Plant Biology and Biogeochemistry Department annual project report 1999

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Annual Project Report 1999

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Webmaster
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Abstract

The Department of Plant Biology and Biogeochemistry is engaged in basic and applied research to improve the scientific knowledge of developing new methods and technology for the future, environmentally benign industrial and agricultural production, thus exerting less stress and strain on the environment. This knowledge will lead to a greater prosperity and welfare for agriculture, industry and consumers in Denmark.
The research approach in the Department is mainly experimental and the projects are organized in six research programmes: 1. Plant-Microbe Symbioses, 2. Plant Products and Recycling of Biomass, 3. DLF-Risø Biotechnology, 4. Plant Genetics and Epidemiology, 5. Biogeochemistry and 6. Plant Ecosystems and Nutrient Cycling.
This electronic version of the annual report from the Plant Biology and Biogeochemistry Department aims to provide information about the progress in our research. Each project summarizes and highlights our results and achievements to give an idea of the research directions in the Department. Some 160 persons, including staff, undergraduate students, postgraduate scientists and visiting scientists from all over the world, address our research goals.
The Department's contribution to education and training is presented. Lists of publications, papers accepted for publications, guest lecturers, exchange of scientists and lectures and poster presentations at meetings are included in the report. Names of the scientific, technical and administrative staff members, visiting scientists, Postdoctoral fellows, Ph.D. students, M.Sc. students and apprentices are also listed.
A printed Annual Report 1999 providing an executive summary of the activities in the Plant Biology and Biogeochemistry Department is available on request from
krista.christiansen@risoe.dk

Introduction

The Department of Plant Biology and Biogeochemistry is engaged in basic and applied research to improve the scientific basis for developing new methods and technology for the future, environmentally benign industrial and agricultural production, thus exerting less stress and strain on the environment.
The Department's expertise covers a wide range of subjects including chemistry, chemical kinetics in the liquid and gas phase, geochemistry, geochemical modelling, hydrochemistry, analytical chemistry, process chemistry, plant molecular biology, plant pathogenicity, plant genetics, bioinformatics, population biology, plant nutrition, nutrient cycling, ecophysiology, terrestrial ecology and ecology of trace elements. The evolution of focus in the Department's research has continued during the year. The revisions take into account the way different programmes have evolved over the last years and the new and exiting possibilities for research which have been opened.

The results of the research and development are disseminated internationally to companies, institutions, organizations and public authorities through scientific publications, research reports, lectures and posters at scientific - and other professional meetings, personal communication with collaborators and through teaching courses at universities.
As always people constitute the most vital asset of the Department. In addition to our scientific, technical and administrative staff, we depend on the support from collaborators and sponsors. The research in the Department is mainly funded directly from the Ministry of Research and Information Technology. However, the Danish Research Councils, the Ministry

of Food, Agriculture and Fisheries, the Danish Directorate for Development, the Ministry of Foreign Affairs, the Council for Development Research, the Ministry of Environment and Energy, the Danish Energy Agency, the Danish Environmental Protection Agency, EU research programmes, private foundations and commercial contracts also make substantial contributions to the total budget of the Department.

Organization

The Plant Biology and Biogeochemistry Department currently incorporates 135 scientific and technical staff members including 20 Ph.D. students. In addition 25 master students work on their theses in the Department. The Department is organized into six research programmes (Figure 1).

Brief Introduction of the Research Programmes

Plant-Microbe Symbioses

The Plant-Microbe Symbioses Programme seeks to identify and characterize genes and processes involved in the molecular interaction and compound exchange mechanisms, operating during the establishment and maintenance of a symbiotic relationship between plants and micro-organisms. The symbioses under study are *Rhizobium*/pea, arbuscular mycorrhizal (AM) fungi/tomato and *Medicago truncatula*, *Blumeria graminis* f.sp. *hordei (Bgh)*/barley, *Bgh/Arabidopsis thaliana*, *Telletia caries*/wheat and *Phytopthora infestance*/ potato. A feature common to all plant-microbe symbioses is the integration of the micro-organism into the interior of a viable plant cell. In all plant-microbe symbioses, a symbiotic interface emerges by formation of a tissue specific, plant-derived membrane. Molecular communication between the symbionts must proceed via this membrane, and the interface thus constitutes the major point of control of the symbiotic relationship. To achieve the research goals the following disciplines were applied as analytical tools: analytical chemistry, biochemistry, plant and microbe physiology, genetics and molecular biology. The overall aim is to develop the technology and the genetic tools for crop improvement in terms of an increased N and P transport to the plant and a lower disease level.

Plant Products and Recycling of Biomass

The programme aims to develop plant varieties of higher quality by use of modern molecular technologies. This included a production of transgenic plants, cereals in particular, in order to create an overproduction of enzymes or to alter specifically the gene expression to modify the nutritional quality (phosphorus and nitrogen) or modulate the polymer content like lignin. In the search for renewable resources as chemical raw materials, carbohydrates available from biomass waste products, such as wood chips and agricultural residues or purposely grown crops, represent one conceivable alternative. The programme contributes to the scientific basis for developing the wet oxidation technique for pre-treatment and conditioning of biomass, straw and wood chips. Wet oxidation readily solubilizes lignin in straw and the product is susceptible to enzymatic treatment and fermentation. In the field of upgrading biomass, the programme aims at converting straw to ethanol by means of wet oxidation followed by fermentation.

DLF-Risø Biotechnology

The programme aims to characterize and control key genes involved in the regulation of the flowering in ryegrass (*Lolium perenne* L.). The overall objective is to develop transgenic high value grass plants, which are incapable of producing stems and flowers during grassland farming (biological encapsulation) and with highly improved quality for agronomic use. Forage grass is by far the largest agricultural crop in the EU, occupying a total of 20 mill. ha in frequent rotation. Compared to other agricultural crops, the grass field has a very positive environmental profile, due to limited use of pesticides, limited seepage of nutrients and the allowance of a large diversity of wild plant species, insects and animals. However, the value of grass as cattle fodder is limited by the fact that especially the grass stems contain high amounts of low digestible, mainly lignin-containing, compounds.

Plant Genetics and Epidemiology

The programme aims to establish the scientific basis for breeding crop plants with new and stronger resistance to diseases and with improved nutrient efficiency, and to analyse the agricultural ecosystem and its interactions with the environment. By use of DNA markers we analyse the genetic basis for important agronomic traits and spread of genes (*e.g.* transgenes) between crops and wild relatives. Fungal disease resistance is studied in detail with respect to genetic and physiological mechanisms of host resistance and the evolution of virulence. Biometric analyses and mathematical models are used to relate experimental results to genetic information and support the making and predictions of hypotheses. The host-pathogen system, barley and barley powdery mildew *Blumeria graminis* f.sp. *hordei,* is used as a model system for an obligate fungus.
Studies of plant population biology are necessary to predict possible consequences of using new genotypes, possessing transgenes. Introgression of genes from crop plants to their wild relatives is studied to assess the risks of using genetically modified plants in the plant production. Oilseed rape and wild *Brassica* species are used as model systems for these studies.

Biogeochemistry

The research programme aims at understanding fundamental chemical processes and developing methods for chemical analysis of agricultural products and organic micro pollutants in the human food chain. The research focuses on the occurrence, transport, turnover as well as effects of trace elements and organic micro contaminants in agricultural and forest ecosystems. Trace elements are followed from the soil or atmosphere to the crop, and through the human food chain. Effects of air pollution and global change are studied, both at the plant and at the ecosystem level. Emphasis is placed on the development of new methods and processes, which can form the basis of an environmentally benign plant production. The main goal of the research is to contribute to an environmental, sustainable and food safety plant production. The laboratory performs chemical analyses for public authorities and private companies.

Plant Ecosystems and Nutrient Cycling

The research emphasizes biological, physiological, biochemical and chemical processes involved in the transfer of plant nutrients through the Soil-Plant-Air-Continuum and the influence of climate changes on the plant ecosystems, the fluxes of volatile compounds.
A better understanding of the biological and chemical processes in the soil-plant-atmosphere system will lead to a reduced requirement for fertilizers and to a reduced loss of nutrients. Special attention is directed towards the genetic background for plant nutrient uptake and processes involved in the physiology of nitrogen and phosphorus. Information about these processes will provide the basic knowledge needed for a sustainable plant production.
In order to fertilize the different parts of the fields in accordance with the natural variation in topography, texture and soil fertility, we are developing systems to continuously measure plant vigour in relation to the position of the machinery in the field.

Research Projects

Detailed information about all research projects in the Department can be obtained from www at http://www.risoe.dk/pbk

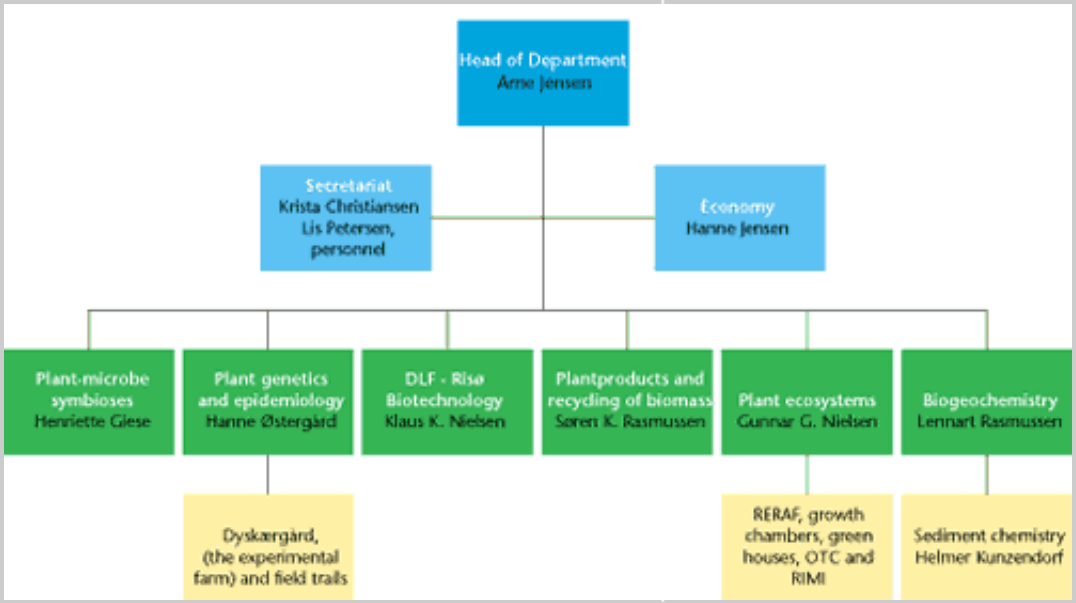

Figure 1: Organization chart

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Plant-Microbe Symbioses

The programme for Plant-Microbe Symbioses seeks to identify and characterize genes and processes involved in the interaction between plants and microbes. The organisms studied include *Rhizobium*/pea, arbuscular mycorrhizal (AM) fungi/tomato and *Medicago truncatula*, *Blumeria graminis* f.sp. *hordei (Bgh)*/barley, *Bgh*/*Arabidopsis thaliana*, *Tilletia caries*/wheat and *Phytopthora infestance/* potato. To achieve the research goals the following disciplines were used as analytic tools: analytical chemistry, biochemistry, plant and microbe physiology, genetics and molecular biology. The overall aim is to develop the technology and the genetic tools for crop improvement in terms of an increased N and P transport to the plant and a lower disease level.

Legume/*Rhizobium* Symbiosis

In Vivo ^{15}N-NMR Spectroscopy – a Tool to Study Assimilation of Symbiotically Fixed Nitrogen

(A.M. Scharff, L. Rosendahl)

In legume root nodules nitrogenase-generated ammonium may be transported from the bacteroid to the plant via an ion channel in the symbiosome membrane. Alternatively nitrogenase-generated ammonium may be assimilated into a carbon skeleton within the bacteroid and then transported to the plant via a recently identified amino acid transporter in the symbiosome membrane (Rudbeck *et al.* 1999; Rosendahl *et al*. (a) and (b), in press). The *in planta* significance of the different transport mechanisms requires non-invasive measurements. We have commenced this task by constructing a gas tight perfusion system connected to a NMR tube, which facilitates *in vivo* ^{15}N-NMR spectroscopy on metabolically active root nodules. In this system we have incubated nodules in $^{15}N_2$ and we have obtained the first ever ^{15}N-NMR spectra, which reflect assimilation of symbiotically fixed nitrogen in actively fixing nodules. The results demonstrate that the first ^{15}N-labelled amino acid to exceed the detection limit is glutamate. Subsequently, ^{15}N is also detected in the amide of asparagine. A limitation to NMR spectroscopy is a rather poor sensitivity and further optimization of the *in vivo* ^{15}N-NMR procedure is required in order to get a complete reflection of the *in planta* assimilation pathway for symbiotically fixed nitrogen.

