Abstract book

8th Conference of the European Foundation for Plant Pathology &
British Society for Plant Pathology
Presidential Meeting 2006

Sustainable disease management: the European perspective

13th-17th August 2006
KVL, Frederiksberg
Denmark
Abstract book

8th Conference of the European Foundation for Plant Pathology &
British Society for Plant Pathology
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Sustainable disease management: the European perspective

13th-17th August 2006, KVL, Frederiksberg, Denmark

Programme and Abstracts

Organising committee:
Lisa Munk, President for EFPP (chair)
David B. Collinge, Chair of Scientific Committee
Dan Funck Jensen, President for Danish Society for Plant Pathology
and EFPP programme secretary
Michael Krogh Jensen, Chair of the PhD committee
Peter Mills, President for the British Society for Plant Pathology
Matthew Dickinson, BSPP programme secretary

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Lisa Munk, David B. Collinge and Dan Funck Jensen with expert assistance from Lene Klem and Maria Busch
Preface

The organising committee welcomes you all to the 8th Conference of the European Foundation for Plant Pathology and the BSPP presidential Meeting at The Royal Veterinary and Agricultural University in Copenhagen.

The main theme of the conference is sustainable disease management in a European context. We believe that the programme reflects both the current dynamic changes occurring in European agriculture with the integration of the ten new states into the EU with all the challenges entailed and the best of European research in plant pathology.

At the time of writing there are approximately 215 delegates from 31 countries. There are more than 90 oral contributions and 115 posters at the time of writing.

The organising committee gratefully acknowledges the support received from the two organising societies, namely The British Society for Plant Pathology and The Danish Society for Plant Pathology. We also acknowledge support from the Research School for Biotechnology (FOBI), Denmark to the PhD educational aspects integrated into the programme.

We thank the Royal Veterinary and Agricultural University for allowing us to use KVL as a venue for the EFPP-BSPP conference and the City of Copenhagen for holding a civic reception in our honour. Finally, we are grateful for the Danish Institute of Agricultural Sciences for hosting the scientific programme of the post conference tour and for providing lunch for the participants.

We sincerely hope that you will enjoy and benefit from the conference and the “wonderful city” of Copenhagen.

On behalf of the organising committee

Lisa Munk
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## Overview

### Sunday 13. August 2006
- 15.00 – onwards: Registration (Marble Hall)
- 18.00 – 19.00: Buffet (Marble Hall)
- 19.15 - 20.00: GARRETT MEMORIAL LECTURE by Prof. Lene Lange, Novozymes, Denmark (3-01)

### Monday 14. August 2006
- 09.00 – 10.00: Conference Opening (auditorium 3-01)
- 10.00 – 10.30: Coffee break (Marble Hall)
- 10.30 - 12.10: Session 01: Induced resistance and defence responses (3-13)  
                    Session 04: Population diversity and dynamics (pt 1) (3-01)
- 12.10 – 14.00: Lunch and Poster sessions 1, 2, 3, 4, 5 & 6 (Marble Hall)
- 14.00 – 15.30: Session 02: Non-host resistance and innate immunity (3-13)  
                    Session 04: Population diversity and dynamics (pt 2) (3-01)  
                    Session 06: Post harvest diseases (pt 1) (3-14)
- 15.30 – 16.00: Coffee break (Marble Hall)
- 16.00 – 17.30: Session 03: Virus (3-13)  
                    Session 05: Epidemiology (3-01)  
                    Session 06: Post harvest diseases (pt 2) (3-14)
- 18.30 – approx 20.00: Civic reception at Copenhagen City Hall. We suggest a subsequent visit to the adjacent TIVOLI pleasure gardens at your own expense.

### Tuesday 15. August 2006
- 09.00 – 10.30: Session 07: Genomes and Transcriptomes (3-13)  
                    Session 10: Plant and seed health and developing countries (pt 1) (3-14)
- 10.30 – 11.00: Coffee break (Marble Hall)
- 11.00 - 12.10: Session 08: Plant pathogen interactions & Cell to Cell communication (3-13)  
                    Session 10: Plant and seed health and developing countries (pt 2) (3-14)
- 12.10 – 14.00: Lunch and Poster sessions 7, 8, 9, 10, 11 & John Colhoun Poster Comp. (Marble Hall)
- 14.00 – 15.30: Session 09: PH Gregory Competition (pt 1) (3-13)  
                    Session 11: Quarantine and legislation (programmed talks) (3-14)
- 15.30 – 16.00: Coffee break (Marble Hall)
- 16.00 – 17.30: Session 09: PH Gregory Competition (pt 2) (3-13)  
                    Session 11: Quarantine and legislation (workshop) (3-14)
- 19.00 – 21.00: workshops
- 18.00 – 19.30: Board meeting for EFPP delegates

### Wednesday 16. August 2006
- 9.00 – 10.00: BSPP PRESIDENTIAL ADDRESS by Professor Peter Mills, Warwick HRI., United Kingdom (3-01)
- 10.00 – 10.30: Coffee break (Marble Hall)
- 10.30 – 12.00: Session 12: Bacterial diseases (3-13)  
                    Session 15: Biological, chemical, cultural & integrated control (pt 1) (3-14)
- 12.00 – 14.00: Lunch and poster sessions 12, 13, 14, 15, 16 & 17 (Marble Hall)
- 14.00 – 15.30: Session 13: Molecular breeding (3-13)  
                    Session 15: Biological, chemical, cultural & integrated control (pt 2) (3-14)
- 15.30 – 16.00: Coffee break (Marble Hall)
- 16.00 – 16.30: Session 14: Strategies for disease control, resistance and host diversification (3-13)  
                    Session 16: Microbial interactions in the rhizosphere (pt 1) (3-14)
- 19.00 - 21.00: Conference dinner

### Thursday 17. August 2006
- 9.00 – 10.30: Session 17: Diagnostics (pt 1) (3-13)  
                    Session 16: Microbial interactions in the rhizosphere (pt 2) (3-14)
- 10.30 – 11.00: Coffee break (Marble Hall)
- 11.00 – 12.00: Session 17: Diagnostics (pt 2) (3-13)  
                    Session 16: Microbial interactions in the rhizosphere (pt 3) (3-14)
- 12.00 – 12.30: Closing of conference (3-01)
Programme

Note that the second column gives the auditorium number (bold) for each session or the abstract number for each talk. Poster abstracts are assigned to specific sessions and follow oral presentations in the abstract book.

Sunday 13. August 2006

15.00 – onwards  Registration
18.00 – 19.00   Buffet
19.15 – 20.00  3-01 GARRETT MEMORIAL LECTURE by Professor Lene Lange, Novozymes, Denmark
The future role of plant pathology - How science can contribute to making a better world?


09.00 – 10.00  3-01 Conference Opening
10.00 – 10.30  Coffee break

A
10.30 - 12.10  3-13 Session 01: Induced resistance and defence responses
Chair: David B. Collinge/ Hans Jørgensen, The Royal Veterinary and Agricultural University, Denmark

10.30 – 11.00  K1.1 Keynote: L.C. Van Loon, University of Utrecht, The Netherlands
Adaptive induced resistance responses to pathogens and herbivorous insects

11.00 – 11.20  O1.1 Philippe Reignault, Université du Littoral Côte d'Opal, France
Efficiency and physiological targets of resistance inducers and natural compounds during the wheat/powdery mildew compatible interaction

11.20 – 11.50  K1.2 Keynote: Frank L.W. Takken, University of Amsterdam, The Netherlands
R proteins, molecular switches of plant defence

11.50 – 12.10  O1.2 David B. Collinge, The Royal Veterinary and Agricultural University, Denmark
Barley genomics and plant defence responses.

12.10 – 14.00  Lunch and Poster sessions: 1, 2, 3, 4, 5 & 6

14.00 – 15.30  3-13 Session 02: Non-host resistance and innate immunity
Chair: Mari-Anne Newman, The Royal Veterinary and Agricultural University, Denmark

14.00 – 14.30  K2.1 Keynote: Uwe Conrath, Aachen University, Germany
Priming: It’s all the world to induced disease resistance

14.30 – 15.00  K2.2 Keynote: Mari-Anne Newman, The Royal Veterinary and Agricultural University, Denmark
Innate Immunity: Plant recognition of bacterial PAMPs

15.00 – 15.30  O2.1 Hans Thordal-Christensen, The Royal Veterinary and Agricultural University, Denmark
Roles of Syntaxins in Disease Resistance.

15.30 – 16.00  Coffee break
16.00 – 17.30  3-13  **Session 03: Virus**
Chair: Mark Stevens, Broom's Barn Research Station, United Kingdom

16.00 – 16.30  K3.1  **Keynote: John P. Carr, University of Cambridge, United Kingdom**
Induced and basal resistance to plant viruses

16.30 – 16.50  O3.1  **Maria Ivone E. Clara, Universidade de Évora, Portugal**
Application of Reverse Transcription - Polymerase Chain Reaction to screen a collection of clones of *Olea europaea* L. for the presence of necroviruses (Tomoviridae)

16.50 – 17.10  O3.2  **Anders Kvarnheden, Swedish University of Agricultural Sciences, Sweden**
Molecular epidemiology of Wheat dwarf virus in Sweden

17.10 – 17.30  O3.3  **Jaroslav Polák, Research Institute of Crop Production, Czech Republic**
Immunity, resistance, susceptibility, tolerance, and hypersensitivity of stone fruits to Plum pox virus, problems of detection

**B**

10.30 – 12.00  3-01  **Session 04: Population diversity and dynamics**
Chair: Mogens S. Hovmøller, Danish Institute of Agricultural Sciences, Denmark

10.30 – 11.00  K4.1  **Keynote: James K.M. Brown, John Innes Centre, United Kingdom**
Natural selection in plant-pathogen interactions: from models to laboratory to field

11.00 – 11.20  O4.1  **Erik Schwarzbach, Miroslav, Czech Republic**
Variability in partial *mlo* virulence in the barley powdery mildew population

11.20 – 11.40  O4.2  **John P. Clarkson, Warwick HRI, University of Warwick, United Kingdom**
Understanding the diversity of *Sclerotina sclerotiorum*; a UK perspective

11.40 – 12.00  O4.3  **Emily Clewes, Warwick HRI, University of Warwick, United Kingdom**
An asexual fungus? The genes say no!

12.00 – 14.00  Lunch and Poster sessions: 1, 2, 3, 4, 5 & 6

14.00 – 16.00  3-01  **Session 04: Population diversity and dynamics**  continued

14.00 – 14.20  O4.4  **Eugene A. Milus, University of Arkansas, USA**
A new and more aggressive population of *Puccinia striiformis* f. sp. *tritici* in eastern United States

14.30 – 14.50  O4.5  **Mogens S. Hovmøller, Danish Institute of Agricultural Sciences, Denmark**
Genetic variability of wheat yellow rust at scales from field to continent

14.40 – 15.00  O4.6  **S. Sreenivasaprasad, Warwick HRI, University of Warwick, United Kingdom**
Population diversity and pathogenicity lifestyles in a broad host range pathogen *Colletotrichum acutatum*: Molecular approaches

15.00 – 15.20  O4.7  **Birgitte Andersen, Technical University of Denmark**
Chemotaxonomy of large-spored *Alternaria* from onion, tomato and potato

15.30 – 16.00  Coffee break

16.00 – 17.30  3-01  **Session 05: Epidemiology**
Chair: Jonathan Yuen, Swedish University of Agricultural Sciences, Sweden & Mike J. Jeger, Imperial College, University of London, United Kingdom

16.00 – 16.30  K5.1  **Keynote: Frank van den Bosch, Rothamsted Research, United Kingdom**
The basic reproduction number, $R_0$. 

3
16.30 – 16.50  O5.1  **Ivan Sache**, INRA-INA-PG, France
Revealing concealed processes in airborne plant disease dispersal

16.50 – 17.10  O5.2  **Neil McRoberts**, Land Economy and Environment Research, Edinburgh, United Kingdom
Matrix projection methods in analysing multiple disease complexes.

17.10 – 17.30  O5.3  **Jonathan Yuen**, Swedish University of Agricultural Sciences, Sweden
Combining the new with old -- a Bayesian view

C
14.00 – 15.30  3-14  **Session 06: Post harvest diseases**
Chair: Ulf Thrane, Technical University of Denmark

14.00 – 14.30  K6.1  **Keynote: Dov Prusky**, Agricultural Research Organisation, Israel
Mechanisms modulating fungal attack in postharvest pathogens interactions and their control

14.30 – 14.50  O6.1  **Tim O’Neill**, ADAS UK Ltd, United Kingdom
Occurrence of latent *Botrytis cinerea* in some cut flower and pot plant species.

14.50 – 15.10  O6.2  **Inge M.B. Knudsen**, The Royal Veterinary and Agricultural University, Denmark
Cavity spot and liquorice rot development in carrots during cold storage

15.10 – 15.30  O6.3  **Arne Hermansen**, Bioforsk, Norway
PCR–based detection of carrot pathogens in soil samples for prediction of disease development in the growing season and during storage

15.30 – 16.00  Coffee break

16.00 – 16.20  O6.4  **Rasmus J. N. Frandsen**, The Royal Veterinary and Agricultural University, Denmark
The biosynthesis pathway of aurofusarin in *Fusarium graminearum*

16.20 – 16.40  O6.5  **Ludmila Slezakova**, Czech University of Agriculture, Czech Republic
Toxigenic micromycetes and their mycotoxins associated with transgenic Bt-maize and nontransgenic hybrids of maize

18.30 -  Civic reception at Copenhagen City Hall. We suggest a subsequent visit to the adjacent TIVOLI pleasure gardens at your own expense.
Tuesday 15. August 2006

A  
09.00 – 10.30  3-13  Session 07: Genomes and Transcriptomes  
Chair: Ph.D students: Michael Krogh Jensen The Royal Veterinary and Agricultural University Denmark & Mary Coates, Warwick HRI, University of Warwick, UK

09.00 – 09.30  K7.1  Keynote: K. Hammond-Kosack, Rothamsted Research, United Kingdom  
Investigating the genome of the cereal attacking fungal pathogen Fusarium graminearum

09.30 – 09.50  O7.1  Friederike Trognitz, ARC Seibersdorf research GmbH, Austria  
Investigating the transcriptome of wheat upon interaction with the head blight fungus

09.50 – 10.10  O7.2  Dale Godfrey, The Royal Veterinary and Agricultural University, Denmark  
Sequencing of a mixed EST library from barley powdery mildew haustoria and barley epidermal cells hosting haustoria

10.10 – 10.30  O7.3  Mette Lübeck, The Royal Veterinary and Agricultural University, Denmark  
Secretome analysis of plant pathogen interactions, based on transposon assisted signal trapping

10.30 – 11.00  Coffee break

11.00 – 12.00  3-13  Session 08: Plant pathogen interactions & Cell to Cell communication  
Chair: Ph.D.-students Michael Krogh Jensen, The Royal Veterinary and Agricultural University, Denmark & Céline Janvier, CTIFL, La Force, France

11.00 – 11.30  K8.1  Keynote: Robert Fluhr, Weizmann Institute, Israel  
Protease control of hormonal cross-talk in the pathogenesis response

11.30 – 11.50  O8.1  Aleksandra Adomas, Swedish University of Agricultural Sciences, Sweden  
Comparative analysis of tissue specific responses of Pinus sylvestris to shoot (Gremmeniella abietina) and root (Heterobasidion annosum) specific pathogens.

11.50 – 12.10  O8.2  Hans J. Lyngs Jørgensen, The Royal Veterinary and Agricultural University, Denmark  
Hydrogen peroxide is important for the defence of wheat against Septoria tritici

12.10 – 14.00  Lunch and Poster session 7, 8, 9, 10 & 11

14.00 – 15.30  3-13  Session 09: PH Gregory Competition  
Chair: Matthew Dickinson, University of Nottingham, United Kingdom

14.00 – 14.15  O9.1  Janathan Danial, Scottish Agricultural Science Agency, United Kingdom  
Application of multilocus sequence typing for characterisation of the brown rot pathogen Ralstonia solanacearum

14.15 – 14.30  O9.2  Mojtaba Mamarabadi, The Royal Veterinary and Agricultural University, Denmark  
Characterization of four chitinase-encoding genes (cr-nagl, cr-ech58, cr-ech42 and cr-ech37) from the fungus Clonostachys rosea (IK726).

14.30 – 14.45  O9.3  Eliana Maffettone, Warwick HRI, University of Warwick, United Kingdom  
The endornavirus AbEV1: a dsRNA element, associated with MVX disease of Agaricus bisporus

14.45 – 15.00  O9.4  Jolyon Dodgson, University of Hertfordshire, United Kingdom  
Epidemiological studies leading to the sustainable control of strawberry powdery mildew
15.00 – 15.15 O9.5 Michael K. Jensen, The Royal Veterinary and Agricultural University, Denmark
Do HVNAC-like transcription factors regulate host defence against the powdery mildew (Blumeria graminis f.sp. hordei) in barley (Hordeum vulgare)?

15.15 – 15.30 O9.6 Gracia C. Ribas-Vargas, University of Nottingham, United Kingdom
Genetic transformation of Lilium for enhanced fungal disease resistance

15.30 – 16.00
Coffee break

16.00 – 16.15 O9.7 Mary Coates, Warwick HRI, University of Warwick, United Kingdom
Searching for Avirulence Genes in Hyaloperonospora parasitica

16.15 – 16.30 O9.8 Triona Davey, Scottish Agricultural Science Agency, United Kingdom
The epidemiology of Potato mop top virus (PMTV) in seed potatoes

16.30 – 16.45 O9.9 Ana Costa, Warwick HRI, University of Warwick, United Kingdom
RNAi as a tool for multifunctional genomics in homobasidiomycetes

16.45 – 17.00 O9.10 Elias Sowley, University of Reading, United Kingdom
Evidence for seed to seedling transmission of Botrytis cinerea in lettuce

17.00 – 17.15 O9.11 Elizabeth Pirie, Rothamsted Research, United Kingdom
Determination of factors involved in resistance in the Brassica napus – Leptosphaeria maculans pathosystem

B
09.00 – 10.30 3-14 Session 10: Plant and seed health and developing countries
Chair: Nicola Spence, Central Science Laboratory, United Kingdom & Jan Torp, DSHC, The Royal Veterinary and Agricultural University, Denmark

09.00 – 09.30 K10.1 Keynote: Andrew Bennett, Syngenta, Switzerland
Plant Health in developing countries – a Foundation Perspective

09.30 – 09.50 O10.1 Brian R. Kerry, Rothamsted Research, Harpenden, United Kingdom
Sustainable management of root-knot nematodes in intensive vegetable production in tropical soils requires local capacity building

09.50 – 10.10 O10.2 Julian S. Smith, Central Science Laboratory, United Kingdom
Implementing quality systems for potato seed with farmers in Uganda: the experience of the Kapchorwa Seed Potato Producers’ Association in Eastern Uganda

10.10 – 10.30 O10.3 Eric Boa, Global Plant Clinic, CABI, Egham, UK
Mobile plant health clinics in Nicaragua and Bangladesh

10.30 – 11.00
Coffee break

11.00 – 11.20 O10.4 Steen Lykke Nielsen, Danish Institute of Agricultural Sciences, Denmark
Napier grass stunt disease in Uganda associated with a phytoplasma

11.20 – 11.40 O10.5 S. Sreenivasaprasad, Warwick HRI, University of Warwick, United Kingdom
Characterisation of the blast pathogen Magnaporthe grisea populations on finger millet and rice in Africa: Towards sustainable disease management

11.40 – 12.00 O10.6 Henrik J. Hansen, Danish Plant Directorate, Denmark
Farm level Rice Seed Health improvement in Vietnam

12.10 – 14.00  Lunch and Poster session

14.00 – 15.30  3-14  Session 11: Quarantine and legislation
Chair: Johan Bremmer, Wageningen University and Research Center, The Netherlands

14.00 – 14.30  K11.1  Keynote: Françoise Petter, EPPO, France
Diagnostic in plant quarantine: review of international initiatives

14.30 – 14.50  O11.1  Stephan Helfer, Royal Botanic Garden, United Kingdom
Quarantine of Wild Collected Plants

14.50 – 15.10  O11.2  Charlotte Thrane, Danish Plant Directorate, Denmark
Quality assurance in Plant Health Diagnostics

15.10 – 15.30  O11.3  Johan Bremmer, Agricultural Economics Research Institute, Wageningen, The Netherlands
Socio-economic aspects of quarantine management

15.30 – 16.00  Coffee break

16.00 – 17.30  Discussion session/ panel discussion addressing Quarantine and legislation in the new EU (for about one hour) chaired by Johan Bremmer.

19.00 – 21.00  Mini-workshops

W1  3-12  Rick Mumford: Port Check: development of “on site” molecular diagnostics for quarantine pests and pathogens

W2  3-13  Michael K. Jensen, Mary Coates and Céline Janvier: Post Ph.D. possibilities

W3  3-08  Jan Torp: Seed Health and agricultural development

W4  3-14  David B. Collinge: The future for teaching in plant pathology

18.00 – 19.30  3-07  EFPP Board meeting (All EFPP delegates are invited)
Wednesday 16. August 2006

9.00 – 10.00  BSPP PRESIDENTIAL ADDRESS by Professor Peter Mills, Warwick HRI, University of Warwick, United Kingdom
25 years of BSPP and 25 years of personal research; what have we learned?

10.00 – 10.30  Coffee break

A:
10.30 – 12.00  3-13  Session 12: Bacterial diseases
Chair: Julian Smith, Central Science Laboratory, United Kingdom

10.30 – 11.00  K12.1  Keynote: Julian S. Smith, Central Science Laboratory, United Kingdom
Patterns of new plant disease spread within Europe and Africa: evidence of a growing problem

11.00 – 11.20  O12.1  Joanna Przysowa, University of Gdansk, Poland
Analysis of the AHL-degrading activity observed in Ochrobactrum sp. A44

11.20 – 11.40  O12.2  Jaana Laurila, MTT Agrifood Research Finland
Characterisation of erwinias causing blackleg and soft rot in Finland by sequencing and virulence tests

12.00 – 14.00  Lunch and poster sessions 12, 13, 14, 15, 16 & 17

14.00 – 15.30  3-13  Session 13: Molecular breeding
Chair: Hans Thordal-Christensen, The Royal Veterinary and Agricultural University, Denmark

14.00 – 14.30  K13.1  Keynote: Beat Keller, University of Zurich, Switzerland
Molecular approaches for characterization and use of natural disease resistance genes in wheat

14.30 – 14.50  O13.1  Ahmed Jahoor, The Royal Veterinary and Agricultural University, Denmark
Application of TILLING for molecular breeding for disease resistance

14.50 – 15.10  O13.2  Friederike Ch. Trognitz, ARC Seibersdorf Research, Austria
Toward positional cloning of a gene conferring resistance to potato late blight, in Solanum caripense

15.30 – 16.00  Coffee break

16.00 – 17.30  3-13  Session 14: Strategies for disease control, resistance and host diversification
Chair: David Pink, Warwick HRI, University of Warwick, United Kingdom

16.00 – 16.30  K14.1  Keynote: Maria Finckh, Kassel University, Germany
How to use diversification strategies for disease control in modern agriculture?

16.30 – 16.50  O14.1  Julia Kinane, Risoe National Laboratory, Denmark
Intercropping Pea with Barley: The Effect on Disease

16.50 – 17.10  O14.2  Mogens S Hovmøller, Danish Institute of Agricultural Sciences, Denmark
Influence of foliar diseases on grain yield of spring barley in low input cropping systems

17.10 – 17.30  O14.3  David A.C. Pink, Warwick HRI, University of Warwick, United Kingdom
Lettuce downy mildew – breaking the ‘boom bust’ cycle

B
10.30 – 12.00  3-14  Session 15: Biological, chemical, cultural and integrated control
Chair: Tim O’Neill, ADAS UK Ltd. United Kingdom
10.30 – 11.00 K15.1  **Keynote: Lise Nistrup Jørgensen, Danish Institute of Agricultural Science, Denmark**
What information do farmers need to optimize disease control in cereals?

11.00 – 11.20 O15.1  **Dan Funck Jensen, The Royal Veterinary and Agricultural University, Denmark**
Biological and technical aspects of developing *Clonostachys rosea* as a fungal biocontrol agent (BCA).

11.20 – 11.40 O15.2  **Céline Janvier, CTIFL, Centre de Lanxade, France**
Towards indicators of soil health: impact of cultural practices on soil characteristics, relationships between these characteristics and with soil suppressiveness

11.40 – 12.00 O15.3  **Birgit Jensen, The Royal Veterinary and Agricultural University, Denmark**
Sustainable disease management in legume rich cropping systems

12.00 – 14.00  Lunch and poster sessions 12, 13, 14, 15, 16 & 17

14.00 – 15.30  Session 15 cont.

14.00 – 14.30 K15.2  **Keynote: Alan J. Slusarenko, RWTH Aachen University, Germany**
Potential for control of plant diseases by natural products - with particular reference to allicin from garlic.

14.30 – 14.50 O15.4  **John Hockenhull, The Royal Veterinary and Agricultural University, Denmark**
Options for managing scab in organic apple production after copper-fungicides are no longer available - some REPCO approaches

14.50 – 15.10 O15.5  **Sandra Wright, Göteborg University, Sweden/University of Molise, Italy**
Biological control of powdery mildew of barley

15.10 – 15.30 O15.6  **Gunn Mari Stromeng, Norwegian University of Life Sciences, Norway**
Factors influencing strawberry flower infection and interactions between *Botrytis cinerea* and its fungal antagonists

15.30 – 16.00  Coffee break

16.00 – 16.20 O15.7  **Piet M. Boonkamp Plant Research International, The Netherlands**
Towards integrated control of *Phytophthora* in potato: results of the Dutch Umbrella Plan

16.30 – 17.20 3-14  **Session 16: Microbial interactions in the rhizosphere**
Chair: John Whipps, Warwick HRI, University of Warwick, United Kingdom/ Dan Funck Jensen, The Royal Veterinary and Agricultural University, Denmark

16.30 – 17.00 K16.1  **Keynote: Jutta Ludwig-Müller, Technische Universität Dresden, Germany**
What can we learn from clubroots: Alterations in host root metabolism and hormone homeostasis caused by *Plasmodiophora brassicae*

17.00 – 17.20 O16.1  **Christopher R. Thornton, University of Exeter, United Kingdom**
Tracking fungi in soil with monoclonal antibodies

19.00 -  Conference dinner
Thursday 17. August 2006

**A**

09.00 – 10.30  3-14  Session 16 continued  
Chair: John Whipps/ Dan Funck Jensen

09.00 – 9.30  K16.2  **Keynote: Kurt Brunner, Vienna University of Technology, Austria**  
Signal transduction in host recognition and mycoparasitism: a case study with *Trichoderma atroviride*

09.30 – 09.50  O16.2  **John M. Whipps, Warwick HRI, University of Warwick, United Kingdom**  
Identification of pathogenicity genes in the sclerotial mycoparasite *Coniothyrium mimitans*

09.50 – 10.10  O16.3  **Małgorzata Manka, The August Cieszkowski Agricultural University, Poland**  
A proposed index of forest environment pollution – effect of soil fungi communities on the growth of *Heterobasidion annosum*

10.10 – 10.30  O16.4  **Sabine Ravnskov, Danish Institute of Agricultural Science, Denmark**  
Arbuscular mycorrhiza affects *Pythium*-cucumber interactions

10.30 – 11.00  Coffee break

11.00 – 11.20  O16.5  **Martina Rickauer, Laboratoire Biotechnologie et Amélioration des Plantes, France**  
Characterisation of root pathosystems involving the model plant *Medicago truncatula*

11.20 – 11.40  O16.6  **Sandra C. K. Carlsen, Danish Institute of Agricultural Sciences, Denmark**  
Flavonoids in white clover in response to presence or absence of two arbuscular mycorrhizal fungi and a pathogenic fungus

B

9.00 – 10.30  3-13  Session 17: Diagnostics  
Chair: Rick Mumford, Central Science Laboratory, United Kingdom

09.00 – 09.40  K17.1  **Keynote: Neil Boonham, Central Science Laboratory, United Kingdom**  
Generic platform technologies for the detection and identification of plant pathogens

09.40 – 10.10  K17.2  **Keynote: C.D. Schoen, Plant Research International, The Netherlands**  
Quantitative multiplex detection of plant pathogens using PRI-lock probes and universal, ultra-high-throughput real-time PCR on OpenArrays™

10.10 – 10.30  O17.1  **Matt Dickinson, University of Nottingham, United Kingdom**  
Use of T-RFLP for the detection of microbes associated with plants

10.30 – 11.00  Coffee break

11.00 – 11.20  O17.2  **Stuart Wale, Scottish Agricultural College, United Kingdom**  
Practical solutions to the control of black dot of potatoes

11.20 – 11.40  O17.3  **Mogens Nicolaisen, Danish Institute of Agricultural Sciences, Denmark**  
Microarrays for identification of *Fusarium* and other fungi

12.00 – 12.30  Closing of conference
Abstracts

GML

GARRETT MEMORIAL LECTURE:

The future role of plant pathology - How science can contribute to making a better world

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Plant pathology is one of the most important disciplines for studies of interaction between microbes and plants. The challenge is to understand it on a molecular level!

In recent years there has been a strong focus on genome sequencing. This inherently led to focus on one organism at a time, and hardly any attention to interaction between organisms. Now it is time to focus on biological interactions to improve our biological understanding, hereby also providing better basis for sustainable use of the resources.

The last century was dominated by use of fossil resources. Energy came from oil, and materials and chemicals came from petrochemistry. Now we are approaching a new era, where solutions will build on biologically produced materials. However, significant biological research efforts must be invested to make this a reality!

Field production as well as microbial production in biorefineries builds on agricultural production of plants. Plant pathology has here an important role to play to ensure that we get the amount and quality of plant products needed, also of the new cultivars, tailor-made to the new products and processes.

Further, plant pathologists’ experience within plant/microbe interaction can be in strong demand also for gene discovery and for development of new biological processes and products, building on microbial processing of plant materials. And last but not least, new progress must be made for control of disease and pest in the marginalized areas of the world.

BSPP

BSPP PRESIDENTIAL ADDRESS 2006:

25 years of BSPP and 25 years of personal research; what have we learned?

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The British Society for Plant Pathology celebrates its 25th anniversary in 2006. The society has changed in many ways during this time and our 25th year is a good opportunity to review our aims and function (to both our members and also the wider plant pathology community) and also the way that we serve these communities.

Over the same period of time, the nature of plant pathology research has changed significantly from an emphasis on aetiology, detection, diagnosis and a reliance on the use of chemicals to a focus, particularly by research funders, on sustainable methods of disease control. I hope to provide a personal perspective of some of those changes with case studies including potato virology, fungal diseases of tropical fruits and global diseases of cultivated fungi.
K1.1  Adaptive induced resistance responses to pathogens and herbivorous insects
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Plants possess both constitutive and inducible basal resistance mechanisms against pathogens and herbivorous insects, now often referred to as "innate immunity". Susceptibility results when the attacker is able to circumvent or suppress these defenses, resistance when defenses are activated sufficiently early and strongly to limit attack. Once resistance responses are activated, plants develop an enhanced defensive capacity against a broad range of attackers, variously named systemic acquired resistance (SAR) or induced systemic resistance (ISR). SAR is typically induced by limited infection with a pathogen, depends on salicylic acid signaling, and is associated with the expression of genes encoding pathogenesis-related proteins. In contrast, the ISR that is induced by specific strains of non-pathogenic rhizobacteria depends on jasmonic acid (JA) and ethylene signaling and involves priming of defense-related gene expression. Wound-induced resistance (WIR) against herbivorous insects is an induced resistance that depends on increased JA levels. Pathogens and insects are differentially sensitive to the resistances activated by each of these signaling pathways, which explains the differential effectiveness of SAR, ISR and WIR against biotrophic and necrotrophic pathogens, as well as specialist and generalist insect herbivores. Extensive cross-talk between the different induced resistance signaling pathways allows the plant to prioritize its response to different attackers.

K1.2  R proteins, molecular switches of plant defence
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Resistance (R) proteins are the main specificity determinants of the plant innate immune system. Most R-proteins belong to the Nucleotide Binding-Leucine Rich Repeat (NB-LRR) family. Our research focuses on the role of the NB-domain for R protein function. Previously, we have shown that the NB domain of the tomato R proteins I-2 and Mi-1.2 binds and hydrolyzes ATP. Specific substitutions in conserved motifs in the NB domain resulted in autoactivating mutants that induce a hypersensitive response (HR) upon expression in planta. Biochemical analysis of the I-2 mutants revealed that they were affected in ATP hydrolysis rather than in binding. The finding that nucleotide-binding mutants are unable to trigger an HR together with the observation that the I-2 protein forms a very stable complex with ADP, let us to propose that the ATP-bound conformation of the protein represents the "on" state and the ADP-bound conformation the "off" state of I-2 (Tameling et al (2006), Plant phys. 140-1233). Support for nucleotide dependent conformational changes of I-2 comes from yeast two-hybrid experiments. Three proteins interacting with the N-terminus of I-2 display an opposite interaction pattern with the mutants compared to wild-type I-2. Based on these data we propose that R-proteins function as molecular switches whose conformation (on/off) is controlled by ATP binding and hydrolysis. Currently, we use a targeted proteomics approach to identify the in vivo composition of the R-signalosome and identify its dynamics in relation to the nucleotide binding state of I-2.
O1.1  **Efficiency and physiological targets of resistance inducers and natural compounds during the wheat/powdery mildew compatible interaction**

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Powdery mildew is caused on wheat by *Blumeria graminis* f.sp. *tritici* and is one of its most damaging foliar diseases. Efforts have been recently made to allow the use of either new molecules of natural origin as alternative fungicides or inducers of plant resistance. The effects of treatments of wheat with chitosan, Iodus 40®, Milsana®, trehalose and salicylyl heptanoate against *B. graminis* were investigated. We will present the obtained protection efficiencies and the effects on active oxygen species (AOS) metabolism, lipid peroxydation, phenolic compounds accumulation and fungal germination. Plants treated with the different products showed differences in the obtained protection levels. The AOS, early markers of defence elicitation, have been histochemically analysed. We focused on H$_2$O$_2$ accumulation at the penetration sites and examined whole cell necrosis. In addition, the rate of lipid peroxides was also measured, altogether with modifications in fatty acid profiles and jasmonic acid accumulation. We sought any direct fungistatic effect both in vitro and in planta at the penetration site on wheat epidermal cells. It appeared clearly that the physiological targets of the different compounds/elicitors were either distinct or overlapping. The extent of defence responses activated by them will be discussed as well as the perspective of their use in Integrated Pest Management.

