEA & ALLAN WEARING. Department of Plant Production, University of Queensland Gatton College, Lawes, Qld 4343, Australia. - Vesicular arbuscular mycorrhizae can increase container plant production of miniature roses.

Two VA mycorrhizae species, *Glomus intraradicis* and *Scutellospora pellucida*, were used to study the growth responses in Miniature Roses to VA mycorrhiza. Both species colonised the rose roots in soilless media, but *G. intraradicis* was more successful. Uninoculated plants remained uncolonised by VA mycorrhiza. For both VA mycorrhiza species, the percentage of roots colonised was lower when grown at higher nutrient levels. *G. intraradicis* in most of the measured parameters generally promoted a significantly greater response than *S. pellucida* or the un-inoculated control. In most cases, *S. pellucida* gave inconsistent responses, and in some cases, actually suppressed growth compared to the control.

In a high nutrient regime, *G. intraradicis* significantly improved growth, but not as greatly as it did when nutrients were less plentiful. In contrast, *S. pellucida* sometimes increased growth at lower nutrient

levels, but often suppressed growth at higher nutrient levels.

The selection of a suitable VA mycorrhizal ecotype (eg. *G. intraradicis*) can be used to successfully increase plant growth, and therefore, throughput of container roses using high nutrient levels in a soilless media.

poster

POLI, RICHARD, VICTOR GALEA & ALLAN WEARING. Department of Plant Production, University of Queensland Gatton College, Lawes, Qld 4343, Australia. - VA mycorrhizae significantly improve germination and growth of Sturt's desert pea (*Clianthus formosus*).

Two VA mycorrhizal species *Glomus intraradicis* and *Scutellospora pellucida* and a VA mycorrhizal blend (*Glomus mosseae* and *Glomus etunicatum*) were used to study the germination, survival and growth responses of a containerised Australian native plant, Sturt's Desert Pea (*Clianthus formosus*). *C. formosus* is classified by nurserymen as a difficult species to produce as seedlings, and then establish as potted colour.

Our studies show that the introduction of VA mycorrhiza into high nutrient soilless media used for growing *C. formosus* increased the seedling survival rate by at least 45% over un-inoculated controls. Not only was the survival rate of these seedlings increased, but they also grew significantly larger than the control plants. The increase in seedling growth was evident by greater lateral shoot production and higher total shoot biomass. Increased survivability, establishment and growth rates of *C. formosus* in high nutrient, soilless media, highlights the need to incorporate VA mycorrhizae as an integral component of containerised plant production systems.

poster session 8 WF AR 31

PRINGLE, ANNE, & JAMES D. BEVER. Department of Botany, Duke University, Durham, NC, 27708, USA. - Seasonal sporulation of eleven VAM fungal species from an old-field in Durham, NC.

Spores of eleven common VAM fungal species from an old field in Durham, NC were counted each of six consecutive months. Nine sites were sampled, each separated by 1m and arranged in a 3x3 grid pattern. At each site, three sub-samples were collected each month. Sub-samples were combined and only healthy spores were counted. Results suggest that there is substantial variation in species composition between sites. There were also distinct seasonal trends in the sporulation of VAM fungal species. For example, *Gigaspora gigantea* was most common in the winter months, and *Acaulospora collosa* was most common in the summer months. The seasonality of VAM sporulation may be correlated to host plant growth and senescence.

talk session 4

PRITSCH, KARIN¹, HERBERT BOYLE², FRANÇOIS BUSCOT¹, JEAN CHARLES MUNCH³. ¹FAL, Institut fuer Bodenbiologie, Bundesallee 50, D-38116 Braunschweig. ²Staatliches Museum fuer Naturkunde Goerlitz, D-02806 Goerlitz. ³GSF, Institut fuer Bodenoekologie, Ingolstaedter Landstrasse 1, D-85764 Oberschleissheim. - Diversity of black alder ectomycorrhizae - anatomical & molecular investigations

Diversity of black alder mycorrhizae (*Alnus glutinosa* (L.) Gaertn.) was investigated within the ecosystem research project "Bornhoeved Lakes" (Northern Germany). Ectomycorrhizae were characterized morphologically and anatomically. Additionally sporocarps were collected at the experimental plot and at two geographically distant alder stands in order to obtain a broad spectrum of potential alder mycobionts for identification. From both, mycorrhizae and sporocarps, polymorphisms within the ITS region of the rDNA were studied by PCR/RFLP-analyses. The comparison of fingerprints led to identification of eight ectomycorrhizal types. Eight more types were characterized but remained unidentified. Identification was only achieved in mycorrhizal and sporocarp samples taken from the experimental plot, whereas none of the geographically distantly collected fruitbodies enabled further identification of mycorrhizal types. Within the experimental plot, above and below ground observations revealed an only 50 % overlap in the appearance of sporocarps and mycorrhizal species. Additional investigations on isolates obtained from some of the sixteen characterized mycorrhizae indicate a possible intraspecific variability. Application of molecular techniques with a higher resolution of polymorphisms is required to elucidate intraspecific diversity and population structure of ectomycorrhizae.

poster session 8 WF AR 57

QUIST, DAVID and JOE AMMIRATI. Department of Botany, University of Washington, Box 355325, Seattle, Washington 98195. - Assessing the mycorrhizal potential of Xylaria species with epiphytic Orchidaceae.

Xylariaceous fungi of tropical ecosystems are known predominantly as saprobes and weak parasites on woody plants. Their overall importance has yet to be investigated. The purpose of this study is to determine the role of Xylarias as possible mycobionts in tropical epiphytic orchids. Several Xylarias were isolated from the roots of epiphytic orchids growing in the Lacandon jungle in southern Chiapas, Mexico. Orchid seeds were germinated with the Xylarias using different nutrient regimes to determine whether a marked growth stimulus occurs in cultures inoculated with the fungus versus a control. Microscopic observation of infected protocorm cells was used to reveal the pattern of fungus colonization within the plant tissue. Earlier work on endophytes of epiphytic orchids suggested the potential of Xylarias as mycorrhizal symbionts, yet no investigations have been conducted to substantiate these claims. Since Xylarias function primarily as decomposers of woody substrates,

it is possible that they breakdown lignin and cellulose to carbon compounds that can be readily transferred to and utilized by orchid-host tissue, much like the peloton forming basidiomycetes. This study provides insight into this possibility based on experimental data.

poster session 6 WF AR 24

QUORESHI, ALI, VICTOR TIMMER. Faculty of Forestry, University of Toronto, Ontario, M5S 1A1, Canada. - Ectomycorrhiza formation and nutrition of containerized Black spruce seedlings under exponential fertility regime

Black spruce (*Picea mariana* [Mill.] B.S.P.) seedlings were grown for 20 weeks in a peat-vermiculite medium and inoculated with two ectomycorrhizal fungi: *Hebeloma crustuliniforme* (Bull. Ex St- Amans) Quel. and *Laccaria bicolor* (R. Mre.) Orton at the time of sowing. The seedlings were reared under conventionally (12.5 mg N seedling⁻¹ season⁻¹) and exponentially applied rates with three levels of N fertilization (12.5, 25.0, 50.0 mg seedling⁻¹ season⁻¹) to examine the effects of fertilization regime and levels of fertilization on mycorrhizae formation and seedling nutrient status. Conventionally fertilized seedlings inoculated with both inoculum types had only 22% - 26% of their short roots ectomycorrhizal. However, exponentially fertilized seedlings inoculated with either inoculum formed 49%-85% ectomycorrhizae. The intensity of mycorrhizae formation was reduced with the high nitrogen (4E) after 20 weeks. Seedlings fertilized exponentially (1E) produced lower biomass than conventional seedlings. The N and P contents were significantly higher in mycorrhizal seedlings than control seedlings. The results suggest that exponential fertilization may be an effective way to produce commercial mycorrhizal seedlings. Since it increases nutrient reserves built up while still maintaining mycorrhizal associations.

poster session 1 ST RR 06

RAGUPATHY, S. & A. MAHADEVAN. Centre for Advanced Study in Botany, University of Madras, Guindy Campus, Madras 600 025, INDIA - Effect of mycorrhizal treatments on the growth of acacias

Fast growing *Acacia holosericea* (Australian) and *A. nilotica* (Indian) inoculated with *Gigaspora albida*, *Glomus aggregatum* and *G. mosseae* grown under pot condition were evaluated. *G. mosseae* treated *A. holosericea* and *A. nilotica* exhibited significant growth and mycorrhizal colonization 3 months after inoculation compared with *G. aggregatum* and *G. albida* treated plants and control. Height and diameter of growth of the two species were compared. The growth of *A. nilotica* was initially slower than *A. holosericea*. However, *A. nilotica* plants of 1 year old retained high growth rates compared with *A. holosericea*. Phosphorus content increased in all the VAM treated plants; especially more in *G. mosseae* treated plant. However, the response slightly varied with different species of VAM fungi. Mixed inoculation did not show any significant difference over

individual inoculum. The extent of infection substantially differed from species to species. The per cent infection was maximum in *Gigaspora albida* inoculated plants.

poster session 5 ST AR 31

RALPH, MARY-LOUISE & JOHN E. HOOKER. Soil Biology Unit, Land Resources Department, SAC, Craibstone Estate, Bucksburn, Aberdeen AB2 9TQ, Scotland. - The influence of plant root exudates on the growth of extra-radical hyphae of arbuscular mycorrhizal fungi

Growth of extra-radical hyphae of the arbuscular mycorrhizal fungus *Glomus etunicatum* was examined *in vitro* using a system which restricted the mycelium to a 2-dimensional growth form. Transverse sections of mycorrhizal roots, used as the inoculum for generation of extra-radical hyphae, were incubated on dialysis membrane overlying molecular grade agarose gel impregnated with the test compound. Observations showed that the exudates of both host and non-host plant roots significantly decreased the length of external hyphae produced by root piece inocula. Host plant exudates appeared to trigger a morphological change in the hyphae, indicating the importance of architecture in hyphal function. Plant flavonoids known to promote the germination of mycorrhizal spores were found to be detrimental to the development of external hyphae, suggesting that different fungal phases are able to react in different ways to environmental stimuli.

poster session 3 ST SH 83

RAMAN, N. & T. SELVARAJ. Centre for Advanced Studies in Botany, University of Madras, Guindy campus, Madras-600 025, INDIA. - Effect of rhizobial and VA-mycorrhizal inoculation on *Prosopis juliflora* treated with tannery effluents.

Heavy metals and toxic elements are being deposited on both soil and in plants in undesirable ratios as a result of industrial activity. These elements in the environment raise genetical, physiological and ecological problems. Studies on the interaction of VAM and *Rhizobium* on legume trees are of immense value. The present study was taken to investigate the effect of *Glomus fasciculatum* and *Rhizobium* sp. in *Prosopis juliflora* treated with tannery effluents. *Rhizobium* and *G. fasciculatum* were inoculated individually or in combination on *prosopis* seedlings in potted conditions. Mycorrhiza and *Rhizobium* combination enhanced the nutrient uptake and improved the nitrogen fixation. Dual inoculation has played a vital role in the amelioration of toxic heavy metals, particularly chromium.

talk session 8

RANGARAJAN, M. Department of Biotechnology, CPMB, Tamil Nadu Agricultural University, Coimbatore 641 003, India. - Synergistic effects of *Glomus fasciculatum* and *Azospirillum brasilense* on the production of *Morus alba* and economic characters of silk worm larvae.

A field experiment was conducted at Regional Research Station Paiyur in Tamil Nadu to find out the synergistic effects of *Glomus fasciculatum* and *Azospirillum brasilense* along with different levels of nitrogen and phosphorus on the biomass production of mulberry (*Morus alba* L.). The experiment consisted of 10 treatments with three replications. Average of two years data on the leaf yield of mulberry indicated that both the organisms at the reduced levels of (75%) N and P gave highest leaf yield of 15.20 tonnes/ha/year, while control with 100% N and P recorded 12.20 tonnes/ha/year, registering 24.9% increase over control. Nutritional assessment of leaf through silk worm rearing indicated that there was significant improvement in economically important characters such as cocoon weight, filament length and silk weight due to the feeding of dual inoculated leaves of *Morus alba* by the silk worm larvae. Response of silk worm larvae to VAM inoculated *Morus alba* L. has not been reported earlier.

poster session 1 ST RR 44

RATTAN, ANUPAMA & ALOK ADHOLEYA. Tata Energy Research Institute, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi 110 003, India. - Effect of mycorrhizal inoculation on the growth and nutrient uptake of three ornamental plants.

Three ornamental plants (*Petunia hybrida*, *Calistephus chinensis*, *Impatiens balsamina*) were tested for their response with mixed indigenous culture in highly alkaline, nutrient deficient soils of northern India. All the three plant species responded positively to mycorrhizal inoculations and resulted to higher reproductive and vegetative growth over the non mycorrhizal plants. *P. hybrida* showed a three fold as compared to the two fold increase in reproductive growth. Mycorrhizal inoculation resulted to increased leaf areas, greater shoot and root weight, number of flowers and early flowering by atleast 15 days in addition to significant increase in P uptake in shoots of all the three ornamentals. Thus, mycorrhizal inoculation at the nursery level can be effective for producing more number of flowers and also early flowering under nutrient deficient conditions.

poster session 10 WF SH 34

RAVNSKOV, SABINE¹, IVER JAKOBSEN¹, LENE KRAGELUND² & OLE NYBROE². ¹Plant Nutrition, Environmental Science and Technology Department, Risø National Laboratory, DK-4000 Roskilde, Denmark. ²Microbiology Section, Department of Ecology and Molecular Biology, The Royal Veterinary and Agricultural University, Rolighedsvej 21, DK-1958 Frederiksberg, Denmark. - Influence of arbuscular mycorrhizal fungi on *Pseudomonas fluorescens* (DF57) in soils with and without roots

The presence and activity of heterotrophic soil microorganisms is influenced by plant roots and their biotrophic mycorrhizal fungi. The effect of two arbuscular mycorrhizal fungi (AMF) on numbers of *Pseudomonas fluorescens* (DF57) was studied in soil

with roots and AMF hyphae and in soil with AMF hyphae only. Cucumber plants were grown in semi-sterile soil in a compartmented growth system; one compartment with roots was separated from two root-free compartments by means of 20µm nylon mesh. The soil was inoculated with *Glomus caledonium* (RIS42, BEG15), *Glomus interadices* (28A) or left uninoculated. The soil in half of the pots was inoculated with *P. fluorescens*. After harvest the soil was examined for hyphal length density and total numbers of bacteria were counted after staining with acridine orange. Numbers of *P. fluorescens* were determined by immuno-fluorescence microscopy. Both mycorrhizal fungi reduced numbers of *P. fluorescens* in soil with roots, whereas they increased the amount of bacteria in soil with hyphae only. The higher number of *P. fluorescens* in root-free soil indicates that hyphae serve as substrate for the bacteria and exclude the possibility that these two AMF should be toxic to the bacteria. The lower number of *P. fluorescens* in root compartments of mycorrhizal plants may therefore suggest that the two microorganisms compete for nutrients in the rhizosphere.

morning talk

READ, DAVID J. Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK. - Mycorrhizas in the real world - the past and the future

This paper takes a brief but critical look backwards at the achievements of the first hundred years of mycorrhizal research and a longer look forward with a view to identifying the priorities and challenges that lie ahead. Considerable progress in description of the structure and occurrence of mycorrhizas has facilitated effective classification of types and awareness of the extent of their distribution through the plant and fungal kingdoms. It is contended, however, that attempts to elucidate the functions of the symbiosis, which have normally been made under simplified laboratory conditions and in which an undue emphasis has been placed upon phosphorus nutrition of the plant, have failed to provide an adequate understanding of the role of mycorrhizas in their natural environments.

While laboratory analyses have adopted a reductionist approach, field studies have revealed the diversity of fungal and plant species forming the symbiosis and the complexity, physico-chemical as well as microbiological, of the environments in which they occur. Simplistic notions of mycorrhizal function are strongly challenged by such observations. Examples of recent studies, which examine mycorrhizal responses in natural or semi-natural conditions, are used to highlight multifunctionality, to stress the need for studies at the community level, and to emphasise that the status of the symbiosis in any ecosystem can only be established in experiments which investigate the full range of its potential functions under realistic conditions.

poster session 1 ST RR 28

REDDY, M.S* & K.NATARAJAN². *Tata Energy Research Institute, Habitat Place, Lodi Road, New Delhi 110 003, India. ²CAS in Botany, University of Madras, Guindy Campus, Madras 600 025, India. - Coinoculation efficacy of ectomycorrhizal fungi on *Pinus patula* seedlings in the nursery.

Forest tree seedlings with multiple mycorrhizas can withstand a wider range of plantation site conditions than plants having only one species of ectomycorrhizal fungus. The majority of fungal inoculations in the world have been developed to produce seedlings infected with only one species of mycorrhizal fungus. In the present study coinoculation efficacy of two ectomycorrhizal fungi viz., *Laccaria laccata* and *Thelephora terrestris* on the growth and mycorrhizal development of *Pinus patula* seedlings was studied and compared with individual inoculation of these fungi. The total number of mycorrhizas increased in seedlings inoculated with the mixed inoculum when compared to the individual inoculum. The percent colonization of *T.terrestris* was more than *L.laccata* when seedlings are inoculated with the two fungi simultaneously. Seedlings inoculated with mixed inoculum showed more shoot height, dry weight when compared to individual inoculated seedlings. From the results it was concluded that coinoculation of ectomycorrhizal fungi improved the growth and mycorrhizal development of *P.patula* seedlings than individual inoculation.

poster session 11 WF RR 33

REDECKER, DIRK¹, HEIDEMARIE THIERFELDER¹, CHRISTOPHER WALKER² & DIETRICH WERNER¹. ¹Fachbereich Biologie der Universitaet Marburg, Karl v. Frisch-Str., 35032 Marburg, Germany. ²Forestry Commission, Northern Research Station, Roslin, Midlothian EH25 9SY, UK - Species identification of arbuscular mycorrhizal fungi by restriction analysis of rDNA internal transcribed spacers (ITS)

The relationship between morphological and genetic diversity within the arbuscular mycorrhizal (AM) fungi (Glomales) was studied. A combined technique of polymerase chain reaction and restriction length polymorphism analysis was used to generate specific patterns from spore extracts of the Glomales. With the primers ITS1 and ITS4, DNA fragments were amplified from *Scutellospora* and *Gigaspora*, that showed an approximate length of 500 base pairs. The apparent length of the corresponding fragments from *Glomus* spp. varied between 580 and 600 base pairs. Within the genus *Glomus*, the restriction enzymes *Mbo* I, *Hinf* I and *Taq* I were useful to distinguish species. Depending on the restriction enzyme used, groups of species with common fragment patterns could be found. Five isolates of *Glomus manihotis* and *Glomus clarum* could not be distinguished by their restriction patterns, which correlates with their morphological similarity. The variation of ITS sequences between the *Gigaspora* species under study was low. Fragment patterns of *Scutellospora* spp. demonstrated their phylogenetic

relationship with *Gigaspora* and only a slightly higher degree of variation.

poster session 6 WF AR 13

RILLIG, MATTHIAS C.¹, JOHN N. KLIRONOMOS² & MICHAEL F. ALLEN¹. ¹Department of Biology, San Diego State University, San Diego, CA 92182, U.S.A. ²Department of Botany, University of Guelph, Guelph, ON, Canada N1G 2W1. - 'Infection intensity' by arbuscular mycorrhizal hyphae as a response variable in mycorrhizal research.

A method for the estimation of the 'intensity' of hyphal root colonization by arbuscular mycorrhizal fungi (and other fungi) is proposed. Infection intensity is measured as the number of hyphae encountered per gridline intersect and is thus an extension of the magnified intersect method (McGonigle et al. 1990) for measuring percent root infection. 'Intensity' is independent of percent root infection, and may be used to get better estimates of fungal biomass in roots. Its usefulness is demonstrated in a case study that examines the effects of elevated atmospheric carbon dioxide on mycorrhizal infection.

poster session 1 ST RR 02

RINCÓN, A., I. ALVAREZ, X. PARLADÉ & J. PERA. Dept. Patología Vegetal. IRTA. Centre de Cabriels, 08348 Cabriels, Barcelona. Spain. - Inoculation of *Pinus pinea* seedlings with four ectomycorrhizal fungi under nursery conditions.

Three methods of inoculation (spore suspension, mycelium inoculum grown in a peat-vermiculite mixture and mycelium entrapped in alginate gel), have been tested at different dosages to determine their effectivity to inoculate containerized *Pinus pinea* seedlings with four ectomycorrhizal fungi (*Laccaria bicolor*, *Melanogaster ambiguus*, *Pisolithus tinctorius* and *Rhizopogon roseolus*) under nursery conditions. Spores of *P. tinctorius*, *R. roseolus* and *M. ambiguus* were applied at 10E3, 10E4, 10E5, 10E6, 10E7 and 10E8 (spores/plant). Mycelial inoculum of *P. tinctorius* was mixed with the potting substrate at the dosages of: 1:4, 1:8, 1:16, 1:32 and 1:64 (inoculum/substrate, v/v). Mycelium of *L. bicolor* entrapped in alginate gel was applied at the rates: 1:10, 1:20, 1:40, 1:80 and 1:160 (inoculum/substrate, v/v). Percentages of short root colonization higher than 70% were obtained with *M. ambiguus* and *P. tinctorius* when spore suspensions were applied at 10E6 or 10E7 spores/plant. Mycelial inoculum of *P. tinctorius* produced similar mycorrhizal infection at all the dosages tested. *Rhizopogon roseolus* spores were also effective at all the dosages tested, colonizing around 50% of the short roots of the inoculated seedlings. Similar level of infection was obtained with mycelium of *L. bicolor* entrapped in alginate gel when applied at the dosages of 1:10 or 1:20 (inoculum:substrate, v:v). The results obtained have permitted to establish the methodology to produce ectomycorrhizal seedlings of *P. pinea* with four fungal species under nursery conditions.

poster session 13 WF SH 55

RIVETTA, BARBARÁ C.V., R.M. MILLER & J.D. JASTROW. Environmental Research Division, Argonne National Laboratory, Argonne, IL 60439, USA. - Succinate dehydrogenase activity of *Glomus mosseae* in *Andropogon gerardi*

We examined the morphologic development and metabolic activity of the fungus *G. mosseae* in the roots of *A. gerardi* grown under controlled conditions. To quantify and visualize the fungus, we used a combination of the vital nitro blue tetrazolium-succinate stain and counterstained with acid fuchsin. We found that once hyphae gained access to the root cortex via an appressorium, lateral branches of actively stained intercellular hyphae penetrated the cortical cells and proliferated as active intracellular coiled hyphae. Within a cell the coiled hypha branched dichotomously to give rise to an active arbuscule that eventually filled the host cell. An increase in activity in the coiled hypha was a prelude to morphogenetic changes associated with branching during arbuscule development. The activity of arbuscules, however, was associated with apparent nutrient exchange. A final stage was characterized by the presence of discontinuous vacuoles that were arranged in a pattern resembling the structure of hyphal coils or arbuscules. Vesicles of different shapes and activities formed terminally from hyphae and were generally observed in groups in root segments other than those usually occupied by coils and arbuscules. These observations will be interpreted in relation to host growth, carbohydrate and nutrient allocation, and phenology.

poster session 12 WF SH 49

RONSHHEIM, MARGARET L. & KRISHNA SHARMA. Department of Biology, Vassar College, Poughkeepsie, NY 12601, USA. - The changing effects over time of mycorrhizae and phosphorous levels on the performance of *Allium vineale*.