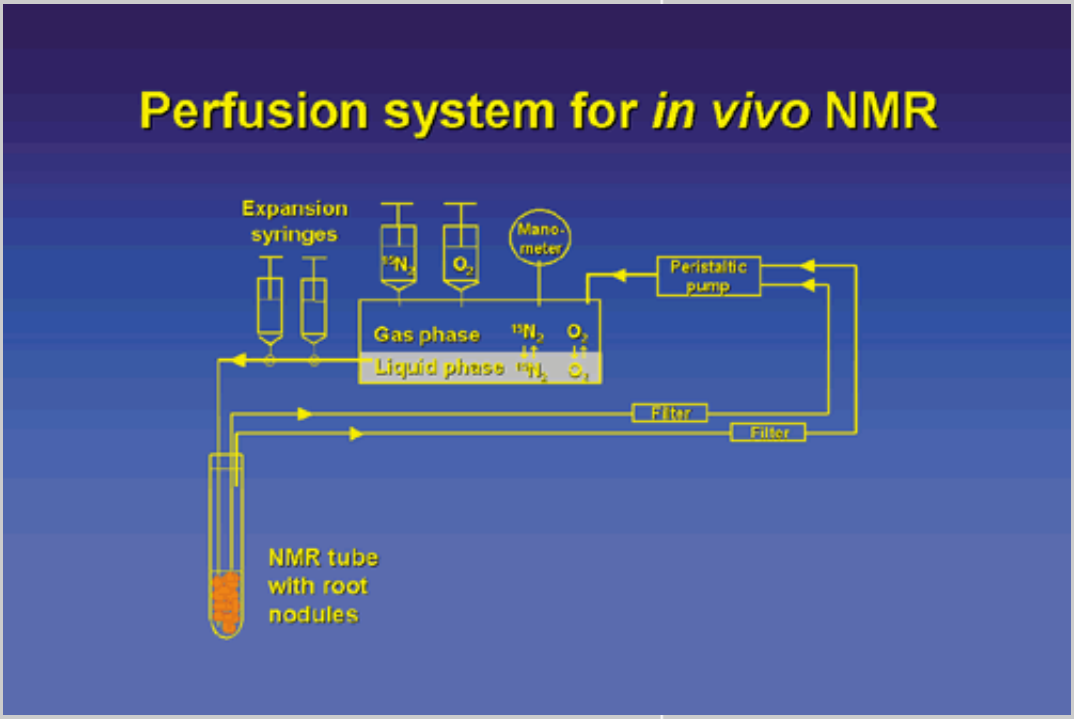

Figure 1: Gas tight perfusion system for *in vivo* ^{15}N-NMR spectroscopy.

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Structural Glycoproteins in Legumes in Response to Colonization by *Rhizobium*

(P.A. Olsson, L. Rosendahl)

Plant structural glycoproteins are likely to be involved in the regulation and function of plant-microbe symbioses: They are abundant on plant cell surfaces, they may be involved in early defence responses, and some are expressed in response to the micro-organism. A group of structural glycoproteins are proline-rich proteins (PRP). We have used immunoblotting with monoclonal antibodies to study the abundance and distribution of PRPs in pea in response to colonization by *Rhizobium*. The PRPs were highly expressed in nitrogen-fixing nodules formed by *Rhizobium* and the pattern of immunoreactive polypeptides was different here from other tissue. The nodules contained an immunoreactive polypeptide of 90kDa, which was enhanced in this tissue. Sub-fractionation of the nodules revealed that the tissue enhanced immunoreactive isoform of PRP was dominating in the interface between the symbionts. The abundance of PRPs was highly reduced in non-nitrogen-fixing nodules when the non-fixing phenotype resulted from a mutation in *Rhizobium*; however, there was no consistent reduction in the abundance of PRP in non-fixing phenotypes resulting from mutation in the plant. The results suggest that bacterial signals related to the bacterial ability to fix nitrogen may be responsible for the regulation of PRP production in root nodules.

Phosphate Transport in Free-Living and Bacteroid Form of *Rhizobium*

(H.M. Christophersen, L. Rosendahl)

Nodulated legumes require high levels of P for optimal symbiotic nitrogen fixation. The basis for the elevated P requirement is poorly understood and little information regarding bacteroid P-acquisition and -metabolism is available. Free-living *Rhizobia* express a low-affinity P-transporter. Under P-limited conditions an additional high-affinity P-transporter, which is

sensitive to phosphonates, is also induced. We have demonstrated that free-living *Rhizobia* take up phosphate at a substantially higher rate than that of the symbiotic bacteroid form. P transport in bacteroids was not sensitive to phosphonates, and thus there is no indication for the presence of a P-starvation induced high-affinity P-transporter in symbiotic *Rhizobium* bacteroids.

Proteome Analysis on Symbiosomes from *Rhizobium*-Induced Pea Root Nodules
(P. Erik, G. Saalbach)
The legume plant-*Rhizobium* symbiosis, known to supply the plant with nitrogen derived from atmospheric nitrogen, leads to the formation of a new compartment in the plant cell, the symbiosome. This compartment harbours the bacteroids and is surrounded by a plasmalemma-derived membrane called the peribacteroid membrane (PBM).
The PBM and the peribacteroid space (PBS) between this membrane and the bacteroid outer membrane fulfil important functions in the maintenance of a continuous and carefully balanced exchange of molecular signals and metabolites between the symbionts. The project is aimed at the investigation of the proteins present at this interface. "Proteome analysis" is used as a new approach to characterize the spectrum of proteins in the symbiotic compartment. This analysis includes 2D-gel electrophoresis to fractionate as well as mass spectrometry (MS) to identify individual proteins.
Symbiosomes have been isolated from pea root nodules via differential centrifugation including a Percoll gradient. Osmotic lysis and a sucrose step-gradient centrifugation have been used to purify PBS and PBM fractions. Soluble PBS proteins are precipitated with acetone/TCA and membrane (PBM) proteins are extracted with phenol, and finally also collected by acetone/TCA precipitation.
For 2D-gel electrophoresis, the Multiphor-system from Pharmacia including pre-cast IPG-strips of different pH ranges (mainly pH 4-7) is used. Analysis of the silver-stained gels using the PDQuest software (Bio-Rad) revealed the presence of hundreds of proteins in the different fractions. These results have been used to establish the first maps for certain gel areas. A high proportion of low-abundance proteins can be found. As revealed by MS analysis many of them represent proteins from broken bacteroids. A procedure for the preparation of PBM with low bacteroid marker-protein content but enriched in a specific membrane marker (ATPase) has been developed.
Spots representing individual proteins were cut out from coomassie-stained gels and digested with trypsin. The resulting peptides were analysed by LC-MS using a LCQ mass spectrometer (Finnigan). Twenty out of approx. 40 spots representing 13 different (mostly bacteroid) proteins could be identified by database search (Sequest).
In this way, the basic requirements for proteome analysis have been successfully established within the past year. Future work will focus on the detection, identification, and cloning of specifically expressed PBM, PBS, and bacteroid proteins. To link the proteome project to genome projects model legume plants will be involved. Experiments with the genetically well-studied *Medicago-Sinorhizobium* system have been started.

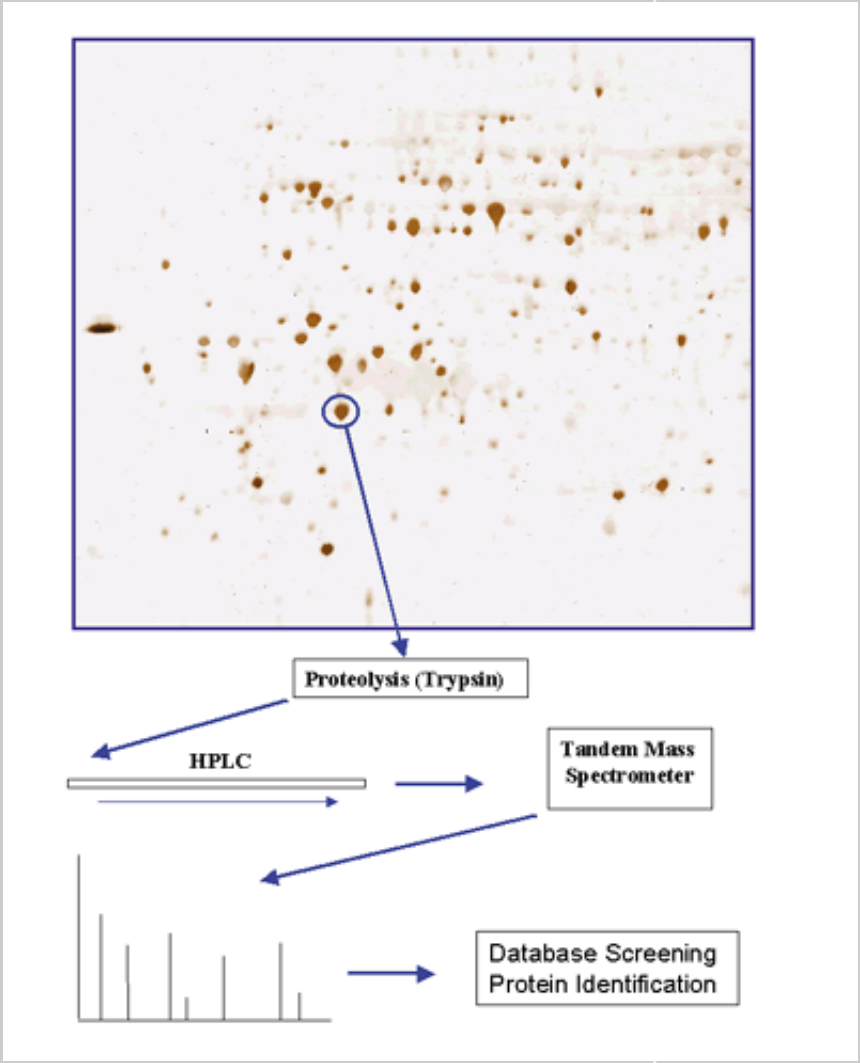

Figure 2: Hundreds to thousands of proteins can be resolved on a 2D gel. The protein spots provide enough material for their identification by mass spectrometry.

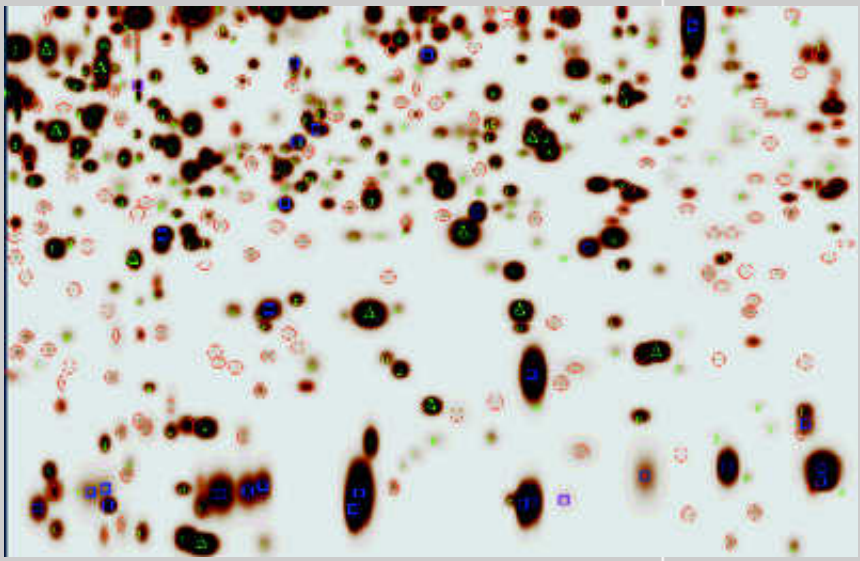

Figure 3: Area from a 2D gel analysed with the "PDQuest" software (BIO-RAD). Total symbiosome preparations were compared with PBM fractions. There are 705 individual spots present in the selected area. Blue and pink rectangles indicate proteins enriched in the PBM fractions. Green letters indicate matched spots. Red circles indicate unmatched spots (not present in PBM).