O1.2  **Barley genomics and plant defence responses**

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Arabidopsis and, to a lesser extent rice are the plants of choice for fundamental studies on the biology of plant-microbe interactions primarily since their genomic sequences are available. However, decades of classic genetic and pathological studies, particularly with the powdery mildew fungus, on barley provide a number of resources which can be used to understanding defence mechanisms and disease resistance.

We have used the combination of public EST data bases, microarray data and differential display to identify potential regulatory genes involved in the activation of basal defences in barley against *Blumeria graminis*. Determination of the role of these genes in defence is determined by gene silencing studies using the transient expression of hairpin loop mRNA’s and constitutive over expression in epidermis tissues followed by infection with *B. graminis*. Our efforts have focussed on NAC proteins and receptor-like protein kinases.
Transcriptional profiling by cDNA-AFLP reveals novel insights between Methyl Jasmonate, Wounding and Insect attack in *Brassica napus*

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Plants exploit a broad range of defense mechanisms to effectively combat invasion by pathogens or herbivores. A detailed transcriptome analysis using the cDNA-AFLP technique was performed to identify genes that are differentially expressed in *Brassica napus* (Westar) leaves upon treatment with Methyl Jasmonate, Mechanical wounding, or feeding by Diamond Back Moth larvae [Plutella xylostella (L.) Lepidoptera: Plutellidae]. In total, 16 different primer combinations were used, generating cDNA fragments ranging from 50 to 500bp in size. Out of 3500 bands, 124 showed qualitative differences among the treated and their respective control samples, including 95 up-regulated and 29 down-regulated bands. A few genes, like Jacalin lectin-MYB, Strictosidine synthase and MD-2-LPS domain were expressed in common among the Meja, wounding and DBM fed plants. Most of the expressed genes were grouped in the categories of signaling pathways, oxidative stress, wound or pathogen response. Genes with altered expression in distal tissue included those in cellular housekeeping functions, indicating that plants modify these vital processes to facilitate a coordinated response to different stress conditions. Several new transcripts were identified that may play a role in insect attack and other signal transduction pathways. Current investigations address effects of pathogen on the transcriptome. These studies were supported by Carl Tryggers Stiftelse and Perssons Fund.

In winter wheat induced resistance to powdery mildew (*Blumeria graminis f.sp. tritici*) under field conditions.

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In small-plot two years experiments the winter wheat Kanzler (susceptible standard to powdery mildew) was tested to inducers of synthetic origin: benzothiadiazole - BTH (Bion ®), salicylic acid - SA, and inducers of biological origin: glycine betain - GB, extracts prepared from oak bark - OB (*Quercus robur* L.), Reynoutria saccharifolia L. - RS, curcuma - CU (*Curcuma longa* L.), ginger - GI (*Zingiber officinale*). Two rows of the cultivar were treated on 7.6.2004 (growth stage 49) and 26.5.2005 (growth stage 39). Infection of powdery mildew was natural. All used inducers incited resistance of wheat to powdery mildew. The most effective was BTH but it seems that the oldest leaves (the fourth leaf from the top) died earlier than in the case of other inducers. Application of BTH was remarkable by a large number of chlorotic spots on most leaves. Less effective results were reached after treatment with OB and SA. The treatment with GI, CU, GB and RS was very effective. Disease severity in whole plants treated by inducers was lower than 50% of control except salicylic acid. Differences between the untreated control and the application of inducers were most obvious on the oldest leaf. Important is the fact that action of inducers was long-term. Our results prove that chemically induced resistance in plants leads to a durable protection against powdery mildew and indicated that even biological inducers could provide long-term protection of wheat.

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**P1.3**

**Induced expression of PR-proteins in barley in relation to infection site**

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Barley (*Hordeum vulgare*) was infected with *Bipolaris sorokiniana* on leaves or roots. The aim was to determine whether the timing of transcriptional changes and protein accumulation of certain pathogenesis related (PR) proteins in leaves and roots depend on the site of infection. Nucleic acid probes and antibodies were used to detect mRNA and proteins corresponding to one acidic, PR-5 and three basic, PR-1b, PR-2c and PR-3 proteins for blot analysis. *B. sorokiniana*, a necrotrophic fungi, is well suited for studying induced host plant responses as it is capable of infecting both leaves and roots of barley. We used ten timepoints, 0-96 hours after infection (hai). After infection of leaves there was a clear peak in transcription of mRNA coding for PR-1b and PR-5 in the leaves at 6 hai, a dip with no detectable transcription at 9 hai, followed by a new transcription peak at 12 hai which then increased at 16 to 96 hai. There were no transcriptional changes in the root when the leaves were infected. When roots were infected there was a slight increase of the transcription level in the roots from 9 hai and onwards. On the other hand, the leaves of root-infected barley showed one clear peak at 16 hai not followed by further increase in transcription. The protein analysis corresponded well with the transcriptional results. The expression pattern of PR-2c and PR-3 differed from PR-1b and PR-5. Results from immunohistochemical localization of the PR-proteins will also be presented.

**P1.4**

**Vitamin B2 (riboflavin) functions as an plant defence activator against *Rhizoctonia* spp. associated with rice sheath diseases**

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The role of riboflavin as an elicitor of systemic resistance and a plant defence activator in rice was demonstrated in the present study. Following treatment with riboflavin, rice plants developed systemic resistance to *Rhizoctonia solani* and *R. oryzae-sativae*, causal agents of sheath blight and aggregate sheath spot of rice, respectively. Riboflavin, at concentrations necessary for induction of resistance (0.01 to 2 mM), did not cause any cell death in rice; also, it did not have any direct effect on the growth of fungi in vitro. Production of hydrogen peroxide was detected at 12 h after inoculation using DAB staining method. Expression of a cationic rice peroxidase, POC1, was induced at 18 h after inoculation in riboflavin treated rice plants. A correlation was found between induction of resistance by riboflavin and up-regulation of POC1 gene. A variety of roles have been proposed for the involvement of peroxidases in the defense response (1). One possible role is the generation of reactive oxygen species (ROS) by peroxidase-oxidative activity. The fact that the production of hydrogen peroxide was upstream of induction of POC1 gene expression, ruled out the possibility of involvement of POC1 in the generation of ROS in these interactions. Another possible function of peroxidases is the formation of structural barriers such as lignin or suberin. Lignin formation was investigated using phloroglucinol/HCl test (2), and lignin was detected in riboflavin treated plants. Therefore, riboflavin-induced resistance can be linked to the induction of defence pathways leading to formation of structural barriers in rice plants.

References:  
P1.5  
The role of Receptor-Like Kinases in Defence Responses Against the Powdery Mildew (Blumeria graminis f.sp. hordei) in Barley (Hordeum vulgare).
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Using classical Differential Display of mRNA isolated from epidermis tissue of Powdery Mildew inoculated barley leaves, we have isolated transcripts, which encode a putative RLK and a NAC-domain transcription factor. RLK proteins are thought to function as receptors that regulate downstream phosphorylation cascades. We have focused on a novel subfamily of RLKs containing cysteine-rich repeats (CRRs) in the extracellular part (Chen, Z., Plant Physiol. 2001; 126:473-6). The cysteines are generally organised in a C-X8-C-X2-C motif, known as the DUF26 domain (Domain of Unknown Function), but the number of amino acids between the first two cysteines may vary between 8 and 10. We have observed up to a 40 fold increase in transcript levels of two RLKs in inoculated epidermal tissues of barley.

The biological functions of RLK1 being are studied by the use of a biolistic transient expression assay. We are applying RNAi as well as over expression studies. Current biochemical studies of the function of RLK candidates include studies of protein–protein interactions using Yeast Two Hybrid screening and Phage display analysis. A Phage display screen using the receptor domain is used to identify potential ligands and agonists in order to obtain insight and tools for studying the signalling mechanisms. We are using yeast two-hybrid screens with the protein kinase and receptor domains separately to identify partners in the signal transduction pathways in which this sequence participates.

P1.6  
Pre-infectional structural basis of resistance against downy mildew in muskmelon
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The downy mildew caused by Pseudoperonospora cubensis (Berk. and Curt.) Rostow is one of the most destructive fungal diseases of muskmelon (Cucumis melo L.) posing a serious problem in the successful cultivation of this crop. The Muskmelon genotypes differ widely in their resistance to the attack by this pathogen. The pathogen penetrates the leaf through stomata. The leaf characteristics may greatly affect the ability of the pathogen to invade the host plant. Besides many other attributes, the difference in resistance has often been attributed to structural differences in leaf anatomy. Keeping this in view, 42 genotypes of muskmelon (19 resistant and 23 susceptible) were evaluated for their leaf anatomical characteristics viz. their resistance/susceptibility to downy mildew disease. The genotypes needed for this investigation were obtained from the Department of vegetable crops, Punjab Agricultural University, Ludhiana, India. Their disease score rating was done by the Deptt. of Plant Pathology, PAU, Ldh., India (using 0-5 scale). The healthy uninfected leaves were collected when the crop was 100 days old and was at fruiting stage. The results revealed that the Stomatal size, frequency and Index were significantly higher in the susceptible genotypes whereas, the resistant genotypes had higher frequency of large sized Trichomes. The thickness of cuticle, palisade tissue, proportion of palisade and palisade Index values were significantly higher in resistant genotypes indicating compact arrangement of palisade cells in resistant genotypes thus preventing further spread of the pathogen. The results indicated that the leaf epidermal and other anatomical characteristics may act as structural barriers against penetration by downy mildew pathogen. The evaluation for these characteristics may prove useful for early and Preliminary screening of newly evolved Muskmelon genotypes for assessing their resistance/susceptibility to downy mildew infection in large breeding populations.
**Induced resistance in watermelon against gummy stem blight**  
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Soaking watermelon seeds with 1 mM Na-tetraborate for 24 hours can protect against gummy stem blight caused by *Didymella bryoniae*. Infection of true leaves and stems were suppressed after seed treatment with Na-tetraborate compared with treatment with distilled water (control). Studies on infection biology in true leaves after inoculation with *Didymella bryoniae* revealed that seed treatment with Na-tetraborate resulted in lower percentages of germinated spores causing successful penetration and larger accumulation of phenolic compounds at penetration sites than after seed treatment with distilled water. Moreover, activities of lipoxygenase and peroxidase were higher in the early infection course after seed treatment with Na-tetraborate than in control plants at 12 and 24 hours after inoculation, respectively. These results indicate that seed treatment with Na-tetraborate for 24 hours can induce resistance in watermelon against gummy stem blight.

**Vesicle trafficking and salt stress**  
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SNAREs drive secretory vesicle targeting and fusion. Syntaxins from the SNARE (Soluble N-ethylmaleide-sensitive factor (NSF) adaptor protein) superfamily have been implicated in both abiotic and biotic stress (Pratelli et al (2004) Trends Plant Sci. 4:187-95).

The Arabidopsis Syntaxin of Plants121 (SYP121) is required for non-host resistance to barley powdery mildew (Collins et al (2003) Nature 2003 425:973-7). NtSyr1 which is the tobacco homolog of AtSYP121 was shown to block abscisic acid (ABA) mediated Cl⁻ and K⁺ channel responses in guard cells (Leyman et al (1999) Science 5401:537-40). In addition, the expression Ntsyr1 is induced by NaCl in leaves (Leyman et al (2000) 3:369-81). Therefore we have been investigating the role of the plasma membrane AtSYP121 and the closely related syntaxin AtSYP122 in abiotic stress. We have found that the syp121syp122 double mutant shows increased salt sensitivity. Thus we are currently studying the molecular basis of these altered salt responses.
Priming: It’s all the world to induced disease resistance

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Plants can acquire enhanced resistance to pathogens after treatment with necrotizing attackers, various natural and synthetic compounds (which include some commercial fungicides) and upon inhibition of a plastid ATP/ADP transporter protein. The induced resistance is often associated with an enhanced capacity to mobilize infection-induced cellular defense responses - a process called ‘priming’ (‘sensitization’). Although the phenomenon has been known for years, most progress in the understanding of priming has been made over the past years. These studies show that priming often depends on the induced disease resistance key regulator protein NPR1, and that priming is likely to affect the regulation of cellular defense responses by enhancing the cellular level of mitogen-activated protein (MAP) kinases.

Innate Immunity: Plant recognition of bacterial PAMPs

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Innate immune defences, or the nonspecific defence mechanisms that plants use immediately or within hours after exposure to non-self molecules, can be triggered by the perception of general elicitors or Pathogen Associated Molecular Patterns (PAMPs). Lipopolysaccharides (LPS) from Gram-negative bacteria, peptidoglycans from Gram-positive bacteria, flagellin, glucans and chitins from fungal cell walls are examples of PAMPs. Their recognition is often mediated by LRR (Leucine Rich Repeat) proteins such as Toll in Drosophila and the Toll-like receptors (TLRs) in mammals. FLS22 is a plant homologue of the flagellin receptor TLR5 from mammals that binds flagellin. Interestingly, in both plants and mammals, recognition of flagellin activates defence related genes.

LPS is an ubiquitous, indispensable component of the cell surface of Gram-negative bacteria. LPS has myriad effects in plants including the ability to prevent the HR induced by avirulent bacteria, priming of some plant defence responses and elicitation of others, induction of the oxidative burst, nitric oxide synthesis, and phosphorylation of mitogen-activated protein (MAP) kinase. Little is known about perception of LPS by plants or the associated signal transduction pathways that trigger LPS-induced plant disease resistance.

We have recently addressed this issue by analysing those structures within LPS from *Xanthomonas campestris* that are required to trigger immune responses in *Arabidopsis*. The challenge ahead is to identify the plant components involved in LPS recognition and subsequent signal transduction.
**O2.1 Roles of Syntaxins in Disease Resistance**  
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Plant disease resistance is the result of the collective activity of several separate defence mechanisms. In genetic analyses, we have previously discovered that syntaxin SYP121 in *Arabidopsis* is required for penetration resistance (Collins et al. (2003) Nature 425: 973-977; Assaad et al. (2004) Mol Biol Cell 15: 5118–5129). SYP121 is probably necessary for vesicle trafficking leading to formation of papillae, which are local cell wall appositions functioning as barriers against fungal penetration. The closely related SYP122 is not required for penetration resistance.

Other defence mechanisms are controlled by different signalling pathways, which are activated upon pathogen attack. The signalling compounds salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) each define such pathways. In addition, plant cells can undergo the "hypersensitive response" (HR), a program cell death (PCD) reaction, that confer resistance to biotrophic pathogens. In mutant studies, we have examined the involvement of SYP121 and SYP122 in these four signalling pathways, and found that the syntaxins act as negative regulators of all four. While SYP121 is the primary regulatory protein, SYP122 is partially able to take over it role. The release of this negative regulation in the *syp121 syp122* double mutant results in strong PCD-mediated resistance to an otherwise virulent powdery mildew fungus.

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**P2.1 How is bacterial lipopolysaccharides (LPS) recognised by plant cells?**  
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Plants are exposed to attack by pathogens and perceive general elicitors both from host and non-host pathogens. These elicitors are often essential structures for the pathogen’s own survival and are for that reason conserved among the pathogens. Such molecules are referred to as Pathogen Associated Molecular Patterns (PAMPs) and the corresponding plant receptors are referred to as Pattern Recognition Receptors (PRRs) (Janeway (1989) Cold Spring Harbor Symp. Quant. Biol. 54: 1-54).

Lipopolysaccharides (LPS) and lipoooligosaccharides (LOS), major components of the cell surface of Gram-negative bacteria, apparently have diverse roles in bacterial pathogenesis of plants and animals. In contrast to the mammalian and insect systems little is known about the recognition of PAMPs and the triggering of innate immunity in plants (Miyake (2004) Trends Microbiol. 12: 186-192).

Our earlier findings showed that LOS and its fragments, the core oligosaccharide and the lipid A part, induced the defence-related genes *PRI* and *PR2* in *Arabidopsis*. The plant defence induced by the core oligosaccharide appeared to be independent from that of lipid A, indicating that the core oligosaccharide and lipid A part may be recognised by different receptors (Alba et al. (2005) J. Biol. Chem. 280: 33660-33668). Future work will focus on carbohydrate microarray and phage display in order to approach an identification of receptors that activate innate immunity in *Arabidopsis* in response to LPS.
Evidence for a correlation between resistance degree to *Armillaria mellea* in *Genista monosperma* Lam. and root isoflavonoid content

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Four cultivars of *Genista monosperma* Lam., an important leguminous plant cultivated for cut flowering branches, characterized by different resistance degrees to *Armillaria mellea*, have been studied from a phytochemical point of view considering the respective root isoflavonoid content.

The evidenced quali-quantitative differences among cultivars in root isoflavonoid composition have been found correlated to the cultivar resistance level towards *A. mellea*, a fungal parasite agent of a root severe disease (Adams (1974) Northwest Sci. 48: 21-28). This relationship is not to be intended as a mere association between a molecular marker and tissue resistance, but it can be rather conceived as a plant constitutive defensive tool (Nigg and Seigler (1992) Phytochemical resources for medicine and agriculture, N.Y., Plenum Presss): *in vitro* trials indeed demonstrated an appreciable antifungal effect of the considered root isoflavonoids towards *A. mellea*.
Induced and basal resistance to plant viruses

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For viruses, it is increasingly apparent that ‘classical’ systemic acquired resistance (SAR) does not operate in isolation from other defensive systems, for example those mediated by RNA silencing, or those regulated by signal chemicals other than salicylic acid (SA). Ongoing experiments are aimed at establishing the relative importance of different SA-induced resistance mechanisms using transgenic plants compromised in various pathways. For example, we are investigating the connection between SAR and resistance mediated by RNA silencing using the cucumber mosaic virus 2b counter-defence protein as a model. Wild-type and mutant 2b gene sequences have been introduced into transgenic plants and modified viral vectors. These are being used to assess whether or not the functional domains of the 2b protein involved in subversion of SA-induced resistance are the same as, or different from, those that function in subversion of RNA silencing-mediated resistance. Finally, it is known that SA also has a role in the maintenance of basal resistance to viruses. However, recent work indicates that a member of another class of signalling chemicals, the inositol phosphates, may be of at least equal importance to SA in maintaining basal resistance and limiting the accumulation of both virulent and avirulent viruses in the early stages of infection.

Application of Reverse Transcription - Polymerase Chain Reaction to screen a collection of clones of Olea europaea L. for the presence of necroviruses (Tombusviridae)

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A collection of 15 clones of olive cv. ‘Negrinha de Freixo’, established 10 years ago for agronomic studies, was subjected to RT-PCR for the detection of necrovirus affecting that crop: Olive latent virus-1, Tobacco necrosis virus D and Olive mild mosaic virus. Double stranded RNAs were extracted from fresh bark tissue collected from young shoots in the spring and from fruits in the autumn, and used as viral targets. Prior to RT reaction, ds RNA preparations were analysed by gel electrophoresis to determine the presence of bands indicative of any RNA virus infection, and all of the available 161 sampled trees tested negative. The same ds RNA preparations were denatured, reverse transcribed and subjected to PCR amplifications, using a set of primers specific for OLV 1 and another set of primers that detect both TNV D and OMMV, as these two distinct viruses share a coat protein gene with 86% homology. Results showed an amplicon sized 760 nt, as expected for OLV 1, in only one tree, and an amplicon sized 260 nt as expected for TNV D and OMMV infections in 34 out of the 161 trees analysed. These results revealed that ds RNA analysis was not a sufficiently sensitive technique as compared to RT-PCR and extend previous data showing that necrovirus are frequently found in nature infecting olive crop. This further stresses the need for their diagnosis in phytosanitary certification and improvement programs of important olive cultivars, which are currently under way in Portugal.
O3.2 Molecular epidemiology of Wheat dwarf virus in Sweden
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Wheat dwarf disease periodically causes significant damage to winter wheat (Triticum aestivum) in Sweden. Characteristic symptoms of disease are dwarfing, yellowing and reduced heading. The disease is caused by Wheat dwarf virus (WDV), which is transmitted by the leafhopper Psammotettix alienus in a persistent manner. WDV has a genome of circular single-stranded DNA and belongs to the genus Mastrevirus, family Geminiviridae. The host range includes wheat, barley (Hordeum vulgare), oat (Avena sativa) and many grasses. There are two strains of WDV: one strain infecting wheat and another strain infecting barley. The complete genomes of two Swedish WDV isolates from wheat were cloned from circular DNA and sequenced. In addition, polymerase chain reaction (PCR) was used to amplify a partial fragment of 1162 bp (excluding primers) for Swedish WDV isolates from wheat, triticale, grasses (Poa pratensis, Apera spica-venti, Avena fatua) and the insect vector. The nucleotide identity for the Swedish isolates was high (>97%). A phylogenetic analysis showed that they all belonged to the wheat strain with otherwise no clear grouping according to host or geographic origin. Thus, the same viral genotype seems to be present in both grasses and wheat. However, only one sample out of 1044 was positive in ELISA when screening wild grasses growing close to WDV-infested wheat fields. PCR tests of leafhoppers caught in yellow traps mostly showed low frequencies of viruliferous insects.

O3.3 Immunity, resistance, susceptibility, tolerance, and hypersensitivity of stone fruits to Plum pox virus, problems of detection
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Different interactions between cultivars of stone fruits and Plum pox virus (PPV) were observed and characterized in studies on resistance of plums, apricots and peaches to PPV. Immunity, resistance, susceptibility, tolerance, and hypersensitivity of stone fruit cultivars was presented in papers published in reviewed and impacted journals. In many cases the description of cultivars was not correct, or was even wrong, especially when objective methods of detection were not applied. Characterization of interactions between cultivar and virus, and examples of interactions will be presented. Immune cultivars cannot be infected with PPV, and the virus is not multiplied in plants. Apricot cultivar Harlayne was proved to be immune to the six different strains and isolates of PPV. Resistant cultivars can be infected with PPV, but the virus multiplication and moving is limited, and artificially or naturally infected trees are recovering from infection during one or two years. Apricot cultivars Leronda, Stark Early Orange and Harval were proved to be resistant to PPV. PPV is multiplied in plant cells of susceptible cultivars and the virus is moving systematically all over the tree and can be detected in flowers, leaves, stems and fruits. Both, resistance and susceptibility are relative. Highly susceptible cultivars to PPV are e.g. plum Prunus domestica, cv. Požegača, apricot cv. Karola, or peach cv. Catherina. Fruits are showing heavy symptoms, often malformations. Tolerant cultivars are susceptible to the virus infection, PPV is multiplied in leaf tissue often in high rate, but the fruit yield and quality are almost not influenced, or in low rate. Several peach (e.g. Suncrest, Canadien Harmony) and plum (e.g. Čačanská rodná) cultivars are tolerant to PPV. The hypersensitive cultivars are extremely susceptible to PPV, leaf cells infected by aphids necrotize and die, infection is eliminated. In case of massive infection by grafting the whole tree is dying. Infection can be also transferred from PPV infected graft through the hypersensitive cultivar e.g. plum cv. Jojo into susceptible rootstock.

Keywords: Virus - host interactions, resistance, susceptibility, Sharka disease, stone fruits, quantitative ELISA.
P3.1 Characterization of two distinct Polish isolates of *Pepino mosaic virus*
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Tomato isolates of *Pepino mosaic virus* (PepMV) first time were observed in 1999, in the Netherlands and U.K. Since then PepMV has spread throughout Europe and many countries of worldwide. Known in the World PepMV isolates can be divided into three strains on the basis of their genetic diversity: European tomato, Peruvian (isolates from *Solanum muricatum*—SM 74 and *Lycopersicon peruvianum*—LP) and US2 (isolates US1 and US2) (Pogan et al. (2005) Phytopatology 96: 274-279). Nucleotide sequences of the most European tomato isolates were similar (ca. 99% identities) but different from Peruvian strain (ca. 96% homology) and US2 strain (79-82% identities).

In 2002 and 2005 in Poland were obtained two isolates of PepMV signed as SW and PK, respectively. Both Polish PepMV isolates are divergent from each other in host range, symptomatology, serological properties and nucleotide sequences. The sequencing strategy was based on RT-PCR with the specific primers generated from the sequence of the RNA polymerase region of U.K. PepMV isolate. PepMV-SW isolate showed ca. 99% sequences homology to European tomato isolates, 96% to Peruvian strain and 80% to US2 ones. PepMV-PK isolate had only 79-81% nucleotide sequences identities to European tomato isolates, 80% to Peruvian strain and 78% to US2 ones. PepMV-SW was similar to European tomato strain but PepMV-PK was highly divergent from PepMV isolates described so far. Detailed comparison between both Polish PepMV isolates and other ones will be presented.

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P3.2 Genetic variability of the wheat and barley strains of *Wheat dwarf virus*

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*Wheat dwarf mastrevirus* (WDV) has a genome of single-stranded circular DNA and belongs to the family *Geminiviridae*. WDV has a broad host range including cereals and many wild grasses. Two WDV strains have been identified, one strain infecting wheat and another strain infecting barley. The wheat strain periodically causes severe damage to winter wheat in Sweden while the barley strain has not been found in Sweden. To assess the genetic variability of WDV, leaf samples of wheat and wild grasses along with individuals of the insect vector were collected from different parts of Sweden and an additional wheat sample from Finland. Partial WDV genomes were PCR amplified from leaves and insects and sequenced. The sequences were used for Neighbor-Joining (NJ) analyses, together with available wheat strain sequences from GenBank. The genetic variability of WDV was low, virus isolated from wild grasses and insects were >97% identical to wheat isolates. No clear grouping according to host or geographical origin could be seen. Full sequences from a Hungarian and a Turkish barley isolate were determined. The nucleotide identity between the two isolates was 94.6% and the identity to the wheat strain was ~84%. A NJ tree with partial sequences of the two barley isolates and barley strain isolates, available in GenBank, showed a clear grouping where the Hungarian isolate grouped with the GS1 isolate from Germany while the Turkish isolate grouped with SP isolates from Spain.
Diagnoses of full blossom disease associated pathogens in certification of Ribes.

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Blackcurrant reversion virus (BRV) and phytoplasmas (Aster yellows group – subgroup 16SrI-B and subgroup 16SrI-C, and Apple proliferation group – subgroup 16SrX-A) were identified to be associated with the Full Blossom Disease (FBD) in red and white currants by PCR, RFLP, sequence analysis and electron microscopy. FBD occurs in many currant cultivars (Špak et al (2006) Crop protection 25: 446-453). We have evaluated FBD symptoms, grafting onto indicators ´Jonkheer van Tets´ and ´Baldwin´ recommended by EPPO, and molecular detection of pathogens by PCR, RFLP and sequencing for certification of Ribes. Flower symptoms which are useful for FBD eradication from germplasm, may vary or be missing in different cultivars and years, in particular in poorly flowering propagating materials and indicators. ´Johkheer van Tets´ is more suitable indicator than ´Baldwin´. Difficult isolation of nucleic acid, erratic distribution and low concentration of pathogens, different sampling periods for phytoplasma and BRV are major obstacles with molecular tests. Choice of optimum primers for nested PCR, verification of results by RFLP and/or sequencing and repeated testing are necessary for phytoplasma detection. BRV RT-PCR was found the most expeditive “marker” for the detection of FBD in the certification procedure. This work is supported by grants OC 853.001 of the Ministry of Education and AV0Z50510513 of the Academy of Sciences of the Czech Republic.

Virus resistance in P. vulgaris and Eukaryotic Translation Initiation Factor, eIF4E

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Bean Common Mosaic Virus (BCMV) and Bean Common Mosaic Necrosis Virus (BCMNV) cause the most serious and widespread seed-borne viral disease in bean (P. vulgaris L.). These viruses are controlled by a combination of cultural practices, seed certification programmes, and most importantly genetic resistance. Recessive genes, bc-1, bc-2 and bc-3 together with bc-u confer specific resistance to BCMV and BCMNV. The bc-genes are found at four independent loci and two functional alleles have been reported at the bc-1 locus (bc-1 and bc-12) and the bc-2 locus(bc-2 and bc-22). Breeding for recessive resistance genes is greatly facilitated if closely linked markers are available and effort are therefore made to identify such markers. It has been found that recessive resistance genes against potyviruses in pepper and pea correspond to eIF4E (Ruffel et al (2002) Plant J 32:1067-1075; Gao et al (2004) Plant J 40:376-385) and the homologous factor eIF(iso)4E has been shown to be a host factor for potyvirus infection in A.thaliana (Leilis et al (2002) Cur Biol 12:1046-1051).

To determine whether recessive resistance in bean is correlated to differences in the eIF4E genes, we have initiated an analysis of these genes in bean cultivars carrying different combination of the bc-genes which will be presented.
Gene expression in Etrog citrus in response to *Citrus viroid IIIb* infection.

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Etrog citrus is the most sensitive indicator for the detection of all characterized citrus viroids. In fact, it shows quite different specific symptoms such as leaf epinasty or bending, stunting, necrosis of petiole, mid-vein or leaf tip. *Citrus viroid III* induces very mild effects on Etrog citrus of leaf drooping as a result of petiole bending and only a variable stunting on trifoliate orange and citrange rootstocks. The regulatory bases of such performance is poorly investigated. Therefore we begun a specific study to detect differentially expressed genes in Etrog citrus Arizona 861-S1 grafted on sour orange and bud inoculated with an isolate of CVd IIIb (CMC-CVd-IIIb) already studied since long time by us. Young, almost fully expanded leaves, showing mild leaf bending were collected and processed, as conventionally, by DDRT-PCR technique, in comparison with healthy leaves. Eighteen genes were identified on the basis of their amino acid sequences. Two of them encoded for proteins with unknown function. Thirteen of them were up-regulated while five were down-regulated in response to the viroid infection. The identified genes were mainly involved in plant defence/stress response, signal transduction, amino acid transport, and cell wall structure. Among the up-regulated genes, a regulator of host RNA silencing and a peroxidase were already reported as involved in viroid and RNA virus pathogenicity.
K4.1 Natural selection in plant-pathogen interactions: from models to laboratory to field
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It is widely assumed that fitness costs of host disease resistance and pathogen virulence generate polymorphism at the respective loci. In theoretical models, however, fitness costs are necessary for polymorphism to exist but they are not sufficient, because the stability of polymorphism also depends on the epidemiology of the disease.

In models which include multiple loci, the stability of polymorphism is also determined by the relationship of costs to the number of resistance or virulence alleles. Polymorphism is most likely when the marginal cost of each additional resistance allele declines and but that of additional virulence alleles increases. This conclusion is supported by emerging data on the organisation and evolution of resistance and avirulence genes.

It is also widely assumed that fitness costs are fixed. However, costs of resistances to strobilurin and triazole fungicides vary with environment conditions, both being more costly in situations which are sub-optimal for the fungus. Costs of different virulences vary and may also be affected by the environment. Knowledge about fitness costs allows predictions to be made about the evolution of virulence and fungicide resistance in the agricultural environment and thus their responses to crop protection measures. Costs are generally small, however, implying that the dynamics of a pathogen’s population structure may be dominated by other factors, such as linkage disequilibrium, rather than fitness costs.

O4.1 Variability in partial mlo virulence in the barley powdery mildew population
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While mildew adapts fast to resistance triggered by recognition of elicitors, adaptation to mlo based resistance proceeds slowly in small evolutionary steps. Although mlo cultivars are grown since 1979 and in the EU the area of spring barley with the mlo gene exceeds 50%, generating selection pressure for mlo virulence, highly mlo virulent pathotypes have not yet been found in Europe. Since mlo resistance depends much on environment, i.e. heat stress (Schwarzbach (2001) Czech J. Genet. Plant Breed. 37: 82–87), drought stress (Baker et al. (1998) Plant Pathology 47:401-410) and inoculum quality, partial mlo virulence (PV) is difficult to estimate. A special technique with standard inoculum quality and uniform inoculation at low density was used to monitor the level of PV (as colony number relative to the compatible interaction) of 16 mildew isolates from different parts of Europe. The results were well reproducible in two laboratories. The PV ranged from 0.1% to 6%. Since the laboratory isolate HL-3 (Schwarzbach (1979) Barley Genet. News. 9:85-88) has now a PV of approx. 50%, long durability of mlo based resistance is likely.
Understanding the diversity of Sclerotinia sclerotiorum: a UK perspective
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S. sclerotiorum isolates were collected from crops of lettuce and carrot in the UK and the internal transcribed spacer (ITS) regions of the ribosomal RNA (rRNA) gene repeat was sequenced for 32 isolates per crop. BLAST searches of the NCBI GenBank database confirmed that all isolates were either the same as, or highly similar (1 or 2 nucleotides different) to deposited rRNA gene ITS sequences for S. sclerotiorum. Mycelial compatibility tests showed that for the 32 isolates from lettuce, there was a dominant group of 5 compatible isolates, three groups of 2 compatible isolates with the remaining isolates all incompatible. The same result was true for the S. sclerotiorum isolates from carrot.

Sequence analysis of the rRNA ITS showed that the dominant carrot and lettuce isolates formed a distinct clade which may represent two genetically distinct populations. Additional molecular studies are ongoing including Southern analysis using pLK44.20, a probe that has been shown to distinguish between MCG groups of S. sclerotiorum (Kohn et al, 1991) Phytopath 81: 480-485), to better discern intra- and inter-isolate diversity.