The effect of VA mycorrhizae and its interaction with phosphorous level was examined over time. Bulbils of *Allium vineale* were planted either in sterile soil or in soil inoculated with VA mycorrhizae collected at the Vassar Ecological Preserve. Six different P treatments were applied to each soil type (0-250 ppm). After 32 days, one third of the plants were harvested, above and belowground biomass was determined, and roots were examined for percent colonization. A second harvest was done after six months, and the remaining plants were harvested 15 months after planting. The plants in this final harvest had reached reproductive maturity, and fitness estimates were obtained. ANOVA's were performed to determine the effects of both VA mycorrhizae and phosphorous levels on biomass, allocation to roots vs. shoots, and allocation to sexual and asexual reproduction. Results demonstrate that mycorrhizal effects on both biomass and allocation to roots vary over time and phosphorous level, with early parasitic effects in high P switching to beneficial effects in low P by the final harvest. Significant fitness

differences were also present for mycorrhizal vs. non-mycorrhizal plants. This study demonstrates the importance of long term studies for understanding mycorrhizal interactions with perennial species.

poster session 3 ST SH 79

ROSEMEYER, M.E.¹ & S.R. GLIESSMAN². ¹Cornell International Institute for Food, Agriculture and Development, Cornell University, Ithaca, N.Y. 14853 USA. ²Environmental Studies Board, University of California- Santa Cruz, 95064 USA. - Phosphorus (P) and nitrogen fertilization decrease VAM of bean (*Phaseolus vulgaris*) more severely in a mulch-based than in a soil-based agroecosystem on a P-fixing Andisol in Costa Rica

Percent VAM, shoot P and shoot nitrogen (N) were assessed in two bean production systems with applied fertilizers: a traditional slash mulch system and a higher-external input, soil-based system (SBS). The same density of seeds were broadcast in the mulch-based system (MBS) as were planted in the SBS. Different levels of 10(N)- 30(P)-10(K) or N and P were broadcast on the mulch and on top of the soil in the respective systems. The randomized complete block design experiment with 5 repetitions was repeated in two cropping seasons. The MBS factorial treatments with 3 levels of applied P and 2 of N showed % VAM decreases only in response to P. Percent VAM of bean roots in the MBS and SBS without fertilization were 98% and 97% respectively, but as N and P applications increased to 65 kg N/ha and 90 kg P/ha, % VAM of MBS beans decreased to 84% and SBS to 94% resulting in significantly different sloped regression lines. Though % shoot P at flowering was not different in the MBS (.255 % P) than the SBS (.274 % P), total shoot P and N, as well as plant biomass, were greater in the MBS. The bean roots in the MBS were growing in the mulch where more of the applied P remained available than in the P-fixing soil.

poster session 15 WF RR 09

ROSENDAHL, SØREN & JOHN W. TAYLOR. Department of Mycology, Botanical Institute University of Copenhagen, Oster Farimagsgade 2D. DK 1353 Copenhagen K. Denmark. - Development of molecular markers for population studies of arbuscular mycorrhizal fungi.

Population studies of arbuscular mycorrhizal fungi are hampered by the difficulty of obtaining enough material from individuals. Allozyme variation in Glomalean fungi is rarely seen, and as several spores are needed to detect each enzyme locus, polymorphism can be hidden. Nucleic acids offer the potential of nearly limitless polymorphic markers. Polymorphism in nucleic acids sequences have been detected among arbuscular mycorrhizal fungi, and it has been possible to amplify DNA from single spores by PCR. But, the direct observation of polymorphisms has not been made in arbuscular mycorrhizal populations. Our use of SSCP

(single strand conformation polymorphism) of ribosomal genes from single spores suggests that polymorphism occur within field populations. The common pattern is two single strands of ribosomal DNA sequence, but some species show four single strands, suggesting that they are more than haploid. Even with this technique, only one marker can be examined from each spore. Possible methods of circumventing this problem will be discussed.

talk session 5

ROSEWARNE, GARRY¹, SALLY E. SMITH², DAVID BIRD³, SUSAN J. BARKER¹. ¹Dept. of Plant Science, ²Dept. of Soil Science, Waite Campus, University of Adelaide, Glen Osmond S.A. 5064 and ³Dept. of Plant Pathology, North Carolina State University. - Overlap in induced gene expression sets for early VA mycorrhizal symbiosis and root knot nematode feeding sites in tomato.

Vesicular arbuscular mycorrhizal (VAM) fungi form a mutualistic symbiotic association with most plant species. Plants benefit through improved mineral nutrition, while the fungus is supplied with fixed carbon via inter and intra-cellular hyphal structures. Almost no host-specificity is demonstrated in the interaction, nor does the fungus induce a significant defence response in host roots. Our research focuses on the early stages of root colonisation by VAM fungi and addresses the question: what are the molecular mechanism/s by which the fungus and plant communicate to enable establishment of such an intimate compatible interaction? We have established a rapid colonisation method that results in extensive, synchronous colonisation of plant roots within 4 days. Using this method to generate staged materials, we have identified root cDNA clones that represent genes induced early in the colonisation process. Sequence analysis included the discovery of an overlap between this induced gene set and that found in feeding sites of the root-knot nematode, *Meloidogyne incognita*. We will present data on the VAM cDNA clones so far identified and the extent and nature of the overlap with the nematode-induced gene clones.

The results of this study are expected to have interesting implications for research on nematode resistance and in addition, underscore the fundamental role that VA mycorrhiza's play in plant root biology.

poster session 9 WF SH 05

ROUSSEAU, ANNIE¹, NICOLE BENHAMOU², ILAN CHET³ & YVES PICHE¹. ¹Centre de Recherche en Biologie Forestière, ²Département de phytologie, Pavillon C.-E. Marchand, Université Laval, Québec, Canada, G1K 7P4. ³Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot, Israël. - A biocontrol agent parasitising AM fungal structures.

The interaction between the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* and the saprophytic fungus *Trichoderma harzianum* was studied by transmission electron microscopy (TEM). An axenic compartments

system, using root organ culture, was selected to delineate the time course of early events occurring with the interaction between the extramatrical phase of *G. intraradices* and *T. harzianum*. TEM observations of samples from these interaction regions showed that spores, main hyphae and subtending hyphae of *G. intraradices* were massively infected by the hyphae of *T. harzianum*. The use of wheat germ agglutinin/ovomucoid-gold complex for the localization of chitin monomers resulted in regular labeling of the AM fungus cell walls, excepted in areas adjacent to the sites of *Trichoderma* penetration. According to our observations, the interaction between *T. harzianum* and *G. intraradices* involves the following events: 1) recognition between both partners. 2) production of cell wall-degrading enzymes by the antagonist 3) local penetration of the antagonist into mycorrhizal spores; 4) active proliferation of antagonist cells in mycorrhizal hyphae; and 5) release of the antagonist. This *in vitro* study provides evidence that the AM fungi is not protected from an attack by antagonistic fungi, but additional research is needed to evaluate this complex interaction that occur in nature.

poster session 4 ST AR 66

ROUSSEL, HELENE¹, VIVIENNE GIANINAZZI-PEARSON¹, DIEDERIK VAN TUINEN¹ & PHILIPP FRANKEN². ¹Laboratoire de Phytoparasitologie, INRA/CNRS, SGAP, INRA, BV 1540, 21034 Dijon cédex, France. ²Max Planck Institute für Terrestrische Mikrobiologie, Abteilung Biochemie, Karl von Frische Straße, D 35043 Marburg, Deutschland. - Investigation of the expression of genes encoding Membrane Intrinsic Channel Proteins (MIP) in arbuscular mycorrhizal and non mycorrhizal roots

There is increasing evidence that arbuscular mycorrhiza and nodule symbioses share some common morphological, physiological, cellular and molecular features. Nodulin 26 (Nod26) is a transmembrane protein of the peribacteroid membrane belonging to a large family of homologous channel-like proteins (MIP) found in plants, animals and bacteria. In studies to investigate whether such a symbiosome membrane protein may be involved in interactions between an arbuscular mycorrhizal fungus and its host plant, we have used a molecular approach to detect and analyse the expression of a MIP-like gene in mycorrhizal roots of different plants. Transcripts of MIP-like genes have been detected by reverse transcription polymerase chain reaction (RT-PCR) using primers corresponding to PcRb7, a Nod26-homologous cDNA fragment from mycorrhizal parsley roots. Amplification products were obtained from roots of tobacco, fababean, pea and leek colonized or not by *Glomus fasciculatum*, *G. intraradices*, *G. mosseae* and *Gigaspora rosea*, respectively. The cDNA fragments amplified from mycorrhizal roots of fababean and tobacco have been sequenced and show high homologies to the MIP family genes. Furthermore, Northern analyses have confirmed expression of the corresponding gene in mycorrhizal and non-mycorrhizal roots. The effect of mycorrhiza formation on MIP-like gene expression in

roots is presently being investigated and results comparing legumes (fababean, pea) and non-legumes (tobacco, leek) will be presented.

poster session 3 ST SH 80

RYAN, MEGAN & JULIAN ASH. Division of Botany and Zoology, Australian National University, Canberra, ACT, 0200, Australia. - Effect of drought on colonisation of crops by VA-Mycorrhizal fungi in SE- Australia.

Colonisation of wheat crops by VAM fungi was examined from 1992 to 1995 on organically and conventionally managed farms in SE-Australia. In 1992 and 1993 rainfall was close to average and the percentage of root length colonised by VAM fungi was related to the level of available phosphorus (P) in the soil and the application rate and solubility of the P fertiliser applied to the crops. Colonisation levels at anthesis ranged from 20% of root length on the conventional farms which used the relatively soluble superphosphate, to 67% on the organic farms where the relatively insoluble rock phosphate was applied. In 1994 a severe drought occurred and colonisation was markedly reduced, being less than 10% in the majority of both organic and conventional crops. Colonisation was particularly low in crop plants adjacent to trees, presumably due to the trees competing with the crop for water. In 1995 rainfall was again close to average but the initial rate of colonisation was slow, indicating that the drought had reduced VAM inoculum levels in the soil. However, by anthesis this effect had been overcome and colonisation reached levels similar to 1992 and 1993. Therefore it is possible that a drought could effect the growth of crops in subsequent seasons if a crop was reliant on VAM fungi at an early stage of growth.

The low colonisation level during the drought indicates that VAM fungi were not making a significant contribution to alleviating the water stress of the crops. Many experiments examining the effects of VAM fungi on host-plant water stress have involved relatively mild drought treatments being applied to plants already heavily colonised by VAM fungi. This study suggests that these experiments have little relevance to drought conditions in countries such as Australia.

talk session 1

RYGIEWICZ, PAUL T.¹, MARK G. JOHNSON², DAVID M. OLSZYK¹, MARTHA E. APPLE³ & JAMES D. LEWIS³. ¹US EPA, National Health and Environmental Effects Research Lab (NHEERL), Corvallis, OR 97333. ²Dynamac Corp., NHEERL, Corvallis. ³National Research Council, NHEERL, Corvallis. - Global climate change: Bridging the gap between the above- and belowground.

Global climate change (GCC) may alter vegetation growth and distribution directly by changing the aboveground and soil environments, and affecting plant processes; and indirectly by affecting soil organisms and organism-mediated processes. Few GCC studies are examining both above- and belowground responses. An

integrated, multi-year project is underway to assess the effects of elevated CO₂ and temperature using native Oregon forest soil and Douglas-fir (*Pseudotsuga menziesii*). Two-year-old seedlings were planted in 1993 and are growing in sunlit, computer-controlled, environment-tracking exposure chambers. Additional seedlings were planted at three sites along an elevational gradient in the Oregon Cascades. Growth, phenology and anatomy of shoots and roots, plant and soil gas exchange, system nutrients and water balance, litter decomposition and nutrient cycling, soil enzymology and foodweb (flora and fauna), ectomycorrhizae colonization and diversity, and quality and quantity of soil organic matter are being followed. Methods used include minirhizotron tubes, PCR-RFLPs, transmission electron microscopy, stable isotopes, and NMR spectroscopy. The project will be described and results of above- and belowground responses will be presented.

poster session 5 ST AR 32

SAINZ, MARIA J.¹, ANTON VILARIÑO², JAVIER SOTO³ & ISABEL IGLESIAS¹. ¹Departamento de Ingeniería Agroforestal y Producción Vegetal, Universidad de Santiago de Compostela, E-27002 Lugo, Spain. ²Instituto de Investigaciones Agrobiológicas de Galicia (C.S.I.i.), Apdo. 122, E-15080 Santiago de Compostela, Spain. ³Departamento de Química Orgánica, Universidad de Santiago de Compostela, E-15706 Santiago de Compostela, Spain. - Deacetilbaccatin III production in mycorrhizal and nonmycorrhizal plants of *Taxus baccata* L.

A greenhouse trial was carried out to study the effect of arbuscular mycorrhiza and phosphorus nutrition on deacetilbaccatin III production in the yew (*Taxus baccata* L.). Deacetilbaccatin III is the most abundant taxane in the plant and a precursor of taxol, an anticancer drug found in the bark and/or leaves of *Taxus* species. Terminal cuttings of 10-12 cm were taken from natural yew populations and inserted in a rooting bed of sterilized sand under intermittent mist in a glasshouse. Five months later, homogeneous surviving cuttings were selected to build up an experiment with the following four treatments, using sterilized sand as substrate: control, inoculation with *Glomus macrocarpum*, addition of 25 ppm of phosphorus, inoculation with *Glomus macrocarpum* + addition of 25 ppm of phosphorus. Ten months later, number, length and arbuscular mycorrhization of roots were determined; leaves were dried, weighed and analyzed for deacetilbaccatin III by HPLC. Root development was improved when plants were mycorrhized and fertilized with phosphorus. In both mycorrhizal treatments, *Glomus macrocarpum* colonized the 50 % of the roots. No significant differences were found among treatments for the deacetilbaccatin III content in the leaves.

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talk session 6

SAITO, MASANORI, TOMOKO KOJIMA & MASAHITO HAYATSU. Department of Ecology, National Grassland Research Institute, Nishinasuno, Tochigi, 329-27, Japan - Fungal origin of phosphatase in mycorrhizal onion roots with *Gigaspora margarita*

Localization of alkaline phosphatase in arbuscule and internal hyphae has been cytochemically demonstrated. It has been suggested that the phosphatase is involved in phosphorus-carbon exchange between host plant and mycobiont. We have been trying to characterize the arbuscule specific alkaline phosphatase. The internal hyphae were isolated from mycorrhizal roots according to the method by Saito (1995). Chitinase and ultrasonication treatment enabled efficient extraction of soluble protein from the isolated hyphae. Alkaline phosphatase activity was detected in this extract from the hyphae. However, the amount of the crude enzyme was too small to be analyzed for further enzymological studies. Therefore, the electrophoretic pattern of phosphatase activities were compared in the crude enzymes extracted from non-mycorrhizal and in mycorrhizal roots, and then a symbiosis specific band for phosphatase activity was found. The relative mobility of the specific phosphatase from roots agreed with that of the phosphatase directly extracted from the isolated internal hyphae. This indicated that the specific band of phosphatase found in the crude extract from mycorrhizal roots was of mycobiont origin. This mycobiont origin phosphatase is now being purified for characterization of the enzyme.

poster session 5 ST AR 34

SANCHEZ, FERNANDO, PILAR TORRES & MARIO HONRUBIA. Dpto. Biología Vegetal (Botánica). Facultad de Biología. Universidad de Murcia. Campus de Espinardo 30100, Murcia, Spain. - Physiological aspects of different pure cultures of ectomycorrhizal fungi from mediterranean forests

A comparative study of the development of fungi species belonging to the ectomycorrhizal genera *Suillus* (*S. collinitus*, *S. granulatus*, *S. luteus* and *S. variegatus*), *Rhizopogon* (*R. roseolus*), *Chalciporus* (*Ch. pierrhuguesii*), *Xerocomus* (*X. subtomentosus*), *Lactarius* (*L. chrysorrheus* and *L. deliciosus*), *Tricholoma* (*T. caligatum*, *T. focale* and *T. striatum*) and *Hebeloma* (*Hebeloma* sp.), in pure culture under different pH values, water potentials and temperatures, is presented. Mycelial growth and structural development are very much influenced by the pH, water potential and temperature of the soil, and consequently mycorrhizal formation. Our main objective was to test the effects of these factors on the mycelial growth of some ectomycorrhizal fungi. The cultures in the pH experiment were prepared in MMN media with a range of pH values between 2.5 and 8.5 fixed with concentrated sulphuric acid, concentrated nitric acid, a mixture of concentrated H₂SO₄ and HNO₃ acids, and NaOH 1N. The different values of water potential were obtained by dissolving polyethylene glycol 6000 (PEG 6000), in liquid MMN to give a range of concentrations. For the determination of

growth rates at different temperatures, the cultures were maintained in incubators at 5, 10, 18, 23 and 30 °C. Biomass production and mycelial growth of each strain were assessed and characterised morphologically. Rhizopogon cultures showed a higher tolerance to high pH values and water stress, whereas Lactarius and Tricholoma were the most sensitive.

morning talk

SANDERS, IAN R.¹, THORUNN HELGASON², ANDRES WIEMKEN¹ & ALASTAIR H. FITTER².
¹Botanical Institute, University of Basle, Hebelstrasse 1, 4056 Basle, Switzerland. ²Dept. of Biology, University of York, PO Box No. 373, York, YO1 5YW, UK. - Genetic and functional diversity of arbuscular mycorrhizal fungi in natural communities

Arbuscular mycorrhizal fungi (AMF) form symbioses with the roots of most plant species in temperate and tropical ecosystems. The symbiosis is ecologically interesting because of its potential to influence ecosystem processes, to determine plant community structure and to influence the number, spatial arrangement and genetic diversity of individuals in plant populations. Results from studies of native AMF from a species-rich calcareous grassland in Switzerland suggest that the diversity of AMF fungi is important in determining these processes. This outlines the need to understand the spatial and temporal structure of AMF communities in the roots of plants and how the external hyphae are distributed in the soil. If the diversity of AMF is an important determinant of plant diversity then we also need to understand the processes that promote and maintain diversity of AMF. At present, molecular techniques provide one of the best opportunities for studying the ecology and diversity of AMF in natural communities and we report on advances in two complementary studies carried out in a calcareous grassland and a woodland ecosystem. Both studies reveal high genetic diversity in natural AMF communities and provide some of the first insights into the distribution of AMF in the roots of different plant species within these communities.

poster session 6 WF AR 73

SANTHANAKRISHNAN, P. and M. ANANDAN. 1996. Department of Agricultural Microbiology, Centre of Advanced Studies in Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore - 641 003, India. - Neem (*Azadirachta indica* A. juss) response to inoculation with VAM fungi, *Azospirillum* and phosphobacterium

A nursery experiment was conducted for neem seedlings to find out the interaction between VA-mycorrhizal fungi with *Azospirillum* and phosphobacterium. The triple inoculation with *Glomus constrictum*, *Azospirillum brasiliense* and *Pseudomonas striata* increased the dry weight of the neem plants. VAM colonization, extramatrical hyphae of VAM, uptake of macro and micro nutrients (N, P, K, Zn, Cu and Mn). Chlorophyll content, indole acetic acid content, total phenol content

and total carbohydrate content significantly than twin inoculants or individual inoculant over uninoculated control. From the above experiment conducted in nursery condition, it was observed that biofertilizer inoculated seedlings attained greater seedling height and collar diameter relative to the uninoculated control at the end of the given period. Hence, it is possible to produce desired size of seedlings in a shorter nursery period by using biofertilizers than by conventional methods. The return per rupee invested was five fold in biofertilizer inoculated.

talk session 3

SAUNDERS, ELEANOR, A.F.S. TAYLOR & D.J.READ. Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, United Kingdom. - Ectomycorrhizal community response to simulated pollutant nitrogen deposition in a Sitka spruce stand, North Wales.

Across large areas of Europe and eastern North America forests have been declining or dying back. One of the main causes of forest decline is thought to be the increase in atmospheric deposition of inorganic pollutant nitrogen (N). The increase in the external supply of inorganic N is in excess of the biological demand resulting in the forest ecosystem becoming N saturated. A European project 'NITREX' was designed to examine the effects of N saturation on forest ecosystems across Europe. At the 'NITREX' site at Aber in North Wales N is applied in weekly chronic doses to simulate atmospheric deposition. A study of the ecological effects of chronic doses of mineral N on the ectomycorrhizal community was carried out using the Aber plots. N has been added to plots in mineral form (NO₃ and NH₄) to a 30 year old Sitka spruce stand over 5 years simulating polluted nitrogen deposition. Root samples were taken from each plot in spring and autumn 1995. The ectomycorrhizal tips were identified and counted to determine the effect of mineral N addition on proportion of roots colonised by ectomycorrhizal fungi, the number of ectomycorrhizal tips and the species composition of the community. This poster outlines the results from the two sets of samples and discusses their implications on the ectomycorrhizal community.

poster session 5 ST AR 35

SCAGEL, C.F.¹ & R.G. LINDERMAN². ¹Dynamac International Inc., Corvallis OR 97333, USA. ²USDA-ARS, Horticultural Crops Research Laboratory, Corvallis, OR 97330. - Correlations between ectomycorrhizal IAA and ethylene production and seedling growth and survival.