Mycorrhiza

Identification and Cloning of Genes Involved in Initiation and Maintenance of the Arbuscular Mycorrhizal Symbioses

(S. Burleigh, J. Nielsen, I. Jakobsen)

The growth benefits of plants in response to colonization by arbuscular mycorrizal fungi (AMF) can be quite variable, ranging from dramatic increases in growth to neutral and even pathogenic reactions. This diversity of response is reflective of the complex interaction between plant and fungal genotypes and is likely to involve numerous genes. Our group is attempting to clone and characterize these genes in an attempt to understand the processes central to mycorrhizal responsiveness.

One gene that may be involved in P transport efficiency of mycorrhizas is a fungal membrane P transporter, which moves P from the soil into the hyphae for transport to the plant. Thus far, these P transporters have only been cloned from *Glomus* species. The construction of a cDNA library from *Scutellospora calospora* by M.J. Harrison, a visiting scientist from Noble Foundation, Oklahoma, USA, will aid the attempt to clone new fungal P transporters and compare their uptake efficiencies.

Differential display methods, previously used to identify transcripts specific for early infection stages in barley powdery mildew, have been optimized for use with RNA extracted from root-external hyphae of AMF. The examination of gene expression profiles from external hyphae under different P nutrition schemes has yielded several transcript fragments from genes seemingly under P control. Several fragments have been cloned and one has been sequenced for further analysis. There is some indication that this fragment might be from a gene in the P-type ATPase family. This would group it with homologues from other organisms that are known to be important in nutrient uptake and proton gradient maintenance.

We have also shown that plant P-transporter expression in mycorrhizas was down-regulated during AMF colonization and that this regulation involved both fungal-derived P as well as another uncharacterized fungal influence. In another study, we found that a low-affinity zinc transporter from *Medicago truncatula* was down-regulated in mycorrhizas, despite its up-regulation by Zn fertilization in uncolonized plants. To assist this research, a relative quantitative reverse-transcription PCR technique was developed to assay gene expression. Using this technique, we showed that a putative nitrate transporter from *M. truncatula* was down-regulated by some, though not all AMF.

Lipid Transport in Hyphae of Mycorrhizal Fungi

(J. Nielsen, I. Jakobsen)

The implementation of a new *in vitro* culture system for arbuscular mycorrhizas has for the first time allowed us to directly study transport processes in living external mycelium. Observation of hyphae vitally stained with lipid-specific flurochromes indicates that the transport of lipids could be via a peristaltic vacuolar reticulum previously observed in ectomycorrhizal fungi. The studies have been carried out in collaboration with L. Lindvold, Optics and Fluid Dynamics Department, Risø.

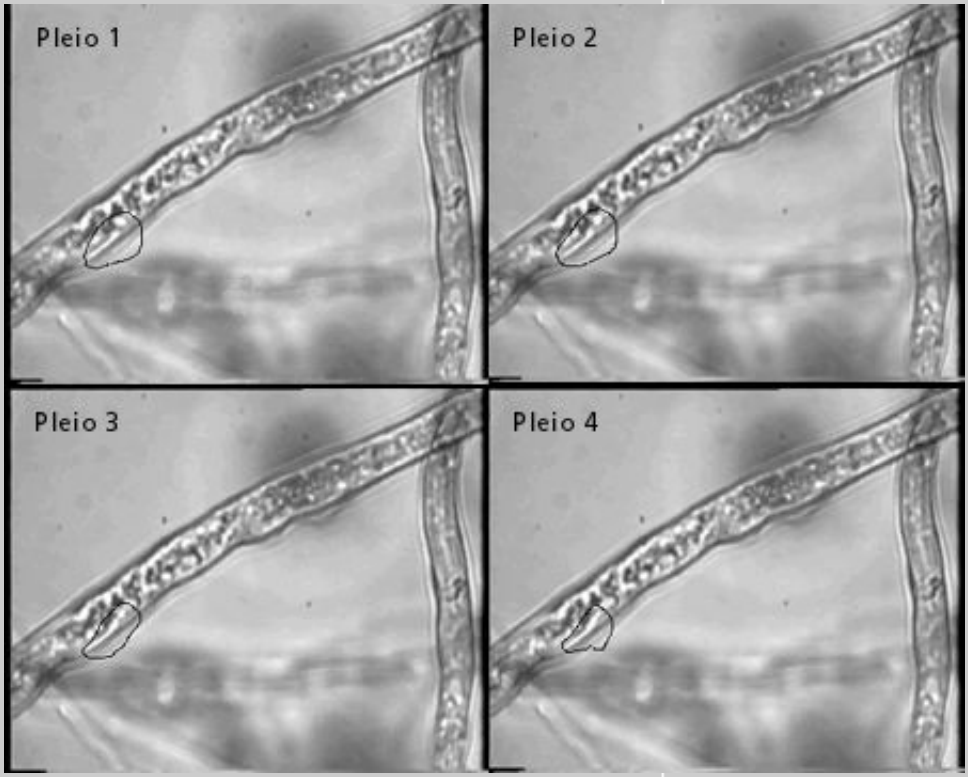

Figure 4: Example of vacuole fusiform fusion in Glomus intraradices, hinting the existence of a pleiomorphic tubule sytem as observed in several other fungal taxa, including ectomycorrhiza. Rounded vacuole becomes drawn out and fuses with lateral tubule (outlined). Frames 0.5 sec. apart.

P Translocation in *Glomus intraradices* Studied by *In Vivo* ^{31}P-NMR Spectroscopy
(N. Rasmussen, I. Jakobsen)
^{31}P nuclear magnetic resonance (NMR) spectroscopy is used to study P metabolism in arbuscular mycorrhizal fungi (AMF) inside and outside roots of cucumber. The *in vivo* NMR method allows biological systems to be studied non-invasively and non-destructively and a major challenge is to provide optimal conditions for the living tissue in the NMR tube. Collaboration with R.G. Ratcliffe, University of Oxford, UK has led to the successful implementation of the so-called airlift system, which aerates and circulates the buffer in the NMR tube by means of an air pressure applied to the tube. The ^{31}P-NMR spectra of excised AMF mycelia and AMF-colonized roots contained signals from polyphosphate (PolyP), which were absent from the spectra of non-mycorrhizal roots. This demonstrated that orthophosphate (Pi) taken up by the fungus was transformed into PolyP with a short chain length. The spectra of excised mycelia revealed only a small signal from the cytoplasmic Pi suggesting a low cytoplasmic volume in this AMF. Excised mycelia had the capacity to synthesize PolyP from Pi, applied after the excision of the hyphae.

P Uptake by *Glomus intraradices* under Monoxenic Conditions
(S. Ravnskov, I. Jakobsen)
The ability of arbuscular mycorrhizal fungi (AMF) to utilize different P sources is difficult to study in soil with its wide range of interacting saprotrophic microorganisms. Monoxenic cultures of mycorrhizas formed with Ri T-DNA transformed carrot roots were used to study the hyphal use of P from the simple organic P compound adenosine monophosphate (AMP). The roots were established in one side of petri dishes where the AMF hyphae crossed a solid barrier and grew into another compartment containing ^{32}P-labelled AMP or orthophosphate. The *G. intraradices* hyphae took up similar quantities of P from both sources and AM fungi therefore appear to be able to hydrolyse simple organic P compounds. This is in accordance with an observed positive relationship between acid phosphatase activity of the mycelium and the hyphal biomass. The study was carried out in collaboration with visiting scientist E. Joner, CNRS, Nancy, France.

Interactions between *Glomus intraradices* and the Biocontrol Agent *Burkholderia cepacia*, and Their Influence on the Root Pathogen *Pythium ultimum*
(S. Ravnskov, I. Jakobsen)
The bacterium *Burkholderia cepacia* is recognised as a biocontrol agent against several root pathogens. Before using this biocontrol agent in plant protection, however, it is important to examine possible negative side effects on plant beneficial organisms such as arbuscular mycorrhizal fungi. Interactions between root-external mycelium of *Glomus intraradices*, *B. cepacia* and the root pathogen *Pythium ultimum* were studied in semi-sterile soil using a growth system with root-free soil compartments in collaboration with J. Larsen, Danish Institute of Agricultural Sciences. Signature phospholipid fatty acids (PLFAs) were used to quantify individual microbes. Phosphorus uptake and biomass (PLFA 16:1ω5) of the *G. intraradices* mycelium were unaffected by five different strains of *B. cepacia*, whereas the length of hyphae was stimulated, unaffected or suppressed by the different *B. cepacia* strains. Biomass of all strains of *B. cepacia* (PLFAs cy17:0 and cy19:0) was decreased by the presence of *G. intraradices* mycelium.
Two of the *B. cepacia* strains, one stimulating and one suppressing hyphal length of *G. intraradices*, were selected to study if *G. intraradices* mycelium affected biocontrol efficacy of *B. cepacia*. The *G. intraradices* mycelium suppressed both *B. cepacia* strains, but this did not impair the ability of the bacteria to suppress *P. ultimum*, as examined with colony forming units on selective media. *P. ultimum* was also suppressed by the *G. intraradices* mycelium alone or in combination with *B. cepacia*. This study shows that the mutual inhibition between *G. intraradices* and *B. cepacia* does not impair their functioning in terms of hyphal P transport and biocontrol efficacy, respectively.

Carbon Allocation in Pea Plants as Influenced by Climate Change and Mycorrhizas
(M. Gavito, T. Mikkelsen, D. Bruhn, I. Jakobsen)
Previous experiments in RERAF with pea and winter wheat showed that elevated atmospheric CO_2 did not stimulate root growth when plants were grown in large pots containing soil with adequate nutrients. Positive effects of elevated CO_2 and mycorrhizas on shoot growth were additive and non-interactive. A further experiment with two harvest times has revealed a significant treatment interaction on growth and rate of photosynthesis in pea. Growth and photosynthesis of mycorrhizal plants were thus increased by elevated CO_2 while non-mycorrhizal plants were unaffected. Neither root-internal nor root-external growth of the mycorrhizal fungus was influenced by CO_2 level. Accordingly, hyphal ^{32}P uptake from root-exclusion bags inserted into the pots was unaffected by the CO_2 level. In conclusion, no evidence has been found for increased nutrient uptake at elevated CO_2. The enhanced plant growth at elevated CO_2 was caused by the higher C assimilation rate and C use efficiency. As expected, the CO_2 effect was highest in the mycorrhizal plants, which represent the largest carbon sink.

Blumeria graminis f.sp. *hordei (Bgh)*/Barley Symbiosis

Molecular Characterization of Nutrient Transporters Involved in Plant-Microbe Symbiosis
(L. Baunsgaard, H. Giese)
The objective of the current project is to clone and characterize transporter molecules involved in nutrient transfer between and within host plant and fungus.
A putative phosphate transporter cDNA was isolated from the barley powdery mildew fungus (*Bgh)* EST-library. The expression pattern of the mildew infection of barley leaves was studied by northern analysis. This indicated differential expression of the putative phosphate transporter at the stage where the mildew haustorium is formed. The corresponding gene was cloned from a *Bgh* BAC library. The function of a full length cDNA will be studied in a heterologous yeast expression system by complementation of yeast mutants and biochemical characterizations of substrate specificity and uptake.
A barley transporter clone was isolated from a barley epidermis/ *Bgh* cDNA library. The amino acid sequence revealed homology to a family of transporter enzymes from plants with

oligopeptide, histidine or nitrate as main substrate. Southern analysis of barley genomic DNA indicated the presence of two or more isoforms of the putative oligopeptide transporter. Furthermore, northern analysis showed different patterns from barley epidermis and mesophyl, respectively. Functional studies of the enzyme in a heterologous yeast expression system are currently approached.
In order to make functional comparisons between pathogenic and mutualistic plant-microbe symbioses, homologous nutrient transporters from the *Glomus intraradices/ Medicago truncatula* symbiosis are attempted cloned by heterologous screening of cDNA and genomic libraries.