An asexual fungus? The genes say no!
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The plant pathogenic Verticillium species are thought to be asexual with no known sexual stage. If this were the case presumably populations would be clonal and identical except for changes due to internal recombination, mutation etc., however this has been proven not to be the case through genetic and molecular methods. Earlier work has suggested that long-spored isolates of Verticillium from crucifers are genetically amphihaploid and interspecific hybrids. However, to date there is no published direct evidence. This presentation reports two molecular markers (partial β-tubulin gene and an intergenic region from 5S rRNA genes) which, through the retention of two sequence types in long- but not short-spored types strongly supports their hybrid origin. These markers, alongside that of mitochondrial cytochrome B gene for most isolates, indicate that whilst one ‘parent’ of the hybrids was probably V. dahliae-like the other was unlike any studied species and not V. albo-atrum as had been suggested elsewhere. The long-spored isolates have previously been divided into two AFLP groups, α and β, but two of these markers suggest that the second group is not homogenous and we propose that some now be known as group γ. Currently group γ is represented by long-spored isolates from horseradish in Illinois, USA, but other sources may be identified.
A new and more aggressive population of *Puccinia striiformis* f. sp. *tritici* in eastern United States

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Before 2000 in states east of the Rocky Mountains, stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici* (Pst) occurred infrequently and rarely caused losses to winter wheat. The predominant races were among the oldest known in the US, attacked only a few differential lines, and remained similar for >25 years. In 2000, isolates with virulence for resistance genes *Yr8* and *Yr9* were found and caused the most widespread and severe stripe rust epidemic in US history. Since 2000, stripe rust has caused significant losses every year and has become the most important wheat disease. The new population of isolates has completely replaced the old population since 2000. Based on AFLP fingerprints, representative old (before 2000) and new (since 2000) Pst isolates had >85% and >94% similarity, respectively. However, old and new populations had only 55% similarity, indicating that the new isolates were a different genetic background. New and old isolates had similar latent periods and spore germination rates at 12°C. However at 18°C, new isolates averaged 2 days sooner for latent period and double the spore germination rate, indicating that the new isolates were better adapted to warmer temperatures and more aggressive. New isolates also appear to have greater fitness to survive over summer and to initiate overwintering infections. Increased fitness, temperature adaptation, and aggressiveness appear to be more important than new virulence for the success of the new isolates.

Genetic variability of wheat yellow rust at scales from field to continent

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Genetic diversity is generally low at scales from field to region in populations of *Puccinia striiformis* f.s. *tritici*. The diversity is higher when taking into account the population changes that may occur during time, and when considering individuals collected at different yellow rust epidemiological zones and/or continents. Diversity for virulence, assayed via an extended set of differential varieties carrying recognised *Yr*-genes, and diversity at the molecular level, measured by Amplified Fragment Length Polymorphism (AFLP), have been analysed in *P. striiformis* samples from Northwest-Europe, Northeast & South Africa, Western-, Central- and South Asia, USA and Australia. The sampling in NW-Europe covered the UK, France, Germany and Denmark in a period of more than 30 years, whereas most samples from other areas were from recent years. The samples were collected from a wide range of host varieties, field trials and farmers’ fields. Diversity in terms of number of clones (defined by AFLP) was generally low within geographically separated populations. However, in some areas and years, highly divergent clones were observed within a local area, even within the same field, suggesting a very different origin of such individuals. Likewise, we observed identical or very similar clones collected at different continents. Both observations may suggest that spores of the yellow rust fungus potentially may move across very large distances within a relatively short period of time.
O4.6 Population diversity and pathogenicity lifestyles in a broad host range pathogen Colletotrichum acutatum: Molecular approaches

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Colletotrichum acutatum is a cosmopolitan pathogen causing anthracnose diseases on a wide range of hosts including citrus, strawberry, olive, peach, almond and lupin. We are using a range of molecular approaches for understanding the population diversity and pathogenicity lifestyles in this pathogen. Based on ITS, tub2 and his4 sequences, C. acutatum isolates from lupins from worldwide locations were genetically homogeneous and formed a distinct group. On the contrary, C. acutatum isolates from olive within Portugal comprised five molecular groups. Interestingly, phylogenetic analyses of 289 ITS sequences of global C. acutatum populations revealed eight molecular groups A1 - A8 with varying biogeographic association patterns. PCR tests based on ITS and tub2 sequences enabled rapid and reliable detection and differentiation of C. acutatum populations. C. acutatum exhibits different pathogenic strategies on various hosts, but the components regulating these processes are only beginning to be understood. We are using forward and reverse genetic approaches as well as a novel Colletotrichum-Arabidopsis model system to investigate the pathogenicity lifestyles in Colletotrichum. A collection of transformants generated by Agrobacterium T-DNA insertional mutagenesis is being tested for alternations in pathogenicity. Further, cloning and characterisation of G-alpha protein, mapK and pkaC genes involved in signalling is underway to investigate their regulatory role.

O4.7 Chemotaxonomy of large-spored Alternaria from onion, tomato and potato

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Epidemics of large-spored Alternaria species can result in considerable crop losses in tomatoes, potatoes, onions and carrots. In the past, these taxa were treated as either formae specialis of A. porri (Neergaard (1945) Danish Species of Alternaria and Stemphylium) or formae speciales of A. dauci (Joly (1964) Le genre Alternaria). Simmons, on the other hand, treats Alternaria solani, A. porri and A. dauci as distinct species and describes several new Alternaria species on Solanaceae including A. tomatophila ((2000) Mycotaxon 70: 1–115)). These different taxonomic approaches to Alternaria classification have resulted in controversy between plant pathologists and morphologists. Qualitative analysis of secondary metabolite profiles and other phenotypic characters have been shown to have high value in differentiation and classification of Penicillium, Stachybotrys and small-spored Alternaria species (Larsen et al (2001) Appl Environ Microbiol 67: 3630–3635, Andersen et al (2003) Mycologia 95: 1227–1238, Andersen et al (2005) Phytopathology 95: 1021-1029). Presently, there are 14 host specific toxins and more than 200 non-host-specific toxins reported from Alternaria in the literature (Kohmoto and Otani (1991) Experientia 47: 755–790). Multivariate analyses of these host and non-host specific toxin data together with colony characteristics at different temperatures discriminate between A. solani, A. porri and A. dauci and show several separate species from tomatoes.
P4.1 Characterization of European *Puccinia coronata* f. sp. *avenae* isolates by plant pathologic and molecular analyses

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Oat crown rust, caused by *Puccinia coronata* f. sp. *avenae*, is an important disease of oat. Totally 60 isolates of crown rust collected in different countries of Europe and Middle East in 2004-2005 were studied for its physiologic specialization using 16 oat differentials with single resistance genes. Seventeen selected isolates were a subject of RAPD analysis. Isolates from the Czech Republic and Estonia possessed 0-8, resp. 0-7 virulence genes. More than 50 % of isolates were virulent on resistance genes Pc40 and Pc45, whereas Pc50, Pc52, Pc59 and Pc68 were effective. Isolates from Hungary and Serbia & Monte Negro possessed 1-8, resp. 1-9 virulence genes. Pc40, Pc45 and Pc46 were overcome more than 50 % of isolates, genes Pc48, Pc52, Pc59 and Pc68 proved to be effective. Isolates from Israel possessing 4-12 virulence genes had the highest range of virulence. Genes Pc40, Pc45, Pc46 and Pc54 were overcome more than 62.5 % of isolates, whereas Pc52, Pc56, Pc62 and Pc68 were effective.

From 50 random primers screened, 46 gave readable results; 45 were found to be polymorphic, 1 primer had monomorphic pattern. No primer alone could differentiate all the isolates. Totally 3326 amplified fragments were scored, 62.9 % were polymorphic. The indexes of similarity ranged from 0.69 to 0.90. The dendrogram showed four associated groups and three detached isolates.

The study was financially supported by the Ministry of Agriculture of the Czech Republic (project MZE no. 0002700603).

P4.2 Study on defoliate and non-defoliate fungal isolates of *Verticillium dahliae* Kleb. from olive trees in orchards of Iran

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Verticillium wilt of olive trees is a fungal agent of serious disease in olive trees orchards of Golestan province northeast of Iran. In infected trees the leaves become brown and weak due to disorder in root and vascular system. Later the trees dry and defoliate. Based on sampling of twigs from different area of olive orchards such as: Hashem-abad, Kordkoy road, Ali-abad road, Shastkalate, Toshan, Karkande and Alang area being collected and various fungal isolates identified. Defoliate and Non-defoliate isolates were determined by significant differences in reaction of optimum temperature, dimension of microsclerotia, reaction of cotton (Cv. Varamin) seedling, and pathogenicity of native olive (Cv.Zard-Roghani) twigs. There was an elongated microsclerotia by defoliate isolate in water-agar medium rather than by non-defoliate isolates. Microsclerotia dimension in olive isolates was 112x 28µ significantly larger than cotton isolates 58x21µ. Optimum temperatures in defoliate and non-defoliate isolates were 27 °C and 23 °C respectively. Defoliate isolates of cotton on varamin cultivar seedlings occurred severe wilting, while non-defoliate isolates showed symptom of chlorosis with mild wilting. In pathogenicity test by defoliate isolate on the twigs of olive trees Zard-Roghani cultivar caused rolling and twisting of leaves.
P4.3 Diversity of fungal pathogens incidence in the Czech traditional regions of caraway cultivation
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During growing seasons 2003 – 2005 the occurrence of biennial caraway pathogens was studied in several localities of the Czech Republic. Monitoring was carried out at one farm which began to cultivate caraway (Supíkovice) and at three traditional ones (Sumperk, Bohuslavice, Maleč). Septoria carvi was the most frequent pathogen observed in each sample but its economical importance in the field was not high. Powdery mildew caused by Erysiphe heraclei was more dangerous pathogen causing complete overgrowing of leaves, stems and seeds before harvest. Seeds infested by powdery mildew are not convenient for direct human consumption and they may be utilised only for processing by distillation. Sclerotinia sclerotiorum is considered to be the most dangerous disease of caraway at present. Importance of this fungus grows in last few years rapidly. It is probably caused by increasing of host plants cultivating areas (rapeseed, sunflower and others). Low incidence of anthracnose (Mycocentrospora acerina) in these seasons is caused by changes of climate or by preventive fungicide applications. Differences among regions were observed in fungal incidence as well as in severity of damages. This work was financially supported by the Ministry of Agriculture of the Czech Republic, Project No. QF4056

P4.4 Investigation on pigweed response to fungal isolates Verticillium dahliae from cotton and olive tree in north east of Iran
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Based on host range distribution of fungus Verticillium dahliae and its survival on various weeds, and symptom determination of pathotypes and its comparison with symptom on cotton could be clear status such host by pathogenicity tests and their susceptibility. Pigweed (Amaranthus retroflexous) is an important host of cotton field and olive orchard, and susceptible to fungus Verticillium wilt. In this survey, pigweed response to fungal isolates of defoliate and nondefoliate determined that their effect is similar on pigweed. In pigweed the first symptom of disease is apparently appeared 20 days after inoculation. But, in cotton the first symptom of disease is observed 30 days after inoculation. Disease symptom of fungal isolates collected from olive trees appeared as defoliate on pigweed and in cotton was chlorosis associated with wilting. Through fungal isolates only G1 from cotton caused seedlings decline of cotton and pigweed. Disease severity of cotton isolates was less on pigweed than cotton plant. Means of colony growth rate of fungal isolates were significantly different by range of temperatures (23,25,27,29°C) that separated from each other. Isolates of B1, D3, and F3 showed disease severity more than 50% on cotton and only 50% on pigweed. But isolates of G1, G2, and G5 showed disease severity respectively, 75-100% on cotton and 8.3-100 on pigweed. There were differences between isolates of microsclerotia by size and their shape as observed irregularly shaped and spherical with small cells.
P4.5  Geographical distribution of Dutch elm disease decline in north of Iran
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In order to determine distribution of dutch elm disease, sampling of elm trees is followed in the forest area of north of Iran. During period of the fungal isolation from twigs associated with symptom of wilting and changing leaves to brown, an asexual stage of fungus observed as conidia and conidiophore with typical dentical hyphae tips identified, as Sporotrix sp. and described. Growth of colony diameter in vitro on the media Malt extract agar was different from various area. The sexual stage of the pathogen associated with production of the perithecia that only produced on elm sapwood agar by crossing of heterotal hyphae from various area. While during sampling in forest area was not observed on the twigs. Both species of the fungal of elm decline, isolated and identified as Ophiostoma ulmi and O.novouulmi from the forest area. Percentage of disease distribution was significantly different in various area of forest. Population of aggressive pathogen O.novo-ulmi is increasing in north of Iran. Pathogenicity of this species is higher than O.ulmi. Since, another species of trees from ulmaceae, called Caucasian tree (Zelkova carpinifolia) is attacked by pathogens in provinces of Guilan and Golestan. Decline of diseased trees between both species of elm trees (ulmus spp.) was more on U. carpinifolia than U.glabra in north forest. Both tree species were susceptible to the pathogen and disease symptom appeared in seedlings after inoculation.

P4.6  Characterization of Phytophthora infestans isolates in the Czech Republic from years 2004 and 2005
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Late blight is a worldwide spread and economically important disease of potato and tomato crops. The causal agent Phytophthora infestans is able to reproduce both asexual and sexual ways, thus mutation and sexual spores (oospores) formation affect genetic variability increase of pathogen populations. Collection of 206 isolates of P. infestans created in 2004–2005 was used for determination of basic markers as mating types, resistance to metalaxyl and dimethomorph and haplotypes of mitochondrial DNA (mtDNA). A1 and A2 mating types were determined by pairing A1 and A2 testers and unknown isolate on medium in Petri dishes and by CAPs method. 120 isolates were determined as A1 mating type and 86 isolates as A2 mating type. Resistance test to systemic fungicides (metalaxyl, dimethomorph) was performed by in vitro test on agar completed by different concentrations of active substance. Among 101 isolates tested to metalaxyl, 97 isolates were sensitive and 4 isolates were intermediate. From 60 isolates tested to dimethomorph all isolates were sensitive. Haplotypes of mtDNA were analysed on the basis of PCR by two sets of primers and subsequent restriction digestion with EcoRI and MspI. From 138 analysed isolates, 118 isolates corresponded to Ia haplotype and 20 isolates corresponded to IIA haplotype. This work was supported by the Grant Agency of the Agriculture Research (NAZV) of the Ministry of Agriculture of the Czech Republic, grant QG 50055.
Willow leaf rust (*Melampsora* sp.) in Estonian willow plantations

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Willow plantations are widespread in many European countries e.g. in UK, Sweden, and Germany. In Nordic countries, *Salix viminalis* L. and *S. dasyclados* Wimm species are mostly used as bioenergy source to provide an alternative to fossil fuels. The most widespread and frequent disease in willow plantations is leaf rust, caused by *Melampsora epitea*, which may lead to premature defoliation and serious yield losses (McCracken and Dawson (1998) *Eur J of For Path* 28:241-250). Furthermore, damaged plants may become more susceptible to other pathogens and frost (Voegele and Mendgen (2003) *New Phytol* 159:93-100).

During three years, the abundance of willow rust has been assessed in different plantations on different clones in Estonia. The most susceptible to willow rust was *S. dasyclados*; *S. viminalis* was generally more resistant. In addition to willow species, the amount of uredinia on leaves also depended on fertilization, year and plantation (Toome et al. (2006) *Biology.Ecology* 3). The isolates from different willow plantations were collected to describe Estonian willow rust population. Rust spores from diseased leaves were inoculated on healthy leaves to get single spore isolates. Harvested spores will be used to test their virulence on the set of willow clones (pathotyping). Additionally AFLP fingerprinting will be used to study the genetic variation of the fungus and to do molecular mapping. In the experiment, two distinct populations (Estonian and Swedish) will be compared.

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Morphological and molecular characterization of *Monilinia* species in Norwegian fruit production

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*Monilinia laxa*, *M. fructigena* and *M. fructicola* cause brown rot of stone and pome fruits, which result in considerable economic losses. *M. fructigena* causes fruit decay in both pome and stone fruit, while *M. laxa* causes blossom wilt, twig blight, canker and fruit rot on stone fruit. A special form of the fungus, *M. laxa* f.sp. *mali*, is found only in apple, where it causes blossom wilt, spur-kill and canker. *M. fructicola* is a quarantine pathogen in the EU, and it is thus of important to have reliable identification methods and to monitor the population of these fruit pathogens. More than 90 isolates of *Monilinia* spp. from the main fruit producing districts in Norway was collected. The isolates originated from cherry, plum, apple, apricot and peach. They were identified by growth characteristics in culture according to EPPO standards (EPPO Bull 33: 245-47) and by a multiplex PCR method (Côté (2004) *Plant Disease* 88:1219-1225). Thus far, the quarantine organism *M. fructicola* has not been detected in Norway. *M. laxa* and *M. fructigena* were isolated from plum, apricot, cherry and apple, however in apple *M. fructigena* was isolated only from fruits and *M. laxa* only from infected fruit spurs (generative shoots). In peach only *M. laxa* was detected. Genetic variation among the isolates is currently being examined using AFLP.
P4.9 Aggressiveness of *Puccinia striiformis* f. sp. *tritici* isolates, relationship to pathotype and AFLP fingerprint group, and implications for durable resistance

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Since 2000, stripe (yellow) rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), has spread to regions in the US where it had not been found previously and has become more severe in other regions where it previously had caused minor damage. New isolates of Pst with virulence for *Yr8* and *Yr9* that first appeared in eastern US in 2000 quickly replaced the old population that had existed there for >25 years. Compared to Pst isolates collected before 2000, isolates collected since 2000 were in different AFLP fingerprint groups and had shorter latent periods and higher spore germination at 18ºC. Isolates with pathotypes and AFLP fingerprints similar to recent isolates from eastern US have been found in diverse regions around the world. The objectives of this study were to determine the relationship of aggressiveness to pathotype and AFLP fingerprint group and to determine if isolates with increased aggressiveness can erode adult-plant resistance that has been perceived as durable. Aggressiveness and temperature adaptation of a diverse global set of old (before 2000) and recent Pst isolates was quantified on seedlings based on latent period and lesion size at cool and warm temperature regimes. A representative subset of isolates was evaluated for aggressiveness at both temperature regimes on flag leaves of three cultivars. The role of temperature adaptation and aggressiveness in recent stripe rust epidemics will be discussed.

P4.10 Settling velocity and dispersion of powdery mildew conidia in still and turbulent air in a settling tower

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The settling velocity of *Blumeria graminis* conidia of 1.2 cm/sec, mentioned by Yearwood and Hazen (1942, Science 96:316-317) and adopted by Gregory (1973, The microbiology of the Atmosphere) underestimates the settling of spores harvested from leaf segments on agar. Fresh *B.g. hordei* conidia from moderately inoculated primary leaf segments, when released in still air from a point at the top of a 65 cm high settling tower, settled at velocities between 1 and 5 cm/sec, following a normal statistical velocity distribution with a mean from 2 to 3 cm/sec, depending on the particular experiment and the inoculum. 95% of the spores settled centrally within a 25 mm radius and an angle of approx. 4º. Slightly slower velocities, but within the same settling angle, were obtained with *B.g. tritici*. Under turbulent conditions, when the inoculum was first dispersed in air and then blown into the tower through circularly arranged holes at the top, the velocity distribution was more flat and slightly skewed towards lower velocity, with 95% between 1 and 26 cm/sec. Spore distribution at the bottom of the tower after turbulent settling approached uniformity, close to the expected Poisson distribution.
P4.11 Variation in Venturia inaequalis between UK and Chinese populations

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A total 92 scab isolates (38 from UK, 50 from China and 4 from India) were screened for AFLP markers to investigate the extent of genetic differentiation between UK and Chinese populations. There were significant differences between UK and Chinese scab populations. The origin (geographical site and variety) of Chinese isolates did not have noticeable effects on the fungal genetic variation. In contrast, UK isolates from four different cultivars differed significantly, especially the isolates from cvs. Gala and Worcester. In addition, scab isolates from Cox were particularly diverse. We have further selected a number of isolates (up to 10) from each of the selected three varieties in India, UK and China and then inoculated these isolates individually against each selected cultivar. Interestingly, none of Indian and Chinese isolates can infect cv. Cox. Furthermore, the selected local cultivars in China and India also appeared to be resistant against isolates from other regions.

P4.12 Genetic drift in the czech population of Blumeria graminis f. sp. hordei.

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Powdery mildew caused by fungus Blumeria graminis f.sp. hordei (=Bgh) is the most frequent trigger of epidemics in both spring and winter barley (Hordeum vulgare L.) in the Czech Republic. Genetic resistance is an economic and safe alternative of the disease control. The resistance of a significant proportion of cultivars of the two barley types is based on specific resistance genes. That is one of the reasons why we study a genetic structure of the pathogen population. Interannual changes in the frequency of individual virulences have been small during the last years. In 2002, the sum of frequencies in 12 selected virulences accounted for 912% (= virulence complexity 9.12). The winter 2002/2003 was extremely unfavourable for surviving winter barley (strong frosts without a snow cover) and a large proportion of the crop stands had to be ploughed in. It also adversely affected the consecutive size of the Bgh population. In the late spring (May/June) of 2003, we collected from the air less than 1% of a usual number of conidia (a bottleneck). In 2004, we examined 262 isolates and found a dramatical decrease in the virulence complexity (7.96, i.e. 1.16 less than in 2002). Though the virulence complexity increased to 8.47 in 2005, it was still bellow the level of previous years. It seems then that unfavourable conditions of the 2002/2003 winter induced a substantial genetic drift in the pathogen population structure which led in this case towards the decrease in frequencies in 10 out of 12 examined virulences.
PCR detection of fumonisin-producing *Fusarium* isolates from Piedmont

Ivan Visentin, Doriana Francia, Danila Valentino, Giacomo Tamietti, Francesca Cardinale

Fumonisins, mycotoxins produced mainly by *Fusarium verticillioides* and *F. proliferatum*, contaminate maize and maize-based products notably in Northern Italy, causing great concern for human and animal health. Diagnostic primers were reported that should afford tracing of fumonisin-producing fungi in kernels, but they await through validation on strains of very different geographical origin. In this study, we collected 100 isolates of *Fusarium* producing catenulate microconidia from maize grown in various localities of Piedmont (North-western Italy), and ascribed them to *Fusarium verticillioides* or *F. proliferatum* based on morphological traits and amplification by species-specific primers for *F. verticillioides* (Patino et al J Food Prot 67:1278-1283). The collection is being analysed also for fumonisin production on maize kernels as well as with a second pair of primers designed on the key fumonisin biosynthetic gene FUM1 (Bluhm et al (2002) J Food Prot 65:1955-1961). This last set had never been tested on non-toxigenic strains from maize; it is indeed not known if, in these strains, the FUM1 gene is deleted, mis-regulated and/or mutated. Preliminary results identify interesting strains whose toxin production is not correlated to the FUM1 amplicon. An ITS-RFLP analysis is also being carried out to the additional purpose of estimating the level of genetic variability in our collection and possibly correlate it to toxin production and FUM1 amplifiability.

Development of molecular markers for the obligate parasite *Hyaloperonospora parasitica* on vegetable brassicas

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Oomycetes cause economically destructive plant diseases in many vegetable and arable cropping systems. Downy mildew of brassica (*H. parasitica*) is a common diseases on seedlings raised in glasshouses for transplanting in the field. Up to nine applications of fungicide are used to control these diseases in the glasshouse, which has often resulted in the appearance of fungicide insensitive variants in the pathogen populations. Any occurrence of fungicide resistance reduces the effectiveness of this type of control option. A strategy based on the deployment of downy mildew resistant cultivars have been used in lettuce to maintain the usage of effective fungicides (such as metalaxyl) in the presence of fungicide insensitivity within the pathogen population. Alternative deployment strategies, which minimizes the dependence on fungicides, have been used successfully in cereals for controlling rust diseases while maintaining the effectiveness of disease resistance. Such approaches rely on advice to growers involving rotation of resistant cultivars that is informed by monitoring of changes in virulence of the pathogen population.

In a new research project it is intended to use downy mildew on vegetable brassica crops as a system for investigating pathogenic population changes in response to cultivar deployment. A key objective in this project is to develop molecular markers from such genes for population studies. Recently new information on the molecular basis of pathogenicity in *H. parasitica* from Arabidopsis has identified an initial group for 25 putative pathogenicity genes (Ppat genes) that could potentially be used to detect genetic variation in populations of downy mildew pathogens in horticultural crops. Non-coding regions are also being investigated to try to identify neutral markers. Progress in developing markers suitable for studying changes in populations of *H. parasitica* will be described.
K5.1  The basic reproduction number, $R_0$.
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The basic reproduction number, $R_0$, number of newly infected individuals arising from the introduction of one infectious individual into a susceptible population during that individual's period of infectiousness, is the threshold quantity determining invasion ($R_0>1$) or non-invasion ($R_0<1$) of plant diseases. $R_0$ can be calculated from information on the pathogen life cycle and a description of the agronomy of the host. A method will be described using matrix notation, which opens the possibility of sensitivity analysis as developed for matrix projection models (Caswell, 2001). It will be shown how to incorporate environmental factors, such as weather, in the calculation of $R_0$. $R_0$ can be used to evaluate disease control strategies. This will be illustrated using a host grown in fields for which planting material is derived from a nursery. Extending the definition of the basic reproduction number slightly, the invasion of a new pathogen strain in a background of an existing strain or mixture of strains can be analysed. This will be shown using the invasion of fungicide resistance stains in a background population of fungicide sensitive stains. The same methodology can be used to calculate the evolutionary trajectories of pathogens strains with the aid of pair wise invasibility plots. This method will be described using the evolution of the multiplication rate of viruses as key example.


O5.1  Revealing concealed processes in airborne plant disease dispersal
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Plant disease dispersal is the result of complex biophysical processes that are often neither directly observable nor accessible to experimentation. Classic models of disease dispersal based on averaged disease measurements poorly account for the actual variability of the field data, which is caused by concealed processes. In airborne diseases like the cereal rusts, the relevant concealed processes may depend on the spatial scale. We consider here the first cycle of disease dispersal from a single disease focus, either in the close neighborhood of the focus (80 x 60 cm) or to remote areas (up to 350 m). We use a new, generic modelling framework to analyze the results of field experiments made either with brown rust (smaller scale) or yellow rust (larger scale) of wheat. We show that the spatial variability of disease resulting from one cycle of spore dispersal can be explained by the heterogeneity of the support (e. g. spatialized differences in leaf receptivity) on the smaller scale and the anisotropy of individual spore dispersal (in distance and direction) on the larger scale. The next step shall be the integration of time-dependent processes in a complete model of the spatio-temporal spread of an epidemic on different scales.
Matrix projection methods in analysing multiple disease complexes

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Despite the fact that crops are routinely attacked by several pathogens simultaneously, plant disease epidemiology has developed largely as a discipline dealing with individual diseases. The situation is slightly different in IPM, where an acceptance of the need to tackle multiple disease complexes is widespread. In low input systems two issues can be expected to be more important than in intensively managed systems. First, interactions among pathogens (and between pathogens and other micro-organisms) are more likely, and might have impacts on disease dynamics and yield loss. Secondly, choices made about intervention in the system to control one pathogen may have effects on other pathogens. In this way, the population dynamics of one pathogen may be subject to exogenous perturbations which are correlated with the dynamics of one of the other pathogens in the disease complex. McRoberts et al. (2003) Austr. Plant Pathol 32: 167-180, highlighted the potential value of generalised Lotka-Volterra equations in framing disease management questions bearing these sorts of issues in mind. Here, we illustrate the potential of an alternative modelling approach – matrix projection – in the same general areas of application. In particular we illustrate how the matrix projection approach generates components describing the intrinsic microbeial community dynamics for pathogens complexes and components describing the effects of exogenous control selected by the crop manager.

Combining the new with old -- a Bayesian view.

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Bayesian methods represent a departure from traditional statistics but provide a framework for data analysis that contains ideas that are easily interpretable. Key concepts in Bayesian methods include the use of random variables to describe parameters, the use of distributions to describe this variability, and the ability to combine existing information with current data to draw conclusions. Whereas analytical solutions in Bayesian analysis are possible only for very simple cases, the use of Markov Chain Monte Carlo methods (MCMC) introduces flexibility to Bayesian data analysis and even offers possibilities not always found in traditional data analysis. The relative roles of the existing information (the priors in Bayesian terms) and the likelihood (derived from the data) in producing the new, updated information is often only viewed in a mathematic form. These concepts also have parallels that are similar to everyday logic in a variety of situations that require combining new information with existing knowledge.
P5.1
In John Colhoun Poster Competition

The role of plant pathogens in altering the population dynamics of a biennial host

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Host-pathogen interactions are important factors in host population dynamics, by altering host reproductive capability, producing debilitating effects and causing mortality in the host, and additionally affecting fecundity and recruitment. Epidemics occur when there are favourable interactions between the host and pathogen, dependent on the plant density or frequency of susceptible (S), infectious (I) and no-longer-susceptible (R) plants. In order to investigate the manner in which a pathogen affects a biennial host population dynamics, an SIR type model was developed with between and within year mortality, seeding, and infection rates, and where susceptible 1st year S plants are infected by infectious 2nd year I plants. During the dormant period the pathogen becomes systemic. With re-growth the infected 2nd year plants (I) transmit the disease to the current generation of S plants, whilst only the R plants set seed to produce the next generation of S plants. All 2nd year plants die at the end of the season. The model is being used in epidemiological studies of the autoecious, demicyclic rust Puccinia hysterium on the biennial plant Tragopogon pratensis in nutrient structured grasslands. It is possible to produce outcomes where infection causes either host population outbreak cycles, stable densities or population crashes. The model is being expanded with different forms of density and frequency dependence, a seed bank and a possible third year in the host life cycle.

P5.2

Sporulation of Bremia lactucae is affected by diurnal periodicity in combination with temperature and light

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Light is a common component of forecasting models for downy mildews because its presence is assumed to suppress sporulation. Our objectives were to evaluate the effects of light intensity and quality, temperature, and diurnal periodicity on sporulation of Bremia lactucae, the causal agent of lettuce downy mildew. We found that the suppressive effect of light on sporulation was strongly dependent upon temperature; there was little suppression of sporulation by light at ≤ 10 ºC. At temperatures where light suppressed sporulation, light in the range from 450-500 nm had the most suppressive effect, although a lesser effect of the wavebands from 450-500 nm was detected. At 15 ºC, a diurnal pattern of sporulation was observed independent of light and darkness. In current forecasting models, the time of sunrise and sunset are used to delimit the dark period when leaf wetness and high RH can induce sporulation. In Nordic countries the effects of short nights and extended twilight conditions should be incorporated into forecasting models. Also, since sporulation may be greatly reduced by light at temperatures above 15-20 ºC, this should be used to modify model predictions of sporulation during the time of day when sporulation can occur. The diurnal rhythm could interact with light and temperature to confound the results of controlled environment studies, and may be the controlling factor in timing of sporulation at low temperatures.
P5.3 Interactions between fungal plant pathogens on leaves
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Multiple diseases are expected to become an increasingly important issue when the demand to reduce pesticide input and organically cultivated areas are increasing. This review concerns foliar pathogens on their host species and covers any plant-pathogen system. It was found that pathogen interactions can affect the total disease level in the field, and that antagonistic as well as synergistic effects may be seen. The arrival time of a pathogen is an important factor in the response of a multiple pathogen system along with the nutritional association of the fungus with the host. Studies under controlled environmental conditions tend to show more pronounced effects than field trials. Therefore, the necessity of further field studies of interactions between naturally developing pathogens is emphasized. For foliar pathogens that depend on leaf area, it is a non-trivial task to distinguish interference competition (antagonism) and exploitation competition (density dependence), and the theoretical background to analysing these interactions is discussed.

P5.4 Forecasting Sclerotinia disease in field grown lettuce
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Sclerotinia disease caused by \textit{Sclerotinia sclerotiorum} is a major pathogen of field grown lettuce and other horticultural crops. Sclerotia of the fungus reside in the soil and germinate to form apothecia. These then release airborne ascospores which infect plants. In the UK, control of the disease relies on foliar fungicides to prevent infection but timing the limited number of sprays allowed is difficult. To address this problem, a forecasting system for Sclerotinia disease was developed based on the effect of environmental factors on appearance of apothecia (and hence release of ascospores) and lettuce infection. Controlled environment (CE) experiments showed that soil temperature and water content were critical for germination of sclerotia while air temperature and humidity influenced infection and disease. A period of cold conditioning was also found to enhance germination of sclerotia. Mathematical relationships derived from the CE experiments were incorporated into models for germination of sclerotia and disease development in lettuce and validated using field data. Work starting in Oct 2006 will investigate how these models can be used most effectively in timing fungicide sprays for control of Sclerotinia in commercial crops.
The Concerted Action on potato late blight ‘Eucablight’ (www.eucablight.org) was launched in 2003 to promote collaboration between researchers across Europe and to collate the previously fragmented data on host resistance to late blight and characterization of its causal pathogen, Phytophthora infestans. Central to the project was the development of standardized protocols, databases and data collection tools with which data from across Europe could be centralized. The ultimate aim was to capitalize on the vast resource of data available in order to allow a pan-European analysis to be conducted. The P. infestans database is currently populated with information relating to over 13,800 isolates from 20 European countries. The host resistance database holds primary disease data and derived statistics from more than 50 field trials assessing mainly foliage blight resistance. The use of seven standard cultivars in these trials facilitated the comparison of resistance information across years and regions. The host database is structured, and made accessible, in such a way that DSS builders can access the model parameters they need to construct locally adapted forecasting systems. In this presentation we describe the data collection and data analyzing tools that have been developed in this project and how they are directly applicable to other host-pathogen systems.
Epidemic development of yellow rust (*Puccinia striiformis*) in a growing wheat crop analysed by a discrete time growth model

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Epidemics caused by polycyclic plant diseases are often observed under conditions not meeting the assumptions of classical growth models. Under field conditions, changes in weather and host canopy size may influence the development of disease. The application of discrete time growth models, which relate disease severity at one time-point (*y*ₜ₊₁) to disease severity at a preceding time-point (*y*ₜ), may be useful in the characterization and estimation of epidemic parameters within sub-intervals of epidemics. In a field experiment, epidemics of wheat yellow rust were initiated by point inoculations centrally in large field plots and observed at growth stages 32, 50, 61, and 75. Within three consecutive periods, the corresponding epidemic development was analyzed by an extended discrete time logistic model: 

\[ y_{t+1}(y_t) = \lambda_t*(y_t+m_t)/(1+(\lambda_t-1)*(y_t+m_t)/K_t^*) \]

where *y*ₜ is the disease severity at time *t*, *λ*ₜ is the finite epidemic rate, *m*ₜ is the disease severity equivalent of spore influx, and *K*ₜ*^* is the amplitude (equal to max. disease severity if *m*ₜ = 0). During the first and the third period the leaf resource changed due to flag leaf emergence and/or leaf senescence. An approximate correction for canopy changes was done by estimating disease severities on all leaves. Relative to analysis by classical continuous time growth models the determination of a period-specific maximum disease severity *K*ₜ is novel. The model was well suited for estimation of period-specific epidemic parameters.
Mechanisms modulating fungal attack in postharvest pathogens interactions and their control
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In the association with pathogen, fruits and vegetables evolved an impressive array of defensive tools. At the same time, pathogen developed mechanisms to comprise fruit and vegetable resistant mechanism in what must have been an evolutionary game. Observations indicate that postharvest host-pathogen interactions are characterized by i. multiple factors of host response affecting resistance and ii. Specific fungal factors that can modulate pathogenicity. Modulation of fungal pathogenicity can be obtained by activating the signal transduction mechanism, metabolizing inhibitory factors and changing the ambient pH where colonization takes place. Host ambient pH and other nutritional factors are important in that they determine the ability of the pathogen to successfully colonize and invade the targeted host, with the aid of secreted pathogenicity factors. Since pH is a critical consideration in the attack strategy of postharvest pathogen, they have developed environmental sensing mechanisms that enable it to tailor ambient conditions, by acidification and alkalinization, to best fit its offensive arsenal. What are the mechanisms used by the pathogen and how could them be modulated to affect fungal colonization? Recent achievements will be summarized and their use as new approach for postharvest disease control will be discussed.