Using GC-MS methodology, in vitro production of IAA and ethylene of different ectomycorrhizal fungi was quantified. Differences in production capacity occurred within and between species of fungi, allowing fungi to be grouped relative to their production potential, as low, moderate, or high producers of IAA or ethylene. Fungi representing each of these production level groups were

used to inoculate conifer seedlings growing under greenhouse conditions, and nursery seedlings grown for field transplant. Responses varied with tree species, mycorrhizal fungus combination and environment. In the greenhouse, fungal IAA production capacity was significantly correlated to several morphological attributes as well as root IAA content of Douglas-fir seedlings, but less correlated with Lodgepole Pine and Englemann Spruce for the same attributes. IAA in roots was also highly correlated with fungus ethylene production capacity in all three conifer species growing in a greenhouse. IAA content of roots of field transplanted seedlings was increased in some inoculation treatments for all conifer species. Both IAA- and ethylene-producing capacity were significantly correlated to several morphological features as well as IAA content of roots for some conifer species. Our results suggest there can be a relationship between auxin and ethylene production by ectomycorrhizal fungi and changes in root IAA content that could affect growth responses and survival of conifer seedlings in the field.

poster session 13 WF SH 46

SHELKLE, MICHELLE, MARGOT KRONICK, MELISSA FARQUHAR & R. LARRY PETERSON. Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1, Canada. - The use of laser scanning confocal microscopy to characterize ectomycorrhizas of *Pinus strobus* L. and to localize associated bacteria

Laser scanning confocal microscopy (LSCM), light microscopy (LM) and epifluorescence microscopy (FM) were used to observe the extramatrical hyphae, mantle patterns and associated bacteria on mycorrhizal tips of *Pinus strobus* L. seedlings grown in pot cultures. *Laccaria* sp. and *Tuber* sp. formed ectomycorrhizas with *P. strobus*, while *Phialophora finlandia* Wang & Wilcox and E-strain (sensu Danielson 1982) formed ectendomycorrhizas. Distinct mantle patterns and cystidia were observed with greater resolution using LSCM, and intracellular hyphae were visualized in three dimensions. Trypan blue penetrated fresh whole mounts to 20 µm and was an excellent stain for visualizing fungal hyphae and bacteria with LSCM. Fluorescein isothiocyanate (FITC) and acridine orange were used in conjunction with LSCM and FM to localize bacteria on ectomycorrhizal tips. With LSCM, bacteria were visible in the surface mucigel, and optical sectioning through the root tip showed that bacteria were also present within the mantle. LSCM is a non-intrusive and fast method for visualizing ectomycorrhizal structures and their associated bacteria on fresh, whole root tips.

poster session 12 WF SH 65

SCHELLENBAUM, LUISA¹, JOACHIM MÜLLER^{1*}, THOMAS BOLLER¹, ANDRES WIEMKEN¹ & HANNES SCHÜEPP². ¹Botanisches Institut der Universität, Hebelstrasse 1, 4056 Basel, Switzerland. - ²Sektion Bodenmikrobiologie, Eidgenössische Forschungsanstalt, 8820 Wädenswil, Switzerland. *Present address: Laboratoire de Phytoparasitologie,

CNRS-INRA, BV 1540, 21034 Dijon Cédex, France. - Arbuscular-mycorrhizal symbiosis and drought stress effects on soluble carbohydrates and amino acid content in maize

One of the major modifications in plant metabolism during drought is the change in soluble carbohydrate content and compatible solutes such as proline. Arbuscular mycorrhizal fungi might affect the plant response to drought by altering its carbon metabolism. In this study we investigated how plant carbon metabolism is affected during drought stress in two cultivars of maize (*Zea mays* L.) with contrasting drought sensitivity, and how the plant's association with the symbiont *Glomus mosseae*, influences the response. After mycorrhizal colonisation had successfully developed, plants were drought stressed for a period of ten days by reducing watering volume to 10% of the control plants. In the roots, glucose and fructose accumulated upon drought except in mycorrhizal plants of the drought sensitive cultivar. Trehalose, in all likelihood a fungal product, increased upon stress in mycorrhizal roots. In the leaf, fructose and glucose only significantly increased with drought in non-mycorrhizal plants while starch and sucrose decreased in all treatments. Accumulation of proline occurred in all treatments as response to drought; it was higher in the leaves of mycorrhizal plants of both cultivars. Our results show that mycorrhizal colonisation can influence the drought-induced sugar accumulation in roots and leaves. They also indicate that mycorrhizal plants accumulate higher amounts of N-containing compatible solutes such as proline and less hexoses under drought stress. Implications for alteration of carbon partitioning and responses to drought are discussed.

poster session 3 ST SH 81

SCHMINCKE, BIRGIT, EDWIN WEBER & REINHARD F. HÜTTL. BTUC Center of Excellence Minesite Recultivation, Brandenburg Technical University of Cottbus, P.O. Box 101344, D-03013 Cottbus, Germany. - Arbuscular mycorrhizal infection potential in a chronosequence of pine forest understorey on lignite mine spoil

Lignite mine spoil is supposed to lack VAM fungi. Thereby, establishment of mycotrophic plant species might be limited in primary succession. Arbuscular mycorrhizal infection potential (IP) of the young soils is hypothesized to increase with time since disturbance. In a chronosequence study on a nutrient poor lignite mine spoil in Lusatia/Germany recultivated with *Pinus* sp. (4-, 11-, 20-, 30-year-old pine stands) bioassay data from diluted soil samples showed an increase of IP at the 11-year-old site if compared to the youngest site. Almost no IP was measured at the 20- and 30-year-old sites. This result rejects the hypothesis of an increase of IP with stand age. However, IP of plots at the older sites with understorey vegetation did not differ from those without. Also, high mycorrhization of understorey plant species did not indicate low IP on old stands. These findings give evidence that the results of the bioassay do not reflect the

actual IP under field conditions possibly due to disturbance of the hyphal network during dilution. Further bioassays using undisturbed cores instead of disturbed soil samples shall investigate whether a fragile hyphal network is the main source of mycorrhizal infectivity at the older sites.

poster session 8 WF AR 69

SCHREINER, R. PAUL & GABOR

BETHLENFALVAY. USDA, Agriculture Research Service, Horticultural Crops Research Laboratory, 3420 NW Orchard Ave., Corvallis, OR, USA, 97330. - Defining the functional roles of endomycorrhizal fungi for sustainable agriculture.

The goal of sustainable agriculture is to balance our need to maximize crop production while maintaining the resource base. We have examined both plant development and soil structure responses to AM fungi in a number of studies in an effort to understand the functional roles of AM fungi within the context of sustainability. A comparison of three AM fungi associated with soybeans in sandy loam, showed that *Glomus mosseae* was superior to either *Glomus etunicatum* or *Gigaspora rosea* in enhancing seed pod development and stability of soil aggregates. These responses were correlated with greater soil mycelial development of *G. mosseae*. In another study comparing fungicide effects on three AM fungi inoculated alone or together as a mix associated with peas in a silty-clay loam, the community of three fungi outperformed each of the single fungi in promoting soil aggregate stability while seed yield was not different among the mycorrhizal plants. The ability of the mixed inocula to increase aggregate stability above that of the single-fungus treatments, but maintain a similar level of seed yield increase over nonmycorrhizal plants was related to a reduction in total root length in the mixed fungus treatment. Our results have shown that AM fungi have differing abilities to enhance soil stability which is affected by the particular plant-soil-fungus combination investigated and is related to soil hyphal development and resource allocation within the host plant.

poster session 3 ST SH 82

SCOTT, ELIZABETH P.¹, WILLIAM R. EASON² and JOHN SCULLION¹. ¹Institute of Biological Sciences, University of Wales, Aberystwyth, UK. ²Institute of Grassland and Environmental Research, Aberystwyth, UK. - Impact of agricultural management regimes on the effectivity of indigenous arbuscular mycorrhizal populations from soils under long-term pasture and grass-arable rotations in western UK.

Arbuscular mycorrhizal (AM) spores, taken from soils under long-term grassland pasture and grass-arable rotations on a large number of farms in the western United Kingdom (England and Wales), were used to inoculate several host plant species. Farms with a history of high input, conventional and long-term organic management were matched in each location. In pasture soils, root infection levels and spore numbers were

generally higher under organic management; in rotation soils the difference was apparent only where these soils had low background levels of plant available phosphorus. In general, AM spore inocula from organic farms were more effective than those from conventional farms in promoting host plant growth. Differences attributable to farming systems were largest where soils of inherently low (P) fertility received high inputs of mineral fertilisers. These results were also repeated when infected root fragments (taken from the original inoculation experiments) were used as inoculum sources, indicating

poster session 8 WF AR 53

SEIDL, MICHELLE¹, JOSEPH AMMIRATI¹ & MEINHARD MOSER². ¹Department of Botany, Box 355325, University of Washington, Seattle, WA 98195 USA. ²Technikerstrasse 25, Institut für Mikrobiologie, Universität Innsbruck, A-6020 Innsbruck, Austria. - Patterns of host/fungus relationships in the ectomycorrhizal genus *Cortinarius* (Agaricales).

The mushroom genus *Cortinarius* is the dominant ectomycorrhizal sporocarp producing component of western North American coniferous forests. Emphasis will be on patterns of host specificity from sporocarp data and their usefulness in exploring relationships among taxa in this genus. Two basic patterns emerge: one represents a strict fungus/host relationship, and the second involves a broader, more general relationship between fungus species and their hosts. Both types of relationships will be evaluated at the species, genus and family level for ectomycorrhizal host plants in relation to individual *Cortinarius* species. Even though most conifer hosts and many *Cortinarius* taxa have broad geographical ranges, it is well known that the presence of a host does not guarantee occurrence of a particular fungus species. Host/fungus relationships will be evaluated at a broader geographical scale where species or subspecies have diverged on the basis of host, for example, association with both conifer and hardwood genera or species. All relationships will be presented in the context of forest/vegetation type and associated ecological parameters will be discussed to better explain patterns of host specificity.

poster session 15 WF RR 11

SELOSSE, MARC-ANDRE, DAMIEN JACQUOT, FRANCIS MARTIN & FRANÇOIS LE TACON. Equipe de Microbiologie Forestière, INRA Nancy, 54280 Champenoux, France. - Genetic structure of *Laccaria* spp. populations; ten-years persistence without introgression of an introduced strain in natural populations.

Laccaria bicolor is commercially used for nursery inoculation of Douglas Fir (*Pseudotsuga menziesii*) in France. The persistence of the introduced strain and its potential introgression (i.e. gene flow) in local populations after transplantation remain questionable. We investigated a *Laccaria* spp. population in Saint-

Brisson (Nièvre, France) where Douglas fir seedlings inoculated in 1985 with an American strain, *L. bicolor* S238N, were transplanted in 1987. 450 sporophores were collected during the autumn of 1995. Strain typing was performed using PCR amplification of rDNA IGS and RAPD markers, whose homo- or hetero-zygosity and reproducibility were previously studied in a *L. bicolor* S238N haploid progeny. As shown by 18 heterozygous markers and 30 homozygous markers, the introduced strain was still present on the inoculated site, producing about 50% of the sporophores collected. We found no evidence of introgression, suggesting a possible incompatibility of this American strain with European *Laccaria* spp. populations. This hypothesis is currently under study, by confrontation of monokaryons. Structure of the *Laccaria* spp. populations on the site suggests a clonal propagation, with little or no recombination between genets.

morning talk

SEN, ROBIN, INGA SARAND, SARI TIMONEN, MINNA RAJAMÄKI, RAINER PELTOLA*, EEVALIISA NURMIAHO-LASSILA, TEIJA KOIVULA, KIELO HAAHTELA & MARTIN ROMANTSCHUK. Division of General Microbiology and *Department of Applied Chemistry and Microbiology, P. O. Box. 56, FIN 00014 University of Helsinki, Finland. - Bioremediation of petroleum derived hydrocarbons in the plant mycorrhizosphere.

The escape of petroleum derived hydrocarbons (PHC) into aquatic and terrestrial habitats represents a major ongoing environmental problem. Attempts at clean-up using specifically tailored micro-organisms, particularly bacteria, often fail due to loss through out-competition with the indigenous microflora. We present a novel approach involving enrichment of PHC degradative bacteria on mycorrhizal roots of Scots pine. Using transparent microcosms it was possible to observe fungal colonisation of introduced PHC contaminated soils from mycorrhizal roots. Bacteria associated with the colonising mycorrhizal hyphae were visualised in the TEM, isolated, characterized using selective media and REP-ERIC PCR and some found to harbour large plasmids containing a catabolic marker gene sequence. Combined GC-MS analysis of the colonised soil indicated that significant bioconversion and degradation of long chain aliphatics and polyaromatics had occurred over the 20 week exposure period. The general implications of the reported mycorrhiza-bacteria diversity, interactions and activities and more specifically for bioremediation technology development will be discussed.

poster session 3 ST SH 84

.SHARMA, MAHAVEER P., NAVEEN P. BHATIA, ATIMANAV GAUR & ALOK ADHOLEYA. Tata Energy Research Institute, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi 110 003, India. - Relative mycorrhizal dependency of *Acacia nilotica* and *Eucalyptus tereticornis* under different levels of phosphorus in marginal wasteland soil

To evaluate the mycorrhizal dependency of two tropical tree species *Acacia nilotica* and *Eucalyptus tereticornis* were grown in the polybags for 12 weeks with and without the indigenous VAM fungi at seven various levels of soil P (0.95, 7, 8, 9, 12, 18 and 30 ppm) in a marginal wasteland soil. Reduction in VAM colonization was noticed with increased soil P levels, the rate of reduction being more pronounced with the decrease in RMD (Relative Mycorrhizal Dependency) of both the species. VAM inoculation had generally higher root and shoot dry weights compared to their counterparts P level in both the species. The dry matter yield of mycorrhizal *Acacia* at soil P level of 12 ppm was comparable to non-mycorrhizal biomass of *Acacia* at soil P level of 30 ppm whereas, in *Eucalyptus* these levels were 8 and 18 ppm respectively. RMD values showed inverse relationship with soil P levels tested. *Acacia* was found to be moderately dependent on VAM for maximum biomass while *Eucalyptus* was found to be highly dependent on VAM. The former species, the non-mycorrhizal plants at higher P levels beyond 12 ppm showed higher shoot P content for dry matter yield when compared to the latter species. The current findings clearly demonstrates that there is an optimum P levels at which the VAM inoculation can draw maximum benefit and thus helps in saving the high input P fertilizer application if the inoculant used with an appropriate protocol.

poster session 8 WF AR 65

SIGUENZA, C¹., I. ESPEJEL² & E. ALLEN¹. ¹Department of Botany and Plant Sciences, University of California, Riverside, CA 92521. ²Facultad de Ciencias Universidad Autonoma de Baja California Apartado postal 1880. Baja California, Mexico 22800. - Mycorrhizae in coastal sand dunes in Baja California.

We observed the mycorrhizal status of eight coastal sand dune species in 1992 at El Socorro, Baja California. The more abundant species were *Haplopappus venetus* and *Helianthus niveus*. In subsequent visits to the field site a switch in the community composition was evident. *H. venetus* was replaced by two species of *Lotus* (*L. distichus* and *L. bryantii*). Seven species are mycorrhizal, with percent infection ranging up to 80 %. One species, *Abronia maritima*, formed no mycorrhiza. The latter species is the only plant that colonizes mobile dunes, while the others are found on stabilized dunes. An experiment was carried out to assess the effect of the mycorrhizae on the two *Lotus* species. Preliminary results show that mycorrhizae can promote growth in both species and flowering and fruiting in *Lotus bryantii*

poster session 3 ST SH 92

SILVA, ELIANE M.R., FRANCISCO A. DE SOUZA & AVILIO A. FRANCO. National Centre for Agrobiological Research (EMBRAPA-CNPAB), Km 47, Seropédica, Itaguaí, 23851-970, Rio de Janeiro, Brasil. - The establishment of arbuscular mycorrhizal fungi associated with nodulated legume trees in land reclamation in the Amazon and Atlantic forest regions of Brazil.

Tree legumes inoculated with rhizobia and arbuscular mycorrhizal (AM) fungi of the species *Glomus clarum* and *Gigaspora margarita* were used in the revegetation of decapitated soils in the region of Angra dos Reis, Rio de Janeiro State, originally covered by Atlantic forest, and in an area of mine wastes originating from bauxite extraction in Porto Trombetas, Pará State, where the original Amazon forest vegetation had been removed. In the Angra dos Reis region, the population of AM fungi before the introduction of the legumes trees, consisted of only a few spores of *Glomus* (*G. macrocarpum*). Forty four months after the establishment of *Mimosa caesalpiniiifolia*, the number of spores increased several times and included the following species: *Glomus macrocarpum*, *G. etunicatum*, *G. clarum*, *Gigaspora margarita*, *Scutellospora heterogama*, *Acaulospora scrobiculata*, *A. mellea* and *A. sp.* At the site in the Amazonia region, there were no detectable AM spores before the introduction of the legume trees. Three years after planting, in the plots of *Acacia mangium*, more than 28 spores.cm⁻³ of soil were found, consisting of the species *G. clarum*, *A. mellea* and *A. foveata*. In plots where top soil was added, apart from the occurrence of these species, spores of *Gigaspora gigantea* and *Acaulospora sp.* were also found, although the number of spores was lower. In the plots of *Sclerolobium paniculatum* a mean of five spores.cm⁻³ of soil were recorded of the species *G. clarum*, *A. mellea*, *A. foveata* and *G. margarita*.

morning talk

SIMARD, SUZANNE¹, DAVID PERRY², MELANIE JONES³, RANDY MOLINA² & DANIEL DURALL³.
¹B.C. Ministry of Forests, Kamloops Forest Region, Kamloops, B.C., Canada, V2C 5Z7. ²Oregon State University, Department of Forest Science, Corvallis, OR, USA, 97331. ³Okanagan University College, Biology Department, Kelowna, B.C., Canada, V1V 1V7. - Ecological significance of carbon transfer in ectomycorrhizal tree species mixtures

Shared compatibility for ectomycorrhizas (EM) and significance of gross and net interspecific carbon transfer were examined in the field and laboratory in south-central British Columbia. A soil bioassay showed that paper birch (*Betula papyrifera*) and Douglas-fir (*Pseudotsuga menziesii*) shared 7 EM morphotypes in common over 90% of their root tips. Reciprocal labelling of paper birch and Douglas-fir with ¹³CO₂(gas) and ¹⁴CO₂(gas) in laboratory rootboxes and the field resulted in bi-directional transfer, with net gain by Douglas-fir. In rootboxes, gross and net transfer represented 29% and 4% of total isotope assimilated by both species. Net transfer was 3 times greater and one-way gross transfer to Douglas-fir 50% greater where interconnecting hyphae were left intact than where severed, but high p-values (p>0.05) leave in question whether hyphal connections facilitated transfer. In the field, gross and net transfer between two-year-old paper birch and Douglas-fir represented 7% and 6% of total isotope assimilated, respectively. Gross and net transfer were 2 times greater where Douglas-fir grew in 5% than 50% or 100%

sunlight, suggesting transfer was affected by changes in photosynthate sink strength of Douglas-fir. Isotope transferred to arbuscular mycorrhizal western redcedar (*Thuja plicata*) represented 18% of gross transfer between paper birch and Douglas-fir, indicating most carbon was transferred between EM species via interconnecting hyphae.

poster session 15 WF RR 24

SIMS, KAREN P. & ROBIN SEN. University of Helsinki Department of Biosciences, Division of General Microbiology, P.O.Box 56 (Viikinkaari 9), 00014 Finland. - Use of PCR and RFLP analysis to detect differences between geographically distinct isolates of *Pisolithus arhizus* and a few species isolates from the related genus *Scleroderma*.

A survey of basidiomycete, gasteroid ectomycorrhizal fungi was undertaken in the Philippines and Indonesia. Cultures of two of the most important genera which can form symbioses with pine, eucalypt and dipterocarp trees, *Pisolithus* and *Scleroderma*, were isolated from fresh basidiomata material collected in the field. Amplification from pure culture mycelium of the fungal-specific primer regions ITS and IGS produced a PCR product which was restricted with several enzymes. Isolates of different geographical origin could be distinguished in the case of *Pisolithus arhizus* and clear species differences were seen within the isolates of *Scleroderma* species. Dilution effects, especially for the more pigmented mycelial cultures were also noted. These molecular methods were in agreement with parallel studies investigating the cultural characteristics of all isolates collected.

poster session 6 WF AR 14

SIQUEIRA, JOSE¹, ORIVALDO SAGGIN-JUNIOR¹, WALDO FLORES-AYLAS¹ & P.T. GUIMARAES².
¹Ciencia do Solo, Universidade Federal de Lavras, MG 37200-000, Brasil. ²EPAMIG, Lavras. - AM fungi and superphosphate application on plant development and yield of coffee in Brazil.

This paper reports a six-year field study relating to the effects of pre-colonization of coffee seedlings with a mix of *Glomus clarum* and *Gigaspora margarita*; and five isolates of *Glomus etunicatum* on initial crop development and bean yield in of coffee in a low-fertility Oxisol amended with P-levels at planting. Inoculated (M) and non-inoculated (NM) outplants were raised in a glasshouse and transplanted into the field in January 1989. Plant height and stem diameter were highly enhanced by P and were greater in M seedlings up to 19 months after transplanting (MAT), but not different at 25 MAT, whereas tree diameter was affected up to 25 MAT. Mycorrhizal colonization was high (43 to 55%) and not different amongst plants, regardless of pre-colonization at nursery stage. However, it was reduced by high P-levels. Bean yield in 1991 harvest, was highly enhanced by P and by pre-colonization. Three isolates of *G. etunicatum* showed yield enhancement above 40% over NM plants.

In 1992 and 1993 yield was affected by P, but not by inoculation. In 1994 and 95, M effects were again in the range of 40% for selected *G.etunicatum* isolates. In the first harvest, P-doses for maximum yield was reduced from 207g P₂O₅-plant⁻¹ for NM plants to little over 100 for the M ones. Such effects was reduced in subsequent years. If adequate phosphorus is applied at planting, pre-colonization of coffee seedlings enhances early crop development and productivity in low-fertility soils in Brazil. (Funded by FAPEMIG and CNPq)

morning talk

SMITH, F. ANDREW¹ & SALLY E. SMITH².
¹Botany, ²Soil Science, The University of Adelaide, SA 5005, Australia. - Selectivity and control: the role of membranes in inter-plant transfer of nutrients

Attention is increasingly focused on the membranes of host/fungus interfaces as sites of control and selectivity of solute transfer in mycorrhizal symbioses. The simplest pattern of transfer - occurring in mycorrhizas other than orchids - involves loss of nutrients such as P from the fungus in return for carbohydrate from the plant. Different patterns of transfer must occur between plants that are linked by mycorrhizal fungi, as revealed by the use of various tracers, measurements of growth, etc. Again, it seems realistic to focus on the membranes at the host/fungus interfaces as sites of control and selectivity of solute transfer between linked plants.