Positional Cloning of an Avirulence Gene
(C. Pedersen, H. Giese)
The initial interaction between barley and the powdery mildew fungus (*Bgh)* is controlled by resistance genes in the plant and corresponding avirulence genes in the fungus conforming to the gene-for-gene hypothesis. To provide the basis for positional cloning, linkage analyses have been carried out on different *Bgh* crosses. Two genetic maps were constructed on the basis of avirulence gene segregation and the use of RAPDs and anonymous RFLP markers. The genetic distances estimated were very large, indicating a very high recombination frequency. To confirm this, another cross was made and a genetic map based on 175 AFLP markers, 67 genomic RFLP markers and 100 EST markers together with 7 avirulence loci has been constructed. Again the map was found to be very long and it was difficult to construct linkage groups. In collaboration with an English group at the John Innes Centre, we have compared *Bgh* maps by the use of common EST markers. These results show that in our cross we have recombination rates about five times higher. We have found close linkage between avirulence gene a6 and two flanking EST markers. Chromosome walking is carried out in this region and screening for YAC and BAC clones containing the EST markers have been carried out. Three BAC clones have been isolated and the BAC ends have been sequenced to allow identification of overlapping clones. This strategy has proved to be difficult due to an abundance of repetitive sequences, but we have now isolated BAC-clones, which extend the BAC-contig. Additional ESTs from this region are isolated and YACs are selected to bridge the gaps.

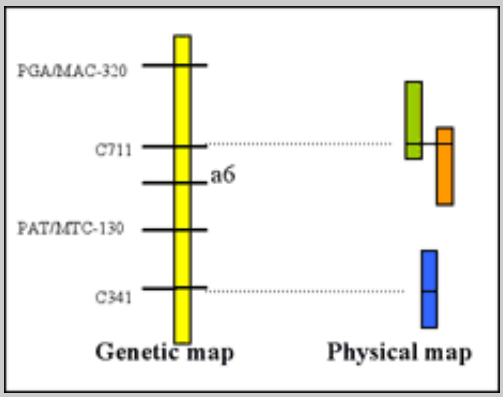

Figure 5: The figure shows a fragment of a genetic map with the avirulence gene a6 and flanking molecular markers and the physical map based on BAC-clones isolated, using the flanking EST markers C711 and C341.

Phage Display Cloning of an Avirulence Gene
(P. Mouritzen, H. Giese)
Bgh genes encoding avirulence and other proteins assumed to interact with barley proteins on the barley plasma membrane may be cloned by phage display technology by performing a panning of *Bgh* cDNA phage display libraries on barley plasma membrane vesicles. cDNA libraries constructed from epidermis, stripped from leaves of barley infected with *Bgh*, will be transferred to phage display vectors for display of the cDNA products on the surface of the phages. A lambda and a M13 phage display vector will be used, which should facilitate display of cytoplasmic and secreted proteins, respectively. The lambda vector has been modified to accommodate subcloning of the cDNA libraries. Culture conditions have been

optimized for barley seedlings to obtain large coleoptiles, which are known to contain a high percentage of epidermis cells. A technique has been established for the purification of plasma membrane vesicles using aqueous polymer two-phase partitioning. These vesicles will be immobilized on cellulose acetate/nitrate filters and used as ligand/bait in panning experiments.

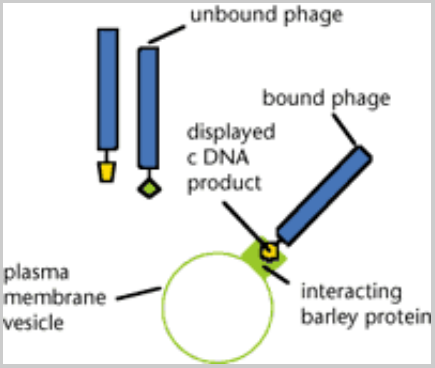

Figure 6: Panning of phage display libraries to plasma membrane vesicles. The covalent bond between the gene product and the phage coat protein facilitate the screening of very large numbers of clones (10^{11} pfu) that would not be possible with a conventional expression library.

Isolation of Genes Expressed during Haustorial Development
(M. Grell, H. Giese)
The products of genes from phytopathogenic fungi, which are expressed during the infection process are targets for the development of pesticides. The objective of the current project is to identify and characterize fungal genes essential for the establishment of *Bgh* on barley. Differential display RT-PCR technology has been used to isolate *Bgh* genes expressed around the moments of the initial penetration (10-12 hours after infection) and the formation of the first haustorium (14-16 hai). The differential expression pattern and fungal origin have been confirmed for about 15 sequences using RT-PCR and southern blot analysis. The *Bgh* sequences represent novel genes. Selected genes have been further characterized. One of them encodes a 172 aa protein highly up-regulated at the time of haustorium formation (14-16 hai). The protein has been expressed in a pET vector and purified. Antibodies have been raised against the product in order to carry out western analyses and to localize the protein *in situ*. Another gene encodes a 385 aa protein induced at the time of spore germination and further up-regulated during the early infection stages. This gene belongs to a gene family with a previously characterized gene member, *Egh16*. Their expression profile during infection in three *Bgh* isolates with differing virulence characteristics has been determined using semi-quantitative PCR. The gene encoding the 172 aa protein is highly expressed at 16 hai in a Japanese isolate, but in a German isolate the highest expression is found at 12 hai. The reason is not known, but we will carry out a parallel morphological study of the isolates to see if there are differences in their development on the plant. The differential display protocol has proved to be effective in isolating differentially expressed genes in the *Bgh*/barley system. The function of the genes remains to be determined. K. Mengden at Konstanz University, Germany, is doing *in situ* localization of the *Egh16* gene product and will get the antibody against the 172 aa protein after affinity purification has been carried out. The results from these studies may indicate a function of the proteins.

Proteome Analyses of *Blumeria graminis*
(S. Lange, H. Giese)
Proteome analysis has been used to supplement our differential display RT-PCR technology approach to isolate genes in the powdery mildew fungus that are expressed during colonization of the plant. Methods have been established to extract proteins from *Bgh* conidia. 2D electrophoresis has been performed on two closely related isolates of *Bgh*. One isolate is unable to infect barley plants carrying the ml-o resistance gene, while the other has been selected from this isolate through 36 generations to become aggressive on this barley. Comparisons of the protein complement in conidia and germinating conidia of the two isolates have resulted in the identification of seven proteins that are differentially expressed.

Two of these have been purified and are now subjected to mass spectrometric analyses to determine their amino acid sequence. Comparison to our EST sequence database will then, hopefully, permit us to isolate the corresponding genes and to carry out expression analyses. 2D electrophoresis has also been carried out on conidia and germinating conidia from 2 *Bgh* unrelated isolates to identify proteins that are differentially expressed.

Functional Studies of Two Differentially Expressed *Bgh* Genes
(P. Mouritzen, H. Giese)
Two cloned *Bgh* genes *Egh7* and *Egh16* were previously shown to be differentially expressed in germinating conidia and in *Bgh* tissue 4-5 days after infection. The function of the genes is unknown and there are still no homologous proteins to be found in the databases. In order to perform *in situ* localization of the two proteins, antibodies were earlier raised in rabbits against the N-terminal part of both proteins (one third) which was expressed in *E. coli*. To improve the specificity of both antibodies, affinity purification has now been performed using the respective antigens, immobilized on nitro-cellulose. The affinity-purified antibodies are specific towards their respective antigens and recognize both truncated and full-length proteins expressed in *E. coli*. However, proteins are not recognized in western blots of protein extracts from germinating spores or *Bgh* infected leaves. This may be caused by a very limited and localized expression of the proteins in specific parts of the fungus. The Pro-rich repetitive structure of *Egh7* might indicate a structural function, *e.g.* in the cell wall, making extraction difficult. Furthermore, the *Bgh* tissue only constitutes a fraction of the infected leaves, which we have used for protein extraction. To test these hypotheses the affinity-purified antibodies have now been handed over to K. Mendgen, Konstanz University, Germany, with whom collaboration has been initiated on *in situ* localization. During the cause of antibody production and affinity-purification a protocol for heterologous protein expression in *E. coli* has been established which allows expression of full-length proteins previously identified as "toxic" to *E. coli*. Nickel affinity chromatography and HPLC techniques for purification of His-tagged proteins, produced in hetereologous expression systems have also been set up.

Transformation of *Bgh*
(L.G. Jensen, S.K. Christiansen)
A transformation system is required for functional analysis of cloned *Bgh* genes. The obligate nature of the fungus requires transformation of intact cells and an *in planta* selection system. Gene transfer is carried out using a hand held gene gun and two strategies for selecting transgen *Bgh* are followed. Using GFP (green fluorescent protein) reporter gene technology it is possible to visually identify genetically transformed green fluorescing *Bgh* from their non-fluorescing wild-type relatives upon exposure to blue light. Following the identification of a green fluorescing spore it is transferred to the surface of a new leaf where it is allowed to develop. In this way the technique provides the basis for a procedure to select spores as well as to follow the growth and development of spores carrying a new genetic trait. In a selection system based on the pesticide Basta the bombarded *Bgh* infected barley leaves are transferred to medium containing the selective agent. Isolates surviving several generations under selective conditions have been contained, but remain to be analysed.

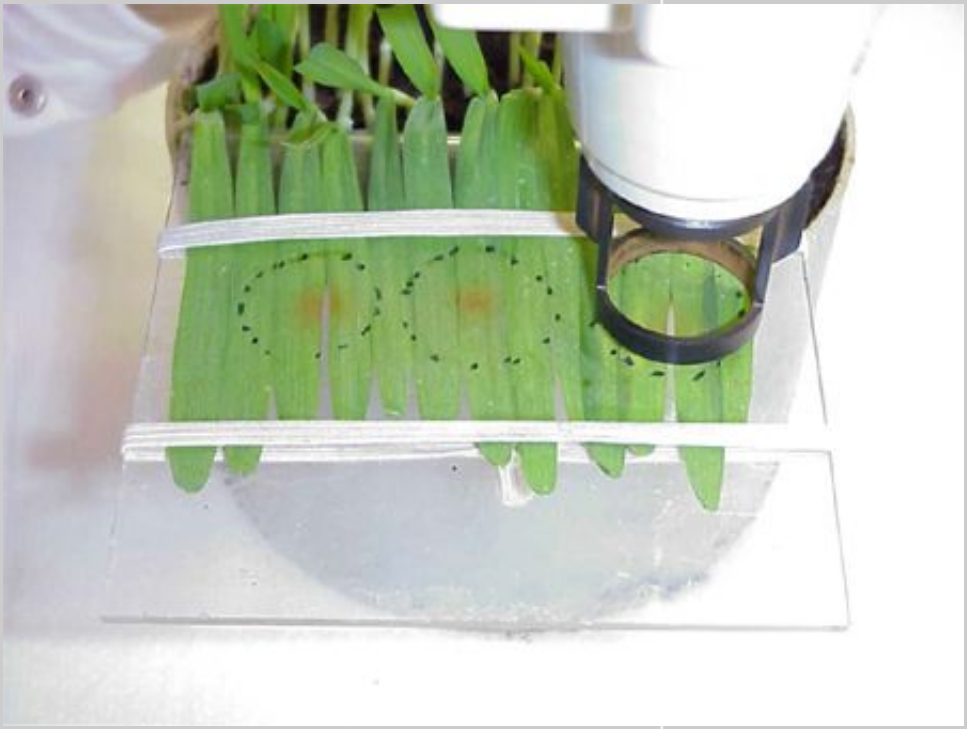

Figure 7: Gene gun transformation of *Bgh* growing on barley leaves.

Defence Mechanisms in Plants

Arabidopsis Mutants with Reduced Resistance against the Barley Powdery Mildew Fungus

(H. Thordal-Christensen, P.H. Fischer, S.C. Somerville)

Arabidopsis thaliana exhibits strong resistance against the barley powdery mildew fungus (*Bgh*). A majority of the conidia is arrested at the stage of penetration, when the host epidermal cell forms papillae. Nevertheless, some conidia penetrate and develop functional haustoria, which allow some secondary fungal growth. Within 2 days after inoculation, the individual epidermal cells, which are penetrated, undergo a hypersensitive response (HR) Figure 8. A microscope-based screening for mutants with altered responses to the fungus was conducted on M_2 lines of EMS treated *A. thaliana*, Columbia. Two mutants were identified with higher penetration rates and therefore higher numbers of single cell HRs. These increases in HR are detectable by the naked eye. Intermutant crosses have demonstrated that the two mutations have occurred in the same gene. These mutant alleles are named *pen*1-1 and *pen*1-2 for higher **pen**etration rates. Histochemical analyses of papilla of *pen*1-1 plants reveal no obvious alterations in the content of callose, structural protein or H_2O_2. Surprisingly, both mutations cause increases in the levels of certain pathogen response transcripts. In order to map-base clone *pen*1, this gene has been mapped to an approx. 50 kb interval.