Occurrence of latent Botrytis cinerea in some cut flower and pot plant species.
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Grey mould (Botrytis cinerea) is a major cause of post-harvest damage in cut flowers and pot plants resulting in losses of over £14 million annually in the UK alone. Infections can occur throughout the supply chain and may remain latent until produce is at the supermarket. Commercial crops of cyclamen, lily and poinsettia were examined for latent botrytis by a paraquat test (Holz et al (2003) Plant Dis 87: 351-358) and by real-time PCR (Suarez et al (2005) Plant Physiol Biochem 43: 890-899) to determine the occurrence of symptomless infection during crop production. Consignments of gerbera flowers received at a packhouse were tested monthly.

B. cinerea was commonly detected in apparently healthy leaves and flowers. In some crops of cyclamen and poinsettia, over 50% of plants were symptomlessly infected at dispatch and there was evidence that incidence of infection, as measured by the paraquat test, and B. cinerea DNA quantity, as measured by PCR, increased with crop age. In gerbera flowers, symptomless B. cinerea was detected at a greater incidence in winter than at other times. The possibility of testing samples to identify crops with greater levels of latent botrytis, as a means of reducing the occurrence of post-harvest botrytis spot and rot, will be discussed. This work was funded by the Department for Environment, Food and Rural Affairs, Carlton Lodge Nursery, Double H Nurseries Ltd, Flower Plus Ltd, Marks & Spencer plc and W J Findon & Son Ltd.
O6.2 Cavity spot and liquorice rot development in carrots during cold storage

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Carrots are cold stored up to eight months in Denmark in order to supply consumers with fresh carrots throughout the winter season. Cavity spot caused by *Pythium* spp. generally reduces quality of stored carrot, and a storage period of more than four months favors development of liquorice rot caused by *Mycocentrospora acerina*. The importance of initial cavity spot for disease development during storage and the succession of fungi in cavity spot symptoms were studied. A time course study with five observations through six months of storage was carried out with three carrot lots and two commercial storage facilities. The study indicated that initial cavity spot symptoms were caused by *Pythium sylvaticum* and *P. violae* and that the spots were invaded by *P. intermedium*, *M. acerina*, *Cylindrocarpon* and *Fusarium* after a few weeks. A general increase in number of cavity spots observed during storage is probably due to latent infections, as spread from initial cavity spots could not be demonstrated. Neither did initial cavity spots extend in size during storage. Fast cooling and stable cooling temperature generally suppressed disease development. Liquorice rot seemed to develop independently of cavity spot lesions, although *M. acerina* frequently had colonized the cavity spots. This indicates deep wounds and carrot maturity to be the most important factors for liquorice rot development.

O6.3 PCR–based detection of carrot pathogens in soil samples for prediction of disease development in the growing season and during storage

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Cavity spot (*Pythium* spp.), liquorice rot (*Mycocentrospora acerina*) and crater rot (*Fibularhizoctonia carotae*) are important carrot diseases. Cavity spot develops during the growing season, while the latter two are post harvest diseases. Five *Pythium* "species" were identified as agents of cavity spot in Norway; *P. intermedium*, *P. sulcatum*, *P. sylvaticum*, *P. violae* and *P. "vipa"* (probably a new species). Based on unique sequence regions in the ITS, PCR primers were designed to identify the five *Pythium* "species" and the two post harvest pathogens. PCR detection was species-specific with no cross-reaction to other *Pythium* species or to other fungal isolates from carrots. At different points in the growing season, soil and carrots were sampled from farm fields in Norway. PCR assays allowed detection of pathogens in both soil and carrot tissue. PCR results from samples of soil adhering to roots late in the growing season corresponded well with the incidence of cavity spot and liquorice rot on carrots after 6 months storage. PCR assay data from soil samples taken within 14 days after sowing carrots also predicted cavity spot incidence reasonably well. There was little incidence of crater rot during the experimental period, which did not allow proper evaluation of the test for *F. carotae*. Commercial testing of carrot soils is now available through the company “Carrotech” and work is in progress to implement quantitative PCR.
The biosynthesis pathway of aurofusarin in Fusarium graminearum

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The plant pathogenic fungi Fusarium graminearum and related species are characterised by their red to pink pigmentation. The colour is due to their biosynthesis of the dimeric polyketide aurofusarin. Ingestion of aurofusarin contaminated feeder by poultry has been shown to reduce the egg quality by affecting the A and E vitamin concentration and the composition of fatty acids. We have successfully characterised the biosynthesis of aurofusarin by targeted gene deletions using Agrobacterium tumefaciens mediated transformation followed by chemical characterization of intermediates using HPLC-UV, high resolution MS and C-NMR. The required genes are found in a 24 kb large gene cluster as known from other secondary metabolite systems. The pathway consists of five enzymatic steps. The initial step is catalyzed by the polyketide synthase PKS12 and results in formation of the monomeric naphthopyrone compound nor-rubrofusarin. The initial PKS product is subjected to modification by tailoring enzymes including an O-methyltransferase (AurJ), a monooxygenase (fmo), an oxidoreductase (OxidoR) and a laccase (Gip1) resulting in the dimeric naphthoquinone aurofusarin. This is the first evidence of a direct link between the biosynthesis of naphthopyrones and naphtoquinones. The function of aurofusarin for the producing fungi is unknown. Infection studies with mutants defect in aurofusarin production has shown that these are as pathogenic as the wild type.

Toxigenic micromycetes and their mycotoxins associated with transgenic Bt-maize and nontransgenic hybrids of maize

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During the period 2002-2005, toxigenic microfungi (Fusarium and Penicillium) associated with preharvest corn (Zea mays) damaged by European corn borer (ECB) were studied on different localities in the Czech Republic. The main aim of this study was to compare a level of contamination by toxigenic micromycetes in 1) transgenic Bt-maize hybrid (protected against ECB with gene for delta-endotoxin from soil bacterium Bacillus thuringiensis), 2) maize hybrid treated with parasitic Trichogramma wasp (biological control of ECB) and 3) maize hybrids without any protection against ECB. In total, 15 taxa of genus Fusarium and 9 taxa of genus Penicillium were recorded. It was found that the occurrence of toxigenic species in Bt-maize and nontransgenic hybrids was similar regardless of different damage of plants, caused by larvae of European corn borer (ECB). However, frequency of Fusarium species in Bt-maize (with mechanical damage only) was significantly lower in comparison with nontransgenic hybrids. Damage of plants caused by ECB differed according to locality. Bt-maize showed resistance to ECB, there were no damaged plants during our survey. Relationship among Bt-maize, occurrence of toxigenic species and content of selected mycotoxins (FUM, DON, ZEA, T-2 toxin) was confirmed by lower occurrence of the mycotoxins in grain of Bt-maize.

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Even though mycotoxicin contamination outbreaks have never been reported on durum wheat (Triticum durum L.) in Sicily, in consideration of the risk that mycotoxin contaminations represent for human health, a study has been undertaken to estimate the presence of potentially mycotoxin fungi contamination of air environments and wheat grain samples in Sicily (South Italy). Samples of air and wheat grains were collected from field, warehouses and farmer’s cooperative storey-buildings and examined to estimate the concentration of total fungi. Fusarium, Alternaria, Aspergillus and Penicillium spp. were isolated and identified. Fusarium and Alternaria spp. were dominant in air and grain samples from field, whereas Penicillium and Aspergillus spp. in mycoflora from warehouses and storey-buildings both in air and grain samples. The highest concentration of total fungi was found in air and grain samples from field. Of all the isolates, Aspergillus and Penicillium spp. were predominant in air and grain samples from warehouses, and among Aspergillus spp., A. ochraceus and A. niger were the most common isolates. Aflatoxin positive A. flavus strains were identified on CAM and were found in field and in storage environments both in air and wheat grain samples. According to the results obtained it’s possible to underline that the mycotoxin fungi contamination is a potential risk present also in Sicily and that the hygienic-sanitary practices are important to maintain uncontaminated grains.

Fungi are the most important spoiling organisms in cereal grains. The mycoflora of winter wheat seeds consisted primarily of Deuteromycetes and some Ascomycetes. As expected, yeasts and Zygomyces were rare found. Winter wheat seeds were analysed to determine their potential transmission of pathogenic and saprophytic microorganisms. The seeds mycoflora of winter wheat was determined by using two methods of fungal investigation on samples collected from different sites of Slovakia. In total 28 genera of micromycetes were found on seeds of winter wheat. Majority of these fungi were identified as fungi sporulating on glume of ears. Mainly Fusarium spp. produced survival structures as sporodochia on glume or other parts of ears and also pycnidia of species from genus Septoria, Phoma and Ascochyta were found on glume of wheat on some localities with high frequency. The most dominant were saprophytic fungi as Alternaria sp., Cladosporium cladosporoides and parasitic fungi from genus Fusarium and Septoria. The prevalence seedborne fungi on winter wheat seeds on samples collected from different sites of Slovakia was Alternaria (38.2%), Fusarium (12.7%) and Epicoccum purpurascens (7.4%). From genus Fusarium were isolated five species F. graminearum, F. poae, F. culmorum, F. oxysporum and F. avenaceum with dominant species F. graminearum in all samples. Fungi as Papularia sp., Nigrospora sp. and Penicillium sp. was also isolated with more than 3.5% of relative frequency.

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**P6.3**

*Fusarium lactis*: cause of internal fruit rot of greenhouse sweet peppers

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Internal fruit rot is a new disease of sweet pepper (*Capsicum annuum* L.) in western Canadian greenhouses. Identification of *Fusarium* isolates from symptomatic fruit showed that most isolates were morphologically similar to *F. proliferatum* (Matsushima) Nirenberg, while a few were similar to *F. verticillioides* (Sacc.) Nirenberg. *Fusarium subglutinans* (Wollenweb. & Reinking) Nelson et al. had previously been reported as the causal agent of a similar disease that occurred in British Columbia. PCR amplification with species-specific primers on genomic DNA allowed us to divide a set of 32 *Fusarium* isolates from infected sweet peppers into two groups. The largest group (28 isolates) was morphologically similar to *F. proliferatum*, yet amplifiable by primers specific to *F. subglutinans* and *F. verticillioides*. The remaining four isolates were morphologically similar to *F. verticillioides*, but could be amplified by specific primers for *F. proliferatum*. Analysis of partial translation elongation factor 1-α and β-tubulin gene sequences of all isolates morphologically identified as *F. proliferatum* and *F. subglutinans* indicated greater similarity (>95%) to *F. lactis* Pirotta & Riboni (1879), a rediscovered species reported by Nirenberg and O’Donnell in 1998 (Mycologia 90: 434-458). Results suggest that *Fusarium* isolates causing internal rot of sweet pepper are phylogenetically distinct from *F. proliferatum* and *F. subglutinans*, and belong to *F. lactis*.

**P6.4**

Detection of the mycotoxin patulin in apparently healthy tissue of three apple cultivars

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*Penicillium expansum* is a common rot causing post harvest pathogen on apples and may produce the toxic secondary metabolite patulin in the fruit. Removal of damaged fruits or trimming of mouldy portions prior to processing can reduce the patulin levels in processed products such as juice and puree. However, patulin contamination of juice/puree made from apparently healthy fruits occurs frequently. In the present project, three apple cultivars were point inoculated with *P. expansum*. When apple rot developed the patulin content in rotten tissue and in different zones of non-rotted tissue were measured. Dept and width of lesions differed only slightly between cultivars. Solid-phase extraction was used for purification of patulin prior to HPLC/UV analysis. The method allowed patulin detected in pieces of apparently sound tissue samples as small as 4 x 4 x 4 mm (~0.5 g). Patulin content differed significantly between cultivars and surprisingly patulin (4 µg/g) could be detected close to the skin opposite the lesion, >2 cm from the border of the lesion. To study e.g. if patulin diffuse in front of the pathogen or vice versa, we have inserted a constitutively expressed gfp-construct into *P. expansum*. Hyphal growth of the transformant could be visualised directly in apple tissue by fluorescence microscopy. This will facilitate observation of fungal growth in apparently sound tissue to rule out if patulin contamination is due to diffusion, de novo synthesis from fine hyphae, or both.
Maize silage is a widely used feed product at Danish cattle farms. Unfortunately growth of filamentous fungi is often seen on and in silage. This may result in mycotoxin contamination of the feed, which may harm the animals. Recent cases of illness among Danish cows are suspected of being related to mycotoxins in the feed. Some mycotoxins are also known to be transferred to milk and blood and thus end up in the human food chain. In this collaborative project we will cover different aspects of maize silage production and utilisation from field to food product and clarify the extent and possible consequences of mycotoxin contamination of maize silage in Denmark. Surveys are conducted on Danish raw maize and silage samples to identify their mycobiota. This is conducted with conventional microbial techniques and DNA chip technologies for monitoring of fungal contamination. Microbial changes during long-term storage of silage will be addressed through silage monitoring and incubation experiments. Mycotoxins in maize and silage will be chemically determined with existing and new methods. In vitro assays are applied to evaluate toxicity of pure mycotoxins, fungal and silage extracts.

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K7.1

Investigating the genome of the cereal attacking fungal pathogen *Fusarium graminearum*

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The trichothecene mycotoxin producing Ascomycete fungus *Fusarium graminearum* (*Fg*) causes ear blight disease of small grain cereals. Infections lower grain quality and safety, and are of increasing global concern. In 2003, the genome was sequenced to ~10 × coverage by the Broad Institute (http://www.broad.mit.edu/annotation/fungi/fusarium). As part of the global initiative to complete the manual annotation of the genome, we have explored in depth *Fg* sequences involved with the transcriptional process (Coulson & Ouzounis (2003) Nucleic Acids Research 31: 653-660), homologues of experimentally verified pathogenicity, virulence and effector genes (Winnenburg, Baldwin et al (2006) Nucleic Acids Res. 34:D459-464, http://www.PHI-base.org) as well as the organisation of gene clusters. Currently, we are exploring the physical distribution of each gene group amongst the four *Fg* chromosomes, their patterns of expression *in planta* and under various *in vitro* conditions using Affymetrix datasets generated by others, and establishing gene function via targeted sequence disruptions.

O7.1

Investigating the transcriptome of wheat upon interaction with the head blight fungus

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A cDNA microarray with 3300 probes was constructed to detect genes differentially expressed in association with resistance against head blight caused by *Fusarium graminearum*. Two sister lines of a biparental cross of wheat differing for resistance against head blight, but sharing 70% genetic similarity were used. Embryonic cell suspension cultures of the lines were inoculated with fungal culture filtrate or with deoxynivalenone (DON) mycotoxin and cDNA was hybridized to the microarray. The microarray scans were analyzed in Bioconductor. The expression of genes appearing differentially regulated in the resistant line was verified by real time PCR with SybrGreen tagging, using the housekeeping gene *α*-tubulin as a control. The expression of the genes in the different treatments could by clustered into 6 groups corresponding to different metabolic pathways. Genes involved in protein biosynthesis are down regulated after DON and fungal culture filtrate treatment. The highest expression change shows a U1 snRNA after 3 hours DON treatment. Genes that were differentially regulated in the resistant vs. the susceptible wheat line included a subtilisin-chymotrypsin inhibitor, cytochromeP-450, glutathione S-transferase, and a cell wall invertase. The *in vitro* system applied was useful to screen for wheat genes that are potentially involved in resistance against head blight.
**O7.2 Sequencing of a mixed EST library from from barley powdery mildew haustoria and barley epidermal cells hosting haustoria**

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The powdery mildew fungus *Blumeria graminis* f.sp. *hordei* (*Bgh*) is an obligate biotrophic pathogen on barley (*Hordeum vulgare*). The haustorium is the only fungal structure that invades the host cell during disease development. It is believed to be fundamental in the establishment of biotrophy via the secretion of effector molecules. Some of these molecules may cross the plant-derived membrane surrounding the haustoria and enter the host cell. Effector molecules are thought to alter host cell metabolism, redirect the flow of nutrients and suppress host defence responses. An important class of effector molecules are avirulence (Avr) gene products. While previous EST sequencing has provided a view of the transcriptome of *Bgh* (Thomas et al. (2001) Fungal Genet and Biol 33: 195-211), these libraries were generated predominantly from early stages of infection and epiphytic hyphae. Consequently, ESTs from haustoria are thought to be underrepresented. We have constructed a mixed cDNA library containing both barley and *Bgh* ESTs. The library was enriched for haustorial sequences, by selective harvesting of haustorial containing barley epidermal cells, in the later stages of infection, when the haustorial density is greater. A significant proportion of the ESTs have not been previously reported in publically available databases, highlighting the functional specificity of *Bgh* haustoria. Haustoria-specific ESTs will be used in future research to identify virulence factors and Avr genes.

**O7.3 Secretome analysis of plant pathogen interactions, based on transposon assisted signal trapping**

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Recent advances in plant pathology clearly indicate that enzymes and other proteins secreted from the parasite cells are main determinants of the outcome of interaction processes. The secretome, which includes secreted proteins and outer-membrane bound proteins, plays an important role in sensing the environment, molecular signalling and communications and negotiations with the host, and in uptake of nutrients. In this project we focus on identifying genes coding for proteins secreted from parasites in interactions with their hosts using the novel genomic approach TAST (Transposon Assisted Signal Trapping). TAST is developed at Novozymes A/S and allows a selective study of cDNA libraries, excluding all other genes than genes coding for secreted proteins. This part of the project is concentrating on interactions between the obligate parasite *Plasmodiophora brassicae* with *Brassica* sp., and interactions between the necrotrophic pathogen *Pythium ultimum* and the biocontrol fungus *Clonostachys rosea*, which has shown multitarget efficacy against several plant pathogens in field experiments. The role of some of the identified genes will be studied in the project. The TAST treatment and subsequent bioinformatics are carried out in tight collaboration with Novozymes A/S.
Identification of expressed *Plasmodiohora brassicae* genes during plant infection

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The soilborne obligate biotrophic pathogen *Plasmodiophora brassicae* is the causal agent of the clubroot disease on crucifers. The life cycle of *P. brassicae* is believed to consist of two phases: Firstly, the resting spores germinate with a primary zoospore which infects root hairs and forms the primary plasmodium and subsequently produces secondary zoospores. Secondly, the secondary plasmodium is developed within the root cortex and stele leading to gall formation and production of zoospores (Ingram & Tommerup (1972). Proc. R. Soc. Lond. Ser. B-Biol. Sci. 180: 103-112).

Due to obligate parasitic nature of *P. brassicae*, identification of expressed genes is very difficult and as a result hereof, only a limited number of *P. brassicae* genes is known to be expressed during infection. Proteins secreted by the cell and outer-membrane bound proteins are believed to play an important role in sensing the environment and molecular signaling. These proteins are therefore potentially important in a host pathogen interaction. Unfortunately, they are difficult to pick up as only a very limited number of the expressed genes are coding for secreted proteins.

In this project different methods are used to identify *P. brassicae* genes expressed *in planta*. These include the newly developed technique Transposon Assisted Signal Trapping (TAST), developed by Novozymes A/S, which selects for genes coding for secreted proteins.
In animals, proteases and their cognate serpin protease inhibitors are associated with innate immunity and defense responses. Although many evidences link protease activity in plants with defense signaling, the regulation of these protease activities in plants is unknown. One member of the *Arabidopsis* serpin gene family, *SAS1* (stress associated serpin1), exhibited properties of inhibitory serpins i.e. it possessed inhibitory activity towards trypsin *in-vitro* and could form a SDS-stable serpin-protease complex. The serpin was detected in intra and extra-cellular locations and found to accumulate in the plant as part of a putative serpin-protein complex in response to BTH. The formation of the complex required NPR1 activity. SAS1 loss-of-function mutants showed enhanced susceptibility to a virulent strain of the bacterial pathogen *Pseudomonas syringae* pv. *tomato* and more abundant marker gene VSP transcript but less abundant marker gene PR-1 transcript than wild-type plants and a gain-of-function SAS1 mutant. The reciprocal effect of SAS1 on these pathways suggests that it acts as a negative regulator of the wound response pathway and as a positive regulator of the salicylic acid pathway. Our results imply a novel junction for salicylic and jasmonic acid pathway cross-talk, through an inhibitory serpin-regulated proteolytic pathway.

Using root and shoot specific pathogens *Heterobasidion annosum* and *Gremmeniella abietina* as experimental model, we investigated whether different organs of conifer trees use the same genetic pathways to respond to microbial attack. The mRNA profiling technology was used to elucidate host response and identify genes differentially expressed during infection of juvenile conifer roots with a shoot pathogen and vice versa. A macro-array containing 384 individual pine cDNAs representing a range of transcripts expressed during different stages of development was examined. Necrotic browning reaction of pine needles infected with the shoot pathogen *G. abietina* was observed but corresponding infection of the root with the same pathogen did not provoke any host reaction. Interestingly both needle and root tissues infected with the root pathogen *H. annosum* responded with strong necrosis. At the transcriptome level, initial results suggest that genes differentially expressed in response to each pathogen were highly specific and might be vital for the tissue specific differences in susceptibility or disease resistance. Additionally, many transcripts of defence related genes (antimicrobial peptide, glutathione-S-transferase and peroxidase) preferentially accumulated in the infected roots in comparison to the needles. Whether such preferential accumulation is of any ecological significance in the co-evolution of the fungi and their host will be discussed.
O8.2 Hydrogen peroxide is important for the defence of wheat against *Septoria tritici*

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The role of several defence responses in wheat against the hemibiotrophic pathogen *S. tritici* was investigated in a compatible (cv. Sevin-isolate IPO323) and an incompatible (cv. Stakado-isolate IPO323) interaction. H$_2$O$_2$ accumulation was a prominent defence response in Stakado, where a strong and early accumulation coincided with restriction of pathogen growth. In Sevin, only very little H$_2$O$_2$ accumulated, except during sporulation, where tissue degradation occurred. This accumulation probably represents a stress response coinciding with tissue collapse and reduction in the photosynthetic activity in Sevin. No decrease in photosynthetic activity and no macroscopic symptoms of infection were observed in Stakado. Further evidence for the role of H$_2$O$_2$ in resistance was obtained from studies of infiltration of catalase (to remove H$_2$O$_2$ water (control) or H$_2$O$_2$. Both early (during initial stages of infection) and late (during the necrotrophic phase) infiltration of catalase enhanced fungal growth and resulted in faster symptom expression in Sevin whereas infiltration of H$_2$O$_2$ reduced fungal growth and delayed symptom expression. Collectively, these data show that H$_2$O$_2$ is detrimental for *S. tritici* throughout its life cycle and has no beneficial role even under the necrotrophic phase of the interaction as has been reported for necrotrophic pathogens. Future experiments to elucidate the role of H$_2$O$_2$ in this host-pathogen interaction will involve the use of catalase knock-out mutants of *S. tritici*.

P8.1 Colletotrichum sublineolum infection induces oxidative cross-linking of cell wall hydroxyproline-rich glycoproteins (HRGPs) in sorghum

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The oxidative cross-linking of Hydroxyproline-Rich Glyco-Proteins (HRGPs) is a general defence barrier in plants against pathogen infection. HRGP accumulation was studied in three sorghum cultivars, *i.e.* cv. 146 (resistant), cv.326 (less resistant) and cv. 623 (susceptible) to *C. sublineolum* isolate CP2126. HRGP was monitored by estimating hydroxyproline (Hyp) in the hydrolyzed cell walls. In the resistant cv.146, there was a significantly larger amount of Hyp compared to the susceptible cv.623, indicating that infection induced HRGP which could be involved in restriction of the pathogen. SDS-PAGE analysis of the acid–ethanol extracted proteins from inoculated leaves followed by periodic acid Schiff staining revealed four HRGPs with molecular masses of ~ 80, 65, 17 and 14 kDa. All these four HRGPs reacted positively in western blotting with polyclonal antibodies against pearl millet HRGP. The 17 and 14 kDa HRGPs markedly increased in all the cultivars after infection, with a larger increase in the resistant cultivars, and data indicate that the 65 kDa HRGP might be involved in infection induced cell wall cross-linking as found in other host-pathogen interactions. The pattern of HRGP mRNA accumulation (using Cassava probe: cMe HRGP1) after infection was also significantly higher at the early time points in the most resistant cultivar. The infection induced oxidative cross-linking as well as papilla and callose deposition in the resistant cultivars at infection sites suggests that these responses are involved in disease resistance.
Post-transcriptional gene silencing (PTGS), observed in eukaryotes (Ding et al., 2004) Viral Res. 102:109), consists in the use of double-stranded RNA (dsRNA) to target specific mRNAs for degradation, thereby silencing their expression. This phenomenon has also been described in fungi and can be used as a functional genomics tool in them (Fitzgerald et al., 2004) Fungal Genet. Biol. 41:963).

*Botrytis cinerea* is a phytopathogenic fungus able to infect more than 200 plant species worldwide, including many commercially important crops, producing the “grey mold” disease. Until now, targeted gene disruption or replacement has been the technique of choice for creating null mutants of specific genes in it. We intend to develop an alternative method to generate strains lacking the products of specific genes, by using the PTGS as a tool.

The *niaD* gene, coding for a nitrate reductase, was chosen to test if PTGS works in *B. cinerea*. Two constructs were made to generate the dsRNA: two different regions of the *niaD* ORF (about 500 bp each one) were cloned between two copies of *niaD* promoter in sense and antisense orientations. The hygromycin resistance cassette was included in the vectors as a marker. Protoplasts were transformed with them and the nitrate reductase activity was measured in 72 hygromycin-resistant transformants. The results showed that 38.9% of the strains had reduced activity with respect to the wild-type strain. Within this percentage, 53.5% showed less than 50% of the wild activity.
O9.1 Application of multilocus sequence typing for characterisation of the brown rot pathogen *Ralstonia solanacearum*
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*Ralstonia solanacearum* is a genetically diverse and geographically widespread plant pathogen with a wide host range. As the causative pathogen of Brown Rot of potato, quarantine measures are in place to restrict its spread within Europe. Incidences of brown rot in Europe are primarily caused by *R. solanacearum* biovar race 3/biovar 2A, which is reported to have strains adapted to cooler climates. Infection of potato crops in Europe predominantly occurs via irrigation with contaminated surface water. The disease has never been found in Scottish potatoes though *R. solanacearum* was discovered in water samples from the Tay river system in 2000 and from its secondary host, bittersweet (*Solanum dulcamara*), growing on the river banks. To investigate the source of this infection, genetic variation was studied within a collection of 106 *R. solanacearum* isolates, mainly race3/biovar 2A isolates from potato, *S. dulcamara* and contaminated water sources. Multi-locus sequence typing (MLST), a molecular strain-typing method principally used in clinical microbiology, was used in a novel application of this technique. Twenty-seven isolates from contaminated water and diseased plant samples, originating in Scotland and elsewhere, were resolved into 18 sequence types. All Scottish isolates were found to be identical and similar to most race 3/biovar 2A isolates tested. These initial results suggest that contamination of the River Tay occurred as a single or limited event.

O9.2 Characterization of four chitinase-encoding genes (*cr-nag1*, *cr-ech58*, *cr-ech42* and *cr-ech37*) from the fungus *Clonostachys rosea* (IK726)
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*Clonostachys rosea* is a widely distributed fungus that often acts as a parasite on other soil fungi. The mycoparasitic activity is thought to be correlated with the secretion of cell wall degrading enzymes, including chitinases. In this work, we identified and characterized four chitinase-encoding genes from *C. rosea* strain IK726. Conserved motif regions among known exo- and endochitinase amino acid sequences from related fungi were used for design of degenerated primers, which were applied for PCR screening of *C. rosea* genomic DNA. The PCR products were used to obtain genes encoding an N-acetyl-β-D-glucosaminidase, *cr-nag1*, and three endochitinases, *cr-ech58*, *cr-ech42* and *cr-ech37*, including their promoter regions by a DNA walking strategy. Enzymatic assays showed that the general chitinase activity of *C. rosea* is repressed in media containing glucose. RT-PCR analysis was performed to confirm that the genes are expressed and to study the expression pattern of each gene. The highest expression of *cr-nag1*, *cr-ech42* and *cr-ech37* were found in media with *Fusarium culmorum* cell walls or chitin whereas almost no expression was detected in media with 1 % glucose. The expression of *cr-ech58* was low in all media tested and seems not to be regulated by glucose. Future work will focus on studies of the role of these four chitinase-encoding genes for *C. rosea* interaction with fungal pathogens by studying the expression of the genes using real time RT-PCR analysis.
O9.3 The endornavirus AbEV1: a dsRNA element, associated with MVX disease of Agaricus bisporus

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Mushroom Virus X (MVX) disease affects the cultivated button mushroom, Agaricus bisporus. The disease comprises various symptoms resulting in reduced crop yield and poor quality. The 26 double-stranded (ds) RNA elements associated with the MVX disease range between 640 bp and 20.2 kbp, three of which (16.2, 9.4 and 2.4 kbp) are routinely found in asymptomatic mushrooms. As part of the effort to characterize the complex of viruses, specific MVX elements are being sequenced. Three functional domains have been identified in a 14.4 kbp dsRNA element (MVX14.4): RNA-dependant RNA polymerase (RdRp), glycosyltransferase and helicase. Phylogenetic analyses based on RdRp sequences suggest that MVX14.4 shares a common ancestor with single-stranded RNA viruses. BLAST and phylogenetic analyses indicate that MVX14.4 is a new Endornavirus. We propose that the MVX14.4 is referred to as Agaricus bisporus endornavirus 1 (AbEV1).

Resistance to MVX disease has not been identified. Post-transcriptional gene silencing (PTGS) is being investigated to improve the understanding of MVXdsRNAs replication and mushroom antiviral defence pathways. To initiate PTGS in A. bisporus, AbEV1 RdRp and helicase sequences have been introduced as RNAi mediating hairpin constructs into MVX-infected and MVX-free mushrooms. Transformants have been recovered containing the appropriate MVX transgenes from MVX-infected mushrooms. Analysis of transformants indicates that MVXdsRNAs are not stable through the transformation process and that this approach cannot be used to “cure” infected mushrooms. Work with MVX-free mushrooms is continuing. Transformants have been recovered and these are being challenged with MVX donors to determine whether PTGS pathway is active.

O9.4 Epidemiological studies leading to the sustainable control of strawberry powdery mildew

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Strawberry powdery mildew (Podosphaera aphanis) is a major pathogen of strawberries, especially when they are grown under protection. Spanish Tunnels are used to extend the growing season. Conditions under Spanish Tunnels are ideal for the production of strawberries and especially at the start of the season (early May), the growth of P. aphanis. This results in growers applying fungicidal treatments at regular intervals. The literature relating to the life cycle of the pathogen was studied to identify where there were gaps in our understanding of its growth and development. The source of primary inoculum had not been identified previously. The growers had assumed it was wind borne but this had not been tested. In this study we examined newly planted fields, where the plants had come from a cold store and plants in established fields where the plants had over wintered.

Initial results suggested that P. aphanis inoculum was planted into new sites. Once the conditions were suitable for the growth of P. aphanis disease developed throughout the tunnel at the same time. The results collected also showed that inoculum over winters within established fields. When the tunnels were covered or the fleece was removed (depending on cultivation method) plants started to show symptoms. These plants were distributed throughout the tunnel. Methods to reduce the initial inoculum and therefore slow the build up of the disease should be incorporated into a sustainable control program.
**O9.5**

Do HVNAC-like transcription factors regulate host defence against the powdery mildew (*Blumeria graminis* f.sp. *hordei*) in barley (*Hordeum vulgare*)?

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NAC proteins constitute a large family of plant-specific transcription factors. They are believed to be involved in the floral and lateral root development in *Arabidopsis thaliana* (Aida et al., 1999; Xie et al., 2000). NAC transcripts have furthermore been shown to be up regulated by different abiotic and biotic stresses, including pathogen attacks and abscisic acid treatment (Hegedus et al., 2003, Greve et al., 2003). Furthermore yeast two-hybrid analyses have revealed the interaction between a viral capsid protein and an *A. thaliana* NAC member (Ren et al., 2000).

We have isolated a NAC transcript from barley by differential display of powdery mildew-infected barley epidermis, and obtained the full length cDNA sequence by RACE PCR. The clone was named HvNAC6 (*H. vulgare*) for its high sequence identity to the rice NAC protein OsNAC6 (*O. sativa*). HvNAC6 was used as bait to screen a cDNA library for other HvNACs, and three additional full length clones were obtained.