In this review lecture we discuss the possible membrane transport mechanisms that are likely to be involved in solute transfer across the interfaces, with emphasis on inter-plant transport. We will emphasize the possible dangers of ignoring diversity in function in considering the benefits and costs of different mycorrhizal symbioses.

poster session 2 ST SH 25

SMITH, JANE E.¹, ARI JUMPPONEN², MICHAEL J. LARSEN³, & DONARAYE MCKAY¹. ¹USDA. Forest Service, Pacific Northwest Research Station, Corvallis, OR 97331, USA; ²Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA; ³USDA Forest Service, Intermountain Research Station, Moscow, ID 83843, USA. - Ecology and taxonomy of *Piloderma* spp.: a golden indicator of old-growth forest soil legacy

A golden-yellow ectomycorrhizal fungus may be an important indicator of old-growth soil conditions in Pacific Northwest forests of North America. Harvest of late successional (old-growth) forests in the Pacific Northwest region has prompted legal challenges to forest management plans resulting in the development of ecosystem management strategies. To evaluate the effect of these management strategies on biological diversity, it is essential to know the identities and community composition of organisms, including ectomycorrhizal fungi, in natural and managed forest ecosystems. Frequency data for *Piloderma* were collected as part of a larger study to determine whether ectomycorrhizal fungal communities vary between successional stages of

Pseudotsuga menziesii forests. Fungal filaments of *Piloderma* spp. were closely correlated with stand age in a recent fungal survey spanning 3 years, 9 forest stands and 1125 sampling points. *Piloderma* spp. occurred in 55% of the old-growth, 6% of the rotation-age, and 2% of the young-growth plots. The potential value of *Piloderma* as an indicator species brought to light the long confusion over species concepts for this genus. A re-evaluation of the taxonomic status, based on microscopic characteristics of nomenclatural types and other specimens, suggests only two distinct species among the four in question, *P. fallax* (syns. *P. bicolor*, *P. croceum*) and *P. byssinum*. These taxonomic views are supported by our preliminary analysis of the internal transcribed spacer (ITS) of the nuclear ribosomal DNA from representative isolates and collections of *Piloderma* spp. from the United States and Sweden.

poster session 3 ST SH 90

SNYDER, SEASON R., MOLLIE WALTON & CARL F. FRIESE. Biology Department, University of Dayton, 300 College Park, Dayton, OH 45469-2320. - Comparative soil and mycorrhizal dynamics associated with small scale disturbances on rangeland ecosystems in Colorado and New Mexico

A comparative study was conducted on the soil dynamics associated with small mammal disturbances on arid and semi-arid rangelands in the United States. The project was undertaken to study whether modifications in physical soil properties of soil would in turn result in alterations in plant community structure and diversity as well as alterations in the soil microbial community. The physical properties of soil examined for this study were temperature, pH, moisture, organic matter, and texture. One factor in the assessment of the soil microbial community consisted of total spore counts for arbuscular mycorrhizae (AM). The results showed there were substantial differences among the physical properties of the soil at the Colorado and the New Mexico site. Within both sites there were no substantial differences in the total number of AM spores between disturbed and undisturbed soils. However, between the two sites there were greater numbers of spores in Colorado in comparison to New Mexico rangeland ecosystems. The results also showed that the differences in physical properties of soils associated with small mammal disturbances were independent of the grazing intensity. The results will provide further insight into the restoration and management of these rangeland ecosystems.

poster session 13 WF SH 45

SÖDERSTRÖM, BENGT^{1,2}, SANDY DICKSON¹, F. ANDREW SMITH³ & SALLY E. SMITH¹. ¹Department of Soil Science, Waite Campus, University of Adelaide, Glen Osmond, South Australia 5064. ²Permanent address: Department of Microbial Ecology, Ecology Building, Lund University, S-223 62 Lund, Sweden. ³Department of Botany, University of Adelaide, South Australia 5005. - Arum and Paris-type of endomycorrhiza in some plant-AM fungus species combinations

Two classes of endomycorrhizas were described by Gallaud (1905): Arum and Paris types, named after the 'type species'. These have now been described from many different plant families. However, very few controlled colonization experiments have been performed with known Paris type plants and known arbuscular mycorrhizal fungi. We report an experiment in which nurse 'pot' cultures of *Glomus mosseae*, *Glomus* sp., (City Beach), *Scutellospora calospora*, *Glomus intraradices* and *Gigaspora margarita* with leek (*Allium porrum*) were planted with Basil (*Ocimum basilicum*), Carrot (*Daucus carota* cv. Topweight), Flax (*Linum catharticum*), Parsley (*Petroselinum crispum*) and Violet (*Viola* sp.). The colonization was followed for six weeks with harvests at 6, 10, 18 and 42 days. Earlier observations suggested that in carrot, parsley, flax and violet Paris-type mycorrhizas are normal. In this experiment all species combinations yielded Arum-type mycorrhizas during the early phases (up to 18 days), with Paris-type characteristics developing later in parsley and carrot. The plant thus seem to govern the development of different mycorrhizal types.

poster session 2 ST SH 26

SPARKS, GRACE¹, JOSEPH AMMIRATI² & ROBERT EDMONDS¹. ¹College of Forest Resources and ²Department of Botany, University of Washington, Seattle, WA 98195. - Influence of conifer forest-clearcut edges on sporocarp production by ectomycorrhizal fungi.

Extensive timber harvesting in the Pacific Northwest has resulted in landscapes dominated by mature forests bordering recently clearcut areas. Anecdotal evidence and non-quantitative observations suggest that certain fungi may fruit more prolifically near edges of paths, roads, and power line corridors than in forest interiors. Ongoing research is being conducted to determine whether a similar edge effect occurs at forest-clearcut boundaries. From September-November 1995, fungal sporocarps were collected along transects that ran from the centers of recent clearcuts into adjacent 60-80 year old conifer stands. Soil moisture and temperature data were collected concurrently. Neither organic nor mineral soil moisture at the time of sporocarp collection appeared to be a dominant factor controlling fungal fruiting. Edge aspect may have exerted a temperature-related, seasonal influence on sporocarp production. In general, biomass of decomposer species tended to be relatively low throughout forests, but much greater in clearcuts. Few mycorrhizal fungi fruited in clearcuts, except near edges (within 15 m of adjacent forests). Individual mycorrhizal genera differed dramatically in both distance from edge at which sporocarp biomass was greatest and in furthest distance into clearcuts that sporocarps were found. Some genera, such as *Cantharellus*, fruited commonly in forest interiors but only rarely near edges. Most others, including *Amanita*, *Dermocybe*, and *Lactarius*, produced greater sporocarp biomass near forest edges than in forest interiors. Because only mycorrhizal fungi exhibited this pattern, the increase could be attributed to some edge-induced change in tree-fungal interactions. Furthermore, different mycorrhizal genera appeared to interact with

their hosts and/or respond to other environmental factors in unique ways along forest edge to interior gradients, at least as reflected by sporocarp production.

poster session 10 WF SH 06

ST-ARNAUD, M.¹, C. HAMEL², B. VIMARD¹, M. CARON³ & J. A. FORTIN¹. ¹Inst. rech. biol. vég., Univ. de Montréal, 4101 Sherbrooke St. East, Montréal, Qc, Canada, H1X 2B2. ²Nat. Res. Sci. Dept., McGill Univ., 21111 Lakeshore Rd, Ste-Anne-de-Bellevue, Qc, Canada H9X 3V9. ³ÉCOVAL Tech., 3600 Tricentenaire Bl., Montréal, Qc, Canada H1B 5M8 - Co-culture with mycorrhizal *Tagetes patula* inhibited *Fusarium* wilt in the non-mycorrhizal host *Dianthus caryophyllus* as well as the pathogen in the soil.

Interaction between *G. intraradices* (Gi) and *F. o. dianthi* (Fod) was studied in the rhizosphere and roots of the nonVAM host *D. caryophyllus* (Dc). *T. patula* (Tp) were sown, inoculated with Gi and 5 wk were allowed for the fungus to become well established. A spore suspension of Fod was then added and 7 d were allowed for the fungi to interact with each other before Dc were sown between the Tp plants. The presence of VAM Tp more than doubled the survival of Dc, significantly lower the disease symptoms, and decreased by four times Fod in the soil while nonVAM Tp had no effect. Dc shoot dry mass was reduced by Fod in nonVAM controls but was not affected in presence of Gi. There was no effect of Gi alone on Tp or Dc shoot dry mass, and no effect of Fod on Tp. Dc shoot content in macro- and micro-elements was not modified by Gi. Without Tp, Gi did not colonized Dc while in presence of Tp, 14-20 % of Dc root length contained abundant vesicles and hyphae but very few arbuscules. Gi presence prior to Dc sowing clearly reduced the disease caused by Fod. Reduction of disease symptoms and pathogen population cannot be attributed to metabolic exchanges between symbionts or to enhance nutrition of the plant, but may result from plant disease resistance mechanisms stimulation by Gi or from direct or indirect effect of the Gi on Fod in the soil prior the Dc sowing.

poster session 12 WF SH 50

STEINKE, EVE & ANNE E. ASHFORD. School of Biological Science, University of New South Wales, N.S.W. 2052, Australia. - The effect of pH on the growth of fungi associated with *Leucopogon parviflorus* mycorrhizas

Leucopogon parviflorus (Andr.) Lindl. (Epacridaceae) is a heath species found on calcareous and siliceous coastal dunes in Australia. The plant is distributed from highly alkaline foredunes to acidic backdunes. Ericoid type mycorrhizas are present in the epidermal cells of the hair roots in all habitats. To compare the properties of fungal endophytes isolated from plants growing in extremely acid and alkaline soils, fungi which were isolated from mycorrhizal hair roots of plants taken from Long Beach, Jervis Bay Australia (35° 02'S 150° 47'E) were grown on Modified Melin Norkrans medium adjusted to various

pH values in the range from 4.4 to 9.0. Differences in the pH optima for growth suggest that different fungi are associated with *L. parviflorus* in acid and alkaline soils. Current investigations are focusing on the nitrogen and phosphorus nutrition of these fungi.

poster session 8 WF AR 20

STEVENS, CHRISTOPHER M.¹, BARBARA L. GOULART*¹, YOLANDE DALPÉ², JAMES F. HANCOCK³, KATHLEEN DEMCHAK¹, & WEI Q. YANG¹. ¹The Pennsylvania State University, University Park, PA 16802 USA, ²The Centre for Land and Biological Resources Research, Ottawa, Canada and ³Michigan State University, East Lansing, MI 48824 USA. - The presence, isolation and characterization of ericoid mycorrhizal symbionts in 2 native and 2 commercial *vaccinium* populations in central Pennsylvania.

Vaccinium (blueberry) roots form an ericoid mycorrhizal symbiosis with certain ascomycetous fungi. Ericoid mycorrhizae increase plant growth and plant yield by enhancing nutrient uptake, sequestering phytotoxic heavy metals, and broadening the optimum pH range for blueberry growth. Over the course of the 1994 growing season, I surveyed ericoid mycorrhizae in 4 different blueberry populations consisting of one native *V. angustifolium*, L. (lowbush) site, one native *V. corymbosum*, L. (highbush) site, one conventional commercial highbush site, and one low-input commercial highbush site. Soil and tissue nutrient levels as well as infection intensity were repeatedly sampled as part of the survey. Fungal isolations were also performed, and isolates were identified using morphological and RAPD analysis. Both native populations had higher mycorrhizal infection intensities than the commercial populations with the lowbush population significantly showing the highest infection levels. The native site soils were lower in pH and nutrient levels than the commercial site soils, infection intensity tended to increase with time during the growing season, and infection intensity differed among commercial cultivars. The results of this survey further indicate the ecological advantage of ericoid mycorrhizae in native populations and its limitations in present commercial practices. This research was supported in part by the DOE/NSF/USDA CRPB Root Biology Program (NSF BIR-922-330).

poster session 11 WF RR 32

STOLL, WILLIAM, KEN CULLINGS, V.T. PARKER. Department of Biology, San Francisco State University, San Francisco, CA 94132, U.S.A. - Diversity and distribution of ectomycorrhizal fungi in young lodgepole pine forests.

Molecular techniques were used to examine the abundance, diversity, and distribution of ectomycorrhizal (EM) fungal species of young lodgepole pine forests. We sampled two six-year-old stands of lodgepole pine (*Pinus contorta*) in Yellowstone National Park that regenerated after wildfires of different intensities. Nine soil cores

(three depths each) (taken in clusters of three cores) and fungal fruit bodies were collected from each stand during July and August 1995. Polymerase Chain Reaction (PCR) was used to amplify the ITS region of fungal DNA extracted from fruit bodies and mycorrhizal roots. EM fungi were distinguished and identified by matching Restriction Fragment Length Polymorphism (RFLP) patterns of fruit bodies and mycorrhizae. Preliminary results indicate that EM fungal populations may be patchily distributed. Initial trends indicate that EM fungal composition may differ both between stands and within stands between coring clusters. The results also indicate that the more intensely burned stand may have a greater diversity of EM fungi and a larger proportion of its mycorrhizal root tips found deeper in the soil. Results from this study will be used to assess the ecological specificity of lodgepole pine's mycorrhizal association and its change over a successional gradient by comparing these results with the findings of a similar study of older mixed species stands of lodgepole pine, subalpine fir, and Engelmann spruce in Yellowstone National Park.

poster session 8 WF AR 59

STREITWOLF-ENGEL, RUTH, IAN R. SANDERS, THOMAS BOLLER & ANDRES WIEMKEN. Botanical Institute, University of Basle, Hebelstrasse 1, 4056 Basle, Switzerland. - Sexual and asexual reproductive traits of two *Prunella* species are influenced by co-occurring arbuscular mycorrhizal fungi (AMF)

P. vulgaris and *P. grandiflora*, like many perennials of species-rich grasslands, have the possibility to reproduce both sexually and asexually. The population structure of these two species will be affected by the proportion and frequency of sexual propagation versus clonal propagation. In both species, clonal propagation is dependent on stolon branching intensity and stolon length. We investigated the influence of the colonization by different AMF, which were isolated from a species-rich calcareous grassland, on plant reproductive traits. Both plant species were totally dependent on AMF for growth in the soil from the study site. The different AMF isolates had a significantly different effect on each of the two plant species. AMF differentially influenced the stolon branching intensity and stolon length in *P. vulgaris* and flowering in *P. grandiflora*. A second experiment, where different concentrations of phosphate were added to mycorrhizal and non-mycorrhizal *P. vulgaris*, indicated that the AMF effects on plant growth were not sensitive to phosphate availability. Our results suggest that AMF have the potential to affect the number and distribution of ramets in *Prunella* populations as well their genetic diversity and also that AMF diversity is potentially an important determinant of plant community structure.

poster session 2 ST SH 85

STUTZ, JEAN C. & CHRIS A. MARTIN. Department of Botany, Arizona State University, Tempe 85287-1601 USA. - Arbuscular mycorrhizal colonization and sporulation in response to high temperatures and elevated atmospheric CO₂.

The interactive effects of elevated atmospheric CO₂ and supraoptimal temperatures on arbuscular mycorrhizal (AM) colonization and sporulation was investigated by inoculating lemon plants with AM fungal populations originally collected from a grassland or from a desert scrub plant community. Inoculated plants were grown in a greenhouse for 6 months at moderate (29/21C day/night) or high (42/32C day night) temperatures and ambient (350 to 380 umol/mol) or elevated (680 umol/mol) CO₂ in a factorial combination. Elevated CO₂ had no effect on the percentage of the root system colonized, although total colonization increased because of increases in total root biomass induced by elevated CO₂. In contrast, high temperatures significantly reduced the percentage of the root system colonized with decreases in both arbuscular and vesicle formation. Colonization patterns were similar for both of the AM fungal populations. Spore abundance was similar when plants were grown at moderate temperature and high temperatures and elevated CO₂ but decreased when plants were grown at high temperatures and ambient CO₂.

poster session 3 ST SH 86

SUBRAMANIAN, K.S. & C. CHAREST. Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, Ontario K1N 6N5, Canada. - Effect of AM-inoculation on drought recovery of maize.

A greenhouse experiment was carried out to examine the influence of arbuscular mycorrhizal (AM) fungus (*Glomus intraradices* Schenck & Smith) on drought recovery of maize. Freshly regenerated seeds of selection cycles 0 (C0, drought-sensitive) and 8 (drought-resistant) of the lowland tropical maize population "Tuxpeno sequia" were used for this study. Maize plants were exposed to drought stress for 3 wks (45-66 DAS) followed by 3 wks of recovery (67-88 DAS). During drought and recovery periods, daily mid-day leaf water potential (LWP), and weekly osmotic potential (OP), osmotic adjustment (OA), and relative water content (RWC) were measured. Organic osmolytes (sugars) in shoots and roots were determined at the end of drought and recovery periods. Mycorrhizal maize plants of cultivars C0 and C8 had higher (less negative) LWP, OP, and RWC, and lower OA throughout the experiment. Sugar contents were higher in AM than nonAM plants regardless of cultivar or drought treatment. According to LWP data, AM plants were able to recover faster from drought stress (C0, 7 d; C8, 4 d) than nonAM (C0, 15 d; C8, 8 d) plants. The response to AM-inoculation was more pronounced in drought-sensitive (C0) than -resistant (C8) cultivars. The study demonstrated that AM-inoculation benefits the host plant to withstand moderate drought conditions and assists in drought recovery.

poster session 3 ST SH 89

SUHARDI, I. Faculty of Forestry, Gadjah Mada University, Yogyakarta, Indonesia. - Mycorrhiza diversity and their compatibility to *Pinus merkusii* and *Schima wallichii* at Merapi valley after new eruption.

Hot cloud from Merapi volcano eruption caused the vegetation and mycorrhiza changed in Merapi valley. Many of grasses, shrubs and trees are extinct Rain or climate and nutrition residue afterward caused development of new vegetation and also spores of mycorrhizae.

Objective of this research is to detect or identify the first natural development of mycorrhiza after hot cloud. Mycorrhizae seem to have an very important role to the next vegetation development. Mycorrhizae inoculation affect the growth of some host trees.

The result showed that only one ectomycorrhiza found namely is *Scleroderma sp* and the rest of fruit bodies are decomposers. Observation of endomycorrhizae has found several genera of endomycorrhizae. The genera are: *Gigaspora* 1, 2, 3; *Sclerocistis* 1; *Glomus* 1, 2, 3 and *Acaulospora* 1.

Inoculation of endomycorrhizae increased 7.3 % height and 55 % diameter growth of *Schima wallichii*, however there have no effect to the *Pinus merkusii* growth.

poster session 5 ST AR 36

SUN, CAMILLA¹, TORGNY UNESTAM¹, STEVEN LUCAS², KARL JOHAN JOHANSSON³, & LENNART KENNE⁴. ¹Department of Forest Mycology and Pathology, SLU (Swedish University of Agricultural Sciences) 750 07 Uppsala, Sweden. ²Department of Surgery, Uppsala University, 75185 Uppsala, Sweden, ³Department of Radioecology, SLU 750 07 Uppsala, Sweden. ⁴Department of Chemistry, SLU 750 07 Uppsala, Sweden. - Do ectomycorrhizal fungi have "kidneys" whose "urine" feeds associated bacteria?

The hydrophobic mycelium of *Suillus bovinus* slowly absorbed [U-¹⁴C]glucose and other tracers along the cords, translocated them out to the peripheral hyphae and exuded them into water drops on the hyphal tips. The exudate was characterized by ¹H NMR spectroscopy and by sugar and amino acid analyses. The exuded compounds consist mainly of carbohydrates (but not trehalose) and peptides. Two organic acids, acetic acid and oxalic acid, were also present in the exudate. Released ions (K, Na, Cl, P, Mg and Ca) were identified by X-ray microanalysis. The ability of the mycelium to reabsorb the exuded compounds was studied using [¹⁴C]-labelled compounds and ⁸⁶Rb. The mycelium was able to reabsorb more than 65% of the exuded ¹⁴C-compounds in less than 2 days. Glucose, mannitol, glutamic acid (pH 3.2), and Rb⁺ were all readily absorbed (apparently joined by other mineral ions) by the mycelium, while oxalic acid at pH 4.2 and glutamic acid at pH 6.5 were not. In dry soil in the field, Rb and water were transferred by hydrophobic cords to the distal mycelium and exuded into the soil.— We hypothesize that water would be absorbed from the root together with plant carbohydrates and initiate the acropetal flow along a concentration gradient of sugars and polyols, at least in dry soil. Exudation of water may represent a necessary kidney function of the peripheral hypha and, furthermore, feed associated weathering and N-fixing bacteria. Plant

transpiration would counteract this flow. Sugar uptake at the source, but not exudation of water, may be one of the driving forces.

Sundaram, M poster session 1 ST RR 10
SUNDARAM, M.D. Department of Microbiology, Annamalai University - 608 002, Tamilnadu, India. - Studies on the mycotrophy of cassava in different soil types of coastal Tamil Nadu

Cassava root colonization by VAM fungi were very poor in the coastal soils of Tamil Nadu. Cassava root associated VAM fungi belonged to the genera *Glomus gigaspora*, *Acaulospora*, *Sclerocystis*, *Scutellospora*, *Endogone* and *Enterophospora*. *Glomus* spp. was found to be the most predominant and accounted for 50 percent root infection in all the soil types followed by *Acaulospora*, *Gigaspra*, *Endogone*, *Enterphospora*, *Sclerocystis* and *Scutellospora*. The VAM fungus, *Glomus fasciculatum* AU-1 best colonized the roots of cassava. Red lateritic soil-grown cassava recorded the maximum root infection (92%) followed by sandy loam (83%), sandy soil (78%) and clay soil (62%). The P concentration was negatively correlated with root infection, while nitrogen and potassium influence varied with all four soil types studied. Critical level of VAM fungi concentration for optimum cassava production was worked out for different soil types. Biocontrol agents did not have negative effect and in fact improved the growth and VAM root infection of cassava. Biofertilizers produced synergistic effect on VAM cassava symbiosis.

poster session 5 ST AR 33
SUNDARI, S. KRISHNA¹, ANIL PRAKASH² & ALOK ADHOLEYA¹. ¹Tata Energy Research Institute, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi 110 003, India. ²Department of Microbiology, Barkatullah University, Bhopal, India. - Lyophilization of EM fungi

Lyophilization though considered to be the most efficient and dependable method for long term preservation of bacteria and other sporulating fungi, has remained a challenge with non-sporulating filamentous fungi. A method has now been evolved to freeze-dry vegetative mycelium of ectomycorrhizal fungi belonging to the genus *Laccaria* and class Basidiomycetes. Three species of the genus namely *Laccaria fraterna*, *Laccaria laccata*, *Laccaria amethystina* were lyophilized and obtained viable at the end of the process. All the three species were tested with a range of cryoprotectants before obtaining the workable protocol. The morphology (appearance and growth pattern) and physiology (growth parameters like pH and temperature) of all the three fungi are found to be similar before and after lyophilization. The fungi are tested for various enzyme activities using enzyme specific substrates in solid media before and after lyophilization and found to be behaviourally same. To confirm the molecular nature of the freeze-dried fungi, protein profiles are being performed using the non-lyophilized fungal mycelium as control. Synthesis experiments are

under way with lyophilized and non-lyophilized fungal mycelium to find out the colonization ability of the fungus. Efficacy tests are also under progress using both of lyophilized and non-lyophilized fungal inoculum. All the experiments are conducted in vitro and showed consistent results on repetition.

poster session 11 WF RR 43
SYLVIA, D. M. and D. J. FARMER. Soil and Water Science Department, University of Florida, Gainesville, FL 32611-0290. - Morphotype diversity and phosphatase activities of ectomycorrhizae in a southern pine plantation

Mineralization is a crucial component of the P cycle and is the dominant process controlling soil-solution concentration of P in the Spodosols of the southeastern US. Phosphatases are produced by ectomycorrhizal (EM) fungi; however, little is known about the ability of native EM to mineralize P in situ. We examined the diversity of EM types over a growing season in a southern pine plantation and determined the effect of EM type on phosphatase production. Diversity was determined from morphological characterization and PCR-RFLP analysis of the internal transcribed spacer (ITS) region of the rDNA gene extracted from mycorrhizal root tips. Phosphatase activities of mycorrhizal roots were assessed by a colorimetric assay. We found that morphotype distribution and phosphatase activities varied widely through a growing season. Those morphotypes with high phosphatase activity were relatively rare, but they may play a crucial role in the P nutrition of plantation pine trees.

poster session 4 ST AR 68
TAGU, DENIS, UWE NEHLS & FRANCIS MARTIN. I.N.R.A. Nancy, Microbiologie Forestière, 54280 Champenoux, France. - Eucalypt ectomycorrhiza differentiation: molecular markers of hyphae attachment and aggregation.