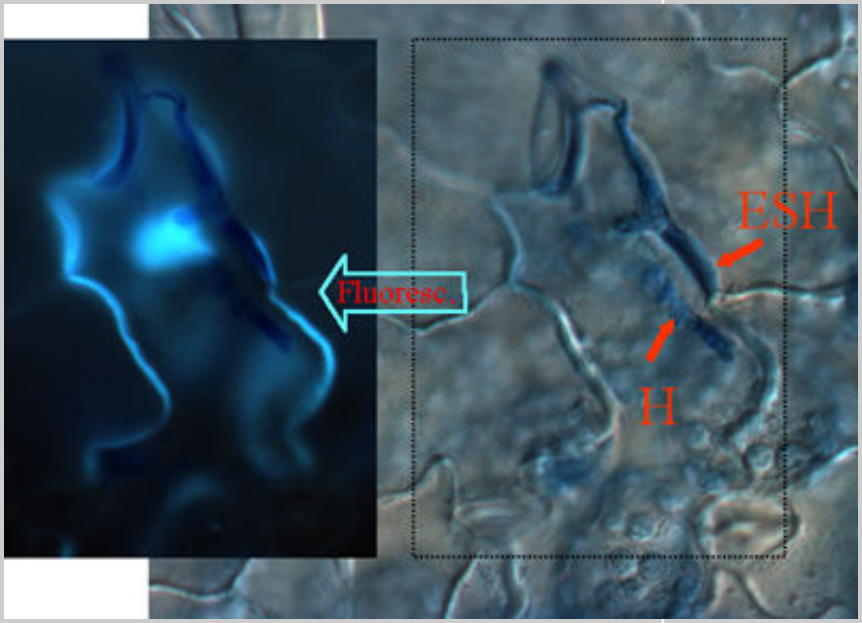

Figure 8: A penetration event in *Arabidopsis* where the barley powdery mildew fungus has been able to grow (indicated by the haustorium (H) and the elongating secondary hypha (ESH)) until stopped by the hypersensitive response (indicated by the fluorescence).

Transgenic Barley with Enhanced Disease Resistance
(T. T. Poulsen, A. Olesen, T. Bryngelsson, D. Collinge, K. Nielsen, R. Oliver, H. Thordal-Christensen)
The purpose of the project is to enhance the disease resistance in barley plants by the use of genetic engineering and thereby create a good resource material for molecular breeding of resistant plants. Antimicrobial pathogenesis-related (PR) proteins are expressed in the leaf by pathogen attack. However, this occurs at the wrong place (mesophyll) in order to be effective towards the powdery mildew fungus (*Bgh*), which is confined to the epidermis. We intend to strengthen the resistance in transgenic plants by altering the regulation of the PR-protein genes towards expression in the epidermis. *In vitro* studies show that three basic PR-proteins (a basic ß-1,2-glucanase, a basic chitinase, and a basic thaumatin-like protein) purified from barley leaves inhibit spore germination of *Bgh*. The corresponding cDNA clones as well as a PR-4 cDNA clone are used for barley transformation. To drive an epidermal expression of the transgenes, we use either an epidermis-specific, pathogen-inducible barley promoter (pOxOLP) or the constitutive actin promoter from rice. The transformation of barley is performed by the use of particle bombardment. We have transformed plants with several of the constructs. These plants are being analysed for integration of the constructs by PCR, BASTA resistance test and southern blotting. Transient transformation experiments suggest that at least the construct for the basic thaumatin-like protein can cause resistance towards *Bgh*.

The Biochemical Activity and Biological Role of a Barley Defence Related Germin-like Protein
(A.B. Christensen, H. Thordal-Christensen)
*Hv*OxOLP is a barley epidermal specific pathogenic induced germin-like protein. It accumulates very early during the interaction with the powdery mildew fungus (*Bgh*), and is potentially very important in the defence to pathogens invading via the epidermis. The protein is related to oxalate oxidase (germin) which is a generator of H_2O_2. Many distinct groups of germin-like proteins exist in plants, but only the oxalate oxidase has an activity ascribed. We speculate whether *Hv*OxOLP generates the H_2O_2 observed in papillae occurring in response to *Bgh*, see Figure 1. Thus, the protein possibly plays an important role in the success of the papillae in stopping the penetration attempts and in triggering other defence mechanisms. Earlier studies have shown that *Hv*OxOLP, just like oxalate oxidase, is a heat stable oligomeric protein. We set out to express His-tagged *Hv*OxOLP in *Arabidopsis thaliana* in order to purify active protein suitable for biochemical studies.

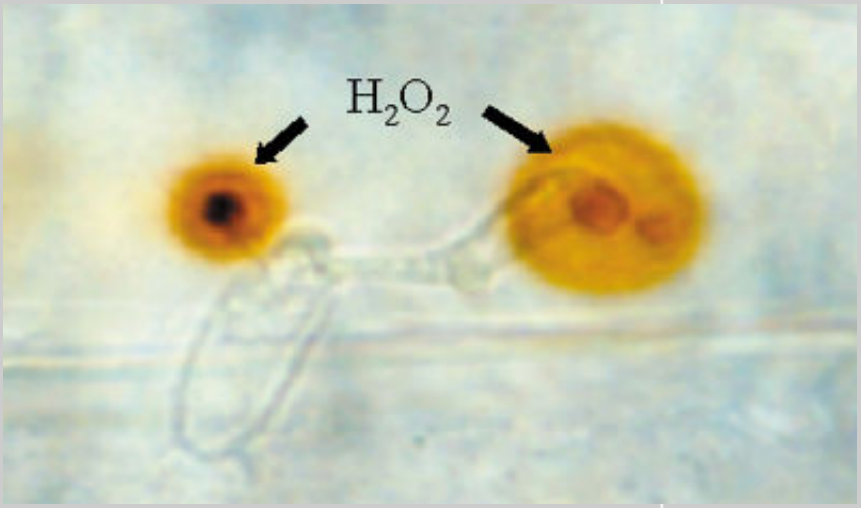

Figure 9: Accumulation of H_2O_2 in papillae induced by *Bgh* attempting to invade a barley leaf.

Molecular Analysis of *Tilletia caries*
(S.K. Christiansen)
In collaboration with the Danish breeders and Research Centre Flakkebjerg, a project aiming at providing resistance towards seed borne diseases is running under the Cereal Network. Risø is responsible for the molecular work on the *Tilletia caries* fungus causing the wheat bunt disease. The molecular marker system, AFLP, has been established and used for analysis of single spore isolates from different locations in Denmark. The results indicate an overall small genetic variation. In addition, we have initiated a project on the development of a PCR based method for early diagnosis of the disease. This would save time in the resistance screening programme.

Alternative Fuels
(L. Frøsig, H. Egsgaard)
Dimethyl ether (DME) is known to be an excellent diesel fuel substitute, since it combines good fuel properties (high cetane number and thermal efficiency) with low NO_x and particulate emissions. Kinetic and mechanistic studies of the combustion chemistry of DME have been conducted using two different approaches.
Laser flash photolysis of Cl_2/DME/N_2/O_2 mixtures in a high temperature flow cell generates methoxymethylperoxy radicals ($CH_3OCH_2O_2^{\bullet}$), one of the primary radicals in the combustion chemistry of DME. The methoxymethylperoxy radicals so formed are allowed to react and the concentrations of stable products produced during the first 1-10 ms are measured by fast, time resolved IR detection (µs resolution).
Molecular beam sampling of premixed DME/O_2 flames with a triple quadrupole mass spectrometer enables us to study ionic species directly in the flames. The ions are formed by proton transfer reaction with strong gas phase acids such as CHO^+. Ion structures are identified by MS/MS techniques and mechanisms for their formations are supported by *ab initio* calculations.

Natural Plant Protection against Pathogenic Fungi
(C. Schou, H. Egsgaard)
Late blight fungus in potatoes caused by *Phytophthoras infestance* (PI) is a serious plant disease with far-reaching social and economical consequences. Foliage reaction of the potato plant leafs is characterized by rapid expansion of lesions, followed by defoliation. Under optimal conditions the infection results in complete death of the vines 1 to 2 weeks after the initial symptoms are observed.
The aim of the project is identifying lipids, proteins and other components related to the infectious process by implementing proteome analysis on expressed components from the fungi and the plant. This is conducted by mass spectrometry with electrospray- (LC-MS) and the more sensitive nanospray-ionization technique, which confers a high degree of sensitivity

into the analysis of peptides and biomolecules in aqueous solution paralleled by GC-MS analysis of lipids, esters and derivatives thereof in organic solution.
Eight strains of PI, mating type A1 and A2, are set up and running in controlled growth chamber on Potato dextrose agar. This material serves as stock in future studies of fungi surface components, and as infection material in *in vitro* models.
Stabile growth of plants from five cultivars with varying degree of resistance towards PI has been established in a growth chamber under controlled growth conditions. Continuous supply of plant material is achieved by cutting breedings in growth chambers.
Cuticle wax of leave surfaces has been analysed with regard to lipid composition, by organic extraction followed by GC-MS analysis. The results point at few - but profound - differences in the wax composition of the cultivars, which subsequently is characterized by NIST database comparison. Unique components will be isolated and used in *in vitro* inhibitory/stimulatory experiments with PI.
An *in vitro* model for study of leave infection by PI has been established. The model has been tested in experiments with coated leaves sprayed with fatty acids, threo-9,10-dihydroxy stearic acid and 16-hydroxyhexadecanoic acid, prior to infection in order to study the effect on the infectious process. Several problems have to be solved before the system can function as a model for study of the infectious process and the mechanisms involved.
A powerful LC-MS machine was implemented in the project for accurate molecular weight and amino acid sequence determination of plant and fungus components. This combined with a powerful sequence database search engine makes identification and analyses of new components possible which will bring detailed insight into the chemistry involved in infectious processes.

Residual Concentrations in Fish
(V. Gundersen, S. Stürup, I. E. Bechmann, L. V. Kristensen, E. Larsen)
Risø has participated in a large project, Residual Concentrations in Fish since 1996. The project was co-ordinated by The Association of Danish Fish Processing Industries and Exporters and carried out in collaboration with BioMar A/S, Denmark, Danish Institute for Fisheries Technology and Aquaculture, Danish Veterinary and Food Administration, Danish Technological Institute and Steins Laboratory, Denmark. The target group was aqua-culture in general, the fishing industry and buyers of fish and fish products. Risø contributed with analyses of inorganic elements in fish and mussels. The project was finished at the end of 1999. The results obtained at Risø indicated unambiguously that multi-element analysis could distinguish between different population sites and different places of breeding.

Projects Sponsored by the Danish Energy Agency
(L. Frøsig, D. Prip, J. Fosskov, H. Egsgaard, E. Larsen)
In relation to the Danish policy to expand the use of biomass for electricity production, consultant assistance and chemical analytical work have been performed first of all to the two plants in Høgild and Harboøre. The project is sponsored by "The Danish Follow-up Programme for Small-scale Solid-biomass Combined Heat and Power Plant", Danish Energy Agency and carried out in collaboration with Ansaldo-Wølund, Denmark, Bioscan A/S, Denmark, University of Lund, Sweden and Department of Energy Engineering, DTU. In addition, laboratory investigations have been performed to identify the tar compounds being responsible for the formation of naphthalene and poly-aromatic compounds (PAH). Those products are thermally and chemically very stable. Emphasis was paid to precursors formed in updraft gasifiers like the plant in Harboøre. Thus, thermal conversions of compounds, derived from the lignin structure were investigated in the temperature range 600-850^{o}C. The results demonstrated that the primary pyrolysis products undergo a series of surprisingly selective reactions, leading to aromatic oxocompounds and hydrocarbons.

Annual Project Report 1999

Plant Products and Recycling of Biomass

This programme was established to develop plants with better quality by use of modern breeding technology. This included a production of transgenic plants, particularly cereals, in order to create an overproduction of enzymes or to alter specifically the gene expression to modify the nutritional quality (phosphorus and nitrogen) or to modulate the polymer content such as lignin.
The growing public interest in the fact that our lives depend upon the availability of the ever-diminishing reserves of petroleum has given momentum to the search for renewable resources to be used as chemical raw materials. The carbohydrates, potentially available from biomass waste products such as wood chips and agricultural residue or purposely grown crops, represent one conceivable alternative. Identification of natural products (metabolites) which are important for plant development and their potential as primers for the production of new high value products is a new challenge for this programme.