Our group is currently working on the biological and biochemical function of these transcription factors. The biological functions of HvNAC genes are studied by the use of a transient expression assay in detached barley leaves. In this assay RNA interference (RNAi) and over-expression is used to determine the role of HvNAC6 upon powdery mildew inoculation. So far RNAi of two of our HvNAC members have revealed an increased susceptible phenotype towards powdery mildew inoculated barley single-cells. QRT-PCR expression profiles HvNAC6 suggest a role for HvNAC6 during basal defence accumulation. The future aim of the current research is to delineate the signalling pathway involving this family of transcription factors and their function in the mediation of defence.

**O9.6**

Genetic transformation of *Lilium* for enhanced fungal disease resistance

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Globally, *Lilium* is a very important floriculture genus after *Rosa* (rose), *Dianthus* (carnation) and *Dendranthema* (chrysanthemum). Consumer preference for floral form changes rapidly, but this requirement cannot readily be met using traditional breeding programmes. Therefore, the direct insertion of specific genes can aid the development, production and marketability of new varieties for novel characteristics such as fungal resistance. *Lilium* is susceptible to several fungal pathogens, including *Botrytis elliptica*, which infects leaves, stem and flowers leading ultimately to a reduction of yield for the cut flower industry.

The aim of the project was to introduce fungal disease resistance into *Lilium* mediated biolistics mediated DNA delivery, using a rice chitinase gene, *CHIT1*. This rice chitinase gene has been isolated from the BAC OSJNBb0028K20 (*Oryza sativa* japonica cv. Nipponbare) and was cloned into the vector pBI101.

This study describes the integration of *CHIT1* in the ornamental *Lilium* ‘Star Gazer’ and its effects on the control of leaf blight caused by *B. elliptica*. 
09.7 Searching for Avirulence Genes in *Hyaloperonospora parasitica*

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*Hyaloperonospora parasitica*, an obligate biotrophic oomycete, is the causal agent of downy mildew on *Arabidopsis* and *Brassica* crops. The interaction between *H. parasitica* and *Arabidopsis* provides us with a model pathosystem for studying plant disease resistance. Resistance to *H. parasitica* is based on a gene for gene relationship, where a resistance gene product and its corresponding avirulence gene product must both be present to trigger a resistance response. I aim to identify pathogen genes involved in the infection process, in particular the avirulence genes that induce a hypersensitive response. To date we have identified 13 candidate avirulence genes by screening spore cDNA libraries. The candidate genes encode for proteins that have no homology to known proteins and contain a RXLR motif with or without a downstream DEER motif or variants of these two motifs. The RXLR motif has been identified in a number of oomycete effector proteins and is speculated to play a role in transport of proteins into host cells. Allele sequencing is being used to determine if these genes are under selective pressure as a result of interacting with host resistance genes. We have generated two pathogen crosses, one between Maks9 and Emoy2 and another between Cala2 and Noks1, in which more than 18 avirulence genes segregate. Candidate avirulence genes will be mapped onto segregating pathogen-cross populations to determine if they co-segregate with known avirulence genes.

09.8 The epidemiology of potato mop top virus (pmtv) in seed potatoes

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Losses attributable to potato mop-top virus (PMTV), a cause of spraing, occur primarily through the rejection of ware stocks by processors and packers, or through the rejection of seed lots by importing countries who have designated PMTV to be a quarantine organism, or who apply a strict tolerance. In order to assess the effectiveness of potential control measures, the transmission and extent of PMTV in Scottish seed potatoes was studied. The rate of transmission of PMTV from seed to daughter tubers was generally less than 50%, often substantially less for some cultivars. The development of symptoms on the growing plant differed with cultivar, varying from severe distortion to no symptoms. The occurrence of PMTV was monitored in seed crops of cv. Cara derived from a common origin source of seed potatoes and grown for 2 years in the seed producing areas of Scotland. The results showed that soil inoculum was much more important than seed inoculum in causing infection of the daughter tubers. In a survey of 128 crops of 5 varieties known to be susceptible to PMTV, the majority were found to be free of PMTV and spraing. PMTV was the main cause of spraing in affected tubers and was more common in some areas of Scotland than others.
RNAi as a tool for functional genomics in homobasidiomycetes
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Gene function studies in homobasidiomycete mushrooms have been hampered by the lack of effective gene silencing. As a result little is known about mechanisms underlying pathogenesis. We aim to identify genes involved in the infection process between the white button mushroom Agaricus bisporus and its pathogen Verticillium fungicola. To expedite functional analysis of disease response genes, we are developing mediated transformation using RNAi hairpin constructs and model genes from A. bisporus and another homobasidiomycete Coprinus cinereus. RNAi targeting of two endogenous A. bisporus genes CBX and URA3 yielded reduced specific transcripts in transformed strains compared with wild type. The GFP reporter was used to test efficacy of hairpin constructs and to quantify down-regulation mediated by RNAi in C. cinereus. GFP was effectively suppressed and the non-fluorescent phenotype was associated with significantly reduced transcripts and protein, as determined by qPCR, fluorimetry and northerns.

We used SSH and cDNA libraries of A. bisporus infected with V. fungicola to identify host genes up-regulated during pathogen infection. Four up-regulated genes belonging to different functional groups have been selected for further studies. Knockdown lines of host response genes have been produced to assess their role in disease development. These studies should reveal the mechanisms regulating fungal-fungal host-pathogen interactions and enable novel approaches for disease control.

Evidence for seed to seedling transmission of Botrytis cinerea in lettuce
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Botryis cinerea was recovered from seeds of lettuce plants which were grown outdoors. Some plants were inoculated with an isolate of B. cinerea at the bud/flowering stage. Seeds plated on Botrytis Selective Medium (BSM) after surface sterilisation in 1% NaOCl showed high levels of internal infection; seeds from inoculated plants had only slightly more infection than seed from uninoculated plants. Q-PCR detected higher levels of infection than the plating test. Immunolabelled and trypan blue stained seeds which were examined with a confocal microscope and a light microscope respectively, confirmed that the B. cinerea recovered from infected seeds was internal. Lettuce plants grown from infected seeds in a sterile environment were non-symptomatic but had B. cinerea growing in them internally, initially confined to the roots. In non-symptomatic open-grown lettuce plants B. cinerea could be isolated from all parts of the plant. In many cases DNA fingerprints of isolates from the root, stem and leaf of an individual plant were identical, suggesting systemic growth rather than repeated infections. It is therefore possible that vertical transmission of B. cinerea is as important as horizontal transmission by conidia in this host.
Leptosphaeria maculans is the causal agent of phoma stem canker, a damaging disease of winter oilseed rape (Brassica napus) worldwide. Wind-borne ascospores released in autumn infect the leaves of the plant, causing leaf lesions. The pathogen then grows asymptptomatically down the petiole to the stem, where it can cause a yield damaging stem canker in the summer. Severity of stem canker in the field varies between cultivars and is assessed just before harvest in July. The importance of the different stages in the L. maculans life cycle in contributing to the severity of stem canker is unknown. Resistance in the leaf to initial infection by ascospores is known to occur and resistance genes have been previously identified. Cultivars known to differ in stem canker severity in the field were chosen to perform a series of field experiments and controlled environment experiments to investigate resistance to the pathogen. Tagged plants in the field were used to determine numbers of leaf lesions on these cultivars over 3 growing seasons and the pattern of leaf emergence and leaf fall was compared between cultivars, as was leaf size and petiole length. In controlled environment experiments, an isolate of L. maculans transformed to constitutively express GFP was used to determine the growth rate of the pathogen in the petiole of these different cultivars, and the quantity of pathogen in the petiole was also measured.
Plant resistance to pathogens involves the accumulation in the plant cell of proteins active in defense mechanisms. Among these are PR proteins including glucanases, chitinases and thaumatin-like proteins (PR-5). Several proteins belonging to the PR-5 group have been used successfully to enhance plant resistance to fungal pathogens. However, to our knowledge, there are few studies on localized accumulation of the PR-5 proteins in the apoplastic space. This seems to be one of the possible strategies for the antifungal activity enhancement of thaumatin II and thaumatin-like proteins since it has been reported that intercellularly secreted antimicrobial proteins are more effective in reducing disease development than intracellularly localized one.

In order to achieve high level apoplastic accumulation of biologically active thaumatin II protein, the chimeric gene lrth encoding the recombinant precursor of thaumatin II protein containing N-terminal signal peptide from radish defensin RS-AP and lacking C-terminal propeptide was constructed. 12 and 16 transgenic tobacco lines harboring thauII and lrth genes respectively were obtained. All transgenic plants exhibited normal phenotypes. Three thauII and four lrth lines were randomly selected for further work. Analysis of the thauII and lrth transcripts using RT-PCR on total RNA was performed, and in all cases specific transcripts were detected. Pre-mRNA transcribed from chimeric lrth gene containing 91 bp intron from radish rs-ap gene is spliced correctly in transgenic tobacco plants. Western-blot analysis of total, apoplast and remnant extracts indicate that plants transformed with the wild-type construct retain most of the produced thaumatin intracellularly. On the contrary, the most amount of thaumatin in lrth tobacco lines was detected in the intercellular washing fluid fraction. Equally important, the activity of thaumatin does not appear to be compromised when the protein is produced and secreted in plants. The secreted thaumatin was bioactive, indicating conservation of the conformational state of the recombinant thaumatin.
Developing countries face many challenges, but transforming their agriculture will be a test for scientists, technology developers and policy makers, it is also a battle ground for ideologies and perceptions. The livelihoods of many poor people depend on agriculture. Agriculture has met current demands in many countries but not in Africa. However the Millennium Ecosystem Assessment reports serious damage to over 60% of the ecosystems on which we all depend. Agriculture must now meet the growing and diversify demands of society and restore these vital ecosystems. Public investments in R&D have declined and business investments focus where farming is profitable.

Diseases continue to reduce the productivity, quality and safety of crops. Pathogens are successful because they are able to be one jump ahead of control systems or only one mutation behind the plant breeder. We will continue to need chemical, biological and physical approaches to the control of disease, particularly in developing countries.

New chemical controls must conform to consumers concerns and legal requirements. Molecular breeding techniques could improve the speed, accuracy and efficiency of plant breeding. A more systematic search of wild relatives might identify new genes and traits which impart tolerance or resistance in crops. Transgenic processes, which have attracted a huge burden of regulation and ‘red flag’ issues, may still be necessary to buy time and strengthen resistance. The European research community must help to rebuild public confidence and trust and work with others to strengthen the capacity of developing countries to develop their own solutions.

A fungal parasite of nematode eggs, Pochonia chlamydosporia, has been developed for use against root-knot nematodes in intensive vegetables. This agent must be used with other control measures such as crop rotation and trap cropping to prevent the build up of large nematode infestations. In Cuba, an infrastructure exists for the delivery of biological control agents, utilising small-scale, dispersed production centres. A pilot plant has been established in the Centro Nacional de Sanidad Agropecuaria, Havana, to optimise methods for the supply of high quality, stable inoculum of P. chlamydosporia to local growers. Applications of the fungus, registered as KlamiC®, have provided > 80% reductions in nematode infestations. Such sustainable management needs local evaluation and advice provided to growers to ensure successful uptake. Transfer of the technology to East Africa has proved difficult, as there are too few trained personnel. In response, the Nematology Initiative for Eastern and Southern Africa (NIESA) has been set up to develop and sustain a critical mass of expertise within the region to develop locally-adapted management methods and provide much needed diagnostic support. Six nematology laboratories have been established in participating African countries and NIESA scientists have attended a 6-week training course. An interactive website has been established (http://www.africannematology.info/index.asp) to provide access to the major nematological journals and to exchange information. Once established, NIESA will provide a core of expertise to provide training and to underpin research inputs to improve crop protection methods in the region.
Implementing quality systems for potato seed with farmers in Uganda: the experience of the Kapchorwa Seed Potato Producers' Association in Eastern Uganda

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Potato is an increasingly important crop to Ugandan smallholders, supporting subsistence and cash needs. However, the opportunity to realise the potential presented by this crop is significantly constrained by the lack of a robust seed production system and seed-borne diseases, notably bacterial wilt. Consequently, farmers recycle seed and concomitantly concentrate disease problems over generations of production.

Research has been initiated with a group of farmers in Eastern Uganda that is pioneering new ways of scaling-up seed potato. Over the course of four years, a core of farmers have worked alongside a local NGO (AT Uganda) and a Advanced Research Institute (CSL) in working through production and marketing constraints. These activities have culminated in the formation of The Kapchorwa Seed Potato Producers’ Association (KASPPA) that now markets seed potato under its brand name KASPPA.

In this presentation some of the salient experiences are discussed as relates to the KASPPAs effectiveness as an association and in the development of a series of Best Practices that support production and marketing. A focus is presented on the management of bacterial wilt and the validation of farm-level assessments that has combined lab research tools with farm-level innovation in producing a decision-making framework of use to farmers. Further attributes of KASPPA will also be highlighted as support the brand claim of quality, schemes of collective marketing and a levy that accrues revenue for the sustainability of the association.

Mobile plant health clinics in Nicaragua and Bangladesh

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There is much that plant pathology can continue to learn from medicine. Trained and registered nurses, doctors and specialists, provide a chain of care that starts with a general diagnosis of a problem then proceeds swiftly to a suggested remedy. Along the way the patient might visit a clinic or be referred to a hospital for further examination. Plant health problems are dealt with less systematically. Services are poorly coordinated, yet there is a huge and persistent demand for advice and help. Since 2003 the Global Plant Clinic has helped run mobile plant clinics and trained plant doctors in Latin America, Asia and Africa. We are testing new ideas in several countries, but with most notable advances in Nicaragua and Bangladesh. The expansion of primary plant healthcare for poor farmers has encouraged diagnostic laboratories in Nicaragua to work together for the first time, making it easier for extension workers to send samples. Nicaragua now has nine mobile clinics, all run independently. They are providing new outlets for integrated pest management methods and sharpening ideas about what is practical for farmers to use. Bangladesh continues to expand their network of plant clinics and plant camps to cope with a daily demand for assistance. Mobile clinics improve surveillance at low cost and increased coverage, and highlight diseases that need more research. Bottom-up, demand-driven can be good for farmers and science.
Napier grass stunt disease in Uganda associated with a phytoplasma
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Napier grass, also known as elephant grass, is an important fodder crop in Uganda. The forage has been increasingly associated with intensive (zero grazing) and semi-intensive dairy cattle production systems to meet the increasing demand for milk in Masaka district in Uganda. Napier grass stunt disease, causing short internodes, bushy appearance, yellow to purple streaking and a low biomass yield, has spread among smallholder dairy farmers in Uganda. Samples of stunted Napier grass were collected in the three major Napier grass growing areas of Uganda, and 17 of 34 samples from two of the three areas were positive for phytoplasma using a quantitative PCR. To investigate possible molecular variation seven samples from different districts were selected for sequencing of the 16S rRNA gene and the 16S/23S intergenic spacer. No sequence variation was found. The sequences showed that the Ugandan isolates belong to the 16SrXI sugarcane white leaf group and, in this, are most similar to Bermuda grass white leaf phytoplasma. The Ugandan isolates further were essentially identical to the 16S rRNA sequences from Napier grass stunt phytoplasmas from Kenya (Jones et al (2004) New Disease Rep 9: Febr –July).

Characterisation of the blast pathogen Magnaporthe grisea populations on finger millet and rice in Africa: Towards sustainable disease management
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Blast caused by Magnaporthe grisea is a major constraint to the production of key crops such as finger millet and rice in Africa. We are using a range of molecular, biological and pathological assays to characterise the pathogen populations. On finger millet in East Africa, more than 190 blast genotypes were identified and these populations were genetically distinct compared to those in Asia. However, we have identified a low level of the ‘Asian pathogen’ in East Africa, suggesting recent introductions. PCR based analysis revealed a near equal distribution (47 – 53%) of mating types MAT1.1 and MAT1.2. In laboratory crosses, these populations showed high fertility, but whether sexual reproduction occurs in nature needs to be investigated. In terms of pathogenicity, genetically similar isolates caused different types of blast under varying agro-ecological conditions. Wild millet harboured pathogen isolates genetically similar to those from the crop. Differences in pathogen aggressiveness were more apparent than compatibility on finger millet underlining the importance of quantitative resistance. On rice in West Africa, blast pathogen populations showed typical lineage based structure. Mating type MAT1-2 was dominant compared to MAT1-1 with high female sterility and low fertility. Clear differences in virulence were observed on rice showing the potential of R gene-based resistance. The knowledge generated provides a framework for developing sustainable blast management.
Subsistence farmers in Vietnam are generally very skilled rice seed producers. They are however frequently without formal agricultural education and for the seed production they may depend on inherited knowledge. Many farmers are aware of the importance of using “good” seed but they lack the knowledge and technical means to establish whether his seed is truly good in terms of germination capacity and health. Laboratory seed health test results of farm saved rice seed are indicating that the seed presently produced in Vietnam contains high levels of seed-borne diseases capable of reducing the germination percent and for some of the field diseases. Several attempts have been made over the years in developing countries to establish seed programmes with the purpose of providing a supply of certified and laboratory tested seed to particularly subsistence farmers. However, several factors have been major obstacles for making subsistence farmers benefit from such seed programmes.

This paper provides a description of an on-farm system to make the farmer able to evaluate his rice seed in terms of seed germination capacity and seed health. The short, practical training programme is designed to reach the subsistence farmers through a Farmer Field School and illustrates through practical training the benefit of using healthy seed as compared to infected seed, the economic benefit of using treated seed compared to untreated seed and how to produce healthy seed.

True yams (Dioscorea spp.) are among the most important staple food in many parts of the tropics particularly in West Africa, the West Indies and the South Pacific Islands where more than 90% of all of global yam production is harvested (FAO, 2002). Although viruses and yam anthracnose caused by Colletotrichum gloeosporioides (teleomorph Glomerella cingulata) are regarded as the most damaging diseases worldwide, recent surveys in all major yam-growing areas have revealed the complexity of yam anthracnose and the importance of Colletotrichum capsici as yam anthracnose agent with disease incidence as high as 60% (Peters et al., 1997). Hence, in the current study, we report the results of detailed studies on the morphology as well as dimensions of reproductive structures of 16 C. capsici yam isolates using both light and scanning electron microscope. We also report the cultural characteristics, growth and sporulation of these isolates under controlled environmental conditions as well as their symptom expression and relative pathogenicity on various yam species. In this study we also investigate host specificity of C. capsici on a number other crops including beans, cowpeas, pepper, tomato and cotton often intercropped with yam in order to identify potential sources of infection and highlight the threat these crops pose to yam plantations when grown as intercrops.

References

Genetically modified (GM) and other cultivated cotton (*Gossypium hirsutum* L.) varieties in Burkina Faso revealed susceptibility to bacterial blight (*Xanthomonas axonopodis pv. malvacearum*) during 2004-2005. The GM cotton varieties are designed to produce insect specific toxins (Bt and Vip) which are naturally occurring insecticides that kill major cotton insect pests; the same varieties without the Bt and Vip genes and FK37 and STAM 59A locally grown cotton varieties were tested under field conditions in Burkina Faso to study the impact of the GM varieties in West Africa. Affected cotton plants were observed in Kouaré and Farako-Bâ. The highest disease incidence (100%) and disease severity was reported during 2004 in the introduced Bt and Vip cotton varieties, in the eastern part of the country at Kouaré and low disease incidence was registered in the traditional varieties FK37 and STAM 59A. The severity of the disease was in general lower during 2005 than in 2004 and the highest disease severity was observed in Bt cotton varieties at Farako-Bâ. The evaluations revealed that the cotton varieties with or without the Bt or Vip genes were susceptible to the bacterial blight disease in the eastern and western regions. Currently, no control measures are applied and only two resistant varieties are cultivated in the country. The impact of the susceptibility to bacterial blight disease on GM varieties with resistance to insect pests is discussed.
Print-PCR in the detection of the bacterial canker pathogen (Clavibacter michiganensis subsp. michiganensis) from tomato plants tissue

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Print Capture PCR has been reported as a method to detect plant viruses first immobilized on blotting paper (Ref. 1). Plant tissue prints conducted on agar (Ref. 2) or blottings (membrane printing) have been used for the detection or isolation of bacteria in the field or in the laboratory (Ref. 1). The method provides convenience in terms of preparation, storing and sending field samples for subsequent analysis at a central laboratory by conventional PCR or other suitable methods. The method also presents the advantage that isolation of the target pathogen is possible. The technique under development at the Danish Seed Health Centre for Developing Countries for the detection of the bacterial canker organism from affected tomato plants material is presented.

Diagnostic in plant quarantine: review of international initiatives
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IPPC DIAGNOSTIC PROTOCOLS
Since the 1990’s, the international phytosanitary context has considerably evolved. The International Plant Protection Convention (IPPC) was revised in 1997 to incorporate principles of the SPS agreement. Provisions regarding a standard-setting mechanism were introduced into the New Revised Text of the IPPC and a Commission for Phytosanitary Measures was established. This Commission recognized that there was a strong interest for members to have international diagnostic protocols. In 2004, mechanism to speed up the development of International Standards for Phytosanitary Measures in specific areas in particular diagnostic protocols, was adopted and a Technical Panel to develop diagnostic protocols for specific pests has been established.

EPPO WORK PROGRAMME ON DIAGNOSTICS
Development of diagnostic protocols
In 1998, EPPO started a programme to prepare diagnostic protocols. The Panel on Diagnostics conducts this work in collaboration with specialized Panels (Bacterial Diseases, Nematodes and the European Mycological Network). An author(s) prepares the first draft of the diagnostic protocol according to a common format. Each protocol is intended to contain the information necessary to detect and identify a particular pest. Relevant Panels and other EPPO bodies review them, thus basing the protocols on the experience of specialists. 72 protocols have been approved since 2000 and are considered regional standards. Approximately 30 protocols are in preparation.

Other activities
EPPO is developing a standard which will interpret ISO Standard 170251 in terms of plant-pest identification and has also prepared a questionnaire to make an inventory of the available expertise on diagnostics and collections in the EPPO region.

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1 ISO/IEC Standard 17025 on "General requirements for the competence of testing and calibration laboratories"
O11.1  

Quarantine of Wild Collected Plants  
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The collection of living plants from the wild for research and cultivation purposes presents an unknown risk to plant health, as the presence of pathogens and pests can only be assessed at a superficial level prior to collection. Effective quarantine measures are therefore an essential part of the plant health strategies for the introduction into cultivation of plants from the wild.  
Currently around 400 accessions per year are imported as living plants from the wild at the Royal Botanic Garden Edinburgh (RBGE). This represents around 25% of all foreign accessions, (the remainder is imported as seed). One of the major advantages of this form of introduction for botanists and horticulturalists is the drastically reduced time it takes for these plants to establish vigorous growth and to produce flowers, an essential prerequisite for identification. In practice, wild plant collections imported at RBGE have proved remarkably free of problems with only 13 interception of pests or diseases during the past five years. Where pests or pathogens were discovered, these could be contained effectively. In this paper the quarantine arrangements in place at RBGE are discussed together with experiences over the past twenty years.

O11.2  

Quality assurance in Plant Health Diagnostics  
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Management of quality of testing is essential for laboratories carrying out diagnostics of regulated plant pests. International trade with plants and plant products largely depend on reliable monitoring for specific pathogens. Thus, diagnostic laboratories are enforced to prove a high level of confidence in their testing results through assuring that the quality of the process of diagnostic testing is harmonised with international legislation and standards. The Diagnostic Laboratory at the Danish Plant Directorate performs diagnostics on pests and diseases in plants and plant products, variety testing in cereal seed, GMO in feed and seed, and Salmonella in feed. Within these areas of diagnostics, the current accredited diagnostics (according to ISO 17025) include the following methods; Immunofluorescence microscopy, ELISA, PCR, real-time PCR, morphological identification, biochemical determination, protein electrophoresis, plant tests, and selective growth of microbes. Development and implementation of a quality system is often met with resistance by staff. However, once developed and implemented the advantages of working quality assures become more obvious. In general, staff become increasingly confident about their work because tasks and responsibilities are defined, and because the work flow is described. In quality management, staff is trained to fulfil their tasks, documentation of critical steps in the diagnostics is carried out, equipment is calibrated, and there is a built-in system for continuous improvement.

Organization and implementation of the quality assurance system will be presented.
Socio-economic aspects of quarantine management
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Recent years have witnessed a renewed interest for socio-economic research in the field of quarantine management. The Dutch Ministry of Agriculture, Nature and Food Quality asked LEI and Wageningen University to develop a socio-economic research programme to support phytosanitary policy. Several topics are subject of research:
2. Chain risk analysis to determine optimal inspection regimes in a production and market chain covering multiple quarantine pests and diseases
3. Developing methods for computing quantitative economic effects within the current EPPO scheme for Pest Risk Analysis
4. Multi criteria analysis of phytosanitary measures. Economic, epidemiological, environmental, implementation, food safety criteria are used for assessing measures.
5. Application of self regulation in quarantine management.

The current research programme is framed by the present national and international political context. The ambition is to extend the research programme with analysis of quarantine management from the trade political point of view. Results can be used for both the development and implementation of quarantine policy. Furthermore, we are challenged to connect the socio-economic discipline to Plant Health Diagnostics and Epidemiology.

Monitoring of Phytophthora ramorum (Sudden Oak Death) in Denmark
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In accordance with the EU provisional emergency phytosanitary measures to prevent the introduction into and the spread within the Community of Phytophthora ramorum (2002/757/EC), the Danish Plant Directorate (PD) has tested plants with symptoms for infection with P. ramorum. The tested samples have their origin in Danish nurseries and garden centres, in public green cites and in forestry sites. So far infected Rhododendron spp. and Virburnum spp. from one public green cite and from several nurseries have been found infected. Sporulation of P. ramorum was only observed after incubation on PARP-H medium. To evaluate the epidemiological potential in Denmark the PD laboratory wanted to investigate if the fungus does produce sporangia under particularly Danish nursery conditions where the young plants often remains for short periods. From a nursery where young plants were found infected in 2005 water samples and samples from soil were taken at different depths. The samples were tested by a baiting technique. P. ramorum was caught from the surface water of one drainage ditch and from sand and soil at 5cm and 10cm depth beneath infected plant material. The PCR method was used for verification of the identification. The result shows that young infected plants in Danish nurseries does produce live inoculum and the inoculum is disseminated to the surroundings. Presence of the fungus in soil may have an impact on the present EU provisional emergency phytosanitary measures.
The purpose of our research was to study the distribution of potato wart in Leningrad region and to characterize the ability of susceptible potato cultivars to form wart by using the inoculum from different populations of Synchytrium endobioicum. Soil samples from 6 quarantine sites of Leningrad region were investigated using the method, developed in the Ukrainian Research Station of Plants Quarantine. The procedure included centrifugation of the soil suspension with NaI. Viable zoosporangia from a supernatant had yellow color and not viable were not colored. The results are shown in Table 1.

Table 1: The presence of viable zoosporangia of Synchytrium endobioticum in soil samples from quarantine sites of Leningrad region

<table>
<thead>
<tr>
<th>№</th>
<th>Location</th>
<th>Number of soil samples</th>
<th>Average quantity zoosporangia in 1 g of soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Viable</td>
</tr>
<tr>
<td>1</td>
<td>Podporozhsky Region The city Podporozhe</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>Podporozhsky Region The village Bereg</td>
<td>6</td>
<td>43</td>
</tr>
<tr>
<td>3</td>
<td>Vsevolozhsky Region The city Vsevolozhsk</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Boksitogorsky Region The village Efimovsky</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Kirshevsky Region The village Gremjachevo</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Vsevolozhsky Region The village Irinovka</td>
<td>1</td>
<td>45</td>
</tr>
</tbody>
</table>

Data of the Table 1 shows that in 3 sites 43-60 viable zoosporangia in 1 g of soil were detected. Taking into account that 30-40 viable zoosporangia in 1 g of soil is optimal for S. endobioticum infection, these 3 locations remain to be in quarantine list. Cultivation of resistant potato varieties on the plots with high inoculum density will stimulated the microevolutionary process of parasite which can provide the increasing of infection type.
There is a general concern that the international movement of agricultural goods due to trade liberalisation and in the international movement of people and materials (globalisation) have been associated with a growing threat of disease introduction. Examples in the UK such as the foot and mouth disease, Newcastle disease, avian influenza, potato brown and ring rot and sudden oak death, exemplify the concern. Likewise, in Africa the spread of plant diseases such as Banana Bacterial Wilt across the Great Lakes region of East Africa presents an additional and serious risk to the livelihoods of vulnerable households.

In this study, we examine the hypothesis that the reported establishment of new, introduced plant diseases has been accelerating over time, by analysing trends in new plant disease introductions that have established over the past century. A particular comparison is made between Africa and Europe. In discussion any trends observed are contextualised against differences in the governance and culture of nations and their capacity to and propensity for monitoring disease. As an example of the data realised it is evident that whilst disease introductions have increased for Europe over recent decades the inverse is apparent for Africa.

Quorum Sensing (QS) allows bacteria to assess their local population density via the secretion of small, diffusible molecules. The ability to interfere with QS offers a potential tool for biocontrol of bacterial diseases. Erwinia carotovora subsp. carotovora (Ecc) is a pathogenic bacterium causing blackleg and soft rot in potato. Control of the pathogenicity factors is QS dependent and regulated by N-acyl homoserine lactones (AHL). In our studies, potato rhizosphere isolates were screened for AHL-degrading activity. Isolate identified as Ochrobactrum sp. A44 was the most active. Four indicator strains were used in signal molecules degradation analyses. E. coli JB534 (luxR-Plux-RBSII-gfpmut3*) generating GFP in the presence of AHL, E. coli pSB401 (luxRI::luxBCDAE) generating light, C. violaceum CVO26 generating violet pigment and A. tumefaciens NT1 (traR, traG::lacZ) with β-galactosidase AHL-dependent activity. The pathogenicity test on potato slices estimate the ability of A44 to inhibit tissue maceration, caused by Ecc. Isolate A44 is able to degrade synthetic AHL and AHL secreted by Ecc. Probably by this phenomenon attenuation of Ecc pathogenicity toward potato tubers is observed. It seems, that A44 could play an important role in the future as a tool in integrate control. Future aims are: characterization of the gene coding AHL-degrading factor and purification of the protein for further investigations and experiments assessing usefulness of the protein in biological control.
Characterisation of erwinias causing blackleg and soft rot in Finland by sequencing and virulence tests

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Contamination of tubers with soft rotted bacteria which cause blackleg and soft rot is one of the biggest problems in seed potato production. Blackleg is caused mainly by Erwinia carotovora subsp. atroseptica (Eca) and rotted of the tubers by the subspecies carotovora (Ecc). E. chrysanthemi (Ech) causes both kinds of symptoms in warm climate. Seed potato fields and harvested tubers are screened visually for rotted in Finland. Because also healthy-looking potatoes can contain latent infections, new diagnostic tools are needed to prevent the spreading of contaminated stocks. To reach that goal we first need to know what erwinias are causing the problem.

Potato stems showing blackleg, rotting tubers and river water samples were collected, and the erwinias were isolated on pectate-containing media. The bacteria were verified as Erwinia by PCR–tests and sequencing the 16S–23S rDNA intergenic region. Among the strains isolated from stems 40% was identified as Eca and 27% as Ech. Majority of the strains isolated from tubers were Ecc (48%) and 7% were Ech. Almost all strains isolated from rivers were identified as Ech. Phylogenetic trees based on the sequences showed that Eca strains were similar, whereas Ecc and Ech groups showed larger variation. Pathogenicity assays on potato tubers and stems showed that virulent Ech strains had been isolated from all sources. The results suggest that Ech is no longer contained to warmer countries but has spread to northern Europe. Therefore, any new methods in Erwinia diagnostics should detect Ech.

Reliability of PCR and BIOLOG BACTERIA techniques for Clavibacter michiganensis subsp. michiganensis and associated bacteria recovered from infected tomato plants

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Bacterial canker of tomato, caused by bacterium Clavibacter michiganensis subsp. michiganensis [(Smith) Davis et al.] (Cmm) is well-known disease resulting serious losses to both glasshouse and field tomato crops in areas where tomato are planted. Cmm was isolated from various parts strongly infected tomato plants, from that a lot of associated bacteria were recovered, too. Reliability of PCR employing our primers (Cmm 1F and Cmm 1R) designed for detection of Cmm was verified. None of concomitant bacteria tested were positive in PCR reaction with these primers. Polymerase chain reaction based on repetitive sequences (rep-PCR) was used for subdividing all strains of associated bacteria. Rep-PCR performed with BOXA1R primer allowed discrimination of the analysed collection into several groups. All isolated plant pathogenic (Pseudomonas aeruginosa, P. syringae, P. corrugata, P. viridiflava and others) and saprophytic (Pseudomonas fluorescens, Pantoea dispersa, P. agglomerans, Stenotrophomonas maltophilia and others) bacteria were identified using Microbial Identification System Biolog Bacteria, but with different reliability. Cmm strains were mostly identified to level species Clavibacter michiganensis, however not always to level subspecies michiganensis.

The work was supported by the Ministry of Agriculture of CR, project No. MZE0002700603 and No. 320/5305 and by the Grant Agency of the Academy of Sciences of the Czech Republic, grant No. AV0Z50510513.
Identification and characterization of *Streptomyces scabies* isolated from common scab lesions on potato tubers by morphological, biochemical and pathogenicity tests in Algeria.

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In summer 2002 and 2003, 35 isolates causing common scab symptoms were collected from tubers presenting superficial and raised corky lesions on tubers surface in number of locations from the Chlef region in western Algeria. The common scab inducing organism was characterized by creamy colonies on yeast malt extract (YMA) and by aerial mycelium which turned brown with age. The organism was Gram positive, non motile, utilized L-arabinose, D-fructose, D-glucose and rhamnose. They degraded the xylose and starch with production of melanin on peptone yeast extract agar-iron (PYI). Additionally, it was determined that most strains obtained from different locations were identical in morphology and biochemical characteristics. Results of pathogenicity tests showed that all isolates were pathogenic on both cultivars (Desiree and Claustar) causing symptoms with aggressiveness of strains varying from mild to moderately severe. Koch’s postulates for isolates were also fulfilled.