Ectomycorrhiza formation induces major changes in protein biosynthesis and gene expression. A shotgun cDNA sequencing was used to clone symbiosis-regulated (SR) genes in the *Eucalyptus globulus* - *Pisolithus tinctorius* mycorrhiza. Two regulated transcripts were identified as hydrophobins, a family of secreted morphogenetic fungal proteins. They were up-regulated during the early stages of mycorrhiza differentiation. Hydrophobins are likely involved in the formation of a hydrophobic layer at the surface of the hyphae for the attachment of the mycelium to the root surface. Fungal SR genes were also characterized by differential screening. The clone d20 - which had no significant homology with any other known sequences - corresponds to an up-regulated transcript during mantle morphogenesis and Hartig net formation. As for hydrophobins, d20 transcripts are not accumulated in immersed hyphae. This suggests that the D20 protein could also play a role in aerial growth of the mycelium. Hydrophobins and d20 represent two molecular markers for fungal adhesion and aggregation. These results (i) strengthened the observations that fungal cell wall

proteins are deeply altered during ectomycorrhiza development and (ii) showed that reorganization of cell wall and extracellular matrices is probably involved in hyphal attachment and aggregation.

poster session 4 ST AR 70

TAHIRI-ALAOUI, A.¹, G. STRITTMATTER², V. GIANINAZZI-PEARSON³ and K. HAHLBROCK².
¹IACR-Rothamsted, CDM. Harpenden, Herts AL5 2JQ, UK. ²Max-Planck Institut, Abteilung Biochemie, Carl von Linne-Weg 10, D-50829 Cologne, Germany. ³Laboratoire de phytoparasitologie, INRA- CNRS, SGAP, BV 1540, F-21034 Dijon Cedex, France. - Induction, repression and derepression of defense-related genes in potato roots in response to an arbuscular mycorrhizal fungus and a susceptible pathogen

The aim of this study was to investigate the expression of 12 defense-related genes during a time course colonization of potato roots by an arbuscular mycorrhizal (AM) fungus (*Glomus* sp.) or during a susceptible pathogenic-interaction of roots with *Rhizoctonia solani*. Results showed three different patterns of gene expression during the development of the AM symbiosis: 1) genes that were expressed at early stages of infection, but were repressed in fully established mycorrhizal association, and unexpectedly activated during the late stages of colonization, 2) genes that had no apparent modifications to their expression during AM symbiosis and 3) a gene that was activated during the late stage of colonization. When the expression of the same genes was studied during susceptible interaction of potato roots infected with the fungal pathogen, their activation increased with infection intensity of the root system. These results suggested that plant defense-related genes were differentially regulated during symbiotic interaction in a way which did not interfere with the development of the fungal symbiont.

poster session 4 ST AR 71

TAHIRI-ALAOUI, A. & J.F ANTONIW. IACR-Rothamsted, Crop & disease management department. Harpenden, Hertfordshire AL5 2JQ UK. - Are specific genes expressed or repressed during arbuscular mycorrhiza symbiosis, responsible for biocontrol of root pathogens ?

Arbuscular mycorrhizal (AM) fungi form a symbiotic relationship with their host by colonizing the cortical region of roots both inter and intracellularly. They benefit the host primarily by increasing the capability of root system to absorb and translocate phosphorus and microelements. AM fungi can also enhance root resistance/tolerance towards root pathogens. The mechanisms mediating AM-pathogen interactions and leading to a better resistance status of the plant against pathogens are not yet known. Hypotheses to explain AM's effect on root pathogens generally include either a physical or physiological basis. Our aim is to clone plant genes whose expression is specifically up or down regulated in AM colonized or pathogen infected roots

and which may play a role alone or in concert with other genes in the biological control of root pathogens. Our experimental model is tomato/*Glomus mosseae*/*Phytophthora nicotianae* var. *parasitica*. Three libraries have been constructed, representing cDNA transcripts from control roots, roots colonized by *G. mosseae* or infected with *Phytophthora*. By differential screening of these libraries we have identified several up and down regulated genes. The role of these specific genes in the establishment of a successful AM symbiosis and their possible involvement in the biological control of root pathogens will be discussed.

poster session 11 WF RR 36

TAMMI, HANNA, SARI TIMONEN, ROBIN SEN. Department of Biosciences, Division of General Microbiology, P.O. BOX 56, 00014 Helsinki University, Finland. - Ectomycorrhizal fungi and their root colonisation dynamics of Scots pine seedlings grown in natural humus and nursery peat substrates

In order to determine indigenous ectomycorrhizal diversity and infection dynamics, non-mycorrhizal Scots pine seedlings were incorporated into transparent microcosms containing natural pine forest humus or nursery peat. Mycorrhization was monitored every 7-14 days over 3 months and individual ectomycorrhiza were characterized on the basis of gross morphology. Six and only one distinct indigenous morphotypes developed on humus and peat substrates, respectively. Brown and a white type (*Suillus bovinus* fingerprint) ectomycorrhiza tended to appear in the humus first but no subsequent successional trends in the developing community was apparent. The success of esterase (EST) isozyme and PCR/ITS/RFLP fingerprinting of these morphotypes was 90% and 50-90% respectively. The need for a species EST/PCR fingerprint library was highlighted in this study. Ecological and practical implications of the observed ectomycorrhizal diversity will be discussed.

poster session 12 WF SH 52

TAWARAYA, KEITARO, KAZUKO HASHIMOTO & T. WAGATSUMA. Plant Nutrition and Soil Science, Faculty of Agriculture, Yamagata University, Tsuruoka, 997, Japan. - Effect of onion root exudates on the colonisation of an arbuscular mycorrhizal fungus, *Gigaspora margarita*

The effect of onion root exudates on the colonisation of an arbuscular mycorrhizal fungus was examined. Onions (*Allium cepa* L.) were grown in solution culture at two phosphorus concentrations (0 (P0) and 2 (P2) mg P l⁻¹) and root exudates were collected. Root exudates were fractionated giving an EtOH soluble fraction and a water soluble fraction with Amberlite XAD-4 resin. Onions inoculated with arbuscular mycorrhizal fungus, *Gigaspora margarita* Becker & Hall were grown with or without (control) these root exudates in a growth chamber. Arbuscular mycorrhizal colonisation, number of appressoria and root length were determined 24 days after transplanting. Arbuscular mycorrhizal colonisation and the number of appressoria were higher in plants

treated with P0-root exudate and P0-EtOH fraction than in the control. P0 and P2 water soluble fractions did not affect the colonisation. There was a positive correlation between number of appressoria per plant and the colonised root length per plant. These results suggest that 1) hydrophobic compounds of P-deficient onion increase appressorium formation and 2) appressorium formation controls the mycorrhizal colonisation.

morning talk

TAYLOR, LEE & THOMAS D. BRUNS. Division of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA. - Myco-heterotrophy in North American orchids: specificity and trophic links.

We demonstrate that seven myco-heterotrophic orchid species associate exclusively with two specific families of ectomycorrhizal fungi. This conclusion is based on congruent evidence from fungal isolation and from RFLP and sequence analysis of two independent genes. Specificity in the *Corallorhizas* was striking. We have found that the sister taxa *C. maculata* and *C. mertensiana* target non-overlapping, but closely related, fungal species even where these orchids co-occur. Furthermore, germination studies show that *Corallorhiza maculata* targets the same fungi as a seedling and as an adult. The distantly related myco-heterotrophic orchid *Cephalanthera austinae* has independently evolved highly specific associations with the Thelephoraceae. We use single stranded conformational polymorphism analysis to show that the fungi which associate with this orchid form standard ectomycorrhizae with photosynthetic hosts at the same place and time. We have also found that a photosynthetic relative of the *Corallorhizas*, *Tipularia discolor*, associates with related ectomycorrhizal fungi, suggesting that the jump away from *Rhizoctonia* to an ectomycorrhizal fungus has preceded the loss of chlorophyll in *Corallorhiza*. We view these myco-heterotrophs as cheaters because they do not supply carbon to associated fungi. We suggest that high specificity is required of cheaters and that connection to an ectomycorrhizal fungus allows access to a larger carbon pool, favoring the independent jumps to these fungi seen in the Orchidaceae.

poster session 12 WF SH 54

THINGSTRUP, IDA, PETER SCHWEIGER & IVER JAKOBSEN. Plant Nutrition, Environmental Science and Technology Department, Risø National Laboratory, DK-4000 Roskilde, Denmark. - Measurement of potential phosphorus transport by field populations of arbuscular mycorrhizal fungi.

Quantification of phosphorus (P) transport to plants by arbuscular mycorrhizal fungi (AMF) under field conditions is of fundamental interest for describing the role of AMF in natural or agricultural ecosystems. Indirect measurements based on comparisons with non-mycorrhizal plants grown in fumigated or fungicide-treated soil are difficult to interpret due to side effects on soil nutrient status and on microflora and fauna. We have developed an alternative method which involves the use

of root free mesh bags as hyphal compartments (HC) in combination with radioactive tracers. Two HC are used: one has ^{33}P mixed into the soil while another contains ^{32}P and carbendazim, which inhibits AMF functioning. AMF transport of P from the HC to the nearby plants is measured as the content of ^{33}P in the plants with the subtraction of the ^{32}P content which provides an estimate of root hair P transport as well as diffusion of radiotracers out of the HC. Growth chamber experiments with seven agricultural soils showed variation in P transport potentials between the natural AMF-populations. Furthermore, variations were observed in the background P transport, probably due to differences in carbendazim interaction with different soils. A field test of the method is carried out in a 20-year-old field experiment on P fertilization in the present growing season.

poster session 6 WF AR 15

THOMPSON, JOHN, DAVID PECK, NICOLE SEYMOUR, TIM CLEWETT & MARK PHILP. Queensland Wheat Research Institute, PO Box 2282, Toowoomba, Qld., Australia. - Integration of the concepts of mycorrhizal dependency and phosphate benefits for field crops

Crops grown in the dark clay soils (Vertisols) of the subtropical Australian grain belt can suffer "long fallow disorder" after weed-free fallowing due to a decline in vesicular-arbuscular mycorrhizal (VAM) fungi. The severity of the disorder depends on the crop species and the nutrient status of the soil for P and Zn. Two experiments (for cool-season and warm-season crops) were conducted on a low P site to determine the relative field mycorrhizal dependency of 18 species of field crops at a range of soil P levels. Nine rates of fertiliser P were rotary-hoed into the topsoil to give a targetted range of soil solution P levels. Part of each plot was fumigated with methyl bromide to kill the native VAM fungi. The crops were sown with and without VAM inoculum (glasshouse-grown *Glomus mosseae* strain 5) in the fumigated and non-fumigated areas of all P rates. Dry matter production by the crops with and without VAM was determined and Mitscherlich response curves of the form $Y = a + be^{cX}$ were fitted against soil P level. With this approach one can determine the mycorrhizal dependency of a crop at any interpolated soil P level and the equivalent phosphate benefit from VAM at any yield level. The crop species varied in mycorrhizal dependency from very high for linseed, fababean, pigeonpea, lablab, cotton, and maize; through high for sunflower, chickpea, navybean and mungbean; to very low for sorghum, oats, wheat, Triticale and barley; to independent for Canola. Such information on crop species requirements for VAM at various P levels is valuable in choosing crop and fallow sequences for farming systems.



TIMONEN, SARI, E-L. NURMIAHO-LASSILA, ROBIN SEN. Department of Biosciences, Division of General microbiology, P. O. Box 56, 00014 University of Helsinki, Finland. - Bacterial colonisation patterns of the pine mycorrhizosphere in natural and nursery soils.

Scots pine seedlings pre-infected with mycorrhizal fungus *Suillus bovinus* were grown in flat microcosms with natural pine forest soil and nursery soil. Samples were collected for electron microscopy and isolation of fluorescent pseudomonads and *Bacillus* spp. from the different parts of pine mycorrhizosphere. SEM showed an increasing diversity of bacteria towards the plant. Sheets of bacteria with similar morphology were found closely associated with fine hyphal fronts whereas mycorrhiza and short roots displayed a large variety of different bacterial morphotypes. The bacteria were located on hyphae embedded in amorphous matrix both in mycorrhizas and in external hyphae. No bacteria were seen in the Hartig net or inside fungal cells. In the bacterial isolations occurrence of bacteria within the respective parts of the mycorrhizosphere was patchy and random suggesting micro scale diversity within the system. Certain trends were, however, observed. Both in numbers and diversity more *Bacillus* spp. could be isolated from external hyphae than from pine roots particularly in the forest soil, and no fluorescent pseudomonads could be found from dry pine forest in contrast with the nursery soil. The observed bacterial colonisation patterns of the mycorrhizosphere will be discussed in relation to their potential roles in plant-soil interactions.

TORO, MARCIA¹, JOSE MIGUEL BAREA² & ROSARIO AZCON². ¹IVIC, Ecology Center, Postal code 21827, Caracas 1020-A, Venezuela. ²Soil Microbiology and Symbiotic Systems Dept., Estación Experimental del Zaidín, Profesor Albareda 1, Granada 18008, Spain. - Assessing the effectiveness of two rock phosphates mixed with Triple Super Phosphate inoculated with P-solubilizing bacteria and arbuscular mycorrhiza applying the isotopic dilution method (³²P).

Rock phosphates are insoluble fertilizers suitable for agriculture because of their low economical value. It's reactivity (amount of soluble P) can be improved when mixed with soluble sources such as Triple Super Phosphate (TSP). P-solubilizing bacteria and arbuscular mycorrhiza also ameliorate the use of these sources. To improve the reactivity of two rock phosphates a greenhouse bioassay with *Agrostis tenuis* inoculated with *Enterobacter* (P-solubilizing bacteria) and *Glomus deserticola* was designed. Riecito and PN-Brut rock phosphates were mixed (1:1) with TSP to obtain 100 ppm P. Both, the mixtures and the rock phosphates alone were tested with the mentioned microorganisms; sterile controls were also set. 50 μ Ci ³²P/0,5 kg soil were added to apply the isotopic dilution method. Microorganisms improved plant growth parameters when the mixtures with TSP were added. Specific activity and Pdfd (68%)

values showed that the combination *Enterobacter-G. deserticola* was the most effective treatment improving the use of Riecito-TSP. Only *Enterobacter* slightly improved the use of the mixture PN-Brut-TSP, indicating less effectiveness of microorganisms maybe due to the low reactivity of PN-Brut. Our results indicate that mixing insoluble sources with TSP and the inoculation of microorganisms contributed greatly to ameliorate the effectiveness of rock phosphates.

TORRES, PILAR & MARIO HONRUBIA. Departamento de Biología Vegetal (Botánica), Facultad de Biología, Universidad de Murcia, Campus de Espinardo, 30100 Murcia, España. - Changes and effects of a natural fire on ectomycorrhizal inoculum potential of soil in a *Pinus halepensis* forest.

A typically mediterranean forest of *Pinus halepensis* which had been affected by a natural fire was selected and a two-year follow-up has been realized studying fruiting fungal species, number of sclerotia in soil, percentages and types of mycorrhizas present from bioassays. Burnt stands presented typical carbonicolous ascomycetes while the basidiomycetes were strongly reduced by fire. Sclerotia extracted from soils were mainly of *Cenococcum*. The number of sclerotia in burnt stands was higher than in control. Seven types of mycorrhizas were recognized in *Pinus halepensis* root systems from bioassays: *Cenococcum*, E-strain, *Rhizopogon*, *Suillus*, *Tuber*, *Xerocomus*, and one non identified. Soils showed a high percentage of mycorrhization exhibiting nearly 100% colonization of the roots. However, these percentages were mainly due to ectendomycorrhizas which represented from 50-90% of the total number. Percentages of ectomycorrhizas were very low. The predominant type are the ectendomycorrhizas formed by ascomycetes included in the denominated E-strain group.

TRAPPE, JAMES¹, EFRÉN CÁZARES¹, ARI JUMPPONEN¹ & CLAUDI HÖFLE². ¹Forest Science, Oregon State University, Corvallis, OR 97331-7501. ²Palmer Research Center, University of Alaska Fairbanks, Palmer, AK 99645. - Permafrozen, 17,600-year-old ecto- and VA-mycorrhizae, dark septate endophytes and *Glomus* spores from the Beringian land bridge, Seward Peninsula, Alaska.

About 17,600 years B.P., when Siberia and Alaska were joined by a massive land bridge, the Devil Mountain Lakes volcanic eruptions covered areas of the Seward Peninsula with tephra. The deposits insulated the underlying soil and covered plant communities growing over permafrost, which rose to embed the roots and associated organisms in ice that has persisted to now. Samples of the frozen soil communities were excavated from several sites with support of the Shared Beringian Heritage Program of the National Park Service. Care was taken to avoid contamination by overlying tephra. The samples were sealed, placed in an ice chest and shipped

within 48 hrs. to the Forestry Sciences Laboratory at Corvallis. The samples are being thawed one at a time for processing roots and extracting AM spores, arthropods and sclerotia. Of the 12 samples collected, three have been processed at time of writing. Two contained abundant roots in good condition, some presumably of willows. Ectomycorrhizae, AM with abundant vesicles and arbuscules, and dark-septate colonizations were common. Spores of *Glomus fasciculatum* and *G. macrocarpum* were common in addition to abundant spores of an ornamented, possibly undescribed species provisionally named *Glomus miocenicum* for the Miocene epoch in which they formed. Approximately 5% of the spores contained cytoplasm. DNA extraction and sequence analysis of these will be attempted to investigate the relationship of *G. miocenicum* to currently existing Glomalean taxa. Single-spore inoculations and pot cultures with sorghum have been started.

poster session 10 WF SH 08

TUINGA, AMY R.¹, J. K. STONE², P. T. RYGIEWICZ³, & M. E. HARMON⁴. ¹Rutgers Pinelands Field Station, New Lisbon, NJ 08064, USA. ²Dept. of Bot. and Plant Path., OSU, Corvallis, OR 97331, USA. ³National Health and Environmental Effects Res. Lab, USEPA Corvallis, OR 97333, USA. ⁴Forest Science, OSU, Corvallis, OR 97331, USA. - Interactions between mycorrhizal and saprotrophic fungi grown on agar and rotted wood.

Interactions between mycorrhizal fungi and saprotrophic fungi are not well understood, but they could alter the function of mycorrhizal fungi, consequently influencing plant growth. Mycorrhizal fungi commonly occur in proximity to saprotrophic fungi in nurse logs in US Pacific Northwest Old-Growth forests. Nine species of mycorrhizal fungi and three species of saprotrophic fungi were paired on four different media, including white- and brown-rotted Douglas-fir wood. Respective colony growth rates were compared for each pairing. Gross morphology was also observed, and hyphal interactions were examined. In general, media affected growth rates of mycorrhizal and saprotrophic fungi more than did the presence of a second fungus. On all media, zones of inhibition formed between most mycorrhizal fungi and all saprotrophic fungi. Inhibition zones did not form between *Laccaria bicolor* or *Pisolithus tinctorius* and any saprotrophic fungi on any media. Intermixing occurred in all treatments regardless of whether a zone of inhibition had been formed earlier. Coiling and vacuolation of saprotrophic hyphae occurred when paired with *Cenococcum geophilum* on any medium. These results indicate that a complex relationship may exist between nutrient status of the substrate and the outcome of organismal interactions.



poster session 11 WF RR 26

UBALIJORO, ELIANE¹, CHANTAL HAMEL², ROBERT MCMCLUNG³ & DONALD L. SMITH¹. ¹Plant Science Department, Macdonald Campus of McGill University, Ste Anne de Bellevue, Canada. ²Soil Science Department, Macdonald Campus of McGill University, Ste Anne de Bellevue, Canada. ³School of Biological Sciences, Dartmouth College, New Hampshire, US. - Detection of microsatellite DNA sequences in AM fungi using a *Glomus* intraradices genomic library.

Research in the molecular genetics of AMF has been hindered by their lack of growth in pure culture. An in vitro-culture system for AM fungi was used to grow *G. intraradices* in the presence of Ri-DNA transformed carrot roots on dual compartmentalized plates. One side of the plates sustained only fungal growth. This technique permitted the isolation of pure fungal material. Fungal material was retrieved after dissolution of the gellan gum and DNA was extracted. A partial genomic library was produced in pBluescript and screened with tetra, tri and dinucleotide repeats. No tetra- or dinucleotide repeats were detected in the partial genomic library but positive clones were isolated from screening with trinucleotide repeats. Sequencing of positive clones verified the presence of microsatellite sequences and permitted the design of primers that amplify these regions. All primers designed were capable of amplifying the variable number of tandem repeat (VNTR) region from crude extracts of *G. intraradices* single spores. These primers are being assayed on other AM species in order to determine their specificity. These primers may permit isolation of taxon-specific markers in the future.

talk session 6

UETAKE, YUKARI, MELISSA FARQUHAR & R. LARRY PETERSON. Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1, Canada. - Changes in microtubule arrays in symbiotic orchid protocorms during fungal colonization and senescence

Microtubule (MT) arrays were studied in *Spiranthes sinensis* (Pers.) Ames protocorms which had been symbiotically cultured in vitro with *Ceratobasidium cornigerum* (Bourdot) Rogers using antibodies to α -tubulin and laser scanning confocal microscopy. Cortical MTs present in uncolonized cells disappeared when cells became colonized by the fungus. In colonized cells MTs were observed, however, between the hyphae forming hyphal coils (pelotons), and a close relationship between pelotons, MTs and nuclei was often observed. These MTs were closely associated with hyphae and formed a net-like structure through the pelotons. During senescence and condensation of the pelotons, MTs were observed surrounding the pelotons, between collapsing hyphae and as circular profiles within the collapsed hyphal masses. In cells which contained completely collapsed hyphal masses and which were not reinfected, cortical MTs reappeared and continued from the cortex through the hyphal remains to the collapsed hyphal masses. MTs associated with hyphae seemed to be

involved in the condensation of hyphal masses in the host cells. Fungal MTs were observed in hyphae except in those which were collapsing.

poster session 6 WF AR 17

UPADHYAYA, ABHA & SARA WRIGHT. Soil Microbial Systems Laboratory, USDA-ARS, Bldg. 318, BARC-E, Beltsville, MD 20705. - A simple and novel method for evaluation of hyphal length and concentration of AM fungi

Measures of hyphal length and density are important to evaluate the contribution of AM fungi to soil stabilization. Based on recent findings, hyphae of these fungi produce a stable glycoprotein, the protein attaches to plastic, the concentration of protein is an indicator of hyphal mass for genera and a monoclonal antibody is consistently reactive across genera and species for equivalent amounts of protein. To test for hyphal protein attachment to plastic as an indicator of hyphal spread in coarse sand 35 cm diameter pots were used. Sudangrass (*Sorghum sudanense* (Piper) Staph.) fed with low phosphorus nutrient solution and inoculated with *Glomus intraradices* UT126 or *Gigaspora gigantea* MA453A was enclosed in 40 µm mesh. Strips of plastic horticultural fabric cut into 3 x 6 cm strips were placed radially at 2 cm intervals beginning at the interface of the mesh and root-free sand. Eight rows of fabric were equally spaced around the pot. At 7-week intervals, two rows of diagonally opposite strips were removed. Protein was extracted using 2 ml of 20 mM citrate (pH 7.0) at 121 °C for 60 min. Quantitative enzyme-linked immunosorbent assay (ELISA) and Bradford protein analyses were performed. *G. gigantea* hyphae moved further, more rapidly and produced more protein than *G. intraradices* hyphae. The quantitative ELISA was 100 times more sensitive than the protein assay. We propose the use of comparative protein values based on ELISA, but mass of hyphae can be calculated using previously measured protein yield/mg of hyphae.

talk session 3

VAN TICHELEN, KATIA K.¹, JACO VANGRONSVELD² & JAN V. COLPAERT¹. ¹Institute of Botany, Katholieke Universiteit Leuven, K. Mercierlaan 92, B-3001 Leuven, Belgium. ²Limburgs Universitair Centrum, Universitaire Campus, B-3590 Diepenbeek, Belgium - Reclamation of a bare industrial area contaminated by non-ferrous metals: occurrence of the AM infection, physico-chemical and biological evaluation of the durability of soil treatment and revegetation

In 1990, 3 ha of a highly metal polluted acid sandy soil at the site of a former zinc smelter was treated with a combination of beringite and compost; beringite is a substance which has a strong metal immobilization capacity. After soil treatment and sowing of a mixture of metal tolerant *Agrostis capillaris* and *Festuca rubra*, a healthy vegetation cover developed. Five years later, we made an evaluation of soil physico-chemical parameters, potential phytotoxicity, floristic and fungal diversity and

mycorrhizal infection of the plant community. Phytotoxicity was shown to be maintained at the low level observed immediately after soil treatment. The water-extractable metal fraction of the treated soil was up to 70 times lower compared to the non-treated soil. Diversity of higher plant species and saprophytic fungi was extremely low in the untreated area due to the high soil toxicity and the absence of metal tolerant ecotypes of plants and fungi. On the treated soil, in contrary, the species richness of higher plants was much higher; several perennial forbs which are not noted as metal tolerant had colonized the revegetated area. Most of these species belong to mycotrophic families so that the presence of a mycorrhizal network in the soil promotes their establishment. The ubiquity of the mycorrhizal fungi in the roots showed that a functioning ecosystem was establishing. In non-treated soil, the mycorrhizal infection rates of the roots were consistently lower during the whole growing season.

poster session 11 WF RR 30

VAN TUINEN, DIEDERIK, BIN ZHAO, EMMANUELLE JACQUOT & VIVIENNE GIANINAZZI-PEARSON. Laboratoire de Phytoparasitologie, INRA/CNRS, Station de Génétique et d'Amélioration des Plantes, INRA, BV 1540, 21034 Dijon Cedex, France. - Development of molecular probes for the study of root colonization by arbuscular mycorrhizal fungi in reconstructed communities

Arbuscular mycorrhiza (AM) interactions can vary from one plant/fungal association to another at the level of both fungal colonization of roots and beneficial effects on plants. The soil community of AM fungal symbionts in term of spore numbers can also be modified by agricultural practices. However, very little data are available concerning the relationship between the composition and distribution of these fungal communities in the soil and in root systems, since hyphal morphology of the fungus does not allow identification at the species level. We are developing molecular tools to identify hyphae of the AM fungal partner at the species level in order to study community composition in plant root systems.