Conversion of Ligno-Cellulose to Bioethanol
(A.B. Thomsen, A.S. Schmidt, H.B. Klinke, A. Woidemann)
The use of bioethanol in fuel is a way to reduce the global CO_2 emission. Risø National Laboratory and DTU work in collaboration with a new concept to produce ethanol from renewable plant resources. Due to a low production of inhibitors, the wet oxidation process was most promising for the pre-treatment of biomass into fermentable sugars. Different types of biomass have been investigated as substrata for fermentation of ethanol. For wheat straw, it was found that alkaline wet oxidation was efficient for fractionating of the polysaccharides into fermentable sugars. For woods, the alkaline treatment should be avoided; hence wet oxidation without alkaline addition was the most efficient. The reaction time needed for efficient fractionation was typically 10 minutes with a reaction temperature of 185ºC. Reaction products found in unconcentrated hydrolysate of wet-oxidized wheat straw had no inhibitory effect upon *Saccharomyces cerevisiae* as the same ethanol production rate was found as in mineral medium. After concentrating the hydrolysate 6 times, the fermentation time doubled (Olsson *et al*. 1999).
A new reactor is under development for demonstrating the wet oxidation process at a larger scale. The continuous reactor with a well-defined reaction time has a process capacity of 100 l/hour. Design, drawings and construction have been carried out in collaboration with Bjørnkjær Maskinfabrik A/S, Brabrand, Denmark. The reactor will be tested and optimized in the year 2000.

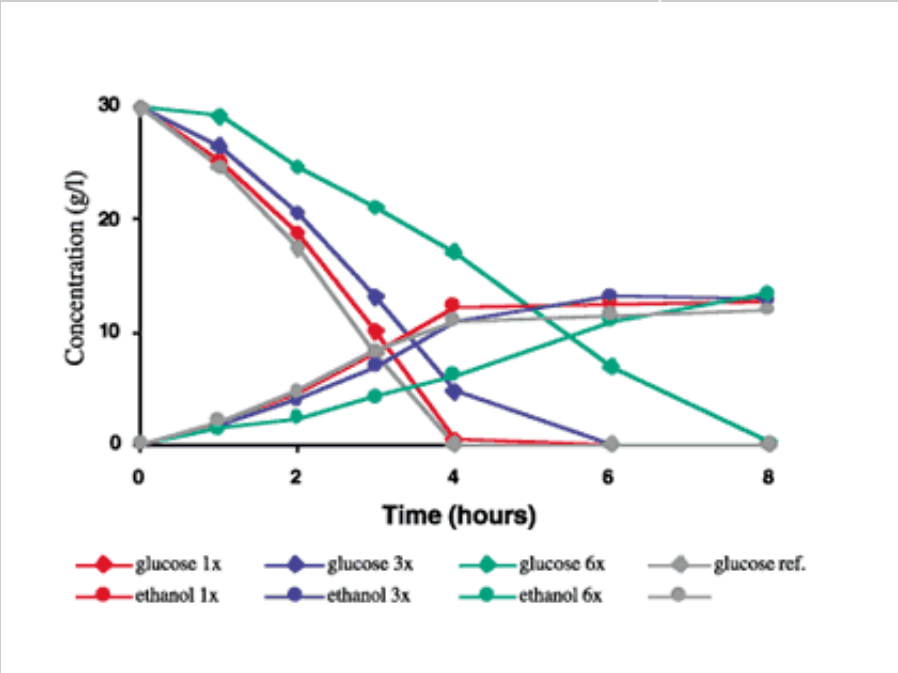

Figure 1: Glucose consumption and ethanol production in concentrated wet-oxidized wheat straw (1x, 3x, 6x) with added glucose. Mineral medium was used as reference. Wet oxidation conditions: 60 g/l wheat straw, 195°C, 10 min., 6.5 g/l Na_2CO_3 and 12 bar O_2 (Olsson *et al.* 1999).

Reference

Olsson, L., Thomsen, A.B., Ahring, B.K., Klinke H.B. (1999) Influence of inhibitors from wet-oxidized wheat straw on Saccharomyces cerevisiae. IEA Workshop - Biotechnology for the conversion of Lignocellulose. Itala Game Reserve, South Africa, 22-26 August.

Plant Fibre Composites

(A.B. Thomsen, A.S. Schmidt, A. Woidemann, S. Mallon)

Natural cellulosic plant fibres are possibly among the strongest fibres known. Besides they have a low density compared with synthetic fibres such as glass and kevlar fibres. Recent research carried out at Risø National Laboratory and RVAU has demonstrated that flax, jute, hemp and wheat straw may be realistic alternatives to synthetic fibres. All plant fibres tested improved the strength of composites based on 50% plant fibres and 50% polypropylene. However, none of these showed that the plant fibres were stronger than *e.g.* glass fibres (Table) (Thomsen *et al.* 1999). This was explained by the low compatibility between polypropylene and the plant fibres. The research is now focused on chemical and biological methods, which improve the compatibility between a plastic polymer and a plant fibre. In order to characterize the plant fibre cell wall, analytical methods are developed to determine the cellulose chain length and reactivity of cellulosic fibres. It appears that flax and hemp with the highest cellulose chain length are potentially those with the highest strength. Flax fibre based composites are presently used as inner door panels in BMW- and Mercedes automobiles.

Fibre	Density g/cm^3	Stiffness GPa	Strength Mpa
Wheat straw	1.51	41	280
Flax	1.54	44	290
Jute	1.52	53	360
Glass	2.62	70	~ 3000

Mechanical properties of plant and glass fibres, estimated from the experimental data of

the composites and the composite theory. (Note: Composites are based on ~300 g/m^2 mats) (Thomsen *et al.* 1999).

Reference
***Thomsen, A.B., Schmidt, A.S., Toftegaard, H., Pedersen, W.B., Woidemann, A., Lilholt, H.* (1999) Natural plant fibre composites based on wet-oxidised wheat straw and polypropylene. Proceedings of 4th European Symposium on Industrial Crops and Products, Bonn, Germany, 23-25 March 1999, p. 762-771.**

Polylactate from Wheat Straw
(A.S. Schmidt, A.B. Thomsen)
The biodegradable plastic polylactic acid (PLA) can be produced from lactic acid by polymerization. L-lactic acid can be produced by fermentation of sugar components. The world-wide production of L-lactic acid through fermentation was 60.000 tons in 1998. Wheat straw contains about 80% sugar components as cellulose and hemicellulose. But in order to utilize these sugars for fermentation, wet oxidation fractionation is needed. The liquid hemicellulose-rich fraction after enzymatic hydrolysis was used as substratum for the production of lactic acid by the lactic acid bacteria *Lactobacillus brevis* and *Lactobacillus pentosus*. Together these bacteria converted all available carbohydrates to lactic acid with a yield close to the theoretical limit (Garde *et al*. 2000). The solid cellulose-rich fraction is utilized in other projects. The wet oxidation process was optimized to obtain a high yield and recovery of hemicellulose using a laboratory scale loop-reactor. The best conditions were 185° C for 15 minutes adding either 6 or 12 bar oxygen and no sodium carbonate or 6.5 g/l sodium carbonate. The optimization was carried out to minimize production cost and still obtain a good lactic acid yield. The project was carried out in collaboration with DTU.

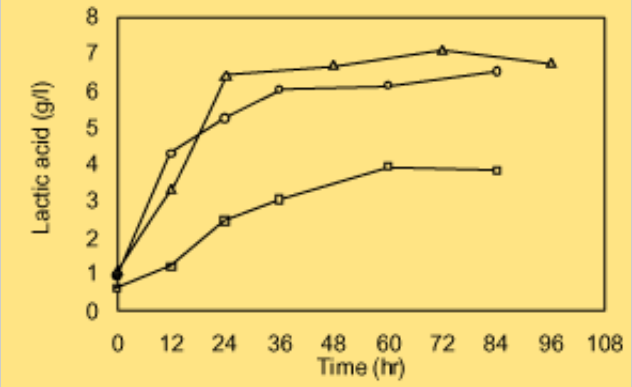

Figure 2: Lactate production from wet-oxidized wheat straw hemicellulose by pure *Lactobacillus pentosus* culture (O), pure *Lactobacillus brevis* culture (□) and mixed culture of *L. pentosus* and *L. brevis* (△) (Garde *et al.* 2000).

Reference
***Garde, A., Jonsson, G., Schmidt, A.S., Ahring, B.K.* (2000) Lactic acid production from wheat straw hemicellulose hydrolysate by *Lactobacillus pentosus* and *Lactobacillus brevis*. Appl. Environ. Microbiol. (in press).**

Peroxidases and Plant Stress Metabolites
(B.K. Rasmussen, K. Burhenne, S.K. Rasmussen)
Plants may contain up to one hundred genes, coding for peroxidases with 35-100% protein sequence identity, all having similar 3D space filling structures. Many of these are expressed throughout the plant life cycle and thus have housekeeping functions in the cell. However, expression of some peroxidase genes is highly regulated during time, spatially to fulfil specialized task or conditionally to counteract biotic (pathogen) or abiotic (ozone) stress. Two barley peroxidases, Prx7 and Prx8, are induced by the pathogenic powdery mildew fungus, and are thus distinct from the more than ten household peroxidases present in barley leaves. They have been extensively characterized with respect to their biochemistry, expression and localization during

infection. Transgenic barley plants and cells were used to monitor the effect on whole plant development and in single leaf cells during the early events of infection. Gene constructs containing Prx8 under control by the constitutive rice actin promotor were delivered to cells in green barley leaves by particle bombardment. Following inoculation with fungal spores a 50% increase in failed penetration attempts could be monitored, which shows that Prx8 contributes to the non-race-specific, partial resistance in barley. Regeneration of fertile transgenic plants constitutively expressing Prx8 failed, indicating that Prx8 interferes with the metabolism needed to develop healthy plants. Transient expression assays, in collaboration with the Carlsberg Research Laboratory, Denmark and the University of Zurich, Switzerland, revealed that expression of Prx8 has a strongly improving effect on the defensive capabilities of cereal cells against pathogenic attacks, but that this effect apparently depends on the genotype of the transgene recipient. Surprisingly, transient overexpression of Prx7 in leaf epidermal cells leads to an increased colonization rate by the powdery mildew fungus. Furthermore, we have shown that this effect is dependent on the correct subcellular targeting of the peroxidase. Our results emphasize the fact that the distinct peroxidases do have distinct functions in plant stress metabolism and very likely in general plant metabolism as well.

Laccases and Peroxidases Involved in Lignin Biosynthesis
(B. Gavnholt, K.B. Holm, S.K. Rasmussen)
Lignin is a very abundant plant polymer, only cellulose is more abundant in the world. Lignin is undigestable to most animals and therefore it is highly interesting to engineer a transgenic plant with reduced lignin content and hence improve the nutritional value. The present project is part of the programme "Fremtidens kulturplanter" (Crops for the Future) working towards designing a transgenic rye grass, *Lolium perenne* L., with reduced alternate lignin content. Laccase and peroxidase are believed to participate in the last step of lignin polymerization. The oxidative enzyme laccase has been cloned from rye grass and southern blot analysis shows that the genome encodes a wealth of different laccase genes (>23). Northern blots show, however, that they can be tissue specifically expressed. In this study, *Asparagus* is used as model plant for the lignin metabolism. Several peroxidase genes have been isolated, and this is expressed during lignification and may hence provide clues to ways of modifying the lignin content or water solubilization.