Effectivity of essential oils against *Erwinia amylovora*, the causal agent of fire blight disease

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*Erwinia amylovora* [(Burr.) Winslow et al. 1920], the causative agent of fire blight disease, appears as threat for some species of family *Rosaceae*. The fire blight pathogen is inscripted in list of quarantine organisms in many countries around the world to them very strict quarantine measures are used. In chemical control, preparations based on copper compounds are applied most frequently, but they can be phytotoxic and not enough effective, hence other alternatives are considered. In our experiment, about 30 essential oils obtained from different plants were tested in antimicrobial effectivity against *E. amylovora*. The screening was conducted *in vitro* on agar plates contaminated by *E. amylovora*. Essential oil as crucial extract was dropped on surface of agar plate, and average of inhibitory zones was measured. Streptomycin was used as control. The weak inhibitory activity against *E. amylovora*, but higher than had streptomycin, was recorded in essential oils from *Lavandula latifolia*, *Melaleuca quinquenervia*, *Mentha spicata*, *Tagetes bipinata* and *Thuja occidentalis* (zones 7-10 mm); the medium inhibitory effectivity was found in essential oils from *Artemisia absinthium*, *Eugenia caryophyllata*, *Mentha citrata*, *M. pulegium* and *Ocimum basilicum* (zones 11 - 20 mm); the strong inhibitory effectivity was shown in essential oils from *Mentha arvensis* and *Origanum vulgare* (zones 21 - 40 mm).

The work was supported by the Ministry of Agriculture of CR, project No. MZE0002700603
High resistance of hawthorn clone (Crataegus x monogyna) to fire blight (Erwinia amylovora)

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Since the first appearance of fire blight of rosaceous plants caused by Erwinia amylovora in the Czech Republic in 1986, shrubs and hawthorn trees (Crataegus spp.) act as the most important reservoir of infectious material from which susceptible cultivars of pear and apple trees may become infected. On the territory where fire blight pathogens are established, susceptible and very susceptible hawthorn plants are prevailing in the natural landscape. V 1994-1996, seedlings growing under the relatively resistant and susceptible hawthorn shrubs in the landscape were removed and transplanted to the experimental plot under insect-proof net. Bedsides, seed samples were collected from non-infected solitary plants adjacent to those showing severe infection. The seeds were germinated in the greenhouse to obtain one-year seedlings suitable for infection with fire blight pathogen. In total more than 60 hawthorn seedlings were inoculated. Shoot tops were cut off using scissors immersed in an E. amylovora suspension of approximately at 1 x 10^9 cfu/ml. A drop of inoculum was placed on wounded tissues. The degree of resistance was scored 30 days after inoculation by measuring the total length of the shoots and visually blighted part of the shoots. The majority-tested seedlings tested were susceptible. Only one of the tested seedlings was highly resistant. Its high level of resistance was confirmed by repeated inoculations (34 to 40 inoculations per season) during twelve growing seasons in 1994 – 2006. The resistant seedling was vegetative propagated. When obtained plants of that clone were tested they exhibited resistance to inoculation of blossoms and bark tissues. The resistant clone was identified as C. x monogyna (Jacq.) having some characteristics of C. laevigata (Poiret) DC.


Usability of the Microbial System Biolog for Identification of Plant Pathogenic Bacteria

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The usefulness of Biolog microbial identification system (MicroLog™, Release 4.2) for phytobacteriological laboratories was evaluated according to the criteria of universality (scope of usability), reliability (confidence, exactness), rapidity, expensiveness (cost-effectiveness) and standardization. The main attention was paid to the evaluation of the scope of usability and reliability of identification. Of a total of 338 world known plant pathogenic bacteria, the Biolog system identifies 28 %. Of a total of 90 plant pathogenic bacteria significant under Central Europe conditions, 60 % are covered by the Biolog system database. An indicator of the reliability of the Biolog system was the percentage of correct identifications to the level of subspecies or pathovar, species and genus from the total number of identifications carried out based on the similarity index of biochemical profile of the tested species with the profile inducted in the Biolog system database. An indicator of the reliability of the Biolog system was the percentage of correct identifications to the level of subspecies or pathovar, species and genus from the total number of identifications carried out based on the similarity index of biochemical profile of the tested species with the profile inducted in the Biolog system database. More than 450 strains of 6 genera were tested. According to the percentage of correct identification (PCI) for the levels genus and species, the identification was quite reliable for genera Erwinia (99 and 99 %), Pectobacterium (82 and 76 %) and Clavibacter (95 and 86 %). However the identification to the level of species for the Rhizobium genus (PCI = 59 %) and pathovars of the Pseudomonas syringae strain (PCI = 27 %) is less reliable.

This work was supported by the Ministry of Agriculture of the Czech Republic, project No. MZe0002700603.
**P12.6 The role of volunteer potatoes in the epidemiology of *Clavibacter michiganensis* subsp. *sepedonicum* under field condition**

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The quarantine bacterium *Clavibacter michiganensis* subsp. *sepedonicum* (Cms) is the causal agent of bacterial ring rot of potato which constitutes worldwide threat for potato growers- and industry. Ring rot is managed through the use of certified seed potatoes, the implementation of a zero-tolerance regulation and strict sanitation practices. The primary source of infectious material is infected seed potatoes. The contribution of other potential inoculum sources, such as non-host weed species, infected volunteer potatoes and contaminated soil, is poorly understand.

The possible role of plants of volunteer potatoes in the epidemiology of Cms was investigated. Surveys were made for presence of plants of volunteer potatoes, i.e. plants arising from tubers derived from previous crops and surviving in soil through one or two winters. Seven sites in Bohemia (the west part of the Czech Republic) were surveyed in 2005. In surveyed fields, the occurrences of Cms were ascertained and officially verified one or two seasons ago. Emerging volunteer potato plants were taken and transplanted in the experimental plot located at Kunratice in the northern Bohemia. After plant senescence, potatoes were harvested by hand.

A total 50 daughter tubers were randomly selected from each 7 samples and stored at 6°C for one month and then at 22°C. The tubers of each sample were randomly sorted into 5 subsamples of 10 tubers each. These subsamples were tested on the presence of Cms by DAS ELISA in four three-week intervals. DAS ELISA tests using polyclonal antibody (LOEWE Biochemica) were carried out by a standard protocol.

Of 7 samples from potentially contaminated fields, Cms in daughter tubers has been found in 6 samples.

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**P12.7 Characterization of Italian *Xanthomonas campestris* pv. *campestris* population by M13-PCR**

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*Xanthomonas campestris* pv. *campestris* (Xcc) is the causal agent of Vascular blackening of crucifers. The symptoms consist of blackening of the vascular tissue and marginal chlorosis of the leaves. In this work 126 Xcc isolates, principally isolated from different regions in Central and Southern Italy, were characterized for DNA polymorphism by PCR, using the primer designed on the minisatellite repeated region of M13 phage. All the Xcc isolates, except two, were distributed in two main clusters: cluster α, with similarity index (SI) of 0.77, containing the major part of the isolates (113) grouped in 36 haplotypes; cluster β (SI = 0.75) containing only 11 isolate grouped in 6 haplotypes. Cluster α was divided in two sub-clusters: α-I (SI = 0.78) containing 61 isolates grouped in 19 haplotypes and α-II (SI = 0.80) containing 52 isolates grouped in 17 haplotypes. Cluster β was divided in two sub-clusters: β-I (SI = 0.80) containing 5 isolates grouped in 3 haplotypes and β-II (SI = 0.78) containing 6 isolates grouped in 3 haplotypes. Sub-cluster α-I contained isolates from Central and Southern Italy whereas sub-cluster α-II contained isolates from Central Italy only; moreover, it contained isolates from France and Great Britain. The four phytopathogenic *Xanthomonas* spp. used as out-groups, fell out the two clusters except *X. vesicatoria*, that fell in sub-cluster β-II. These results show very large variability of Xcc.
P12.8 Pseudomonas syringae DC3000, is it pathovar tomato, maculicola or what else?
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The first completely sequenced Pseudomonas syringae strain was P. syringae pv. tomato (Pto) DC3000, a rifampicin resistant derivative of P. syringae strain NCPPB1106 isolated from tomato on the island of Guernsey (UK) in 1960 by R.A. Lelliott. PtoDC3000 was chosen to be sequenced because it causes disease not only on tomato, but also on the model plant species Arabidopsis thaliana. PtoDC3000 also causes disease on cauliflower, while most other P. syringae pv. tomato strains do not cause disease on either A. thaliana or cauliflower. Based on previous RFLP and sugar utilization studies, it was suggested that DC3000 is a pathovar maculicola strain. We sequenced several genes in the genome of strains that are known to be closely related to PtoDC3000 based on previous studies. We identified several strains with host ranges identical to that of strain DC3000 that were isolated from weeds in the USA and New Zealand. These strains have chromosomal regions that are identical to pv. maculicola strains, regions similar to pv. tomato strains, and regions distinct from both pv. tomato and maculicola strains. This suggests that PtoDC3000-like strains evolved through recombination events between pv. maculicola-like strains, pv. tomato-like strains, and at least one more group of strains, that are not pathogenic on any major crop.

P12.9 Characterization of plant pathogenic bacteria associated with premature dying of young grapevine plants in the Czech Republic
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Twenty two isolates of Rhizobium (Agrobacterium) and Pseudomonas genera were isolated from grapevine xylem fluid during the spring bleeding on three localities in central Bohemia and south Moravia. Semiselective media for Rhizobium radiobacter (Rra) and Rhizobium vitis (Rv) were used for isolation. Of the 12 Pseudomonas isolates, 33% were identified by Biolog system as Pseudomonas fluorescens (Pf), 33% as Pseudomonas syringae (Ps) pathovars and 34% as others Pseudomonas species. In the tobacco hypersensitivity test, Ps pathovars and Pseudomonas savastanoi pv. glycinea were positive. In the test on tomato seedlings and on the segments of grapevine canes only one Pf isolate was pathogenic. All of Pseudomonas isolates were nonpathogenic in tests on chrysanthemum. Of the Rhizobium 2 isolates were identified as Rv, 6 as Rhizobium rhizogenes (Rrh) and 2 as Rra. All of them were pathogenic in the test on tomato seedlings. Both S40/2 and S40/3 Rv isolates caused tumors on tomato seedlings and were pathogenic in the pathogenicity tests on chrysanthemum and grapevine canes. Tumor on tomato seedlings and lesion on the grapevine canes was caused with one of the Rrh isolate too. It can be concluded, that bacteria of Rhizobium genera were associated with grapevine plants dying in new planted vineyards in the Czech Republic in 2002 – 2004.

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K13.1  Molecular approaches for characterization and use of natural disease resistance genes in wheat
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In the large gene pool of the wheat crop species, there are a few lines which show resistance against some races of powdery mildew, a fungal pathogen. This resistance is caused by single genes which directly or indirectly recognize pathogen effector molecules. We have recently isolated by map-based cloning one of these specific powdery mildew resistance genes, the \textit{Pm3b} gene. Comparative genomics was an essential tool for map-based gene cloning in the large, hexaploid wheat genome. At the \textit{Pm3} locus, there are 10 known \textit{Pm3} genes, each conferring resistance against a specific group of fungal races carrying the matching avirulence gene. Based on haplotype studies in wheat lines carrying different \textit{Pm3} alleles, we have developed molecular tools from conserved up- and downstream sequences to isolate the functional alleles. We found that the \textit{Pm3} genes form a true allelic series and that they are highly conserved at the molecular level. The low divergence, as well as the absence of these genes in the tetraploid wheat gene pool, indicate that the \textit{Pm3} resistance alleles are evolutionary young and that they evolved after wheat domestication. Domain swap experiments have allowed a detailed insight into the molecular basis of specificity as well as the intramolecular interactions required for protein activity. These data provide the basis for a rational strategy to study naturally occurring variability of \textit{Pm3} genes as well as the potential creation of new functional alleles in vitro.

O13.1  Application of TILLING for molecular breeding for disease resistance
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The existing molecular genetic technologies to analyze crop plant genomes did not match the progress that had achieved in the field of DNA sequence information where a huge amount of sequence data are available now in particular from model species such as \textit{Arabidopsis} and rice. Therefore, TILLING (Targeting Induced Local Lesions IN Genomes) is a procedure of reverse genetic strategy introduced to fill the gap between structural genomics and functional genomics in crop plants (Colbert et al., (2001) Plant Physiology 126: 480-484). The TILLING permits the identification of mutations in target genes without production of genetically modified organisms. In crop plants, it is an enormous advantage that mutations found with TILLING do not involve introducing any foreign DNA. Therefore, plants identified by TILLING technology are completely non-GMO. Ecotilling is a variant of TILLING, and is a high throughput method to detect and discover new point mutations and InDels (Insertions and Deletions) in DNA (Comai et al. (2004) Plant Journal 37. 778-786). Ecotilling differs from TILLING in that natural populations are used, as opposed to mutated populations, and the samples are not pooled. As natural populations are used, different polymorphisms can be expected, including large insertions and deletions, with several polymorphisms often occurring in the same gene. Ecotilling was employed to identify the alleles of \textit{Mla} and \textit{mlo} locus controlling powdery mildew resistance in barley.
O13.2  Toward positional cloning of a gene conferring resistance to potato late blight, in Solanum caripense
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We detected resistance to potato late blight in a wild relative, S. caripense (crp). Progenies segregating for dominant resistance were exposed to P. infestans, the causal agent of late blight, and the resistance proved intact against all the samples representing strains of the pathogen occurring worldwide. For an initial framework map, we used two reciprocal cross progenies of just two parental crp genotypes. For the resistance, a single QTL was detected. However, insufficient polymorphism within that mapping population caused us to develop a second population by crossing one individual of the first population with an unrelated S. caripense plant. We expected to obtain in this self-incompatible species sufficient polymorphism for the development of a dense genetic map. However, again, rather low genetic variability amenable to the detection of molecular markers (AFLP, SSR, candidate gene) was apparent. The putative resistance locus was linked to flanking markers at a large genetic distance not yet allowing for cloning of the gene. Progress in mapping of chromosome-specific markers was achieved through the application of consensus sequence (cos) primers (http://www.sgn.cornell.edu/documents/markers/cosii.xls). Single nucleotide polymorphisms (SNP) were detected. Using primers specific for these SNPs we could obtain and map markers and, in that way assign tentative Solanum chromosomes.

P13.1  Is the yellow rust resistance in an old European wheat durable?
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The resistance in an old European wheat cultivar (Parent 1) to the yellow rust pathogen Puccinia striiformis has been found to be conferred by two major APR QTLs located on linkage groups 1 and 2. These major QTLs were identified in a DH population between Parent 1 x Brigadier using field scores collected in 2004 and 2005. A minor QTL on linkage group 3 (Parent 1) may also confer some resistance. Brigadier, the adult plant susceptible parent, also contributes some residual resistance with effects being found on linkage groups 4 and 5. Currently no PCR-based flanking markers are available for the 2 major QTLs. AFLP-Bulked Segregant Analysis (AFLP-BSA) is being carried out, to identify bands linked to both QTLs. Nine bands identified will be sequenced and converted into locus specific PCR markers. Growth stage experiments using NILs have shown that the group 1 QTL has an effect on resistance at an earlier plant growth stage than the group 2 QTL and that its full effect is also expressed earlier. This, along with evidence from the QTL analysis, suggests that the group 2 APR QTL may offer a more durable form of resistance individually when compared to the group 1 QTL. To give a better insight into the potential durability of the major QTLs, the phenotypes of the two resistances are currently being examined cytologically in the NILs. This will determine the developmental stage at which the pathogen’s growth is arrested.
Characterization and detection of resistance donors in potato germplasm using DNA markers

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In recent time is a resistance breeding against pathogens one of the most important way, how economically eliminate yield looses in potato crops (\textit{Solanum tuberosum} ssp. \textit{tuberosum}). By the methods of molecular biology is it possible to locate and determine genetic resources of required features, thus we can improve a new resistant varieties develop. One of many followed descriptions is a resistance to endoparasitic root cyst nematode \textit{Globodera rostochiensis}. The most important H1 resistant locus locates on distal end of the long arm chromosome V. This gene confers resistance to \textit{G. rostochiensis} pathotypes Ro\textsubscript{1} and Ro\textsubscript{4} by the activation of a hypersensitive reaction and is closely linked to RFLP markers CP113 and CD78 (Gebhardt et al. 1993, Pineda et al. 1993). Additional instrument used to determination of H1 are CAPS markers CT51\textsubscript{CAPS} and CP113 (Niewohner et al. 1995). Markers 239E4left\textsubscript{CAPS} (designed by) and CT51\textsubscript{CAPS} (mentioned above) can be used to screen for recombination due to position on chromosome V. Finally, newest technique applied to detection resistance gene against PCN is AFLP, especially AFLP markers EM1 and CM1 (Bakker et al. 2004). The aim of our work is to find applicable CAPS markers (alternatively AFLP markers convert to CAPS), cheaper and easy to use for MAS. This work is support by the research project of the Ministry of Agriculture of the Czech Republic: MSM 6010980701 Molecular and technological basis of quality potato production.
K14.1 How to use diversification strategies for disease control in modern agriculture?
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While diversity for resistance has been recognised for more than 60 years as a key factor in disease management and diversification strategies such as cultivar mixtures and multilines are presented and advocated in almost every plant pathology textbook, the general view in modern agriculture is that it would be too difficult and expensive to implement on the one hand. On the other hand, difficulties in marketing the produce are also feared. The question thus arises if and how such strategies can be designed to find a place in modern agriculture. Considering the general ecological benefits of diversification and the possible economical benefits for the growers and society a variety of possible approaches to the solution of actual and perceived problems in modern agriculture will be discussed. These include (i) strategies in genetic resources development: Approaches such as the selection for inducibility of resistance and competitive ability as well as an evolutionary breeding approach leading to highly adaptable plant populations (composite cross approach) will be discussed. (ii) Local and regional management strategies: Possibilities for growers to use diversification strategies on-farm with the existing equipment will be presented. (iii) Technical solutions: These are integral to the future use of diversification strategies and they reach from more or less simple adjustments to machinery for planting and harvesting to separation devices of the harvested goods.

O14.1 Intercropping Pea with Barley: The Effect on Disease
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Intercropping is the simultaneous cultivation of more than one crop species in the field. Levels of diseases were compared in barley and peas grown as sole crops or in an intercrop. On barley net blotch (Pyrenophora teres), leaf rust (Puccinia hordei) and powdery mildew (Blumeria graminis f. sp. hordei) were observed. All three diseases were reduced in the intercrop. On pea, ascochyta blight (Mycosphaerella pinodes) and mildew (Erysiphe pisi) were also observed at reduced levels in the intercrop compared to the sole crop. The reduction in disease observed appeared to vary with the disease observed, the crop variety used (both the host and the accompanying crop), the amount of each crop in the intercrop, the sowing pattern and the level of inoculum. The mechanisms underlying the reduction in M. pinodes on intercropped pea were investigated. Reduced pea biomass in the intercrop (compared to the sole crop) appears to account for part of the reduction in disease observed in the intercrop. The remainder of the reduction appears to be caused (in part at least) by the barley forming a physical barrier to the movement of M. pinodes. There appears to be little interaction between the pea and barley influencing response to M. pinodes in pea. Differences in microclimate between the sole and intercrop were small, and could not be related to the disease reduction.
O14.2 Influence of foliar diseases on grain yield of spring barley in low input cropping systems
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Variation in grain yield was studied in 52 spring barley varieties and 17 combinations of location, growing system and year (‘environments’). Choice of variety was found to be as important a factor for grain yield as other factors in the management. In the absence of disease free control plots, we used external variables for variety traits as regression coefficients, i.e., the official grouping for disease susceptibility in ‘Plant Protection Online’ and grouping for yield potential under low or absent disease based on Danish variety trials. The environments were characterised by yield potential and disease intensity and used as covariates in the model. The disease resistance grouping of the varieties coupled with the environmental disease load of powdery mildew, leaf rust and net blotch had a highly significant effect on grain yield. Scald occurred at low levels in most environments, resulting in a relatively poor basis for drawing conclusion about the influence of this disease. The slopes of the regression lines varied for each disease between varietal resistance groups, i.e., increased susceptibility resulted, in general, in increasing yield losses. The results confirm the power of using environmental variables and external variety trait variables as covariates in yield-loss analysis, in particular in biological systems with highly variable environments and potential large host genotype effects on biotic stresses.

O14.3 Lettuce downy mildew – breaking the ‘boom bust’ cycle
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Much effort has been expended breeding for resistance to \textit{Bremia lactucae} (the causal organism of lettuce downy mildew). However, there is a gene-for-gene relationship between \textit{B. lactucae} and lettuce and breeding for resistance to lettuce downy mildew has followed a classic boom-bust cycle. This has recently accelerated and often new resistances ‘breakdown’ within one or two season of being released. There is an alternative form of resistance in lettuce. This is a quantitative rate reducing resistance characterised by fewer plants becoming infected, fewer leaves infected and smaller lesions. It has been termed field resistance and it is apparently race non- specific. The resistance was identified in the 1980’s but to date only one cultivar has been recorded as being specifically bred for field resistance. The quantitative nature of field resistance, the poor morphology of the source of resistance and the conditions needed to carry out effective selection have made it difficult combine high levels of resistance with good morphology. We have carried out a genetic analysis and located quantitative trait loci (QTL) determining field resistance. We have identified molecular markers linked to each of the QTL which can be used for marker assisted breeding for field resistance. Currently we are working with a lettuce breeding company to put this into practice and break the boom bust cycle of lettuce downy mildew resistance breeding.
Ramularia leaf spot (RLS) has reached high severity levels in many spring- and winter barley cultivars in recent years at several sites in Denmark but the importance of the disease for Danish barley production is still unclear. Data from multi-environment trials indicated large varietal and environmental differences in RLS severity. Of the variation in RLS severity observed on individual barley cultivars, about 71% and 66% could be explained for spring- and winter barley, respectively, based on effects of the cultivar and the environment. The spring barley cultivars consistently exhibiting lowest levels of RLS severity were Power, Isotta, Cruiser and some others while Lonni, Lomerit, Nobilia, Chess and Carola were the winter barleys generally least affected by the disease. Such cultivars may be promising RLS resistance donors. Genetically closely related cultivars exhibited similar pattern with respect to statistical parameters describing their RLS reaction. The results suggest that varietal resistance can be an efficient means for controlling RLS. The apparently most RLS-susceptible spring barley cultivars were those possessing Mlo-resistance against powdery mildew while the apparently most RLS-resistant ones such as Power, Isotta and Cruiser do not. Associations with varietal resistance properties regarding other diseases were not apparent. More work is needed to determine the nature and genetic basis of RLS resistance and to develop reliable methodology for efficient resistance testing.

A double haploid wheat population (91 lines from the Winter x Spring wheat cross Arina x NK93604) was phenotyped and mapped to understand and characterise the genetic basis of PDR components and their relationship to FHB. The map construction using SSR, AFLP and DarT markers is still in progress and identification of linkage groups is still tentative. Fusarium resistance has been assayed in detached leaf bioassays (latent and incubation periods), and in the field for 3 years. Relationships have been analyzed by interval mapping (IM) and the principal component analysis-derived method Partial Least Squares Regression (PLSR). In general, the latter method identified more QTL than IM and with higher (adjusted or calibrated) R² values. For incubation period IM identified 2 QTL, close to markers Dup004 (4A) and gwm 161 (3DS), explaining 19.4%. For latent period only one QTL was identified, gwm698 (7AL, 12.1%). Both were derived from the Arina parent, contributing to longer incubation/latent periods. Using PLSR the same were identified, in addition to others from both parents, with ca. 26%R². Interestingly a QTL in Arina was identified in both traits close to markers gwm389 and barc75, which both map to the 3BS region known from Sumai-3. However, since the two traits were only weakly correlated, the QTL explained only part of the variance. Molecular markers for FHB resistance were generally not coincident with those for incubation and latent periods, indicating that PDR components and FHB were largely under separate genetic control. However, PLSR analysis of DON also identified a QTL at barc75 (3BS), indicating an overlap with incubation and latent periods.
Evaluation of *Fusarium* head blight resistance in US soft red winter wheat germplasm using a detached leaf assay

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This research investigated if a detached leaf bioassay, inoculated with isolates of *Microdochium nivale* var. *majus*, identified components of FHB resistance among thirty entries of United States soft red winter wheat in the 2002 Uniform Southern FHB Nursery (USFHBN). FHB resistance of the USFHBN entries was evaluated at 10 locations in eight states during the 2001-02 season. Incubation period was significantly correlated across all FHB resistance parameters accounting for 45% of the variation in FHB incidence, 27% of FHB severity, 30% of *Fusarium* damaged kernels and 26% of the variation in grain deoxynivalenol (DON) concentration. These results contrasted with previous studies of moderately resistant European cultivars in that longer incubation period was correlated with greater FHB susceptibility, but agreed with previous findings for the Chinese cultivar Sumai 3 and CIMMYT germplasm containing diverse sources of FHB resistance. The results indicate the bioassay has potential for use in distinguishing between specific sources of FHB resistance when combined with data on FHB reaction and pedigree information. For example, entry 28, a di-haploid line from the cross between the moderately resistant US cultivar Roane and the resistant Chinese line W14, showed leaf parameters that suggested a combination of both sources of FHB resistance. While the USFHBN represents the combination of adapted and exotic germplasm, with the exception of Ernie, the moderately resistant US commercial cultivars (Roane, McCormick, NC-Neuse and Pat) showed long incubation and latent periods and short lesion lengths in the bioassay. Similar relationships between incubation period and FHB resistance were observed in the 2005 USFHBN (data not presented).
P14.4  Yellow Rust and Powdery Mildew Resistance in Scandinavian bread wheat
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All accessions were resistant to Yr and over 50% had only unidentified Yr resistance. The Yr genes postulated in the material were Yr3 (40%), Yr4 (40%), Yr2 (11%), Yr7 (5%) and Yr6 (0.7%). The genes Yr3 and Yr4 were the most prevalent in all countries, somewhat more in spring wheat than winter wheat. Resistance to Pm was found in 39% of the material, including postulated genes Pm1a (14%), Pm2 (14%), Pm9 (13%), Pm4b (6%), Pm5 (3%), Pm8 (2%) and Pm6 (0.7%). Norway had a high relative frequency of Pm resistant accessions (94%), followed by Sweden (35%), Finland (23%) and Denmark (20%). The genes Pm1a+2+9 were only present in spring wheat, and were most common in Sweden and Norway. The gene Pm5 was only found in winter wheat, and was most frequent in Denmark and Finland. The difference in Yr vs. Pm seedling resistance is most likely reflective of different pathogen dynamics, and breeding programmes in the respective countries.

P14.5  Influence of variety mixtures on potato late blight in organic potato production
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In organic potato production, yield and quality of potatoes are threatened by Phytophthora infestans causing late blight, which is the most devastating potato disease worldwide. A field study was conducted to determine how efficient mixtures of potatoes with different levels of partial resistance are in reducing late blight under organic conditions. At two locations and two years, four varieties and their four-way mixture were grown in large plots and late blight was assessed weekly. In all four year-site combinations the percent severity of late blight in the mixture was significant lower or equivalent to the level in the varieties Oleva, Danva and Producent and higher in the variety Kuras. Percent severity of late blight was slightly reduced in most mixture plots compared to the mean level of the varieties in monoculture, but only significant in one year-site combination. There was no statistical significant mixture effect on yield or on the amount of blight infected tubers. It is concluded that the late blight reductions in the mixtures are of a magnitude which can not justify introduction of potato mixtures in practical organic potato production.
What information do farmers need to optimize disease control in cereal?
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The number of media, which can be used today to spread information to end-users have increased significantly over the last decades. Today, information is commonly disseminated to farmers by farmers magazines, specific newsletters from advisors received by mail or e-mail, home-pages or sms on mobile phones. For generations, scientists have aimed at clarifying and understanding specific diseases' life cycles, factors influencing epidemiology, yield loss potential and host-pathogen interactions in order to be able to minimize the disease risk, build warning systems or recommend specific control thresholds in relation to applying fungicides. The decision support system Crop Protection Online (CPO) is an example of a threshold-based system, which determines environmentally sound and economically viable fungicide strategies based on thresholds. The number of end-users among farmers has been stable but low during the last 10 years (1000 farmers). Major hurdles in getting more users are believed to be: 1) the requirements of carrying out assessments in the field, 2) farm sizes getting larger leaving less time for decision making in the individual field, 3) lack of economical incentives to change from today’s standard treatments. 4) the decision support systems’ inability to interact with other computer based programmes on the farm. 5) CPO does not appeal to the farmer’s way of making decisions on crop protection in general. A sociological investigation of the farmers’ way of making decisions in the area of crop protection has shown that arable farmers can be divided into 3 major groups a) system-orientated farmers b) experienced-based farmers and c) advisory orientated farmers. The information required by these 3 groups is different and has to be looked at individually from the end-user’s perspective rather than from the scientist’s perspective.
The volatile antimicrobial substance allicin (diallylthiosulphinate) is produced in garlic when the tissues are damaged and the substrate alliin (S-allyl-L-cysteine sulphone) mixes with the enzyme alliin-lyase (E.C.4.4.1.4) (Cavallito and Bailey (1944) Am. Chem. Soc. 66: 1950-51).

\[
\text{alliin} + \text{alliinase} \rightarrow \text{alllicin} + 2\text{pyruvate} + 2\text{NH}_3
\]

Allicin undergoes thiol-disulphide exchange reactions with free thiol groups in proteins. It is thought that these properties are the basis of its antimicrobial action. We tested the effectiveness of garlic extract against a range of plant pathogenic bacteria, fungi and oomycetes in vitro and in planta in diseased tissues (Curtis et al (2004) Physiol. Mol. Plant Pathol. 65: 79-89). Allicin effectively controlled seed-borne Alternaria spp. in carrot. Allicin in garlic extracts was quantified spectrophotometrically and by HPLC and a rapid bioassay was developed for routine use.

In Arabidopsis the reduction in disease was apparently due to a direct action against the pathogen since no accumulation of salicylic acid (a marker for systemic acquired resistance, or SAR) was observed after treatment with garlic extract. We see a potential for developing preparations from garlic for use in organic farming, e.g. for reducing the pathogen inoculum potential in planting material such as seeds and tubers. In addition we are employing a biotechnological approach to incorporate the alliinase gene into Arabidopsis to assess if resistance can be increased under appropriate circumstances.
O15.1 Biological and technical aspects of developing *Clonostachys rosea* as a fungal biocontrol agent (BCA).

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Isolation, selection and field performance: *In planta* screening was used for selection of candidates with multifactor or plant related mechanisms of control and well adapted to where they are to act. A strain ‘IK726’ with high biocontrol efficacy was selected. A bioassay with plants we developed reflects the performance of IK726 in the field. This has been a unique tool for further development of the BCA. Production, formulation and delivery: IK726 is produced by wet and dry fermentation. Our experience is restricted to small-scale production although production of *C. rosea* in large scale has been reported. Formulation of IK726 has resulted in a good shelf life. Coating or biopriming of carrot seeds with IK726 effectively controlled *Alternaria* spp. and enhanced seedling establishment. There have also been indications of control of soilborne diseases and IK726 can be incorporated in greenhouse mix for use with protected crops. The biology of IK726: DNA reporters transformed into IK726 (GFP, DsRed) were used to study IK726 *in situ*. In this way, we have observed conidial germination, colonization and conidiogenesis in soil, in greenhouse media and on seed, roots and leaves. Moreover, *in situ* interactions with pathogens have been studied. Work focusing on enzymatic activities and gene regulation in biocontrol interactions is in progress.

EU-registration: This still needs to be addressed although relevant information has been gathered. This will be done in close collaboration with industry.

O15.2 Towards indicators of soil health: impact of cultural practices on soil characteristics, relationships between these characteristics and with soil suppressiveness

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The aim of this study is to find the relationships between different descriptors and the pathological potential of soils towards plants in order to identify indicators of soil health. Three cultural practices were applied in an experimental field before carrot culture (integrated, cattle manure amendment and biofumigation with fodder radish). Eleven soil samples were collected in each plot at 3 key points during the cropping sequence (before treatment, before and after carrot culture), for 2 years. Physicochemical, biological, microbial diversity and plant-pathological properties were measured and correspondences were analysed by Principal Component Analysis. Inter-class PCA enabled us to distinguish any effect between treatments and dates. Relations between physicochemical, nematological, biological and molecular datasets, provided by co-inertia analysis, are discussed and dealt with the receptivity of the soil samples to *R. solani* damping-off. In both years, biofumigation resulted in a significant increase of the microbial densities, biomass and basal respiration, together with a decrease of the soil receptivity to *R. solani* damping-off. Organic amendment, and even more biofumigation, modified the bacterial and fungal community structures. Co-inertia analyses revealed significant co-structures between the nematological and physicochemical characteristics datasets. The other analyses are in progress. This strategy will enable us to propose a bunch of soil health indicators.
Sustainable disease management in legume rich cropping systems
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In feed self-sufficient organic farming systems, the proportion of grain legumes in a rotation should exceed 30% to meet the protein requirement. The current major constraint for a high frequency of grain legumes in organic rotations is soil and seed borne diseases. Many fields in Europe are e.g. no longer suitable for pea production due to high levels of \textit{Aphanomyces euteiches} and effective control measures are currently unavailable. The aim of the investigation was to identify major soil and seed borne pathogens, host specificity, plant host resistance and thresholds values in grain legumes. The level of \textit{A. euteiches} resistance in commercial pea varieties was insufficient. Among a range of legume species, vetch and alfalfa were infected and could maintain the infestation level of \textit{A. euteiches}. In contrast, faba beans and lupines did not host \textit{A. euteiches} and therefore can be considered as alternative crops in such fields. These crops might, though, multiply highly root damaging \textit{Fusarium avenaceum} and \textit{F. oxysporum} with a broader host range among the grain legumes. However, highly \textit{Fusarium} spp.-resistant varieties of both species were identified. Anthracnose caused by \textit{Colletotrichum gloeosporioides} is the most important seed borne disease in lupine with a recommended 0-tolerance threshold. Anthracnose resistance was identified in genotypes of \textit{Lupinus augustifolius}. Furthermore, early varieties appeared less infected but more field trials are needed to adjust the threshold value.
Options for managing scab in organic apple production after copper-fungicides are no longer available - some REPCO approaches.