For this, we have developed a set of primers (LR1 and NDL22) for amplification by PCR of the 5' end of the large ribosomal sub-unit of AM fungi. Precautions in their design were taken to avoid amplification of bacterial DNA, often associated with AM fungal spores. The 700 bp fragment amplified with the LR1 and NDL22 primers from single spores of different Glomales species has been cloned and sequenced. PCR primers with different levels of specificity (species or genus) have been obtained by sequence comparison. These primers used in combination with LR1 or NDL22 enabled specific detection, by nested PCR, of AM fungi within root systems of plants inoculated with mixtures of different glomalean species.

talk session 3

VANDENKOORNHUYSE, Ph.¹, LEYVAL C.¹ & WALKER C.². ¹Centre de Pédologie Biologique, CNRS UPR 6831, 17 rue Notre Dame des Pauvres, 54500 Vandoeuvre-les Nancy (France). ²Forestry commission, Forestry authority, Northern research station, Roslin, Midlothian EH25 9SY (United Kingdom). - Impact of heavy metals on the diversity and genetic variability of AM fungi in soil.

To study the effect of heavy metals on AM fungi diversity, three plots of a field experiment at Braunschweig (Germany) were examined: highly contaminated (metal spiked sludge), intermediary contaminated (unspiked sludge) and uncontaminated control plots. A classical taxonomic study has been carried out. A total of six different species (two undescribed species), mainly *Glomus* sp., was found on the different plots of the research field.

The number of spores of the different species in the different plots increase in the low polluted soil compared to the control plot but significantly decrease in the highly polluted soil. This may be due to toxicity effect in the latter.

Interspecific studies using PCR-RFLPs on 18S rDNA have been undertaken in order to verify and validate the classical taxonomic identification, and to define the phylogenetic position of the undescribed species. Intraspecific studies using 2 different methods of microsatellite-primed PCR will be used for the characterization of different genotypes.

The molecular diagnostic of the different species and the level of genetic variability will be defined for the glomales in interaction with metal pollution effect.

talk session 9

VÄRE, HENRY¹, RAUNI OHTONEN¹ & MAURITZ VESTBERG². ¹Department of Biology, University of Oulu, P.O.Box 333, FIN-90571 Oulu, Finland. ²Laukaa Research and Elite Plant Unit, The Agricultural Research Centre of Finland, MTT, FIN-41330 Vihtavuori, Finland. - Mycorrhizas in Fennoscandian oroarctic.

Northern Fennoscandia is located along a transition from oceanic to continental sectors, where the vegetation pattern is further complicated by altitudinal zones. The tree line is formed generally by birch (*Betula pubescens* subsp. *czerepanovii*) here. As in most subarctic ecosystems the mycorrhizal status of plants is poorly known. Soil samples and plant roots were collected for a mycorrhizal survey in front of a receding glacier (450-520 m a.s.l.) and along an altitudinal gradient from the low to middle oroarctic belt (600-900 m a.s.l.) above tree line. The distance between collection localities is about 50 km. As in the true arctic, no AM fungi or AM colonization could be detected in front of the receding glacier, not even in its older parts, although graminoids which are generally considered as AM were growing there. Along the altitudinal gradient average colonization varied little from 600 to 800 m, but at 900 m root colonization dropped significantly, due to the greater abundance of non-mycorrhizal plants. Mycorrhizal

colonization varied a lot between species. Seven AM fungal species were identified, none previously reported from the Kiolen mountains in Scandinavia, *Acaulospora scrobiculata* being the most common. Ericoid and ectomycorrhizal plants had a typical mycorrhiza of their own type at both sites, but in front of receding glaciers these have not yet been adequately studied.

poster session 10 WF SH 24

VARESE, GIOVANNA CRISTINA & ANNA MARIA LUPPI-MOSCA. Department of Plant Biology, University of Turin, Viale Mattioli 25, 10125 Torino, Italy. - Surface and inner microfungus communities of *Fagus sylvatica* L. ectomycorrhizae

Microfungal communities from serially washed and serially washed plus H₂O₂ sterilized ectomycorrhizae were compared. The samples were collected from nine randomly selected trees approximately 50 years old in a pure beechwood in northern Italy. Unsterilized serially washed mycorrhizae showed a percentage of colonization of about 65% and the fungi isolated belonged to 30 taxa. Sterilized mycorrhizae showed a significantly lower percentage of colonization (about 36%) and the fungi isolated belonged to 26 taxa, nine of which were common to the unsterilized mycorrhizae. The genus *Mortierella* sensu lato (and in particular *M. parvispora*), the genus *Verticillium* (and in particular *V. psalliotae*), *Cylindrocarpon magnusianum* and *Gliocladium roseum* were significantly (Wilcoxon's signed rank test) associated with unsterilized mycorrhizae. By contrast, dark and hyaline sterile mycelia, *Oidiodendron griseum* and *Absidia glauca* were significantly associated with sterilized mycorrhizae. The significance of the most common species on and inside mycorrhizae is discussed. The multivariate analysis (Principal Component Analysis and Cluster Analysis) differentiated two major fungal assemblages related to unsterilized and sterilized mycorrhizae. These results suggest that the surface and the inner part of *Fagus sylvatica* mycorrhizae seem to be two microhabitats that host microfungus communities with presumably different degrees of association with the host plant.

poster session 11 WF RR 28

VARGA, ANIKO M. and HUGUES B. MASSICOTTE. Forestry Programme, Natural Resources and Environmental Studies, The University of Northern British Columbia, Prince George, B.C., Canada. V2N 4Z9. - Ectomycorrhizae characterization and fungal linkages between Sitka Alder (*Alnus viridis* ssp. *sinuata* Regel) and Lodgepole Pine (*Pinus contorta* Dougl. ex Loud var. *latifolia* Engelm.) from young and mature stands in the Sub-Boreal Spruce (SBS) biogeoclimatic zone using morphological and PCR RFLP approaches.

In north-central B.C., lodgepole pine forests often naturally regenerate with sitka alder co-dominating the site for the first 20 years until pine and alder become over- and under-story species respectively. Research has shown that the genus *Alnus* interacts with a limited number of fungal symbionts whereas *Pinus contorta* is

potentially able to form hundreds of possible fungal associations. This study will characterize the ectomycorrhizal diversity of sitka alder and lodgepole pine growing in mixture in two young regenerating seral stages and two mature pine-alder stands within the SBS and determine the potential for existing mycorrhizal linkages. The young sites are 9-year old naturally-regenerated forests following winter harvest whereas the mature pine-alder stands are approximately 100-120 years old and are most likely fire regenerated. Sitka alder and lodgepole pine roots will be sampled in spring and fall along random transects and ectomycorrhizae will be characterized using a combination of morphological and PCR-amplified RFLP DNA analyses to assess the mycorrhizal diversity. Temporal and frequency distributions of mycorrhizae will also be determined. Preliminary results are discussed here.

poster session 5 ST AR 37

VIERHEILIG, HORST, CATHERINE ALBRECHT, BERTA BAGO & YVES PICHE. Université Laval, Faculté de Foresterie, CRBF, Pavillon Marchand, Québec G1K 7P4, Canada. - Do flavonoids play a role in root colonization by AM fungi?

Certain flavonoids have shown to stimulate arbuscular-mycorrhizal (AM) spore germination and hyphal growth, indicating a role in the precolonization phase (1,2). However, in the AM colonization phase the role of flavonoids is still unclear. It has been reported that application of certain flavonoids, as biochanin A, to AM host plants increase the colonization of roots (3,4), but do not change the mycorrhizal status of the AM nonhost plant rape(5). We treated two species of the AM nonhost plant lupin with biochanin A and looked for AM root colonization. Internal fungal structures could be observed in roots of one lupin species. Albrecht et al. (in preparation) proposed the induction of specific new chitinase isoforms as physiological markers for a successful colonization in AM host plants. In our experiment a new chitinase isoform could be detected in the lupin roots with internal fungal structures, showing the colonization of the lupin also on a physiological level. These results indicate, i) flavonoids might play a mayor role in root colonization by AM fungi, ii) newly induced chitinase isoforms might be used as markers for a successful root colonization.

1) Giovannetti et al. (1994) in: Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems; S. Gianinazzi and H. Schuepp (eds.) Birkhaeuser Verlag, Basel/Switzerland. 2) Poulin et al. (1993) J. Chem Ecol. 19: 2317-2327. 3) Siqueira et al. (1991) New Phytol. 118: 87-93. 4) Siqueira et al. (1991) Plant and Soil 134: 233-242. 5) Xie et al. (1995) Plant Physiol. 108: 1519- 1525.



poster session 10 WF SH 33

VILLEGAS, JAVIER, PETER MOUTOGLIS & J. ANDRÉ FORTIN. Institut de recherche en biologie végétale, Université de Montréal, 4101 est rue Sherbrooke, Montréal, Québec, Canada H1X 2B2. - Bacteria, mycorrhizal roots or external mycorrhizal mycelium, who is the best phosphorous solubilizer?

AM-mycorrhizal transformed carrot (*Daucus carota*) roots, mycorrhizal external mycelium and *Pseudomonas aeruginosa* and its effect, either alone or in interaction, on phosphorous solubilization and the pH of the media was studied. A two sided Petri plate system was used. Mycorrhizal transformed carrot roots colonized with *Glomus intraradices* were inoculated on one side on a minimal growing medium with N in the form of NO_3^- . The second compartment contained the same media but insoluble phosphorous was added. In this compartment, mycorrhizal roots or just the external mycelium was allowed to be established and was tested alone or in combination with *P. aeruginosa*. In addition, *P. aeruginosa* was tested separately in the second compartment. The pH as well as the soluble phosphorous concentrations in the media were measured at the beginning and 13 weeks after inoculation. An increase in the pH of the media was observed where external mycelium and bacteria were growing alone. A decrease in the pH was observed where mycorrhizal carrot roots were maintained alone. By contrast, a notorious decrease of the pH in the media was observed when external mycelium and bacteria were interacting. Mycorrhizal carrot roots associated with bacteria showed a higher decrease of the surrounding pH compared to treatments containing mycorrhizal carrot roots alone. Significant differences in the concentration of soluble phosphorous were detected in the various treatments. External mycelium and bacteria, were less effective phosphorous solubilizers than mycorrhizal roots. The levels of soluble phosphorous detected in the media were significantly higher where both mycorrhizal roots and external mycelium interacted with bacteria. However, the latter combination produced the highest concentration of soluble phosphorous in the media. This study shows that the effect of mycorrhizal roots, external mycelium and *P. aeruginosa* on pH and the phosphorous dynamics is different if the organisms are alone or in direct interaction.

poster session 8 WF AR 72

VIMARD, BRIGITTE¹, MARC ST-ARNAUD¹, VALENTIN FURLAN² and J. ANDRÉ FORTIN¹. ¹Institut de recherches en biologie végétale, Université de Montréal, 4101, Sherbrooke St. East, Montreal, Québec, Canada, H1X 2B2. ²Research Centre, Agri-Food Canada, 2560, Hochelage Blvd. Sainte-Foy, Québec, Canada, G1V 2J3. - In vitro monoxenic spores of *Glomus intraradices* used to produce endomycorrhizal plants: a solution to potentially contaminated inoculum

Use of a reliable inoculum free from other microorganisms to produce arbuscular mycorrhizal (AM) plants is of the greatest importance to study the interaction between AM plants and soil microorganisms. We

investigated the colonization of leeks from in vitro produced monoxenic *Glomus intraradices* spores. The large amount of isolated spores required were produced using a two compartment in vitro growth system previously described (Mycol. Res. 100: 328-332). A suspension of monoxenic spores was used as inoculum and compared with an inoculum of chopped roots of pot-grown AM leeks. The plants were grown in controlled environment and two types of sterilized growth media were evaluated: calcined montmorillonite clay (Turface) or a mix of soil, peat and inorganic additives. Root colonization progressed faster in the partially organic medium. In this medium, after a initial delay, root colonization from monoxenic spores was similar to that observed with the conventional inoculum, reaching respectively 43 % and 54 % after 8 wk. An interaction between media and inoculum type was obtained. Leek roots colonized from the monoxenic spores were free from other fungi while the roots colonized from the chopped roots regularly shown contamination by other fungi.

poster session 3 ST SH 88

VODNIK, DOMINIK, NADA GOGALA. Department of Biology, BF, University of Ljubljana, Vecna pot 111, 1000 Ljubljana, Slovenia. - Phytotoxicity of lead and the use of ectomycorrhiza during reforestation.

A mycorrhizal symbiosis must be competitive when inoculated seedlings are planted, particularly if they will be under stress. Apart from compatibility studies, previous laboratory tests of tolerance and the function of the symbiosis during stress are necessary if the choice is to be successful. Heavy metals in the forest ecosystem are a frequent stress factor. Thus, we compared the tolerance of various ectomycorrhizal fungi to increased Pb concentrations and followed its transport in the mycelium. We measured the effect of 5µM Pb on photosynthesis, mineral content, and pigments in the symbiosis *Picea abies*-*Pisolithus tinctorius*. We compared our laboratory results with those obtained in field studies in Zerjav, Slovenia, where there are approximately 9000 ppm of Pb in the upper 5 cm of soil, and studied growth of 3-year seedlings previously inoculated in the nursery with *Laccaria laccata*, *Lactarius piperatus* or *P. tinctorius*. Those inoculated with *Laccaria laccata* were found to be unsuitable for reforestation after 18 months of growth in this region, but the effects of inoculation were still seen in the others. All experiments demonstrated the advantages conferred by inoculation with a suitable fungus, such as *P. tinctorius*, in the presence of Pb phytotoxicity. Several parameters show that the advantage of mycorrhizae lies in well-known positive effects, for example such as improved P uptake, and not in decreased Pb transport in the mycorrhized seedling.



VOSÁTKA, MIROSLAV, LENKA SOUKUPOVÁ. Institute of Botany, Academy of Sciences, CZ-252 43 Pruhonice, Czech Republic. - Extra- and intra-radical organs of AMF in acid rain stressed ecosystems

Decline of Norway spruce forests in Central European mountains induced by acid rain (AR) is accompanied by expansion of grass *Calamagrostis villosa*. Associations of grass with arbuscular mycorrhizal fungi (AMF) are influenced by soil acidification, namely by pH about 3.5 and increased release of toxic Al³⁺. Differentiated reduction of intra- and extra-radical organs of AMF has been observed for four years on six plots in Krkonose Mts. exposed to different pollution. The distribution of AMF is related to the grass expansion and differs in young and mature grass stands. As the extra-radical mycelium (ERM) and sporulation of AMF might be reduced by AR, the AMF propagate probably by infecting of neighbouring roots rather than by spores. The negative effects of simulated AR on indigenous or inoculated AMF associated with *C. villosa* have been verified in four laboratory experiments. Substantial decrease of spore production, ERM growth and its dehydrogenase activity were found after AR treatment but the root colonisation decreased slightly. Results of field and laboratory studies suggest the important role of AMF in these ecosystems. AMF are present even on the most polluted sites, however, their life strategy might be changed by chronic pollution stress.

poster session 5 ST AR 38

WALLANDER, HÅKAN¹, TONIE WICKMAN² & PÅL-AXEL OLSSON¹. ¹Department of Microbial Ecology, Ecology Building, Lund University, 223 62 Lund, Sweden. ²Department of Civil and Environmental Engineering, Land and Water Resources, Royal Institute of Technology, 100 44 Stockholm, Sweden - Apatite as P source and microcline or biotite as K sources in ectomycorrhizal *Pinus sylvestris* seedlings.

Mycorrhizal (M) pine seedlings grew less than non-mycorrhizal (NM) seedlings when grown with apatite as the sole P source. However, seedlings colonized by *Suillus variegatus*, *S. bovinus* or *Paxillus involutus* had higher P contents of shoots compared with NM seedlings, indicating improvement of plant P uptake by these fungi. *Hebeloma longicaudum*, *Rhizopogon vulgaris* and *Piloderma croceum* gave no P uptake improvements in apatite soils. *P. involutus* colonized pine seedlings grew better than *S. variegatus* colonized or NM seedlings when microcline was used as a K source. On the other hand, *S. variegatus* colonized seedlings grew better than other seedlings when biotite was used as a K source. The K uptake from biotite in *S. variegatus* colonized seedlings was correlated to the amount of fungal biomass (ergosterol) in the soil. The amount of citrate in the soil solution was 3-5 times higher with *S. variegatus* than in all other treatments, indicating high weathering capability. *S. variegatus* raised bacterial activity (as thymidine incorporation) and biomass (specific fatty acids and microscopical counts) in biotite

soil, while in the other fungus/soil combinations, bacterial activity was decreased compared to NM soils.

poster session 12 WF SH 69

WALLEDA, THOMAS¹, ASTRID WINGLER², CHRISTOPH SCHAEFFER¹ & RÜDIGER HAMPP¹.

¹University of Tübingen, Botanical Institute, Physiological Ecology of Plants, Auf der Morgenstelle 1, D-72076 Tübingen, Germany. ²University of Sheffield, Robert Hill Institute, Department of Animal and Plant Sciences, Sheffield S10 2TN, U.K. - Effect of inorganic nitrogen supply on carbohydrate metabolism of ectomycorrhizal fungi.

Increased availability of nitrogen in forest ecosystems can negatively affect growth of ectomycorrhizal fungi and mycorrhizal development both in natural conifer forests and in pot experiments. However, the physiological basis is still unclear (Wallander 1995). Therefore, we investigated the effect of inorganic nitrogen on the carbohydrate metabolism in the ectomycorrhizal fungus *Amanita muscaria* (L. ex Fr.) Hooker in liquid culture. When *A. muscaria* was supplied with 5 mM NH₄⁺, fructose-2,6-bisphosphate contents increased, indicating an enhanced flux through glycolysis. This high glycolytic flux is important for providing carbon skeletons for the assimilation of ammonium (Wallander 1995). In addition, this carbon sink eventually also affected the content of the fungal disaccharide trehalose: It was clearly reduced when inorganic nitrogen was supplied to the cultures. Additional carbon sources in the medium decreased this effect. Obviously, pool sizes of fungus-specific carbohydrates, such as trehalose, can indicate the physiological condition of ectomycorrhizal fungi with regard to carbohydrate and nitrogen supply. Thus, they can be useful tools for investigating the allocation patterns of carbon in ectomycorrhizal systems grown under high inorganic nitrogen availability. Examples from field studies will be presented.

Reference: Wallander, H. (1995) Plant and Soil 168/169: 243-248.

poster session 3 ST SH 93

WALTON, MOLLIE & CARL F. FRIESE. Biology Department, University of Dayton, 300 College Park, Dayton, OH 45469-2320. - Assessment of mycorrhizal and soil indicators in rangeland ecosystems subject to different disturbance intensities

Processes which alter soil-plant nutrient dynamics in rangeland ecosystems can be better understood by studying the impact of disturbance intensity between two intrinsically different ecosystems. A study was conducted to examine the effects of cattle as disturbance agents on the overall health of mycorrhizal and soil components of the semi-arid shortgrass prairie in Northeastern Colorado and the arid rangeland of Southeastern New Mexico. These two systems are thought to respond differently to grazing pressure based on adaptation to the presence or absence of large herbivores over evolutionary time. Both of these systems have been subject to controlled grazing

regimes for many years, with heavily grazed areas and areas where cattle have been excluded. Total spore numbers of arbuscular mycorrhizae (AM) in the soil were used to compare the below-ground health of systems under different grazing intensities. Plant community structure was recorded to document any shift in plant species composition within sites due to grazing. Total spore abundance was greater in the Colorado site as compared to the New Mexico site. The spore abundance at the New Mexico site is lower in the more heavily grazed area as compared to an area that has been protected from grazing. At the Colorado site, spore numbers were lowest in a heavily grazed regime, but highest in a lightly grazed regime. Spore abundance follows the perennial plant species abundance at the Colorado site between different grazing regimes.

poster session 6 WF AR 74

WANG, CHUN-LI. Dept. of Plant Industry, National Pingtung Polytechnic Institute, Nei-Pu, Pingtung, Taiwan 91207, R.O.I. - Response of papaya inoculated with mycorrhizal fungi to drought stress

Vesicular-arbuscular mycorrhizal fungi (VAMF) can assist plants to take up more water, phosphorus and minerals, and to increase the drought tolerance of many plants. Hence it has been applied as a biofertilizer in crop production. Papaya is one of the major economic fruit trees in Taiwan, where a great number of excellent seedlings were demanded commercially every year. The purpose of this research was to study effects of VAMF on the growth and response of papaya plants to drought stress. Papaya seedlings were raised in plug trays and were inoculated with a mixture of *Glomus mosseae* and *G. etunicatum* spore soil during sowing. The growth of inoculated seedlings was investigated after 40 days. The results showed that VAMF promoted the quality of papaya seedlings. The plant height, stem diameter and leaf area of mycorrhizal seedlings were better than those of non-mycorrhizal. When seedlings were transplanted to pots, either normal irrigation or drought stress were performed. The results showed that the growth of irrigated plants was better than those of drought stressed. Plants inoculated with VAMF were better than those of non-inoculated at the beginning and during mid-growth period. The efficiency of photosynthesis of inoculated plants was better than those of non-inoculated. When withholding water for a long period, papaya plants showed negative CO₂ assimilation rate. After irrigation for 15 min, the CO₂ assimilation rate of inoculated plants restored continuously until 145th min, while non-inoculated plants restored slowly and declined at 55th min. The photosynthetic efficiency of inoculated plants was always higher than those of non-inoculated plants. Our results suggest that upon drought stress, VAMF inoculated papaya plants would have a better adaptability.



morning talk

WATKINS, NAOMI¹, ALASTAIR FITTER¹, JON GRAVES¹, TESSA BROWN¹ & DAVID ROBINSON².
¹Department of Biology, University of York, York YO1 5YW, UK. ²Scottish Crop Research Institute, Dundee DD2 5DA, UK. - Quantifying carbon transport between mycorrhizally-linked plants using natural abundance ¹³C measurements.