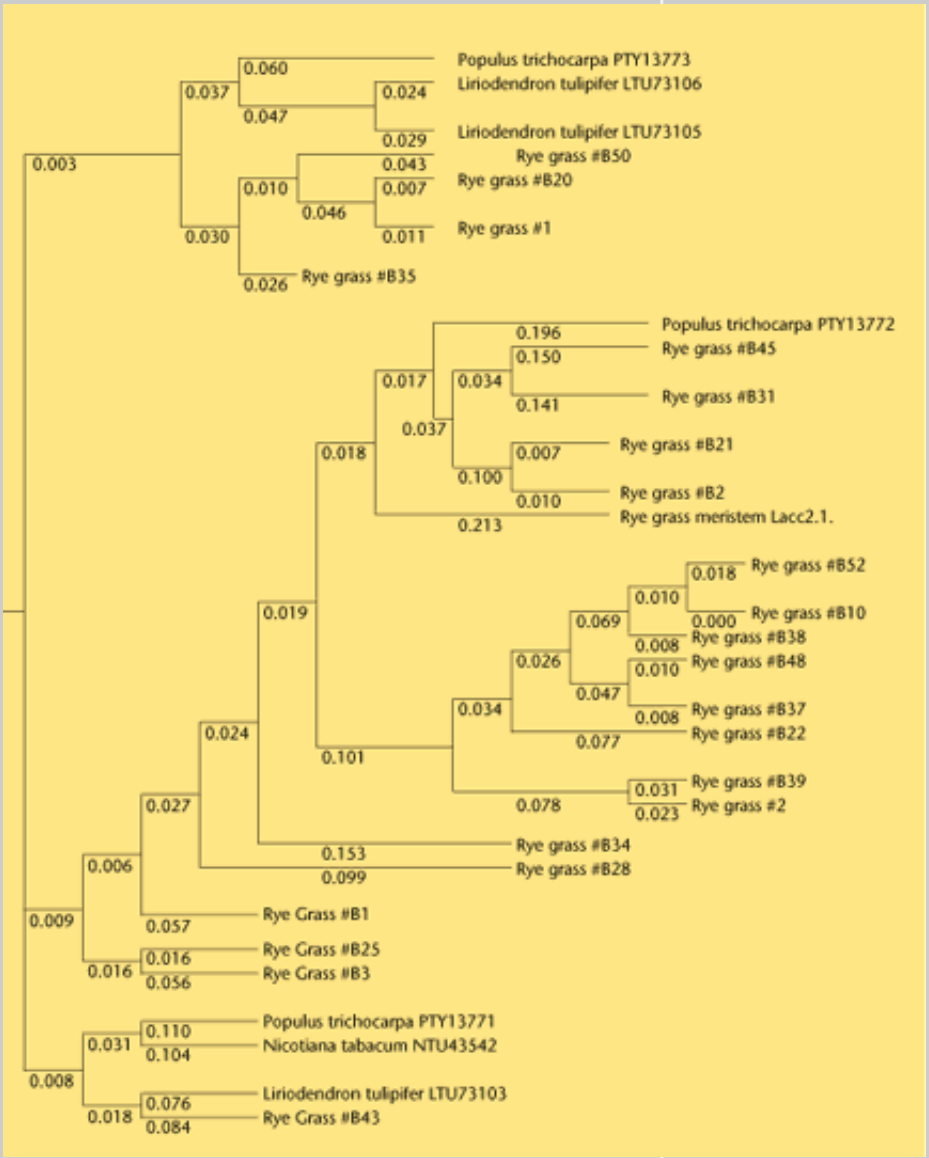

Figure 3: Phylogenetic tree based on sequence data from a laccase DNA-fragment in rye grass. Sequence data from other species are included in the alignment.

Low Phytate Barley Mutants: Genetics, Biochemistry and Nutrition
(F. Hatzack, K.S. Johansen, S.K. Rasmussen)
The objective of this mutational breeding programme is to improve the nutritional availability of phosphorus (P) from barley grains. The first mutants containing high amounts of soluble grain P and low amounts of indigestible phytate ($InsP_6$) were identified in 1996. Since then, our understanding of the genetics and biochemistry of these mutants has made significant progress. Experiments have been carried out to locate and estimate the number of structural genes in the biosynthetic pathway from *myo*-inositol to phytate. These experiments were performed in Alexis low-phytate mutant lines as well as in back crosses with the Alexis cultivar. With regards to biochemistry, grain material from Pallas-3A mutants was used for quantitative isolation of Ins(1,3,4,5)P_4 - a compound assumed to play a crucial role in plant phytate synthesis. Structural analysis of this compound by NMR was conducted in collaboration with Roskilde University Centre, Denmark. Apart from this work, steady improvement of thin-layer chromatography protocols as an attractive alternative to paper electrophoresis led to publication of a new high-performance thin-layer chromatography-method for inositol phosphate analysis.

Reference
***Hatzack, F., Rasmussen, S.K.* (1999) High-performance thin-layer chromatography**

analysis method for inositol phosphate analysis, J. Chromatog. 736: 221-229.

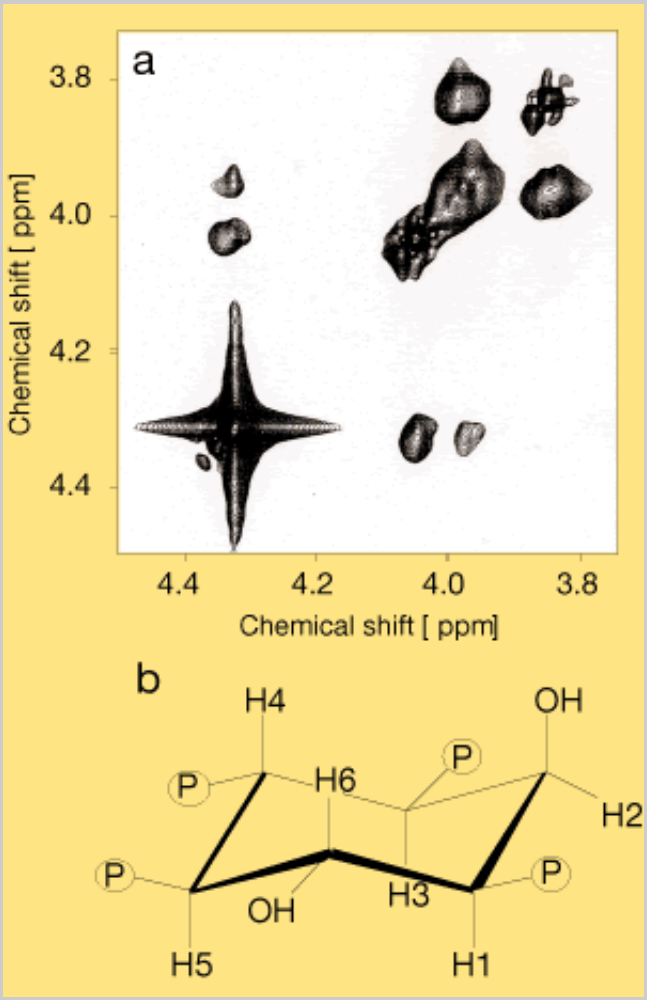

Figure 4: 2D-TOCSY NMR spectrum (a) and chemical structure (b) of Ins(1,3,4,5)P_4 - a putative intermediate of phytate synthesis isolated from barley mutant grains.

Purification of a Wheat Bran Phytase
(K.S. Johansen, S.K. Rasmussen)
During the last few years we have worked towards the purification and subsequent cloning of a wheat bran phytase. The aim is to explore this enzyme in our plant transformation programme in order to obtain high value crops. This strategy is followed in analogy with the mutational breeding programme of low phytate barley mutants. As only one plant phytase has been cloned previously, it is also of fundamental scientific interest to learn more about the nature of the wheat bran phytase. We have successfully purified the enzyme activity and obtained N-terminal as well as internal amino acid sequence information. This has enabled us to begin the cloning of the cDNA, which is the coding for the phytase. A synthetic peptide corresponding to the N-terminal amino acid sequence has been used to raise rabbit antibodies and the diluted serum has been shown to recognize a protein of the expected size in western blot analysis. The antibodies have provided us with a good tool for future analyses of phytase expression and localization. This work (Johansen *et al*. 2000) has lead to the identification and cloning of several wheat bran proteins, including a new monomeric glyoxalase (1 unit/mg protein) with duplicated domains. The presence in dry wheat bran led us to suggest a new role for glyoxalase I to protect cells during severe water deficiency or during re-hydration when the tissue becomes metabolically active.

Reference
***Johansen, K.S., Svendsen, I., Rasmussen S.K.* (2000) Purification and cloning of glyoxalase I from wheat bran. Plant Science (in press).**

Animal Feeding Trails with Low-Phytate Barley
(K.S. Johansen, F. Hatzack, S.K. Rasmussen)
The nutritional effects of two barley mutant lines with an altered phosphorus (P) profile and phytate content (13 and 43% of total P, respectively) have been studied in collaboration with the Foulum Research Centre, the Danish Institute of Agricultural Sciences. Because grain quantities were limited, rats served as a model for the pig. Four groups of five Wistar rats (weighing 65 g) were fed the low-phytate lines, the mother variety and a mixture of barley varieties. All diets were supplemented with vitamins and minerals except P, calcium, zinc and copper. The apparent digestibility of P was improved by up to 13% in the mutant lines. Although all diets were very similar in zinc content, only rats fed the mutant lines had a net absorption and a positive zinc balance indicating improved zinc availability. Rats appear to be a suitable model for P utilization in pigs when testing new breeding lines and thus meet the demands by plant breeders for mineral bio-availability tests which may be used at an early stage.

Phosphorus Nutrition Evaluated in Pot Cultures
(K.C. Engvild, F. Hatzack, K.S. Johansen, S.K. Rasmussen)
Low-phytate mutants of barley, three of which derived from Pallas cultivar (1st year) and three from Alexis cultivar (2nd year) have been investigated for response to phosphorus nutrition in 20 litre pots with either pure quartz sand (1st year) or rockwool (2nd year) as substrata. Pallas and Alexis served as controls. The plants were grown to maturity out of doors with an automated siphon air lift watering system with a 10 litre reservoir. Two types of phytate mutants were used: A-type with almost no phytate in the seed, and B-type with about 50% phytate and 50% inorganic phosphorus in the seed. The pots received 3 g N, 2 g K and 0.1 or 0.3 g P (1st year) or 0.2, 0.4 and 0.8 g P (2nd year). Fertilizer additions were split and given in the reservoirs. The yield of the phytate mutants was reduced at all phosphate levels compared to wild type. A-type mutant yields were lower than B-type yields (Engvild *et al*. 1999). The mutants were more sensitive to late addition of P than the wild type.

Figure 5: Pot cultures of low-phytate barley grown under limited P-fertilizer in an antivoliere.

Reference
Engvild, K.C., Hatzack, F., Johansen, K.S., Rasmussen, S.K. **(1999) Response of low-phytate barley mutants to phosphorus nutrition in pot cultures. Risø-R-1150: ISBN 87-550-2634-6.**

PR Transgenic Plants for Quality Improvements
In a stand at Roskilde fair with the theme of future foods, DLF-Trifolium had organized a joint contribution of posters from several research institutes working on transgenic plants. We participated with two posters, describing our visions of how genetically modified plants may benefit the environment. We outlined the prospect of strengthening the plants' defence against pathogens by the genetic modification of the peroxidase content, which could reduce or abolish the need for application of fungicides. Also the possibility of reducing the environmental impact of pig and poultry production through improving the digestibility of grain phosphorus was taken as an example. As delineated

above, we are working with several strategies to reach this goal, one of them being the genetic transformation of crop plants with fungal or plant phytases.

Plant Serpins: Serine Proteinase Inhibitors
(H.Ø. Jensen, T. Roberts, S.K. Rasmussen)
Serpins are a superfamily of proteins with a range of physiological functions and they have been identified in humans, animals, insects, plants and viruses, but not in prokaryotes. The majority of serpins are inhibitors of serine proteinases, but some have evolved other functions such as hormone transport and control of blood pressure and even inhibitors of cysteine proteinases. Over the last 15 years we have cloned and characterized a number of serpins from barley and wheat and in collaboration with the Technical University of Denmark demonstrated that they are active inhibitors *in vitro*. Their expression throughout plant growth has pointed to their presence in other tissues than grains, but still remains to be found in plantar function.