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The European Union (EU) works towards a reduction in the use of copper fungicides (Council regulation 2092/91, annex II), because copper has negative side effects on soil organisms. Organic producers of a number of crops including organic apple growers are much dependant on sulphur, lime sulphur and especially copper-containing fungicides to control diseases. Of these, especially copper is highly effective against scab. Improved prevention and control of apple scab (\textit{Venturia inaequalis}) in organic production without the use of copper is a primary goal of the EU 6th framework project REPCO* (www.REP-CO.nl/). There are several novel windows of opportunity for controlling apple scab that are being investigated in the REPCO project and REPCO targets several points in the life cycle of \textit{V. inaequalis}. Screening of new fungicidal and resistance-inducing compounds, from natural sources including plant extracts is carried out on apple seedlings in the growth chamber and - for the most promising - this is followed by small scale testing against summer epidemics in orchards. Patent applications have been submitted for several materials. Selection of biocontrol agents against the summer stage is also underway. Another focus is the prevention of winter inoculum formation. For this, endophytes are isolated and tested for their ability to prevent ascospore development, and leaf degradation is stimulated by addition of organic materials to leaves in autumn, and by improvement of leaf consumption by earthworms. The most promising of the various methods are to be tested together under orchard conditions.

*REPCO (Replacement of Copper Fungicides in Organic Production of Grapevine and Apple in Europe) is partly funded by the sixth EU-framework program (Projekt Nr. 501452). The information in the publication is the authors’ responsibility. EU does not take responsibility for how this information is used. Project staff: Danièle Tissot Boireau, DG RTD Unit E03 Safety of Food Production Systems, SDME 8/22 Belgien, e-mail: daniele.tissot@cec.eu.int. Projektkoordinator: J. Köhl, Plant Research International, Wageningen, NL, e-mail: jurgen.kohl@wur.nl
Biological control of powdery mildew of barley
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Twenty-eight diverse bacterial strains representing different genera isolated from Swedish and Moroccan soils were tested for induction of resistance to powdery mildew of barley (Blumeria graminis f. sp. hordei). Seeds coated with bacterial culture broth resulted in plants that either were more susceptible or more resistant to powdery mildew. The five strains that induced the highest level of resistance to powdery mildew were identified and tested more stringently for induction of resistance. The same five strains were also tested for direct biocontrol activity by applying them to leaves. Four strains were particularly effective in preventing attack by powdery mildew. The mode of direct inhibition was studied first on microscope slides and later on leaves. In both cases, supernatants of these strains inhibited the development and elongation of the appressorial germ tube. The effect was non-reversible; the powdery mildew fungus did not recover, even a week after the application of the supernatant. We also tested application of supernatants to leaves that had previously been inoculated with powdery mildew. Even as long as 120 hours after powdery mildew inoculation, when first symptoms started to appear, the supernatants applied were effective in controlling and curing the plants from mildew. The potential of bacterial suspensions or supernatants of ‘spent’ culture broth as novel plant protection agents holds promise for the future.

Factors influencing strawberry flower infection and interactions between Botrytis cinerea and its fungal antagonists
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The gray mould pathogen, Botrytis cinerea, infects strawberry flowers, but symptoms may first appear at later stages of fruit development. Biological control using fungal antagonists in the growing season is aimed at inhibiting flower infection. However, field trials in Norway have shown that the effect of such treatments is unpredictable and biological control of B. cinerea cannot yet be recommended for commercial farmers. Flower infection by B. cinerea, as well as interactions between pathogen and antagonist, are influenced by spore concentrations, nutrient availability, temperature, and humidity. We are currently testing newly isolated indigenous fungi as antagonists of B. cinerea in the field, as these may be better adapted to the environmental conditions than antagonists previously tested. Preliminary results indicate that indigenous strains of Epicoccum nigrum and Aureobasidium pullulans have potential as control agents of B. cinerea. Improved biocontrol efficacy through better timing of antagonist applications could be achieved by knowledge of how climatic conditions and flower stages affect latent flower infection. Using a Botrytis-specific monoclonal antibody (Meyer et al (2000) Mycol Res 104: 979-987), we were able to detect infection in strawberry flowers 48 h after inoculation under laboratory conditions. We are currently evaluating real-time PCR as a method of monitoring flower infection by B. cinerea and activity of antagonists under field conditions.
Towards integrated control of Phytophthora in potato: results of the Dutch Umbrella Plan
Piet M. Boonekamp

In 2003 the Dutch Umbrella Plan Phytophthora was launched with the aim to reduce the negative impact on the environment of the use of fungicides to control *P. infestans* in potato by 75% in 2012. All research on Phytophthora in potato was integrated, focused on the reduction strategy, and brought together in six themes: genomics of Phytophthora, genomics of Phytophthora – potato interactions, new sources for resistance, epidemiology, population biology, and a Toolbox. The Toolbox is important as results from the other five themes are translated into practical solutions for integrated control. As the Umbrella plan is about half way, the first results from the different themes, and the integration in the toolbox will be presented. In addition the outlines of research in the next phase will be reported. Finally the use of the Umbrella plan for a further integration of European research on Phytophthora will be discussed. Reference: Boonekamp P.M. (2005) In: Proceedings of Potato 2005 Conference (A. Haverkort, P. Struik, eds).

A model for fungicide applications in winter wheat
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A new model for optimising fungicide applications in winter wheat has been developed on the basis of field data collected over five years. The model integrates varietal resistance classes and fungicide application strategies (timing, active ingredients, and dosage) with dose-response functions, quantifying the efficacy of individual fungicides with respect to controlling rust, powdery mildew and Septoria diseases. The model indicates average yield gains of 1.03 t/ha from using fungicides targeted against Septoria. An additional 0.5 t/ha yield gain was indicated for cultivars susceptible to Septoria. The yield gain from controlling powdery mildew and rust was approximately 0.2 and 0.1 t/ha, respectively. In 90% of the trials, the yield gain from controlling septoria leaf blotch exceeded the economical threshold (0.35 t/ha). The potential yield gain from combined mildew and rust specific fungicide applications rarely exceeded this threshold. The potential need for fungicide treatments differed much across regions. The standard deviation of the net yield gain was calculated as an indicator for the economic risk of using fungicides. Calculations showed that reduced dosages, independent of varieties, strategies and optimal dosage level, significantly stabilise the net yield gain. The model can become a useful tool for farmers to determine environmentally sound and economically viable fungicide strategies. Like all models, it needs regular updates with new data on varietal responses and fungicide efficacies.
P15.2 Plasma Membrane as the Target Site for the Antifungal Activity of the Herbicide Sethoxydim
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The fungicidal effect of sethoxiydim (Pakdaman et al, 2002, The Proceedings of the 53rd German Crop Protection Conference, p. 91, 16-19 Sept., Bonn, Germany) on the canola (Brassica napus var. Olifera) white stem rot pathogen (Sclerotinia sclerotiorum) encouraged us to conduct a series of studies on the mechanism of antifungal activity of this herbicide commonly applied in Iranian fields under canola cultivation. Our preliminary studies on the changes in the level of malondialdehyde (MDA) as the main product generated through peroxidation of polyunsaturated fatty acids indicated the disintegration of fungal bilayer of plasma membrane as the result of herbicidal treatment. Also, it was demonstrated that the amount of hydrogen peroxide in the treated samples was higher than the control samples with no herbicidal treatment. Therefore, our present results confirm the disintegration of the plasma membrane as one of the mechanisms for the antifungal impact of sethoxydim. As with weed plants, the phytotoxic impact has been attributed to the inhibition of the first enzyme in the lipid biosynthesis pathway, acetyl-CoA carboxylase, therefore, it would be very interesting to study on this subject and the relations between the sensitivity of different fungi and their DNA and protein sequences of acetyl-CoA carboxylase.

P15.3 Screening of cruciferous catch crops for club root resistance using biomarker fatty acids
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Cruciferous plants are commonly used as green manure catch crops due to their extensive root development, which can prevent plant nutrients from leaching between main crops. However, concern has been raised whether use of cruciferous catch crops will increase levels of the club root pathogen Plasmodiophora brassicae in the soil increasing the risk of club root development in a subsequent cruciferous crop such as rape or cabbage. In this scenario it is important to identify catch crops with P. brassicae resistance and in the present project nine different cruciferous catch crops were screened for P. brassicae resistance both in greenhouse pot experiments and in the field. For this purpose a new method to quantify P. brassicae in roots using biomarker fatty acids were developed showing that especially the fatty acid arachadonic acid can be used to quantify P. brassicae in roots. Among the tested cruciferous catch crops Raphanus sativum was less susceptible to P. brassicae both in pot experiments and in the field.
P15.4  Impact of various spray strategies with pyraclostrobin and epoxiconazole on sensitivity of Septoria tritici
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Field experiments involving various spray combination strategies of pyraclostrobin and epoxiconazole, were carried out in 2004 to monitor the fungicide sensitivity of Septoria tritici on different localities, leaf levels, and points of time. Sensitivity towards epoxiconazole and pyraclostrobin was assessed on pure-cultured isolates. No correlation between sensitivity towards epoxiconazole and pyraclostrobin was observed. Selection for less epoxiconazole sensitive isolates was observed as a result of fungicide application. Fungicide application did not result in any significant shifts of the population median (EC50) over time compared with the starting population. Under unsprayed conditions, the population median sensitivity declined over time. High input spray strategies resulted in significantly higher variability of epoxiconazole-sensitive isolates compared to unsprayed treatments, although no significant differences in sensitivity of the population median were observed between any of the sprayed strategies. Due to the high initial level of pyraclostrobin resistance, only minor or no impact on pyraclostrobin resistance was observed following the various combination treatments. Several spray applications with a mixture that included pyraclostrobin selected most heavily for strobilurin resistant mutants, although data for disease levels show that this strategy resulted in the best control.

P15.5  Intense development of snow mould in Latvia in 2005
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Snow mould of winter cereals (especially rye) has been observed sporadically in Latvia. Nevertheless, it is not a serious problem, especially in winter wheat sowings under an intensive regime of management. In Latvia, a sharp development of snow mould (caused by Microdochium nivale (Fries) Samuels & Hallett, previous term Fusarium nivale, teleomorph Monographella nivalis (Schaffnit) E. Müller) was noticed in the year 2005. Snow mould was observed in different conditions – on grass plots near houses, in sowings of production winter cereals, and also on experimental plots of wheat cropping systems. Three varieties (‘Tarso’, ‘Cubus’, ‘Zentos’), four different times of sowing (30.08; 07.09; 16.09; 29.09), and three different seed rates (300, 400, and 500 seeds per m2) were tested in the trials. The assessment of snow mould was carried out on April 14, at starting of vegetation. The incidence of snow mould fluctuated from 5 to 60 %. Seed rates did not influence the spread of snow mould, but incidence of the disease depended on the sowing time and the variety. The main reason for the sharp development of snow mould was the uncommon meteorological conditions during the winter of 2004/2005. The autumn was warm; therefore the density of wheat sown in normal time was too high. In addition, January was warm and life processes started early in some places, which weakened the plants. Investigations are still being continued for better understanding of this phenomenon.
Effect of compost fertilization on the control of corky root on canned tomato in open field
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Corky root, caused by Pyrenochaeta lycopersici, is an important soilborne disease of tomato in Italy. Effect of compost, obtained from the organic fraction of municipal solid wastes, on the reduction of corky root damage on canned tomato, was evaluated in a field naturally infested in Southern Italy. At harvesting, disease severity measured on roots collected from plots treated with 15 (C 15) and 45 (C 45) t ha⁻¹ of dry compost was significantly lower than that measured on roots collected from plots manured with chemical fertilizers (MIN) only. Detection by PCR, performed on symptomatic root fragments, confirmed P. lycopersici as the causal agent. The number of potentially antagonistic bacteria was evaluated on soil samples collected during tomato growth. Mean values (c.f.u. g⁻¹ of soil) of Pseudomonas spp. were 50×10⁴ for C 15, 81×10⁴ for C45 and 31×10⁴ for MIN; mean values of Bacillus spp. were 34×10⁴ for C15, 14,2×10⁵ for C 45 and 15×10⁴ for MIN; mean values of Pseudomonas spp. and Bacillus spp. in plots not manured with compost and chemical fertilizers were 45×10⁴ and 9,6×10⁴ respectively. The lowest value of Pseudomonas spp. and Bacillus spp. in the soil samples not treated with compost and the higher number of these bacteria, especially Bacillus spp., in the compost used, seems to indicate a direct role of these bacteria in the control of P. lycopersici. Further investigations on a larger number of Bacillus spp. isolated from the plots manured with compost are in progress.

Potential use of α-tomatine to control phytopathogenic microrganism
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Plants produce a lot of antimicrobial molecules useful to control phytopathogenic agents in agriculture. The ecotype “San Marzano” of Solanum lycopersicum produce high level of steroidal saponin α-tomatine. In this work, Trichoderma viride bioassays with crude extracts from leaves, roots and callus tissue of “San Marzano”, were performed to confirm if leaves contain the highest content of α-tomatine and to verify if this compound is produced at high concentration in callus tissue culture. Moreover, 14 phytopathogenic fungi and 15 phytopathogenic bacteria were evaluated for their susceptibility to pure α-tomatine. The crude extract from San Marzano leaves was the most active (66 % of growth reduction of T. viride with respect to control after 72 h) whereas crude extract from callus tissue culture was less active. All fungi, except Cladosporium sp., showed growth inhibition by pure α-tomatine used at concentration of 250 µg ml⁻¹. The highest growth inhibition was observed for Rhizoctonia solani, Fusarium oxysporum and Botrytis sp. (75 %, 69 % and 66 % of growth reduction respectively) whereas the lowest inhibition was observed for Sclerotium rolfsii, F. solani and F. semitectum (33 %, 35 % and 40 % of growth reduction respectively). No inhibition by α-tomatine was observed on bacteria. Further investigation is in progress to test the in vivo control of phytopathogenic fungi and to analyze the expression of the cycloartenol synthase, involved in α-tomatine biosynthesis.
**P15.8** Bacillus as beneficial bacteria for plant protection

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Use of microorganisms for protection of plants is an interesting approach to support a more sustainable agriculture. Bacterial protection can be due to activation of defence mechanisms in the plant e.g. induced resistance (IR), competition for nutrients and growth space and/or production of antibiotics. *Bacillus subtilis* is a naturally occurring soil organism and many strains are common as plant endophytes. Several Bacillus strains have been shown to function as PGPR and increase tolerance to a wide array of pathogens in species as diverse as bean and rapeseed. Bacillus produces a wide spectrum of antibiotics that are effective against fungal and bacterial pathogens. Bacillus produce spores that are much more tolerant than vegetative cells to different kinds of stress. These are important traits to support a persistent establishment of the bacteria under field conditions. We have identified several Bacillus strains that vary in their protective ability to fungal pathogens on *Brassica napus* (oilseed rape) and *Arabidopsis thaliana* in a controlled environment. Two bacterial strains have been identified that mediate protection towards the serious fungal pathogens *A. brassicaceae V. longisporium, B. cinerea, and L. maculans* on *B. napus*. Bacillus strains were also tested for production of antibiotics against pathogens under in vitro conditions, which showed that some strains provide direct growth inhibition.

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**P15.9** Micromycetes of genus *Fusarium* on barley grown after maize

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In 2005, after three years of maize growing as a forecrop in two localities in the Czech Republic (Praha-Ruzyně and Ivanovice na Hané), the health situation of spring barley against micromycetes of genus *Fusarium*, was determined. Three different strategies of protection were used: 1) susceptible and resistant varieties of barley to attack of *Fusarium* species, 2) varieties of maize as a forecrop (transgenic Bt-maize and its non-transgenic izolinie) and 3) fungicide protection and a control. The spectrum and the occurrence of potential toxicogenic micromycetes on barley in all variants of field treatments grown after maize were determined. The content of selected mycotoxins (DON, NIV) in all variants of barley grain was detected.

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Occurrence of *Rhizoctonia cerealis* and *Rhizoctonia solani* on winter cereals under conditions of various cropping systems and depending on forecrop
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The soil-borne fungi from the genus *Rhizoctonia* are found on numerous plant species in different climatic zones. In cereals they infect roots and stem bases (sharp eyespot). Infection of cereals with *Rhizoctonia* spp. depends to a large extent on the previous crop. The aim of this paper was to evaluate the occurrence of sharp eyespot on winter cereals under conditions of various cropping systems, depending on forecrop and weed control. Also the colonisation of its stem bases and roots by pathogenic fungi, especially from the genus *Rhizoctonia*, was examined. The health status evaluation was carried out at the shooting phase and at the milk maturity stage. The macroscopic estimation was accompanied by the analysis of fungal species identified on stem bases and roots which showed disease symptoms. Mycological analysis of roots was carried out at shooting phase and stem bases at the milk maturity stage. Infection caused by *Rhizoctonia* spp. was confirmed by a molecular method (PCR). Effect of weed control and forecrop (spring cereals and fallow) on occurrence of sharp eyespot was evaluated in Mochelek, north–western Poland. Previous crop and weed control did not affect occurrence of sharp eyespot and composition of fungal species isolated from roots and stem bases. In Osiny, south-eastern Poland, winter wheat was cultivated in four systems: organic, integrated, conventional and monoculture. The research showed differentiated occurrence of sharp eyespot depending on system. The highest infestation was noted in organic system and the lowest in monoculture. Cropping systems did not affect species composition of fungi isolated from roots and stem bases. Stems of cereals were colonised mainly by *R. cerealis*, and roots by *R. solani*.

Stem-base diseases in winter wheat under conventional and organic farming systems
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The stem-base disease complex includes several causal agents, especially eyespot (*Oculimacula yallundae* and *O. acuformis*) and brown foot rots (*Fusarium graminearum, F. culmorum, F. avenaceum* and *F. poae*). The aim of this work was to observe how the pathogen colonisation of wheat stems was affected by farming systems (organic/conventional) and previous crop (cereal/pea/oilseed rape). Another goal of the work was to demonstrate whether these fungal species are really present in one niche (stem base), together creating the so-called disease complex, or whether they occur separately. The experiment was designed on two varieties of winter wheat (Sulamit and Ebi). The pathogenic fungi were identified by molecular methods (PCR) using species-specific primers. In our experiments, *O. acuformis* and *O. yallundae* were the most frequent species detected on stem bases of wheat and in some cases they were found together on one stem. One species of the *Fusarium* genus was found in our experiments only, *Fusarium avenaceum* in the variety Sulamit under the conventional farming system after a previous crop of peas.

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P15.12 Prospecting for new fungicides to control apple scab (*Venturia inaequalis*) in organic fruit growing

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Apple scab (*Venturia inaequalis* (Cke.) Wint.) causes serious losses in quality and yield of organically as well as conventionally grown apples. Organic apple growers are highly dependent on the use of sulphur, lime sulphur and copper fungicides to control apple scab. Whereas only elemental sulphur is permitted for scab control in organic apple production in Denmark, and in addition lime sulphur in The Netherlands, copper fungicides are used in organic production in many European countries. However, the use of copper fungicides in the EU is now being phased out (EU Council Regulation (EEC) No. 2092/91) and alternative fungicides for control of apple scab and other diseases in other crops are increasingly needed. As part of the EU project REPCO (Replacement of Copper Fungicides in Organic apple and grapevine production in Europe, 2003-7) we are prospecting for plant extracts and other materials, including resistance inducers, to be used for apple scab control in organic fruit production. In the routine screening systems, potential materials are evaluated for control efficacy against scab on apple seedlings grown under controlled conditions in growth chambers. Promising compounds are further tested in small-scale orchard experiments and finally trialled in a modern integrated organic orchard system, in which application of the compounds is combined with sulphur and other control measures. Several interesting materials have been identified as potential organic fungicides.

P15.13 Effects of recycled substrates on severity of crown and root rot of tomato soilless grown

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*Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) is one of the most damaging soil-borne diseases of tomato (Ozbay, N. and Newman, S.E. (2004) Plant Pathology Journal 3 (1): 9-18). The disease occurs both in greenhouse and in open field and causes significant losses in tomato production. In closed soilless systems, with recirculation of nutrient solution, the disease is reported to cause serious problems. The suppressiveness of used rockwool and perlite against FORL infections were evaluated. New substrate and once-used substrate, sampled from closed soilless systems, was autoclaved or not, artificially inoculated or not and, finally, sown with tomato seeds cv Cuore di Bue. The effects of autoclaved/non autoclaved and new/once-used substrate on FORL incidence were assessed by evaluating the symptoms of crown-rot on the root-shoot transition zone of tomato seedlings. Not autoclaved and inoculated once-used rockwool significantly reduced FORL incidence when compared with not autoclaved and inoculated new rockwool. Autoclaved and inoculated used rockwool did not suppress FORL. These results are in accordance with other research that demonstrated the key role of resident microflora in suppressing root rot diseases in soilless systems (Postma et al. (2000) Phytopathology. 90: 125-133). In contrast, autoclaved and not autoclaved and inoculated used perlite significantly reduced FORL incidence when compared with not autoclaved and inoculated unused perlite.
In most areas throughout the world, canola and wheat are grown in rotation. To omit the competitive and harmful effects imposed by the narrow-leaved weeds in the canola fields, one of the most commonly applied herbicides, especially in Iran, is sethoxydim from the herbicidal family cyclohexanediones. *In vitro* studies on the effect of this herbicide have shown that not only is *Fusarium graminearum* able to tolerate this herbicide but also it is able to increase its growth rate, however, the carmine red color decreases to pale pink, indicating the reduction in the activity of the pathway for the biosynthesis of naphthoquinone pigments namely polyketide biosynthesis pathway (Kim et al, 2005, Appl. Environ. Microbiol. 71(4): 1701-8).

Sethoxydim inhibits acetyl-CoA carboxylase, that is the first enzyme in the pathway for the biosynthesis of lipids. The higher growth rate of the fungus under herbicidal treatment can be regarded as a sign of its plasma membrane integrity which is in contrast to the very reduced or no growth of *Sclerotinia sclerotiorum*. If the same mechanism that occurs *in planta* is also to some extent active in *Fusarium* species, then the polyketide biosynthesis pathway will be weakened in the favor of the pathway for the biosynthesis of trichothecenes. Trichothecenes are among the main virulence factors responsible for the host plant yield loss, and human and livestock mycotoxicoses.

**Evaluation of Pseudomonas isolates for biological control of charcoal stem rot of melon caused by Macrophomina phaseolina**

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Charcoal stem rot of melon caused by (*Macrophomina phaseolina*) is one of the most important diseases of this crop in Iran. Samples of soil surrounding melon root were randomly chosen from melon fields in the Garmesar area of Iran. Over eighty hundred and seven *Pseudomonas fluorescens* isolates were obtained using the soil dilution method on King B medium and screened against *M. phaseolina in vitro*. Twelve promising isolates were tested further in the greenhouse for their ability to control charcoal stem rot. Dual culture, volatile metabolite and cell-free culture tests showed that all 12 selected isolates of *Pseudomonas* inhibited growth of the pathogen. Inhibition varied from 28 to 58% in dual culture, from 16 to 54% in volatile metabolite and from 25 to 73% in a cell-free culture test. The results of seed soaking with bacteria indicated that the percentage of healthy plants in treatments *M. phaseolina* + *Pseudomonas* isolates or *Pseudomonas* isolates alone, was significantly greater than those of pathogen alone (p<0.05). Isolates p6 and p5 of *P. fluorescens* biovIII and isolate P3 of *Pseudomonas putida* biov B caused a lower incidence of the disease and also increased plant height and fresh and dry weight of shoot and root in these treatments. Similar results were obtained when soil of pots incorporated with pathogen and bacterial antagonist was used.
P15.16 Preliminary evidence on the use of essential oils for seed sanitation
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The control of bacterial diseases of plants is a considerable problem in agricultural practice because of the limited availability of bactericides. Besides, antibiotics are forbidden in agriculture in many Countries, and copper compounds, which will be restricted and controlled in the European Union, no other active principles are available. Furthermore, the control of bacterial diseases is complicated by the fact that a large number of phytopathogenic bacteria are seed-transmitted. The presence of a few contaminated/infected seeds may lead to highly damaging epidemic outbreaks. The above consideration prompts the need for a search for alternative active compounds for control of plant bacterial diseases including eradication of pathogens from seed. Recently our studies showed antibacterial activity of essential oils of coriander, cumin and caraway against Gram-positive and -negative phytopathogenic bacteria. Assays with the main components of the above essential oils showed a high activity of oxygenated monoterpenes with phenol and alcohol functions, moderate activity of oxygenated monoterpenes with aldehydes, ketons, ethers, esters functions and the reduced or no activity of non-oxygenated monoterpenes and sesquiterpenes. Preliminary results show the potential use of the above essential oils for seed sanitation procedures. In fact, the application of eugenol solutions to bean seeds artificially contaminated with *Xanthomonas campestris* pv. *phaseoli* var. *fuscan*, significantly reduced the bacterial population. At the assay concentrations, negligible or limited effects on seed germination was observed.

P15.17 The Influence of Calcium on the Diseases Occurrence in Winter oilseed rape (*Brassica napus*)
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Stem canker (*Leptosphaeria maculans*) and white mold (*Sclerotinia sclerotiorum*) are serious pathogens of oilseed rape in the Czech Republic. Disease occurrence was strongly influenced by year, position of rape in crop rotation system, weather conditions, characteristics of locality and growing technology (fungicidal treatment). This work issued from hypothesis that calcium foliar application can reduce disease attack on rapeseed. Damages to leaves caused by *Leptosphaeria maculans* on the treated plants was about 5% in autumn. Damage to leaves on the untreated plants was higher, about 10%. Calcium application led to a reduction of damage caused by *Sclerotinia sclerotiorum* before harvest and an increase in yield. Damage caused by white mold was 14-24% lower on treated plants than on untreated ones. Spraying applications were applied before flowering and at full flowering stage. The test was conducted in the field.

Key-words: winter oilseed rape – calcium – occurrence of diseases – yield – field-test

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Five strains of bacteria with biocontrol activity against powdery mildew of barley, caused by *Blumeria graminis* f. sp. *hordei*, were studied more closely as they had shown promising results in earlier studies (Azarang et al. (2004) BCAs IOBC/wprs Bulletin 27: 57-62). The present study aimed at characterizing the effects of the biological control agents on the pathogen, microscopically as well as macroscopically. The liquid bacterial cultures were centrifuged and supernatants were filtered. The filtered supernatants protected barley as well as the bacterial liquid cultures against powdery mildew, demonstrating that active substance(s) was/were secreted in the culture medium during growth. The supernatants were applied to 8-day old leaves of barley prior to inoculation, as well as at different time points after inoculation of spores. In order to observe the inhibition of fungal differentiation, treated leaves were cleared and the fungus was stained with Evan’s blue. Four bacterial supernatants showed good ability to prevent and cure the disease, even when applied as much as 120 hours after inoculation. One of the strains was less effective, due to inefficient adhesion of the supernatant. The effects of the treatments were inhibition of germ tube development and the decomposition of the fungus. In the future, these supernatants could be tested for biological control activity in larger field trials. Their active substances and mechanisms could be determined on a molecular level.

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*L. maculans* and *L. biglobosa* are the causative agents of the highly damaging stem canker disease in oilseed rape. Stem canker is responsible for significant yield-loss and given the projected importance of oilseed rape as a fuel-crop it is important that the best methods of controlling it are identified. The primary aim of this project is to investigate the interactions between *L. maculans* and *L. biglobosa* on oilseed rape (*Brassica napus*). The effects of the commonly used triazole fungicide flusilazole on this interaction will also be investigated. The experimental research for this project will be done using a combination of *in vitro*, controlled environment and field experiments. The relative fungicide sensitivities of the two pathogen species will be examined in a series of *in vitro* assays. Colonisation of leaf, petiole and stem tissue by the two species will be examined using quantitative PCR (qPCR) analysis of selected tissues over a time-course in controlled environment experiments. The effects of fungicide on the rate and pattern of colonisation will then be examined using a similar method. Field experiments will provide data about the impact of different spraying times on the pathogen population under natural conditions.
K16.1  What can we learn from clubroots: Alterations in host root metabolism and hormone homeostasis caused by *Plasmodiophora brassicae*

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The clubroot disease of Brassicaceae is one of the most damaging within this plant family. Caused by an obligate biotrophic pathogen, the interaction between *Plasmodiophora brassicae* and its host is difficult to analyze. Since *Arabidopsis thaliana* is a good host for *P. brassicae* we have used the ATH1 Affymetrix array to investigate host gene expression during the development of the disease at two different time points. At the early time point young plasmodia of the pathogen were visible, but the host tissue showed limited change of host cell and root morphology. At a later time point different developmental stages of the pathogen such as secondary plasmodia but also developing sporangia and mature resting spores were present and host root morphology was severely altered. Plant hormones such as auxins and cytokinins contribute to cell division and enlargement during gall development. Data are presented how the levels of these two hormones could be altered in root galls. In addition, the growing root gall constitutes a strong metabolic sink. Assimilates from the leaves are transported to the club where starch is stored in amyloplasts. Many of the genes involved in sugar or starch synthesis, especially invertases and invertase inhibitors are differentially expressed during clubroot. Their importance for gall development has been tested using knockout mutants and transgenic plants. A model to explain the function of plant hormones and primary metabolism in clubroots is presented.

K16.2  Signal transduction in host recognition and mycoparasitism: a case study with *Trichoderma atroviride*

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*Trichoderma atroviride* is a mycoparasite commercially applied as biological control agent against several fungal pathogens. The mycoparasitic interaction is host specific including recognition, attack and killing of the host through production of infection structures, cell wall lysing enzymes, and antifungal metabolites. Investigations on the signal transduction pathways of *T. atroviride* and *T. viride* revealed that alpha subunits of heterotrimeric G proteins and MAP-kinases are involved in host recognition and activation of the mycoparasitic response. Tga1 and Tga3, the *T. atroviride* subgroup I and III G alpha subunits, were shown to affect mycoparasitism-related processes like chitinase gene transcription, antifungal metabolite production, and infection structure formation. Although tga1 deletion resulted in a complete loss of mycoparasitic overgrowth and lysis of *Rhizoctonia solani*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*, a delta-tga1 mutant displayed an enhanced growth inhibition of the hosts by over-producing antifungal metabolites and showed an increased protection of bean seeds against *R. solani*-caused root rot.

O16.1  Tracking fungi in soil with monoclonal antibodies
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Studies of the interactions between Trichoderma species and other fungi in soil and compost have been hampered by the absence of methods that allow the unambiguous detection of these species in mixed populations of fungi. Conventional methods of quantification do not satisfactorily differentiate between Trichoderma species and other fungi and are unable to discriminate between biomass derived from spores and from mycelial fragments. Transformation of Trichoderma strains with β-glucuronidase and GFP-encoding genes have provided useful tools for ecological studies in the rhizosphere and bulk soil, but studies have so far been restricted to individual recombinant isolates. Hybridoma technology allows the production of monoclonal antibodies (mAbs) that are specific to genera, species or even isolates of fungi. Furthermore, they are able to discriminate between mycelium and spores. This paper will describe the development and use of mAbs to quantify the saprotrophic growth dynamics of Trichoderma species in soil and peat. In combination with mAbs specific to the plant pathogen R. solani and saprotrophic Aspergillus and Penicillium species, I will show how hybridoma technology can be used to quantify the dynamics of pathogens, biocontrol agents and other soil saprotrophs during antagonistic interactions in soil and peat-based systems. The use of mAbs to develop rapid lateral flow devices for the detection of the fungi in soil and in the plant rhizosphere will also be described.

O16.2  Identification of pathogenicity genes in the sclerotial mycoparasite Coniothyrium minitans
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Coniothyrium minitans is a fungal biocontrol agent of the plant pathogen Sclerotinia sclerotiorum, which attacks sclerotia of the pathogen in the soil. We are using a range of gene identification and characterisation approaches to dissect the mechanisms of sclerotial mycoparasitism in this interaction. The first approach used suppression subtraction hybridisation between cDNA from C. minitans grown in culture and C. minitans colonising sclerotia of S. sclerotiorum. A subtracted library of 672 clones containing cDNA fragments of putative upregulated genes was established. Sequencing of these cDNA clones and bioinformatics analysis led to the identification of 251 ESTs and assignment of putative functions. Dot blot and virtual Northern analysis showed different levels of upregulation of various C. minitans genes during sclerotial colonisation. The second approach involved insertional mutagenesis of C. minitans using REMI and T-DNA tagging. Eight pathogenicity mutants were obtained from a panel of over 4000 transformants. Genes with similarity to the PIF1 helicase of Neurospora crassa and PTH11 of Magnaporthe grisea were amongst those identified. Molecular characterisation and analysis of these pathogenicity mutants is now underway. The final approach has been to isolate genes putatively involved in signalling and colonisation from sequence information available in other pathogenic systems. Using PCR based methods and a genomic macroarray, pkaC, pmk1, and cmg1 genes have been obtained from C. minitans. Characterisation of some potentially key genes has now begun and gene silencing and complementation studies to investigate their role in sclerotial parasitism have been initiated.
Scots pine (*Pinus sylvestris* L.) forests in Poland suffer from air pollution to considerable degree. In six forest districts in the Polish Lowlands, representing various degrees of coniferous forest pollution, communities of soil fungi were investigated in 2003 and 2004. The communities were examined for their effect on the growth of *Heterobasidion annosum*, a severe pathogen of tree roots. The 2003 vegetation season was extremely dry. All the communities of fungi supported the pathogen’s growth. The communities from two districts in unpolluted area supported (on average) the pathogen’s growth only slightly, while the communities from a district located in severely polluted area supported it very much. The three districts located in the region of average pollution had fungal communities supporting the pathogen growth to an extent similar to that in severely polluted area. The 2004 vegetation season was rather humid. All the communities of fungi suppressed the pathogen’s growth. Those from the districts in severely polluted area suppressed it (on average) to the smallest extent. The communities from the district located in severely polluted area supported it very much. The three districts located in average pollution region had fungal communities supporting the pathogen growth to an extent similar to that in severely polluted area. Besides pollution degree, weather seems an important factor effecting soil fungal communities in the districts investigated.
O16.5  **Characterisation of root pathosystems involving the model plant Medicago truncatula**

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Medicago truncatula, an omni-Mediterranean species, has been established as a model for genomic studies of legume plants. It is a host for root symbiotic microbes as well as for various pathogens, making it a good model plant for comparative studies in order to understand the mechanisms which regulate acceptance of a symbiotic microbe without diminishing its ability to defend itself against pathogenic microorganisms. As a first step towards such studies we established two root pathosystems, involving a bacterium and a fungus. *Ralstonia solanacearum* and *Fusarium oxysporum* are soil-living plant pathogens, causing bacterial wilt disease and Fusarium wilt disease respectively on a wide range of crops. We tested a large number of bacterial strains and *M. truncatula* lines, and identified compatible and incompatible interactions with *Ralstonia*. The study of *M. truncatula* recombinant inbred lines allowed to localise a major QTL responsible for resistance. Disease development required the *hrp* cluster in *Ralstonia*. Strains of *F. oxysporum f.sp. medicaginis* (Fom) isolated from alfalfa were shown to infect *M. truncatula*. We introduced the GFP marker gene into *Fom* by Agrobacterium-mediated transformation to study root colonisation. First results on the characterisation of both pathosystems and ongoing projects will be presented.