Although it has long been known that carbon can move between plants linked by a common mycorrhizal network, measurements using ¹⁴C could not determine whether there was net movement or what amounts of carbon were moving. We have used both the natural difference in ¹³C discrimination of C3 and C4 plants, and the artificial signal obtained from commercially available CO₂ to detect and quantify this movement. When the C3 *Plantago lanceolata* and the C4 *Cynodon dactylon* were linked, typically around 10% of the carbon in the roots of *C. dactylon* was derived from *P. lanceolata*. We also found net C transfer when pairs of *Festuca ovina* plants were linked and an artificial ¹³C signal was generated from commercial CO₂. The main index of transport was based on the difference between ¹³C signals in root and shoot material, implying that transferred carbon stayed in roots. Clipping experiments, aimed at forcing root carbon into the shoots, confirmed this, suggesting that it remained in fungal structures; it appeared to be associated with vesicles. Roots with extensive hyphal rather than vesicle development appear to contain fungal carbon derived from the same shoot, supporting the concept that hyphae are involved in carbon transfer.

poster session 2 ST SH 28

WATSON, DOROTHY M. H.¹ & PATRICIA D. MILLNER². ¹Department of Soil, Crop and Atmospheric Sciences, Cornell University, Ithaca, New York, USA 14853. ²USDA-ARS Soil Microbial Systems Laboratory, Beltsville, Maryland, USA 20705. - Assessment of Glomalean species biodiversity as influenced by trapping method.

Assessment of Glomalean fungal spore populations is not a straightforward procedure. To be detectable, the fungi must first sporulate and sporulation requirements differ between species. More than one method should be used to assess the spore population and avoid underestimating the total biodiversity of the ecosystem. Direct observations of field picked spores can be supplemented by various trap culturing techniques to yield more information about the total population. Although this concept is not new, it merits emphasis for anyone interested in Glomalean biodiversity, particularly for studies evaluating the effects of environmental stresses on these fungi. The following example illustrates this concept. Four approaches were used to assess the Glomalean fungal population of an agricultural field in Beltsville, Maryland and each approach yielded different answers. The four approaches were: #1. observation of field picked spores; #2. standard trapping technique of a 1:1 dilution (field soil to pasteurized soil mix) #3. MPN bioassay utilizing five soil dilutions (1:25, 1:125; 1:625;

1:3125; 1:15625) and #4. use of chopped bait-plant roots as inoculum or transplanted bait-plants that had been grown in undiluted field soil for four weeks. The greatest number of species were obtained through the MPN method. However, the other approaches yielded additional species.

poster session 8 WF AR 43

WATSON, DOROTHY M. H.¹, PAULA M. DAVIS² & SHARON B. COLEMAN². ¹Dept. of Soil, Crop and Atmospheric Sciences, Cornell University, Ithaca, New York, USA 14853. ²Dept. of Entomology, Cornell University, Ithaca, New York, USA 14853. - Interaction of arbuscular mycorrhizae and corn rootworm development in a greenhouse rotation study

A greenhouse study was set up to investigate a possible interaction between arbuscular mycorrhizae and the development of corn rootworm. Mycorrhizal treatments were established before the study began, by inoculating a precrop of soybean or corn (maize) plants with 50 spores each of *Glomus mosseae* (INVAM # SC226). After two plantings of the precrop, a time period of 6 months, the shoots were removed and the corn rootworm study was initiated. Corn was planted in each pot and at the same time, approximately 200 corn rootworm eggs were introduced to the pot. Corn rootworm development was assessed at 4, 5, 6 and 7 weeks, and adult emergence was monitored at the end of the study. Precrop history strongly influenced the growth of the corn plants, while mycorrhizal treatment affected the development of the corn rootworm larvae, resulting in an increased number of emerging adults.

poster session 3 ST SH 94

WEBER, EDWIN, BIRGIT SCHMINCKE, ALEXANDER FRENS & REINHARD F. HÜTTL. BTUC Center of Excellence Minesite Recultivation, Brandenburg Technical University of Cottbus, P.O.Box 101344, D-03013 Cottbus, Germany. - Mycorrhization - dominant strategy in primary plant succession on nutrient-poor mine spoils?

Open-cast lignite mining results in extremely nutrient-poor substrates. According to READ's hypothesis on succession after a disturbance event mycorrhizal species should immediately dominate early succession if mineral nutrients are scarce from the start. On young, unrehabilitated open mine spoil in Lusatia/Germany, typical non-mycorrhizal species were rare. Pioneer species sampled were always found mycorrhizal if the respective species is potentially mycorrhizal. Similarly, on rehabilitated land (4 years since disturbance, 3 years after afforestation), dominating understorey species were mycorrhizal. Mycorrhization within a typical early succession species (*Corynephorus canescens*) showed spatial dependency as indicated by semivariogram analysis. A simple function of nutrient availability could not be confirmed so far. However, methods used for estimating nutrient availability might not have been appropriate for spoil substrates. Methods are currently tested. Mycorrhization of dominant species as well as

mycorrhization of species that are not always found mycorrhizal in the field suggest that mycorrhization is a dominant strategy in early succession on nutrient-poor mine spoils in Lusatia.

poster session 13 WF SH 85

WEISS, M.¹, S. MIKOLAJEWSKI¹, H. PEIPP¹, V. WRAY² & D. STRACK¹. ¹Institut für Pflanzenbiochemie, Weinberg 3, D-06120 Halle, Germany. ²Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, D-38124 Braunschweig, Germany. - Localization of secondary metabolites in roots of *Larix decidua*

In roots of *Larix decidua* Mill. (Pinaceae) a tissue-specific localization of secondary metabolites was found. Compounds were analyzed by HPLC and by histochemical methods. Both, specific distributions and age-dependent changes were observed with several metabolites. Two anthocyanins we found to be restricted to the calyptra. The levels of two flavanols, catechin and epicatechin, were high around the meristem and within the apical part of the cortex and decreased in older parts of the roots. On the other hand we found an increase of these metabolites within the stele. Within the cortex of mycorrhizal roots catechin and epicatechin and wall-bound ferulic acid were restricted to the inner part of this tissue, which is not colonized by the fungus. With aging of the roots an increasing content of maltol glucoside could be detected, with highest levels in the stele. Quercetin rhamnoside and a tetrahydroxystilbene glucoside were restricted to the phloem tissue of older roots. It is postulated from the distribution of the flavanols and wall-bound ferulic acid within the root that these metabolites are involved in the restriction of the colonization by the mycorrhizal fungus.

poster session 8 WF AR 71

WHITBECK, JULIE L.^{1,3} and TJEERD BOUMA². ¹Root Biology Program and ²Department of Horticulture, The Pennsylvania State University, University Park, PA 16802, and ³EEO Biology, Tulane University, New Orleans, LA 70118 USA. - Outrunning roots? - the morphometrics of arbuscular mycorrhizal hyphae.

When considering the advantages to a plant of forming a symbiosis with an arbuscular mycorrhizal fungus, the morphology of the fungal hyphae is implicated in increasing the efficiency of soil resource extraction. In comparison with plant roots, hyphae of arbuscular mycorrhizal fungi are characterized as having smaller diameters and incorporating less biomass per unit of extension growth. These attributes enable hyphae to explore tight spaces into which roots cannot penetrate and, given equal amounts of photosynthate, to extend further from the plant stem than an organ of greater diameter. Despite the ubiquity of such claims in the mycorrhizal literature, it is challenging to extract hyphae from soils and there is little data on hyphal morphometrics. Employing pot cultures of *Glomus intraradices* grown with *Sorghum bicolor*, we measured

hyphal distance growth, diameter distributions, total length proliferation and total biomass. We compare these values with literature reports of root diameter distributions and specific root length and discuss the utility of these estimates in evaluating patterns of carbon allocation from the whole plant to the ecosystem.

morning talk

WHITHAM, THOMAS. G.¹, CATHERINE. A. GEHRING¹, TAD THEIMER¹, STEVE TRAVIS¹, NEIL COBB¹, CHERYL R. KUSKE², & PAUL KEIM¹. ¹Department of Biological Sciences, Northern Arizona Univ., Flagstaff, AZ 86011, USA; ²Environmental Molecular Biology, Los Alamos National Laboratory, Los Alamos, NM 87545 USA. - Using molecular techniques to understand community structure from the bottom up: interactions of environmental stress, pinyon pine, mycorrhizae, soil microbes and insect herbivores at Sunset Crater.

Since the eruption of Sunset Crater in 1064AD, colonizing pinyon pines have encountered a new stressful environment for which they are not yet adapted. These stressful conditions have resulted in an altered mycorrhizal and microbial community, and increased susceptibility to a keystone insect herbivore. RFLP analyses of the mycorrhizal community show a pronounced shift in taxa, and greenhouse experiments suggest that the ability of pinyons to survive in this new environment is in part due to the acquisition of mycorrhiza better suited to cinder soils. Genetic analyses of pinyons show that trees resistant to insect attack are differentiated from susceptible trees, and both tree types differ quantitatively in their mycorrhizal community. Furthermore, the interactions of environmental stress with the plant-herbivore-mycorrhiza complex has ripple effects throughout the community that affects seed dispersing birds and mammalian seed predators. We believe that the application of molecular techniques to the trees, their mycorrhiza, soil microbes and insect herbivores represents a key aspect of integrating the ecology and evolution of this system, and is likely to show how a diverse community maps onto the underlying genetic structure of the pinyon population. Also, the responses of the 350+ species of this community to this recently altered environment suggest how global climate change could affect biodiversity.

poster session 2 ST SH 32

WIDDEN, PAUL. Biology Department, Concordia University, 1455 de Maisonneuve W., Montréal, Québec, Canada, H3G 1M8. - Morphological diversity of vesicular-arbuscular mycorrhizal fungi in maple forests.

Vesicular arbuscular mycorrhizal (VAM) fungi are usually pictured as penetrating the outer cells of the roots via intracellular coils, and then spreading through the cortex via intercellular hyphae. Arbuscules and vesicles are normally produced as terminal, intracellular branches from these hyphae. The morphology of the VAM fungi in roots of both sugar maples and the herbaceous plants of

the forest floor often have very different morphologies. While some plants (eg. *Maianthemum canadensis* and *Smilacina racemosa* have VAM as described above, others (*Acer saccharum*, *Trientalis borealis*) spread through the cortex via intracellular coils, from which the arbuscules develop (composite arbuscules) and have no intercellular hyphae. In *Viola pallens* and *Trillium* species there are no intercellular hyphae, but terminal, simple arbuscules are produced. In another series of plants (*Clintonia borealis*, *Erythronium americanum* and *Medeola virginiana*) the penetration patterns vary, but the spread is via a series of banana-shaped projections (bobbits), from which the arbuscules, and, sometimes, vesicles form. Such diversity leads one to question just how diverse the VAM morphology is globally, and to speculate concerning the functional consequences of this diversity.

poster session 9 WF SH 12

WIEDMER, ERIC, BEATRICE SENN-IRLET, Geobotanisches Institut der Universität Bern, Altenbergrain 21, CH-3013 Bern, Switzerland. - The ectomycorrhizal root colonisation of dominant plant species in alpine snow-bed communities in relation to environmental factors.

Plants of alpine snow-bed communities are stressed in terms of nutrient availability as a result of long snow cover and short vegetation period (< 8 weeks). Under these conditions mutualistic symbiosis are a prerequisite for a successful long-term colonisation by dwarf shrubs and other perennials. The aim of this study is to determine the influence of ecological parameters such as soil temperature, soil texture, pH, nutrient availability (nitrogen, phosphorous, carbon and calcium) on the degree and the diversity of ectomycorrhizal root colonisation of dominant host plants i.e. *Dryas octopetala*, *Polygonum viviparum*, *Salix herbacea*, *S. reticulata*, *S. retusa*.

Roots were harvested in ten plots at two sites (subalpine: 1850 m; alpine: 1950 m a.s.l.) in the Bernese Oberland (Switzerland) on soils derived from crystalline and calcareous parent materials respectively. 24 morphotypes were distinguished with methods described by Agerer (1987-1994). In an attempt to quantify the observed mycorrhizas, 12 m roots per host plant have been analysed. With <0.1% bare roots are almost lacking in all investigated host plant species. Instead ectomycorrhizal root colonisation reaches 80-100%. Pseudomycorrhizas are present mainly in the subalpine plots whereas *Rhizoctonia* is found mainly in the alpine plots. According to the results of a canonical correspondence analysis (CCA) the different morphotypes of *Salix retusa* can best be explained by selected environmental factors, first of all by a limestone gradient and the N/P-ratio. Different shapes of the emanating hyphae characterise the morphotypes along a gradient of soil development.

poster session 3 ST SH 95

WIEMKEN, VERENA, KURT INEICHEN & ANDRES WIEMKEN, Department of Botany, University Basel, Hebelstr. 1, CH-4056 Basel, Switzerland. - Spruce and pine mycorrhizae under elevated atmospheric CO₂.

The effect of elevated atmospheric CO₂ concentration on the growth of roots, mycorrhizas and extraradical mycorrhizal mycelium of *Pinus silvestris* (L.) and *Picea abies* (L. Karst) was examined. *Pinus silvestris*-*Pisolithus tinctorius* ectomycorrhizae were synthesized at ambient and elevated CO₂ in an axenic Petri dish model system. Three times more mycorrhizal clusters were formed and the extraradical mycelium produced had twice the biomass at elevated than at ambient CO₂ after three months.

In addition, the effect of elevated CO₂ was investigated in model ecosystems: six cloned spruce trees were cultivated in large boxes (1.00/0.70/0.35 m, length/width/depth) in growth chambers under a realistic scenario of atmospheric CO₂ concentrations (280, 420, 560 ppm) and nitrogen input (0, 30, 90 kg ha⁻¹ yr⁻¹) for three years. The soil used was taken from a spruce forest and was layered in the original order. Fungal markers, ergosterol and mannitol were determined in the roots/mycorrhizae and in the soil. Fungal markers per root (dw) and per volume of soil was found to be higher at the elevated CO₂ levels. This difference was apparent after the first and second year of exposure but levelled out after the third year indicating a more rapid occupation of the given soil volume by the root/mycorrhizal fungus system at elevated CO₂, particularly by the fungal component.

poster session 2 ST SH 33

WILSON, GAIL & DAVID HARTNETT, Division of Biology, Kansas State University, Manhattan, KS 66506, USA. - Mycorrhizal influence on floristic composition and diversity in tallgrass prairie

Arbuscular mycorrhizal fungi (AMF) are ubiquitous and abundant in tallgrass prairie. Mycorrhizae may significantly influence plant community structure through differential host plant species responses to infection and altered competitive relationships. To assess effects of AMF on tallgrass prairie plant species composition, abundances, and diversity, replicate 2x2 m native tallgrass prairie plots were maintained under mycorrhizal (untreated control) or mycorrhizal-suppressed (fungicide treated) conditions for 5 growing seasons. In addition, vegetation responses were measured in mycorrhizal suppressed and control microcosms (40 x 52 x 32 cm) containing an assemblage of eight prairie grass and forb species. The fungicide benomyl successfully reduced mycorrhizal root colonization in microcosms to only 6.4%. Suppression of AMF resulted in a reduction in total new aboveground plant production and a decrease in biomass but increase in reproductive allocation of the warm-season tall grasses. Forbs showed variable responses to mycorrhizal suppression, and the 2 legumes studied showed significantly reduced survivorship in fungicide-treated microcosms. Although total estimated

aboveground biomass was not affected, long-term suppression of AMF in the field resulted in decreased abundances of the obligately mycotrophic warm-season grasses, increases in the relative abundances of C₃ graminoids and forbs, and significant increases in the evenness of species abundances and plant species diversity.

poster session 3 ST SH 96

WIMER, ARLENE A. & JOANNA B. MOTT. College of Science and Technology, Texas A&M University - Corpus Christi, 6300 Ocean Dr., Corpus Christi, Tx. 78412. - The occurrence and distribution of mycorrhizal fungi in association with halophytic plants on a dredge materials island.

The study site is a small dredge materials island [234 m X 212 m X 1.5 m] located on the Gulf Intracoastal Waterway, in the upper Laguna Madre of south Texas. The construction and maintenance of navigational channels annual requires the disposal of thousands of cubic meters of dredge materials. These soils are characteristically low in nutrients and high in pH and salinity which may hinder plant growth. The study objective is to investigate the occurrence and distribution of mycorrhizal fungi in association with halophytic plants on the island. Three transects have been established. Soil composition ranges from shell hash to fine sand. Analysis of soil samples shows salinity ranging from 780 ppm at the benchmark to 15080 ppm at the vegetation high tide interface. The pH is consistently alkaline [8.1-8.8] and nitrogen levels very low [1-2 ppm]. Phosphorus levels are moderate [9-15 ppm], except for one location with a 41 ppm level. The island's dominant vegetative species are: *Prosopis glandulosa*, *Schizachyrium scoparium*, *Eragrostis secundiflora*, *Aristida longespica*, *Opuntia lindheimeri*, *Heterotheca subaxillaris*, *Paspalum monostachyum*, and *Andropogon glomeratus*. Mycorrhizal fungal spores have been isolated from all soil samples. Distribution is not homogeneous. Lower numbers of larger size spores were found in soils of reduced salinity. Larger number of small diameter spores were isolated in higher salinity areas. The dominant grasses on the island are heavily infected with VAM fungi. Other dominant species examined are also infected with mycorrhizal fungi.

poster session 11 WF RR 27

WIPF, DANIEL¹, BERNARD BOTTON¹ & FRANÇOIS BUSCOT². ¹University Henri Poincaré-Nancy I, Faculty of Sciences, Laboratory of Forest Biology, U.A. INRA 977, BP 239, F-54500 Vandœuvre Cedex, France. ²FAL, Department of Soil Biology, Bundesallee, 50, D-38116 Braunschweig, Germany. - Polymorphism analysis in morel: a facultative secondary mycorrhizal fungi.

In forest ecosystems, morels parasites slightly mother roots of trees and forms ectomycorrhizas on rootlet primarily mycorrhized with a specific mycobiont. The mycorrhizal capability of morels seems to be related to

formation of heterokaryons released by the lowered nutrients availability in forest soils. Testing of this hypothesis necessitates to monitor behaviour of nuclei populations during strains confrontation assays under different trophic conditions. Due to the large coenocity of morels, a condition to such monitoring assays is to develop an adequate molecular analysis tool. Another difficulty for such studies is the debatable morel systematics. In order to develop the investigation tool of heterokaryons and to improve the species notion in morels polymorphisms of isozymes and of ribosomal DNA of isolates were studied in *Morchellaceae* and *Helvellaceae*. The ribosomal internal transcribed (ITS) and intergenic (IGS) spacers were PCR-amplified from mycelia of *Morchella*, *Mitrophora*, *Gyromitra*, *Verpa*. Variations in length of both amplified DNA sectors, and number of restriction sites of the less variable ITS sector allowed discussion of systematics. The isozymes polymorphism was consistent with the phylogenetic relationships between the investigated strains only at a high systematic level (genus). This method allowed characterization of heterokaryons interactions of obtained by non-self pairing experiments of monosporal strains of *M. esculenta*.

poster session 12 WF SH 70

WRIGHT, DEREK, JULIE SCHOLES & DAVID READ. Animal & Plant Sciences, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK. - Sink effects of mycorrhizal colonisation of clover plants.