O16.6  **Flavonoids in white clover in response to presence or absence of two arbuscular mycorrhizal fungi and a pathogenic fungus**

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The effects of two arbuscular mycorrhizal (AM) fungi (*Glomus claroideum* and *G. mosseae*) and a pathogenic fungus (*Pythium ultimum*) on the production of selected flavonoids from two cultivars of white clover were evaluated. The fungal colonisation was determined with the line-intercept method and the detection of *P. ultimum* was performed by ELISA. Eight identified flavonoids and 14 unidentified flavonoids were determined with LC-MSMS analysis. The production of flavonoids in clover root varied, not only as a result of presence of beneficial and/or pathogenic fungi but also depending on fungal isolate and plant cultivar. *P. ultimum* infection affected the concentrations of a number of hitherto unidentified flavonoids. The flavonoids that showed increased concentrations in response to infection with *P. ultimum* are supposedly stress metabolites, synthesised or produced from glycosides in response to the attack. However, the presence of one or both AM fungi resulted in most cases in a suppression that overruled the induction by the pathogen. The antagonistic potential of the AM fungi against the pathogen must therefore be through another mechanism.

The present results clearly emphasise the need for more intensive chemical characterisation of flavonoids. More research into the influence of flavonoids in plant defence responses and into the interaction between plant and plant-related microorganisms is needed as well.
P16.1  

**Infection of tomato rootstocks by Colletotrichum coccodes**  
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During 2003 root rots were observed on grafted tomatoes grown in Northern (Liguria, Piedmont), Central (Campania) and Southern Italy (Sicily). The root tissues became blackish, showing cracks as in the case of corky root (*Pyrenochaeta lycopersici*) infections. However, contrary to corky root infections, the necrotic roots appeared with a cortex easy to be removed and the remaining internal root tissues assumed a blackish colour. The symptoms were particularly evident on older roots of rootstocks and the tissues turned grey to blackish. On the basis of several analysis a fungus producing blackish round sclerotia of a diameter less than 1 mm was constantly observed. Tests carried out to confirm Koch’s postulates confirmed the pathogenicity of the isolated fungi recognized as *Colletotrichum coccodes*, the causal agent of the brown root rot of tomato. Tests carried out in pot with artificial soil infestation demonstrated the susceptibility to this disease of standard tomato plants and interspecific and intraspecific tomato rootstocks. These observations show that grafting tomatoes on resistant rootstocks cannot be considered the only practice to control soilborne pathogens in soils infested by *C. coccodes*.

P16.2  

**Development of a Real-time PCR method for quantification of Trichoderma atroviride 122F in soil and comparison with soil dilution plating and qualitative PCR methods**  
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Fungi of the genus *Trichoderma* are widely used in industry and agriculture. The Council directive 2005/25/EC requires that an assessment of fate and behaviour in the environment should be made, if a microbial biocontrol agent has to be registered and used as fungicide in Europe. The potential for persistence and multiplication of micro-organisms is usually assessed with the classical microbiological method of counting the colony forming unit (CFU). This method is slow and time consuming. New molecular techniques can provide more rapid tools with a high throughput potential to monitor the presence of a particular microorganism in the environment. The aim of the present study is to develop a real-time PCR based method to detect and quantify the presence of a strain of *Trichoderma atroviride* (F122) used as biocontrol agent (BCA) in soil against Armillaria root rot. Among available strategies, we selected a particular Single Nucleotide Polymorphism (SNP) specific probe for the identification and quantification of *T. atroviride* F122 in a pool of fifteen *Trichoderma* species (CBS collection, Belgium). The method was tested for specificity, efficiency, linearity, limits of detection and quantification of *T. atroviride* F122. Several DNA extracts were used originating from single *Trichoderma* spp. isolates, total DNA from soil treated with *T. atroviride* F122 under controlled and field conditions. Finally, the method was compared with the traditional CFU counting and qualitative PCR methods, in term of efficiency, reliability, time and money costs.
P16.3  
**Evidences on mycoparasitism in spores of AM fungi isolated from sugar cane fields of Iran**  
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Mycoparasites are extremely common in soil. Chlamidospores of the arbuscular mycorrhizal fungus *Gigaspora gigantea* can be parasitized by different fungi and actinomycetes such as *Acremonium*, *Chrysosporium*, *Trichoderma*, *Verticillium*, *Exophiala*, *Streptomyces* and *Nocardia*. This study was carried out to identify arbuscular mycorrhizal fungi in sugar cane fields of Iran (Khuzestan and Mazandaran provinces) during 2002-2004. Spores of arbuscular mycorrhizal fungi were extracted from soil by wet sieving (25-80-400 mesh) and decanting method followed by centrifugation using 55% sucrose aqueous solution. Finally, 17 species of AM fungi belonged to two genera including *Glomus* and *Acaulospora* were identified. However, some evidences on mycoparasitism were observed in AM spores extracted directly from the field soil. A few *Pythium* species are aggressive parasites of other fungi. In this study spiny-walled oogonia of *Pythium oligandrum* were detected inside the chlamidospores of *Glomus lamellosum* and *Glomus ambisporum*. Oogonia of this *pythium* species were echinulate and around 20 µm in diameter. We could also find chlamidospores of *Glomus claroideum* formed within chlamidospores of *Glomus ambisporum*. This was an interesting example of mycoparasitism within an AM population. Additionally, internal projections and radial canals in the spore wall of some species such as *Glomus liquidambaris* and *Glomus sinuosum* were probably correlated with mycoparasitic hyphae activities.

P16.4  
**Mycoparasitism of Penicillium pinophilum toward Rhizoctonia solani**  
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Antagonism between soil fungi regulates the biocenotic organization of fungal populations. Therefore understanding the ecological relationships between fungal species is quite relevant to exploit the suppressive properties of cropped soils in the management of soil-borne plant diseases. *Rhizoctonia solani* is a widespread and polyphagous pathogen, known to cause damping-off and sore shin of tobacco. In Italy such diseases occur in all tobacco-growing areas except Salento. Presence of fungal antagonists was investigated as a possible explanation of suppressiveness of field soils in that area, and actually several mycoparasites (e.g. *Verticillium biguttatum*, *Gliocladium roseum*, *Lecanicillium psalliota* and *Trichoderma* spp.) were recovered. Isolates of *Penicillium pinophilum* were also collected. Unlike the former species, whose isolates were able to penetrate or coil *R. solani* hyphae in dual cultures, at first no evidence of mycoparasitism was observed for *P. pinophilum*. In fact, hyphae of the antagonist grew approached to *R. solani* hyphae for short stretches only. As we observed an inhibitory capacity of culture filtrates *in vitro*, antagonistic properties of *P. pinophilum* appeared to be rather dependent on antibiosis. Observations by scanning electron microscopy showed that when *P. pinophilum* hyphae grow leaning against those of *R. solani*, hyphal walls tend to coalesce and *R. solani* hyphae assume a flattened and empty appearance; at a later stage, they completely loose their cylindrical structure and typical branching pattern. However, less frequently a definite evidence of wall boring and hyphal penetration could be observed which demonstrates unequivocally the mycoparasitic aptitude of this soil fungus.
**P16.5**

**Wheat straw competition between *Rhizoctonia solani* and two *Trichoderma* isolates. I: cellulose exploitation.**

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Members of the genus *Trichoderma* are known for their ability to secrete cellulose degrading enzymes. In antagonistic isolates these enzymes are supposed to be related to their saprophytic lifestyle or to their direct action against plant pathogens. We have evaluated the Competitive Saprophytic Ability (CSA), expressed as competition for wheat straw, between *R. solani* and two antagonistic strains of *Trichoderma*. Both antagonists were able to compete with *R. solani* for wheat straw possession even if their CSA seemed to be limited in time. On this basis the antagonistic strains appeared to be ruderal and combative, whereas *R. solani* appeared to be ruderal and stress tolerant. As the major components of wheat straw are cellulose and hemicelluloses, we evaluated cellulosolytic activities as a possible mechanism for capture and maintenance of the resource. The evaluation of mycelial protein content allowed the estimation of the fungal growth of the three isolates in presence of sucrose or straw as carbon source. *R. solani* did not show any difference in the growth in presence of sucrose or straw, whereas the antagonistic fungi preferred straw as growth substrate. Extracellular protein produced by the three isolates changed significantly in relation to the carbon source, being higher in straw in all tested fungi. The three isolates showed similar levels of enzymatic activities (cellulase, exoglucanase, endoglucanase and -1,4-glucosidase), suggesting that other mechanisms need to be investigated to understand competition for straw possession.

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**P16.6**

**Role of arbuscular mycorrhiza-associated bacteria from the genus *Paenibacillus* in biocontrol of *Pythium***

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Several studies have shown that arbuscular mycorrhiza (AM) can reduce root diseases caused by *Pythium*. Antagonism from bacteria in the mycorrhizosphere has been proposed as a mode of action. In the present study, the *Pythium* biocontrol features of seventeen strains of *Paenibacillus* spp. from AM and non-AM systems were examined. Thirteen strains significantly increased the percentage of seedling emergence of seeds inoculated with *P. aphanidermatum*. The two best strains of *Paenibacillus macerans* not only reduced pre-emergence damping-off incidence with 73%, but also gave full protection against *P. aphanidermatum* so that 68%-82% of the emerged seedlings remained healthy seven days after sowing. The two best strains of *Paenibacillus macerans* and the best strain of *Paenibacillus polymyxa* also significantly increased the percentage of seedling emergence after inoculation with 10⁷ zoospores of *P. aphanidermatum*. Our results demonstrate a potential among bacteria from *Paenibacillus* spp. to control pre- and post emergence damping off in cucumber caused by *Pythium*. 
**P16.7** Auxin-inducible GH3 gene family is upregulated in host roots during *Plasmodiophora brassicae* colonization and gall development.

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*Plasmodiophora brassicae* is the causal agent of clubroot disease which is one of the most damaging among *Brassicaceae*. This disease was analysed among others with a microarray experiment using the model plant *Arabidopsis thaliana*. One interesting result were changes in auxin metabolism and homeostasis during the infection. So the GH3-gene family, of which some genes adenylate auxins to form aminoacid conjugates (Staswick et al.. 2005), is upregulated by *Plasmodiophora brassicae* during colonization of host roots. Single Knock-out lines and double knock-out lines of GH3 genes were analysed in regard to clubroot colonization and gall development. In order to overcome side effects of knock-out lines and to repress the expression specifically in roots and galls we took the two most upregulated GH3-genes and made antisense constructs combined with root specific promoters from *Arabidopsis thaliana*. Due to homologies within the GH3 gene family these antisense constructs will probably influence some of the GH3 genes simultaneously in roots. Thereby we try to elaborate a proof-of-concept for the importance of auxin homeostasis during gall induction. With a pathogenesis-inverse control of auxin homeostasis it is expected to produce an unspecific resistance against the obligate parasite which can also be transferred in rapeseed and cabbage.

**P16.8** Clubroot development is hampered in cytokinin receptor double mutants of *Arabidopsis thaliana*

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*Arabidopsis thaliana* is a host plant of the obiligate biotrophic root parasite *Plasmodiophora brassicae*, the cause of clubroot. Local changes in the cytokinin homeostasis by up-regulation of cytokinin receptor and down regulation of cytokinin-oxidases has been shown to be linked to pathogenesis (Siemens et al. 2006, Mol. Plant Microbe Int. 19:480-494). Three sensor histidine kinases, AHK2, AHK3, and CRE1/AHK4 of *A. thaliana* have been shown to be cytokinin receptors, which revealed partially redundant functions (Riefler et al. 2006, Plant Cell 18:40-54). The interaction with *P. brassicae* of loss-of-function mutants of all three receptors has been analysed. Single mutants showed wildtype clubs, whereas the development of clubroot in the root of the double mutant AHK3/AHK4 is hampered. These double mutants showed a reduced gall size. Histological analysis revealed an inhibition of the development of the pathogen. Thirty days after infection the root of the double mutant AHK3/AHK4 is colonised by vegetative secondary plasmodia of the pathogen but no mature spores can be detected, whereas in control plants mature spores can be already found 17 days after inoculation. This apparent locking is a hint, that the pathogen development itself is dependent on cytokinin-mediated signal transduction of the host cells.
Gnomonia fragariae is a poorly studied ascomycete, which recently has been demonstrated to be a cause of severe root rot and petiole blight of strawberry in Latvia and Sweden. In this study the green fluorescent protein (GFP) was used as a marker to visualize the pathogen in host plant tissues and to monitor different stages of infection process. Several GFP-tagged transformants of G. fragariae revealed intense fluorescence in mycelium, ascomata, asci and ascospores. They remained stable for at least seven subcultures and after the re-isolation from the host plant tissues. The transformation did not affect the morphology and growth rate of the fungus, although wild type was slightly more aggressive in colonization experiments in comparison to the transformant strain used in the this study. Ascospores were attached all along the roots and germinated from both ends 24 h after inoculation. No specific infection structures, such as appressoria, were formed. The specific penetration sites on epidermal cells and preferences in colonization for certain root and petiole tissues were observed. Several weeks after the inoculation the mature ascomata were formed on infected petioles for both transformant strain and wild type. The results obtained in this study showed that GFP is an efficient marker to visualize G. fragariae in the strawberry root and petiole tissues and the use of GFP-tagged fungus was an effective approach to study plant-pathogen interactions.
K17.1  **Generic platform technologies for the detection and identification of plant pathogens.**

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The detection and identification of plant pathogens currently relies upon a very diverse range of techniques and skills, including both traditional culture and taxonomic skills to modern molecular based methods. The wide range of methods employed reflects the great diversity of plant pathogens and the hosts they infect. The well-documented decline in taxonomic expertise, along with the need to develop ever more rapid and sensitive diagnostic methods, has provided an impetus to develop technologies that are both generic and able to complement traditional skills and techniques. Real-time PCR is emerging as one such generic platform technology and one that is well suited to high-throughput detection of a limited number of known target pathogens. The relatively high capital cost of the required instrumentation and the laborious nature of nucleic acid sample extraction methods currently limits uptake of real-time PCR as a generic tool in routine plant pathogen detection. Progress with developing generic techniques for plant pathogen identification, particularly of unknown samples, has been less rapid. Diagnostic micro-arrays and direct nucleic acid sequencing both offer potential as generic methods for the identification of unknown plant pathogens but are unlikely to be suitable as high-throughput detection techniques. This presentation will review progress in both detection and identification techniques for plant pathogens and highlight the trend towards multi-disciplinary studies.

**Acknowledgement**
The authors acknowledge support from the Plant Health Division of Defra UK and the European Union.

K17.2  **Quantitative multiplex detection of plant pathogens using PRI-lock probes and universal, ultra-high-throughput real-time PCR on OpenArrays™.**

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Current technologies for multiplex, quantitative analyses frequently suffer from compromises between the level of multiplexing, throughput and accuracy of quantification. In general, for the detection of nucleic acids, microarrays provide very high levels of multiplexing, but less accurate quantification and usually low throughput. Real-time PCR provides the most reliable means of target quantification and is suitable for the analysis of a relatively high number of samples. However the achievable level of multiplexing is low. Nano-scale technology provides high-density, low-volume microchambers, which could accommodate very high number of reactions, performed under standard conditions. Yet many of these systems are still experimental and are incapable of real time fluorescent monitoring required for quantification. However recently, a conceptually new, ultra-high-throughput platform has become available for real-time PCR; capable of accommodating more than 3000 reactions per array. The OpenArray™ has 48 subarrays, allowing parallel testing of up to 48 samples, with each subarray contains 64 microscopic (33 nL) through-holes. The primers are pre-loaded into the holes, while the sample and reagents are auto-loaded due to surface tension. PRI has developed PRI-Lock probes for multiplex detection; providing flexibility and bridging the gap between target-specific recognition and high-throughput amplification. These probes are long oligonucleotides (similar in structure to Padlock probes) and contain artifically selected primer sites and a TaqMan probe region, flanked by target complementary regions.  

*In this paper, we report the development of a high-throughput, quantitative multiplex diagnostic assay based on above technologies.*
Use of T-RFLP for the detection of microbes associated with plants
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Terminal restriction fragment length polymorphism (T-RFLP) is a method for the analysis of bacterial and fungal communities. It is based on the amplification of DNA from samples using ‘Universal’ primers, one of which is fluorescently tagged, followed by restriction digestion. The resultant fragments are then resolved on an automated DNA sequencer to identify the species present in the sample. Most methods to date have used primers that amplify the 16S rRNA for bacteria and the ribosomal ITS1 region for fungi. However, there are limitations in the resolution based on these primers. We have been developing primers for the 23S rRNA gene for bacteria and the ITS2 region for fungi, to improve the resolution of species identification. In addition, we have selected primers that allow simultaneous amplification of fragments from the host plant DNA, which can be used as a reference point for identifying fluxes in bacterial and fungal populations on and in plants. We will discuss the use of this technique in three areas of research. Firstly, for analysing changes in bacterial and fungal populations on salad vegetables from harvest, through processing to sale as ready-to-eat produce on supermarket shelves, and to determine the effectiveness of different decontamination regimes. Secondly, for monitoring the establishment and survival of potential biological control microbes on the roots of wheat plants. Thirdly, for detecting and identifying phytoplasmas in plant material.

Practical solutions to the control of black dot of potatoes
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With a sophisticated washed potato market in the UK, tuber blemish diseases have become more significant. Black dot caused by Colletotrichum coccodes has become one of the most important of the blemish diseases. A study funded by the British Potato Council over three years set out to understand how inoculum affects disease development and the epidemiology of the pathogen within the context of practical control measures. SCRI has developed a robust Realtime PCR test for soil and tuber inoculum. Using this test on soil samples from 4ha blocks, it has been possible to quantify soil-borne inoculum. The test uses a 60g sub-sample and tests have shown that provided the soil is well mixed a single sub-sample gives a good estimate of soil contamination. In field trials over four sites across the UK and in two seasons a strong relationship has been found between the level of soil contamination and black dot development. Thresholds have been set to indicate the risk of disease developing from soil inoculum. Disease developing from seed-borne inoculum has been limited in trials and whilst this source of inoculum is important for contaminating previously uncontaminated soil, it appears to less significant in disease development than soil-borne contamination. Avoidance of fields with high levels of soil contamination is the most effective single control measure. An in-furrow application of azoxystrobin has reduced disease development but is most effective where soil contamination is not high. Irrigation has been shown to increase the level of black dot and it is important that irrigation is scheduled to avoid over use. A delay in harvest also increases black dot and where the disease risk is high, harvest should be achieved as soon as the crop is ready to lift.
Microarrays for identification of *Fusarium* and other fungi

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Microarrays are useful not only for gene expression studies but also for multiplex identification of pathogens. Such diagnostic arrays are already being developed for a number of plant pathogens including viruses, bacteria and fungi. The aim of this study was to investigate the feasibility of oligonucleotide based arrays for detection and identification of *Fusarium* spp. The main genomic target for discrimination was the ribosomal internal transcribed spacer (ITS), but also functional genes such as *Tri12* involved in mycotoxin production were tested. The use of short (18 – 32 nt) oligonucleotides was shown to be efficient in discriminating most *Fusarium* species, however some species could not be resolved due to low sequence variation between species and therefore other gene targets may have to be used in future studies. Using *Tri12* derived oligonucleotides it was possible to identify different chemotypes of *Fusarium graminearum*. Preliminary results from similar studies for other fungi such as *Aspergillus* spp. and *Penicillium* spp. will also be presented.

Quantification of common bunt and dwarf bunt in plant tissue by qPCR

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*Tilletia* [*Tilletia caries* (DC.) Tul., syn. *T. tritici* (Bjerk.) Wint (causative common bunt) and *Tilletia controversa* Kühn (causative dwarf bunt)] hyphae penetrate plant tissue in growing phase and infection process is latent until plant full maturity. In that time it transformates grains into sori filled in *Tilletia* teliospores. *Tilletia* product strongly smelly trimethylamin, that causes unavailability of contaminated crop for food and keeping products. Bunt presence degrade harvest quality index and faces to total disposal of crop, it means loss of all costs expended on na crop creation, fertilization, treatment and harvest or crop disposal. Basic precautions against bunts include use of resistant variety. Already there are resistant varieties in the world collection and the others are breeding constantly because pathogen constantly overcome plant resistance. Optimal inoculation methods and plant infection by bunts are tested. All methods need visual classification of bunt presence in full maturity and comparing of number of infected plants to non-infected plants. None of scientific teams used molecular diagnostics or other modern methods for resistance testing time reduction. Nevertheless in this case all tests including plant growing need expensive several-months cultivation. Molecular biologic methods give problem solution. They are rapid and precise and they are routinely used for the fast and easy detection of pathogens in plant tissues or biologic materials in the world. Bunt mycelium quantification in seedling tissue and its comparision with infectious intensity in full maturity can give useful information about wheat variety resistance against bunt.
Pathogens of the genus *Neofabraea* (*N.alba* and *N. malicorticis*) (Ascomycetes, Helotiales) cause cancer on pear and apple branches and postharvest bull’s eye rot of apple and pear, which is an important postharvest disease in Europe, the United States and other growing areas. According to EPPO certification schemes, propagating material of *Malus* and *Pyrus* should be inspected for the presence of these pathogens. But isolation and determination of these pathogens is laborious and time consuming. Therefore we are focused on preparation of specific antibodies for the detection and determination of these pathogens. Intracellular proteins extracted from mycelial mass, cultivated on mineral media, were used as an immunogen. IgG fraction were isolated from sera of immunized rabbits by affinity chromatography on protein A. Specificity and sensitivity of the obtained antibodies (Abs) were assessed by PTA-ELISA. Both Abs were evaluated as sensitive with the detection threshold 0.2µg of fungal proteins.ml⁻¹. Calibration curve was constructed for the range of concentration 0.5 - 10µg fungal proteins.ml⁻¹, in which the curve has logarithmic relationship with equation $y = 0.8\ln(x) + 0.9$. Abs were assessed as genus specific with cross-reactions up to 15% for other pathogens of *Malus* and *Pyrus* and other micromycetes associated with pome fruits branches. Further, detection of the pathogens in artificially infected host tissue is discussed.

The work was supported by the grant MZe CR QF 4074.
P17.4  Sensitivity of Detection Apple Mosaic Virus in Apple Trees by ELISA
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Different apple tissues (phloem, dormant buds, leaves and flowers) in different vegetation periods were tested on the presence of ApMV by ELISA. Symptomless positive apple trees of two varieties were evaluated. The sensitivity of detection ApMV was assessed by determining the relative concentration of the viral protein by Double Antibody Sandwich (DAS)-ELISA. The highest virus concentration and therefore the highest sensitivity of virus detection were obtained when young leaves before flowering (phenological stage of "little mousy ear") had been examined. Flower petals and leaves sampled during May were also positive. The reliability of the detection was proved by repeating tests of eighty apple trees of four varieties from two orchards in two following years and by RT-PCR. We can recommend DAS-ELISA as the routine method of testing apple trees on the presence of ApMV.

Keywords: Apple Mosaic Virus, ELISA, detection, sensitivity, tissue, apple tree

P17.5  Detection of Potato virus Y (PVY), Potato mop top virus (PMTV) and Tobacco rattle virus (TRV) using different diagnostic assays
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In our present study a range of PVY, PMTV and TRV isolates was investigated using biological, serological and molecular genetic methods. Because of the economic importance of PVY to potato production, isolates mostly identified biologically as PVY-N, causing necrotic rings on the tubers, were selected for this study. PVY, PMTV and TRV are important potato diseases that are difficult to distinguish by visual symptoms. There were compared various detection techniques of the viruses causing necrosis in or on potato tubers e.g. ELISA, RT-PCR event. IC- RT-PCR. The results of this study are standard laboratory protocols for detection of these pathogens used in PRI Havlickuv Brod and offered also to Phytosanitary Service of Czech Republic. The attempt to set the multiplex detection of three viruses was unsuccessful and currently we have been working on the protocol by means RT-qPCR.

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**P17.6**

**Occurrence of double-stranded RNAs indicating the presence of putative cryptoviruses and endornaviruses in Capsicum species**

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The presence of an endornavirus and a cryptovirus in *Capsicum annuum* has been reported previously. We investigated whether these endogenous dsRNA-viruses also occur in other *Capsicum* species and whether the dsRNA pattern of the putative genomic dsRNAs parallels the *Capsicum* phylogenetic tree. Samples were taken from a collection of eight pepper species (*C. annuum*, *C. baccatum*, *C. chacoense*, *C. chinense*, *C. eximium*, *C. frutescens*, *C. praetermissum*, *C. pubescens*) kept at ABC. High-molecular weight dsRNA (>10 kbp), which may represent the genomic dsRNA of endornavirus was detected in every/almost every cultivar of *C. annuum*, *C. chinense*, *C. frutescens*, *C. praetermissum*, *C. baccatum* var. *baccatum* and var. *pendulum* species; in *C. chacoense* the molecular weight of dsRNA was lower than in the other species. No dsRNAs could be detected in *C. eximium* and *C. pubescens*. For cryptic viruses, a segmented genome consisting of 2-3 dsRNA-species in the size range 1-3 kbp is characteristic. Such dsRNA-pattern was observed only in *C. chinense*, *C. frutescens*, *C. chacoense* and *C. annuum*. On the basis of the length of dsRNA pairs at least three different cryptoviruses may occur in these *Capsicum* species. It will be shown that the dsRNA-pattern observed in our experiments runs parallel to the *Capsicum* phylogenetic tree established on the basis of microsatellite markers. Further experiments are underway to prove the endoviral and cryptoviral origin of the dsRNAs detected in our experiments.

**P17.7**

**Colletotrichum acutatum on strawberry and other host plants in Norway**

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Colletotrichum acutatum, the cause of strawberry black spot, was detected in Norway on strawberry for the first time in 1999. Bitter rot has caused damage in a variety of fruit crops in Norway for decades. The causal pathogen of bitter rot was assumed to be *Glomerella cingulata* (anamorph: *C. gloeosporioides*), but identification of the species by PCR has revealed it as *C. acutatum*. During the years 1999-2005, we collected 98 isolates from various host plant species in Norway, all of which were identified to be *C. acutatum*. The fungus is a quarantine-organism in strawberry, and it is thus important to clarify whether strawberry can be infected by isolates from other hosts. Preliminary results of laboratory inoculation tests in strawberry fruits using isolates from strawberry, highbush blueberry, raspberry, pear and cherry, showed that all of them could cause anthracnose on strawberry fruits. Genetic diversity among isolates from different hosts was examined by AFLP, and so far we have not found that isolates with the same host origin are more closely related than isolates from different hosts. We have not been able to fully identify *C. acutatum* based on morphology, because these characteristics seem to overlap with other *Colletotrichum* species. *C. acutatum* isolates grown on PDA varied greatly with respect to colony colour, colour of the spore masses, and conidial shape. A few of the *C. acutatum* isolates were self-fertile and produced the teleomorph *G. acutata* in culture.
Phytoplasmas are wall-less bacteria that are transmitted between plants by insect vectors. They are phloem-limited and infect a wide range of plants to cause symptoms such as yellowing, phyllody, witches broom and general decline, examples being aster yellows and coconut lethal disease. Because they cannot be cultured, identification and classification is based primarily on sequencing of rRNA genes. *Candidatus* categories are then used, such that strains within a ‘*Candidatus* phytoplasma’ species share at least 97.5% of their 16S rRNA gene sequence. Primers based on the 16S rRNA gene are also the main method for diagnosis of phytoplasmas in plants, but the uneven distribution of these organisms within plant tissues, and the requirement for sequencing to unequivocally identify an organism to a *Candidatus* species makes these techniques cumbersome for routine use. We have been developing the use of T-RFLP (terminal restriction fragment length polymorphism) to improve these diagnostic techniques. Primers have been developed for part of the 23S rRNA gene which not only amplify any phytoplasmas present, but also amplify part of the plant chloroplast genome. Use of specific restriction enzymes followed by fragment analysis then allows us to assign any phytoplasmas present to different *Candidatus* species. In addition, the plant chloroplast gives a specific fragment which is used as an internal control to show that the DNA has been successfully isolated and amplified.
Port Check: development of ‘on site’ molecular diagnostics for quarantine pests and pathogens
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3 Institut National de la Recherche Agronomique, Sophia Antipolis, France.
5 Invitrogen (formerly DNA Research Innovations), Sophia Antipolis, France.
6 Bioreba, Reinach, Switzerland.
7 Cepheid Europe, Maurens-Scopont, France.
8 Takara Bio Europe, Gennevilliers, France.
9 Other partner details are given at
http://www.portcheck.eu.com/participants.cfm

Port Check (www.portcheck.eu.com) is an EU-funded project aimed at delivering the tools and procedures to allow EU Plant Health laboratories and inspection services to perform molecular diagnostic assays on-site and at points of entry. Using field portable real-time PCR platforms (in particular the Cepheid Smartcycler), the project has developed assays (using TaqMan or Takara Cycleave chemistry) for the detection of a number of key quarantine organisms: Phytophthora ramorum, potato brown & ring rot, citrus black spot, potato wart disease, Potato spindle tuber viroid, Karnal bunt, Thrips palmi and a range of statutory nematodes (pinewood, root knot, potato cyst). In addition, the project is investigating stabilised reagent formats, including beads, in order to make the system more robust and user-friendly. Particular attention has also been paid to the problems associated with sampling and nucleic acid extraction under field conditions. This includes the development of a portable homogenisation system (Bioreba ‘baby’ Homex) and novel on-site nucleic acid extraction using Charge Switch® Technology (Invitrogen). In addition to the academic and industry research partners, the Port Check consortium (39 partners in total from 18 countries) also includes a comprehensive network of official Plant Health laboratory and inspection service organisations. These partners will play a key role in ring testing the methods developed as part of the R&D effort.

Acknowledgement
Port Check is funded by the European Union under the Framework 6 program. In addition, some partners also acknowledge national funding: UK Defra Plant Health Division (CSL), Bundesamt fur Bildung und wissenschaft, Bern/Schweiz (Bioreba), Dutch Ministry of Agriculture, Nature and Food Quality (PPS).
W2  Post-PhD Possibilities
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All PhD candidates should start thinking about job prospects well before defending their dissertation. The topic of future jobs always comes up in yearly evaluation talks and especially during the final year of your studies. Crucial steps will have to be taken with the purpose of meeting the demands of the world beyond your studies. At this years conference a Post-graduate workshop will be held on the evening of Tuesday 15th Aug. We hope that the workshop will facilitate students understanding of the career choice process and enable them to make effective decisions regarding their careers. The workshop will include three short presentations; one provided by a representative of industrial research (NN), one provided by Professor Jim Beynon, representing the opportunities within academia, and finally Celine Janvier will present her own personal case story of her experience from academia to industry. The workshop mainly aims at PhD students, but all delegates are welcome to attend and provide input for 'PhD students on the go'. The event is free for all delegates attending the conference and both drinks and a light buffet will be arranged.

W3  Seed Health and agricultural development
Jan Torp

Losses in yield and quality caused by seed borne diseases are considerably higher in developing countries compared with industrialised countries. In these, the use of strict seed multiplication, field inspection and chemical seed dressings have controlled the majority of important seed borne diseases. In the developing countries most seed is still farm saved seed, often multiplied on farm for several generations. Is this because it is actually economically unfeasible for subsistence farmers to safeguard their seed or impossible for them to source high quality seed? How can this vicious circle best be broken; what are the key actions to be implemented?
We will initiate this workshop by presenting a few cases to illustrate some of the seed problems faced by the poor farming communities in developing countries, and hope to stimulate a mutually inspiring discussion, and to strengthen the networking between researchers working with seed health and agricultural development.

http://www.shc.kvl.dk
http://www.africancrops.ipbhost.com/
https://www.ippc.int/IPP/En/default.jsp
The future for teaching in plant pathology
David B. Collinge

In common with many natural science educations, where the numbers of students are falling, Agricultural sciences and Plant Pathology are under threat as university subjects in many European countries.

How are we tackling the problem of reduced enrolment in the different EFPP countries? Which educations (agriculture, biotechnology, biology, food sciences, forestry, horticulture...) have plant pathological components? How do we ensure that plant-microbe interactions have a role as inspiration in plant physiology and microbiology courses? How do we prioritise plant pathology teaching in departments with several faculty members versus departments with limited teaching resources? Is there a role for distance learning in plant pathology? Experiences?

We will start this workshop with a couple of case stories briefly describing the structure and aims of plant pathological teaching in different European universities. We hope to use this workshop to gain mutual inspiration for the future of teaching, and to encourage the development of a teaching network in European universities, for example, using Erasmus-Mundus and Tempus programmes for teacher and student mobility, and for the implementation of the Bologne structures.

Some links
http://www.plbio.kvl.dk/Undervisning/Kurser.aspx
http://www.nova-university.org/
http://www.euroleague-study.org/index.html
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