Whilst enhanced mineral nutrient supply is conventionally seen as the 'benefit' to a host plant infected by vesicular-arbuscular mycorrhizal (VAM) fungi, the 'cost' is seen as a loss of carbon to the fungus. However, few studies have defined this 'cost' in terms of the effect on host photosynthetic metabolism. Whilst increased rates of photosynthesis have been observed following mycorrhizal colonisation, these observations have usually been confounded by parallel changes in the mineral (phosphorus and nitrogen) nutrition of the host. Using VAM and non-mycorrhizal (NM) clover in which the foliar phosphorus and nitrogen concentrations have been manipulated to be similar in both sets of plants we have examined the hypothesis that mycorrhizal plants will show increased rates of photosynthesis which may compensate for the carbon 'cost' of the fungus. This paper describes the response of such clover plants, grown in the VAM and NM condition, in terms of growth, whole plant carbon partitioning and the carbon demand of the fungal symbiont with a view to determining the magnitude of the sink effect.

poster session 12 WF SH 27

WRIGHT, WENDY¹, ALASTAIR FITTER¹, DALE SANDERS¹ & ANDREW MEHARG². ¹Department of Biology, University of York, PO Box 373, York YO1 5YW, UK. ²Institute of Terrestrial Ecology Monkswood, Abbots Ripton, Huntingdon, Cambs. PE17 2LS, UK. - Regulation of mycorrhizal colonisation in grass populations polymorphic for phosphate uptake kinetics

We are investigating a novel approach to measuring the functioning of the arbuscular mycorrhizal (AM) symbiosis under natural field conditions, based on the discovery that arsenate (As) tolerance in the common grass *Holcus lanatus* is achieved by a reduction in As influx. In terms of plant nutrient uptake, the As ion is an analogue to the phosphate (P) ion and uptake occurs via the same carrier system. Tolerant *H. lanatus* plants have a constitutively suppressed high affinity uptake system (HAUS) that is insensitive to plant P status, and which leads to reduced influx of both P and As. Suppression of the P_i carrier is under major gene control. Some *H. lanatus* populations growing on normal soil are polymorphic for this gene and tolerance frequency is approximately 50%. Tolerant individuals can have up to 40% greater AM colonisation than non-tolerants. The implication is that suppression of the HAUS and its associated reduction in P influx is, at least in part, compensated by increased mycorrhizal infection. We are monitoring AM colonisation, P nutrition and performance of tolerant and non-tolerant plants in natural populations over two seasons. The fitness of genetically defined plants is being measured in glasshouse and garden experiments. The P_i flux of experimental plants, both mycorrhizal and non-mycorrhizal will be characterised by electrophysiology.

poster session 12 WF SH 72

WRIGHT, SARA, & ABHA UPADHYAYA. Soil Microbial Systems Laboratory, USDA-ARS, Bldg. 318, BARC-E, Beltsville, MD 20705. - Glomalin - a stable glycoprotein produced by hyphae of AM fungi

The community of AM fungi is involved in soil stabilization and aggregation by mechanical means and possibly by the contribution of cementing agents. A very stable glycoprotein, glomalin, is produced in abundance by hyphae of all glomalean fungi studied to date. Stability of the protein is indicated by the procedure necessary to solubilize the protein (20 mM sodium citrate at pH 7.0 for 90 min at 121°C) and the glycoprotein nature of the molecule. Bulk soils were tested for the protein using twelve soils common to the Mid-Atlantic area of the United States collected from undisturbed areas. The amount of protein extracted ranged from 4.4 to 14.4 µg protein/mg of air dried soil. Electrophorograms of soil and hyphal protein were identical for denatured protein on sodium dodecyl sulfate polyacrylamide gels, native protein on isoelectric focusing gels and N-linked oligosaccharides by capillary electrophoresis. Percent of aggregates (1.2 to 2 mm size range) in dry soil was correlated with ($P \leq 0.05$) silt and immunoreactive glomalin. The concentration of protein in aggregates between 3.3 and 2 mm was 3.27 to 10.49 µg protein/mg soil and 4.64 to 13.27 µg protein/mg soil for aggregates between 1.2 and 2 mm in size. Aggregate protein was not necessarily related to total soil protein for these soils. Further work is in progress to determine the relationship between protein in aggregates and protein in bulk soil.

poster session 14 WF RR 46

WU, CHI-GUANG & SUH-JEN LIN. Soil Microbiology Lab, Agricultural Chemistry Department, Taiwan Agricultural Research Institute, Wu-feng, Taichung, Taiwan, Republic of China. - Glomales of Taiwan: VII. *Jimtrappea* and *J. macrospora*, new taxa of Acaulosporaceae (Glomales)

A new genus, *Jimtrappea* (Acaulosporaceae) was isolated from the mountain area of National Yu-Shan Park, Central Taiwan that includes one species, *J. macrospora*. *Jimtrappea* is characterized by its unique spore ontogeny producing spores directly within the sporiferous saccule and the type of spore germination. At maturity, a spore is appressed by a hyaline, ephemeral outer wall which is part of sporiferous saccule. After the stalk of the saccule collapses, the spores of *Jimtrappea* are closely similar to those of *Acaulospora* and *Entrophospora*. spore germination is by germ tubes that grow through the spore wall and produce vesicular structures in the end of germinating hyphae. Comparative spore ontogeny among the genera of Glomales is discussed. A key to the genera of Acaulosporaceae is provided.

poster session 6 WF AR 18

WU, TIEHANG & WENYING HAO. Institute of Soil Science, Academia Sinica, P. O. Box 821, Nanjing, 210008, P. R. China. - The management of arbuscular mycorrhizal fungi(AMF) in red soils of subtropical region of China. I. Indigenous AMF under different utilization ways and their response to plant growth

Red soils under tea garden, orange garden, forest land and two kinds of cropfield in the subtropical region of China were surveyed by the spore number method to determine biodiversity and species composition of arbuscular mycorrhizal fungi. 13 species of 4 genera in the Glomales were identified in 200 soil samples collected between period 1991-1994. *Glomus manihot* and *Scutellospora heterogama* occurred in 53% and 67% of soils respectively. The predominate genus and species under site of different utilization are significantly different. *Glomus* is the dominant genus, and *Glomus manihot* and *Glomus versiforme* are predominated respectively in 2 kinds of cropfield of peanut. In forest land, the dominant genus was *Scutellospora* in which *Scutellospora heterogama* and *Scutellospora calospora* were the dominate species. *Scutellospora* was also the dominant genus in orange garden, but the dominant species are *Scutellospora heterogama* and an unidentified species of *Scutellospora*. In tea garden, *Acaulospora* is the dominant genus and *Acaulospora laevis* and *Acaulospora denticulata* are the dominant species. Infectivity measured as root colonization rate and effects on plant growth of indigenous AM fungi varied among soils of different utilization. Field situations for the feasibility of arbuscular mycorrhizal inoculation by *Glomus caledonium* is also given by glasshouse bioassay. Comparing with the introduced AMF inoculum *Glomus caledonium*, the effectiveness of indigenous AM fungi under tea garden, forest land and cropland dominated by *Glomus versiforme* is much lower than that

of the introduced inoculum, so the inoculation will be effect under these utilization ways, but which under orange garden and crop land dominated by *Glomus manihot* is higher than introduced inoculum. This study provided an important basis for assessing the potential of arbuscular mycorrhizal fungi inoculation in different utilization and screening more efficient AM fungal inoculum for application in red soils in subtropical region of China.

poster session 6 WF AR 19

WU, TIEHANG¹, N. MALAJCZUK² & MARK BRUNDRETT². ¹Institute of Soil Science, Academia Sinica, P. O. Box 821, Nanjing, 210008, P. R. China ²Division of Forestry, CSIRO, Private Bag, P. O. Wembley, W. A. 6014, Australia. - Competition and Effectiveness of Arbuscular and/or Ectomycorrhizal Fungi on Seedlings of Bluegum/(*Eucalyptus globulus*)

Eucalypts can form both arbuscular mycorrhizas(AM) and ectomycorrhizas on the same root system. There are numerous reports in the literature of increased eucalypts growth following inoculation with ectomycorrhizal fungi. However the importance of arbuscular mycorrhizas on eucalyptus root in growth promotion has not been extensively studied. The response of arbuscular mycorrhizae on eucalyptus growth and the question of whether arbuscular mycorrhizal fungi compete for infection site on the roots and influence the effects of ectomycorrhizal fungi on eucalypts growth remains unclear.

This study examine the contribution of arbuscular and ectomycorrhizal fungi when inoculated simultaneously or separately on the growth and nutrient accumulation of *Eucalyptus globulus* seedlings in a glasshouse experiment. Two arbuscular mycorrhizal fungi i.e. *Glomus invermaium*[WUM 10(1)] and *Acaulospora laevis*[WUM 11(4)] were tested. The ectomycorrhizal fungi were *Pisolithus* sp. and *Descolea maculata*. The total dry weight of plants inoculated with *Pisolithus* sp. and *Descolea maculata* increased 44% and 54% respectively comparing with the non-inoculated. Mycorrhizal infection by AM fungal inoculation were high, however there was no significant difference in dry matter between plants inoculated with AM fungi and non-inoculated plants. When AM fungi were dual inoculated with the ectomycorrhizal fungi *Pisolithus* sp., the AM fungi had a depressive effect on plants growth when compare with the plants only inoculated with *Pisolithus* sp.. AM fungi had no significant depressive effects on ectomycorrhizal plants of *Descolea maculata*. This suggest that *Descolea maculata* is a more competitive fungus able to compete with AM fungi on plants growth.

poster session 2 ST SH 47

YAMADA, AKIYOSHI & KEIZO KATSUYA. Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan. - Seasonal change and species composition of ectomycorrhizal fungi in a stand of *Pinus densiflora*.

Mycorrhizas and fungal propagules were investigated in a reforested stand (plot size: 625 m square) of *P. densiflora* Sieb. et Zucc. (age: 45 years), which is the most common pine in Japan. The pine ectomycorrhizal tips were collected monthly from June 1995 to April 1996, and they were classified into 61 distinct morphological types (presumably the same number of species). Soil samples collected every other month from February 1994 to August 1995 were incubated in vitro with axenic seedlings of the pine for 4 to 8 months. The formed ectomycorrhizal tips were classified into 56 morphological types.

Seasonal changes in the number of mycorrhizal tips and mycorrhizal types differed between field collected mycorrhizas and in vitro ones. Although almost dominant mycorrhizal types in vitro were also recovered from field collected mycorrhizas, 26 mycorrhizal types were restricted to in vitro. These results indicate that seasonal changes of mycorrhizas under field conditions do not necessarily affect the mycorrhizal propagules which are estimated in vitro. The in vitro analysis exhibits a great variety of mycorrhizal species which are probably present in the field as living saprophytes or dormant fungi.

poster session 2 ST SH 34

YOSHIDA, LIDIA C. and EDITH B. ALLEN. Department of Botany and Plant Sciences, University of California, Riverside, California 92521, USA. - The effect of nitrogen deposition on the growth and colonization of mycorrhizal plants

Anthropogenic nitrogen deposition is causing vegetation changes to plant communities but less is known about its effect on mycorrhizae. We hypothesize that N deposition may be influencing the decline of coastal sage scrub in favor of exotic grasses by affecting the functioning of the mycorrhizal fungi. The goal of this study was to determine the effect of ammonium (NH₄) and nitrate (NO₃) on the growth and mycorrhizal infection of a coastal sage scrub species, *Artemisia californica* Less. and an exotic annual grass, *Bromus madritensis* L. ssp. *madritensis*. NO₃ is the dominant form of deposition in southern California. Seeds of each species were germinated in pots of sterile soil, inoculated with native soil containing mycorrhizal spores and received 50 ppm of either NO₃ or NH₄. Preliminary results indicate that the presence of mycorrhizae negatively influenced the growth of the exotic grass, *Bromus* to NO₃ addition. However, mycorrhizal inoculation did not change the growth of the *Artemisia* after 9 weeks with either form of nitrogen. The implications of this result are that N deposition could change the competitive balance between the two species as mediated by their mycorrhizal response. In a related study, mycorrhizal spores and percent infection of coastal sage plants were significantly lower in polluted sites.



YUN, WANG and IAN R. HALL. New Zealand Institute for Crop & Food Research, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand. - Matsutake and its cultivation

Matsutake (pine mushroom) are the edible fruiting bodies of a few species of *Tricholoma*. The principal species are *T. matsutake*, *T. magnivelare*, and *T. caligatum*. Related species are *T. bakamatsutake*, *T. quercicola*, *T. fulvocastaneum* and *T. robustum*. These occur naturally throughout the Northern Hemisphere associated mainly with softwood trees but also with Fagaceae. They are held in very high regard in Japan which consumes approximately 3,000 tonnes per annum. Annual production of matsutake in Japan is less than 1,000 tonnes with the balance imported from China, North and South Korea, Canada, USA, Morocco and Taiwan. Wholesale prices in Japan range between ¥3,350 and ¥70,000/kg with the price reflecting quality, origin and availability. Retail prices at the start of the season can be up to ¥160,000/kg. Although methods have been developed for increasing the yields of matsutake forest, despite considerable research in Japan and elsewhere methods for the cultivation of matsutake remain elusive. Information will be presented on various aspects of matsutake in particular its ecological requirements and will highlight those areas that warrant study.

YUN, WANG & IAN R. HALL. New Zealand Institute for Crop & Food Research Limited, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand - Morphology and anatomy of Matsutake mycorrhizas.

The morphology and anatomy of naturally occurring infections found by *T. matsutake* and *T. magnivelare* were observed and compared with those synthesized in the laboratory between *P. densiflora* and *P. radiata* and 20 isolates of *T. matsutake*, *T. magnivelare* and *T. caligatum*. In both, infected rootlets and short laterals were monopodial, light brown, swollen and clavate. In the laboratory some strains penetrated deep into the cortex producing masses of densely interwoven sometimes swollen hyphae which sometimes had chlamydospores. Eventually the roots were destroyed although hyphae were never found inside the endodermis. Other strains less aggressive established infections similar to middle aged infections on roots collected from forests where there was no mantle, an ill defined structure which resembled a poorly developed Hartig net, and a system of longitudinally running intercellular hyphae.

It was concluded from these and other studies that during early stages of the infection by *T. matsutake* and *T. magnivelare* there appeared to be a mycorrhizal relationship but this was soon succeeded by pathogenic and saprobic phases.

ZAMBONELLI, ALESSANDRA¹, ANNAMARIA PISI², ELENA TIBILETTI¹ & DAWA PENJOR³. ¹Dipartimento di Protezione e Valorizzazione Agroalimentare, via Filippo Re 8, I-40126 Bologna, Italy. ²Istituto di Patologia Vegetale, via Filippo Re 8, I-40126 Bologna, Italy. ³National Mushroom Centre, Simtokha, Thimphu, Bhutan. - Anatomical-morphological characterization of *Tuber indicum* Cooke & Masee mycorrhizae on *Pinus pinea* L. and *Quercus cerris* L.

Mycorrhizae of *Tuber indicum* Cooke & Masee obtained by spore inoculation on *Pinus pinea* L. and *Quercus cerris* L. seedlings are described in order to provide useful data for their identification. The techniques adopted for the observations were those proposed by Agerer (1987-1995). The morphological and biometrical characteristics of the external elements (hyphae and cystidia) and the anatomical features of the mantle layers were observed using a light microscope. Micromorphological characteristics of the mantle surface and cystidia (spinulae) were studied with a scanning electron microscope (SEM). The results show that the external elements observed (in particular the cystidia) in *T. indicum* ectomycorrhizae are very similar to those of *Tuber melanosporum* Vitt.

ZAMBONELLI, ALESSANDRA¹, MIRCO IOTTI¹ & ANNAMARIA PISI². ¹Dipartimento di Protezione e Valorizzazione Agroalimentare, via Filippo Re 8, I-40126 Bologna, Italy. ²Istituto di Patologia Vegetale, via Filippo Re 8, I-40126 Bologna, Italy. - Dynamics of ectomycorrhizal association in a *Pinus pinea* experimental field.

The dynamics of ectomycorrhizal association were studied in a experimental plantation of *Pinus pinea* L. set up in a natural *Tuber borchii* Vitt. production area at Marina di Ravenna, Italy, in the autumn 1990. In our experiment seedlings inoculated with *T. borchii*, *Laccaria bicolor* (Maire) Orton. (strain S238), *Hebeloma sinapizans* (Paulet) Gill. and uninoculated seedlings weakly infected with *Sphaerospora brunnea* (A. & S. ex Fr.) Svrcek & Kubicka were raised under controlled greenhouse conditions and then transplanted in the field. *T. borchii* was the only mycorrhizal fungus that produced fruit-bodies and production was 12.2 kg/ha, in 1995 and 10.8 kg/ha in 1996. Examination of seedlings roots showed that *L. bicolor*, *H. sinapizans* and *S. brunnea* ectomycorrhizae had disappeared, whereas the mean percentage of *T. borchii* infected root tips was 43%. In the root samples examined five other ectomycorrhizal morphotypes were observed and described, two of these were also observed in 30-40 year old pine forests near our experimental plantation.

morning talk

ZELMER, C. D., S. HAMBLETON and R. S. CURRAH. Dept. of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada. - Getting to the root of the matter: mycoheterotrophy in Orchidaceae and Ericaceae from a fungal perspective

A large number of mycoheterotrophic plant species are found in the families Orchidaceae and Ericaceae (s. l.). Both families contain photosynthetic and achlorophyllous representatives. Apart from a cataloguing of mycorrhizal types, most mycoheterotrophic Ericaceae and Orchidaceae have not been examined from a mycological perspective. Chlorophyllous species in the Ericaceae form ericoid mycorrhizas with inoperculate ascomycetes (e.g. *Scytalidium*, *Hymenoscyphus*) and anamorphs of Myxotrichaceae (e.g. *Oidiobondrium*), or arbutoid mycorrhizas with ectomycorrhizal basidiomycetes. Chlorophyll-producing orchids are associated with basidiomycetes in the anamorphic genera *Ceratorhiza*, *Epulorhiza* and *Moniliopsis*. In both families, adoption of mycoheterotrophy has paralleled a change in mycorrhizal fungi, with both groups favouring ectomycorrhizal fungi of woody plants, or vigorous saprophytes or plant pathogens. However, this trend is composed of an extremely small sample of representatives from both groups. Few studies have identified and compared the fungi of related auto- and mycoheterotrophs, or examined the change in endophytes during the lifetime of the host. Orchids present unique opportunities to explore endophyte species shifts as many have an extended (or reiterated) mycoheterotrophic phase in their life histories. Although still preliminary, *in situ* germination experiments indicate that the early, mycoheterotrophic phase of some terrestrial orchids may be associated with different fungi than the mature, chlorophyll producing adult plants. New methods for the isolation and identification of endophytes and ecological and physiological examinations of the mycorrhizal fungi are needed to aid our understanding of mycoheterotrophy in the Ericaceae and Orchidaceae.

poster session 3 ST SH 91

ZETO, S. K., & R. B. CLARK. Appalachian Soil & Water Conservation Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, P.O. Box 867, Beckley, WV 25802 USA. - Growth and root colonization of mycorrhizal maize grown on acid and alkaline soil.

Arbuscular mycorrhizal fungi often have wide adaptability over many soil environments. This study was conducted to determine the effects of the AM fungi *Glomus etunicatum* WV579A (Ge), *G. diaphanum* WV579B (Gd) and *G. intraradices* WV894 (Gi) on growth and root colonization of maize (*Zea mays* L.) grown on two acid (pH 4.2 and 4.5) and two alkaline (pH 7.8 and 8.0) soils. Shoot and root dry matter (DM), leaf area (LA), and root length (RL) were higher for mycorrhizal (AM) than for non-mycorrhizal (nonAM) plants grown on all soils, and plants grown on alkaline soil had higher DM than plants grown on acid soil. Shoot

and root DM followed a sequence of $Ge > Gd \geq Gi > nonAM$ for plants grown on the alkaline soil. The AM isolates enhanced DM of plants grown on acid soil, and differences among isolates were minor. Differences between AM and nonAM plants for total LA and RL were greater than for DM. Specific LA values for AM plants were consistently higher than for nonAM plants, while specific RL values for AM plants were only slightly higher than nonAM plants. Roots with arbuscules and/or vesicles was nil to low for plants grown on acid soil and relatively high for plants grown on alkaline soil. Roots with hyphal infection varied with type of soil and AM isolate, and was considerably higher than arbuscules/vesicles for plants grown on acid soil. Root hyphal infection appeared to be more important than arbuscules for growth enhancement of plants on acid soil. The AM isolates used were effective in enhancing growth of plants on both acid and alkaline soils showing wide adaptability to broad soil pH conditions.

talk session 2

ZEZE, ADOLPHE¹, MICHEL HOSNY², HUBERT DULIEU², VIVIENNE GIANINAZZI-PEARSON², DIEDERIK VAN TUINEN² & SILVIO GIANINAZZI². ¹Department of Soil Science, University of Adelaide and CRC for Soil and Land Management ; Glen Osmond, 5064 South Australia. ²Laboratoire de Phytoparasitologie, INRA/CNRS-SGAP-BV 1540-21034-Dijon-Cedex-France. - Repeated DNA Sequences as tools for VAM fungal detection in colonized roots

The classification and identification of VAM fungi are mainly based on the structure and the morphology of the vegetative spores. However morphological features can be influenced by environmental conditions, which can be crucial to the identification of the fungus, especially in field populations. Moreover, it is difficult to distinguish different species of VAM fungi in the root system because they all develop arbuscules or vesicles which are similar for most species. Genetic studies of VAM fungi have been hampered by the fact that the fungi cannot be grown without the host plant. The use of DNA technology offers some possibilities for the detection and the identification of VAM fungi, because DNA techniques do not rely on culture of fungus. Different approaches have been developed for the detection and identification of VAM fungi at the molecular level (Simon et al., 1993, Lanfranco et al., 1995). It is generally accepted that the major source of eukaryote genomic variation is the divergence of their repeated DNA sequences. As another approach, we used repeated DNA sequences to develop a reliable tool for a VAM fungus detection in the root system. Genomic cloning of the DNA from a VAM fungus *Scutellospora castanea* Walker (Zézé et al., 1994) enabled the screening of repeated DNA sequences. Using Dot blot and Southern analyses, *Scutellospora*-specific probes were isolated. The characterization of one of them showed that it was a tandemly organized element. The sequencing of this element allowed us to design two primers (SC1-1, SC1-2) which were shown to be *S. castanea*-specific. Using these primers, we could detect

specifically the fungus *S. castanea* with DNA from either spores or *A. porrum* infected roots.

poster session 9 WF SH 07

ZHANG, YONGCAN, MITIKU HABTE & DONALD P. SCHMITT. Department of Agronomy and Soil Science, University of Hawaii, Honolulu, Hawaii 96822, USA. - Suppression of the activity of the plant parasitic nematode *Meloidogyne incognita* in white clover by *Glomus mosseae*

The ability of *Glomus mosseae* to suppress the plant parasitic nematode *Meloidogyne incognita* in white clover was determined in a green house study using a mixture of an ultisol and Sunshine Mix. A phosphorous sorption isotherm was used to establish target P levels of 0.05 and 0.1 mg/l. One-month old white clover seedlings not colonized and colonized by *G. mosseae* were transplanted into the medium contained in 6.25-by 25-cm deepots. Just before transplanting, 20,000 eggs of *M. incognita* were pipetted into 5 cm deep holes made at the center of the deepots. After 49 days of further growth, root wet weight, shoot dry matter yield, the proportion of root system occupied by galls, numbers of nematodes and nematode eggs in roots, and extent of mycorrhizal colonization were determined. At soil P concentration of 0.05 mg/l, *G. mosseae* reduced nematode damage by 70%. There were fewer nematodes inside the roots of mycorrhizal plants than in nonmycorrhizal ones. At the higher P level, mycorrhizal infection levels were significantly lower if nematodes were absent than if they were present. Nematodes caused serious damages to mycorrhizal plants as well as nonmycorrhizal ones at this soil P concentration. The suppression of *M. incognita* by *G. mosseae* in white clover could not be explained by improved P nutrition.

poster session 12 WF SH 61

ZHAO, B.¹, A. TROUVELOT², V. GIANINAZZI-PEARSON² & S. GIANINAZZI². ¹Department of Microbiology, Huazhong Agriculture University, Wuhan 470070, China. ²Laboratoire de Phytoparasitologie, INRA-CNRS, SGAP, INRA, BV 1540, 21034 Dijon Cedex, France. - Comparison of two arbuscular mycorrhizal fungi in their production of total and viable mycelium with soybean and *Astragalus*

Two arbuscular mycorrhizal fungi (*Glomus mosseae* and *Glomus intraradices*) were tested to compare their ability in production of intraradical and external mycelium associated with *Astragalus sinicum*, a small seeded plant, and *Glycine max*, a large seeded plant. *A. sinicum* was much more dependent than *G. max* on mycorrhizal formation especially at early growth stages. Hyphal development was estimated by means of three staining procedures. External hyphae were extracted by the membrane filter method. Total fungal biomass was assessed by trypan blue staining and viable amounts of hyphae inside and outside roots were assessed after staining for succinate dehydrogenase (SDH) or alkaline phosphatase (ALP) activity. The total amount of fungus

increased steadily in roots and soil with harvesting time until eight weeks. Staining with SDH and ALP methods indicated that only a proportion of hyphae were enzymatically active. The length and percentage of viable hyphae varied with the AM association. *G. mosseae* produced a much greater length of external hyphae during the first two harvests (5 and 7 weeks) when it was associated with *A. sinicum*. The viability of hyphae formed by the two fungi varied considerably depending on the host plant used. Percentage of viable hyphae in association with *A. sinicum* was significantly different from that with *G. max*, even though the total amount of hyphae was relatively similar, and the viability of hyphae decreased after 5-7 weeks planting for both host plants used. The results show that the host plant influences the pattern of hyphal production and activity of AM fungi.



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