Molecular Markers in Medicinal Plants
(S.K. Rasmussen)
The *Apiaceae (Umbelliferae)* is one of the best known plant families with peculiar botanical characters such as the typical umbellate inflorescences and the specialized dry fruits splitting into two one-seeded mericarps. They are widely distributed in temperate climate regions where they often are used as spices or drugs due to the presence of useful secondary metabolites such as coumarins, essential oils and sesquiterpenes. Natural medicines used in Korea and other Asiatic countries include extracts from a large number of plant species from this family. Dried roots of *Angelica*, for example, are widely used as drugs in Korea, Japan and China. During 1993, in South Korea 6,631 mt of *Angelica* were cultivated on 2,688 ha, 526 mt of *Bupleurum* on 409 ha and 735 mt of *Peucedanum* on 149 ha. Due to a world-wide interest in oriental medicine, production of those plants has grown even more over the recent years. Since many species and varieties exist, development of molecular markers would be important for quality assessment in the medicinal industry. RAPD analysis of four *Angelica* species, *Bupleurum falcatum* and *Peucedanum japonicum* showed that the 10-basae primer OPC-17 gave polymorphic banding patterns. Cloning of the two ribosomal intergenic transcribed spacers, ITS1 and ITS2, allowed us to include this in the phylogenetic tree of *Apiaceae* and to identify the satellited chromosomes of *Thapsia garganica*.

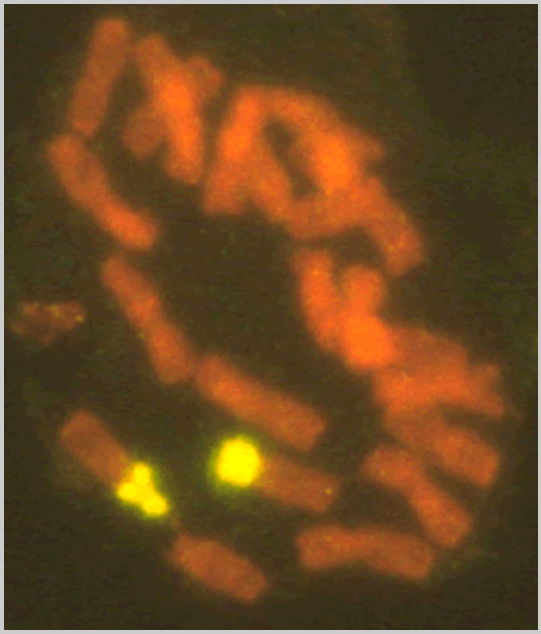

Figure 6: Flourescent *in situ* hybridization to root tip metaphase chromosomes of *Thapsia garganica.*

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DLF-Risø Biotechnology

DLF-Risø Biotechnologyhe establishment of a biotechnology consortium between DLF-Trifolium A/S and the Risø National Laboratory in 1998 is a new initiative to optimize the interplay between plant research activities at Risø and the research needs of Danish plant breeders. The main objective of this collaboration is to acquire the basic knowledge necessary for developing a new type of genetically engineered grasses (*Lolium perenne*) unable to produce stems and flowers during grassland farming (biological encapsulation) and with highly improved quality for agronomic use. In order to satisfy society's demand for safety concerning use of genetically modified organisms, the development of a conditional non-reproductive grass, which avoids dispersal of active transgenes into nature, is currently one of the main tasks of the programme.
In order to control the flowering of ryegrass the key regulator genes important in transition from vegetative to reproductive growth have to be identified. By implementation of advanced screening techniques and direct cloning approaches we were able to isolate more than 100 genes potentially involved in flowering in ryegrass and selected candidate genes are currently investigated in transformed ryegrass lines.
The following strategies were applied to achieve the development of stem and flowerless ryegrass varieties.

Isolation of Key Genes Involved in Stem and Flower Formation
(C.H. Andersen, H. Richter, C.S. Jensen, K. Petersen, S. Broeng, I. Dræby)
In order to identify ryegrass genes which are up or down regulated as a consequence of the induction to flowering, different plant tissues and organs were harvested at various time-points during vernalization (an essential cold treatment for 3 months in which *Lolium perenne* acquires the competence to flower) and during secondary induction (increased temperature and long day conditions). The mRNA (expressed genes) from tissues, collected at the different points was isolated and served as the starting material for the different methods used to isolate differentially expressed genes.

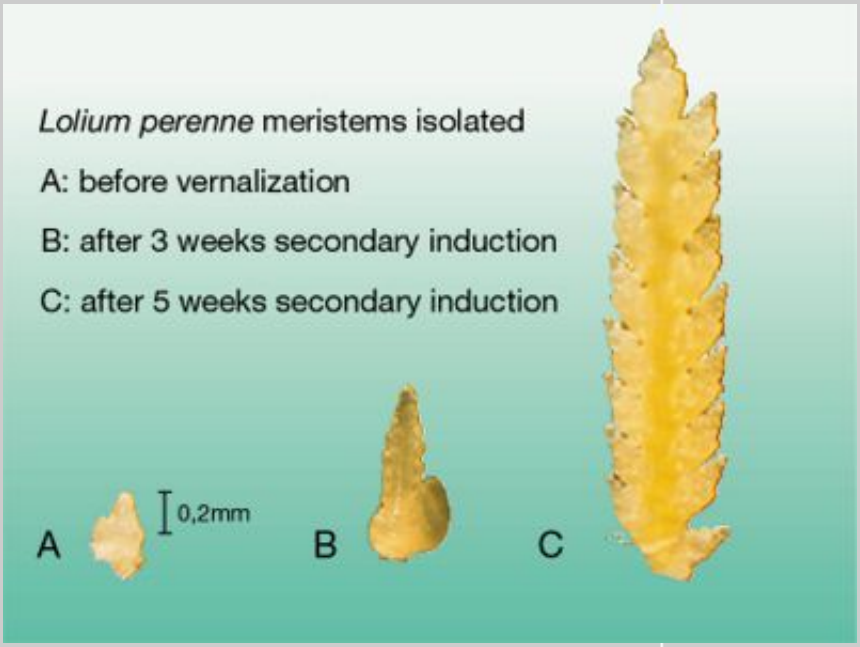

Figure 1: *Lolium perenne* meristems isolated. A: before vernalization. B: after 3 weeks secondary induction. C: after 5 weeks secondary induction.

Using PCR-based subtractive hybridization techniques, **D**ifferential **D**isplay **R**everse **T**ranscriptase **P**olymerase **C**hain **R**eaction (DDRT-PCR) and **F**amily **S**pecific **D**omain Display (FSD Display) we were able to identify more than 20 differentially expressed APETALA1- and AGAMOUS-like MADS-Box transcription factor genes which are known to play crucial roles in flower development in other plant species.
Using the genetic information gained on the characterization of flowering genes from other plant systems (*Arabidopsis*, Rice etc.) we succeeded in cloning several orthologues to flowering genes from other plant species like the orthologues to LEAFY, TFL and GA-MYB from *Arabidopsis*.
In a further project the DLF/Risø consortium is an active participant in an EU funded research programme under the 5th Frame Work: QLG2-CT-1999-00351. The aim of this research programme is to initiate a large-scale analysis determining the expression patterns of approximately 5,000 genes from *Arabidopsis thaliana*, and our group will specifically examine flowering related genes. The knowledge generated in this project is then the basis for the isolation of the corresponding genes from ryegrass.

Figure 2: Research scientist T. Didion and Ph.D. student K. Petersen analysing the

transcription pattern of regulator genes involved in flower induction with the help of the Family Specific Domain Display technique.

Characterization of *Lolium* Flowering Genes
(C.H. Andersen, K. Salchert, D. Aubert, H. Richter, C.S. Jensen, K. Petersen)
Once the differentially expressed genes are identified they are investigated using northern analysis, southern analysis and *in situ* Hybridization or *in situ* PCR. For low expressed genes we use RNase Protection Assays or semi-quantitative RT-PCR to verify the differential expression pattern. As a method to get an understanding in the complex protein-protein interactions among the flowering specific transcription factors we implemented the Yeast Two-Hybrid analytic tool. We were able to show protein-protein interactions of several of the MADS-Box transcription factors from *Lolium* in direct interaction studies and we are currently screening a special Two-Hybrid fusion library to identify protein-protein interactions with the orthologues to LEAFY and TLF.

Figure 3: Research scientist K. Salchert and Lab. technician R. Bonde working on *in-situ* PCR.

We also succeeded in the isolation of several meristem specific genes that are only expressed upon secondary induction and at the moment we analyse the corresponding genomic sequences for regulatory elements, which we will use in an ablation-based strategy. The aim is to block stem and flower formation via ablation of the flowering programmed meristems by expressing a gene deleterious to the meristem by means of the identified meristem specific, flowering induced promoter sequences.
Furthermore, these regulatory promoter sequences will be analysed using the Yeast One-Hybrid tool to identify new regulator proteins, which upon binding cause the flowering specific regulation of this promoter region.

Development of Novel Conditional Gene Expression Systems
(T. Didion, M. Storgaard)
For certain applications in basic as well as in applied plant sciences the expression of a gene of interest is desired only in a specific tissue, at a specific time point in the plant development or even only in a specific generation or progeny of the plant. We therefore work on the development of conditional expression systems in plants in general and especially in ryegrass for this project. One strategy is the isolation of a tissue specific promoter region to restrict expression to certain tissues. Another strategy is the use of a chemically inducible promoter, which allows expression of the gene of interest only upon treatment with a specific chemical compound. We are currently adapting an ethanol inducible expression system, which proved efficient in *Arabidopsis* and Tabacco, for the use in ryegrass. Using this system it is possible to induce the expression of the gene of interest upon ethanol application. Ethanol used in these low concentrations turned out to be environmentally benign, which favours this system

compared to systems using hormones or environmentally harmful chemicals.
In an additional strategy we investigate the possible use of chimeric transcription factors and promoters. Expressing each part in different plant lines the system only becomes active upon crossing the parent lines with each other, thereby generating a hybrid plant with a complete and active expression system. The Hybrid-based expression system is currently under investigation in transgenic *Arabidopsis* and *Lolium* plants.

Ryegrass Transformation Systems, *Brachipodium* as a Model Plant
(M. Folling, P. Christensen)
Transformation of *Lolium perenne* with sense and anti-sense constructs of selected flowering genes is performed via particle bombardment or PEG mediated transformation into embryogenic suspension cultures initiated from meristem cells or immature embryos. The role of the isolated candidate genes and their potential in flower induction/development will be examined in plants over-expressing (sense) or repressing (anti-sense, co-suppression) the specific gene function. Regenerated transgenic plants will be investigated during vernalization and after secondary induction with a specific attention to phenotypic changes in organ structure or flowering behaviour in order to unravel the function of the examined genes in the transition from vegetative to reproductive growth.
However, due to the time consuming transformation and regeneration procedure of transgenic ryegrass plants we initiated the development of the grass *Brachypodium distachyon* as a model system for monocot plants. With a life cycle from seed to seed of 15 weeks, its small genome as well as its self-fertility, this grass species would make the analysis of the isolated flowering gene much easier and faster.

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Plant Genetics and Epidemiology

The aim of our research is to analyse the agricultural ecosystem and its interactions with the environment. By means of DNA markers we analyse the genetic basis for important agronomic traits and how genetic diversity changes in time and space when crops are grown under different input systems. Also spread of genes (*e.g.* transgenes) between crops and wild relatives are studied. Much emphasis is put on genetic and physiological mechanisms of host resistance and on the evolution of virulence. Biometric analyses are used to relate experimental results to genetic information, and mathematical models support the formulation of hypothesis and predictions. Our research is organised in four main projects.

Disease Resistance against Fungal Pathogens
(H. Østergård, M. F. Lyngkjær, L. Eriksen)
Durability of race-specific resistance against cereal powdery mildew caused by the fungus *Blumeria graminis* f. sp. *hordei* in the field is often compromised due to adaptation of the pathogen population. Therefore we try to identify and characterize mechanisms involved in more durable forms of resistance. Using double inoculation techniques we have in collaboration with IGER, Wales, UK examined cellular-level consequences of sequential attacks in the cereal powdery mildew system (Lyngkjær and Carver 1999a,b; Carver *et al*. 1999; Lyngkjær and Carver, in press). In two sets of isogenic barley lines with and without the race-specific powdery mildew resistance gene *Mla1*, induction of cellular resistance mechanisms was studied. Here we found that successful infection (haustorium formation) by a virulent powdery mildew isolate of barley with the *Mla1* resistance gene suppressed the ability of attacked host cells to initiate the resistance mechanisms (Figure 1). This mechanism would normally lead to a hypersensitive response when infected by an avirulent powdery mildew isolate. Correspondingly, infection attempts by avirulent powdery mildew lead to almost complete cellular resistance to later attack from normally virulent powdery mildew. This work has been partly financed by the Veterinary and Agricultural Research Council and the Danish Directorate for Development.

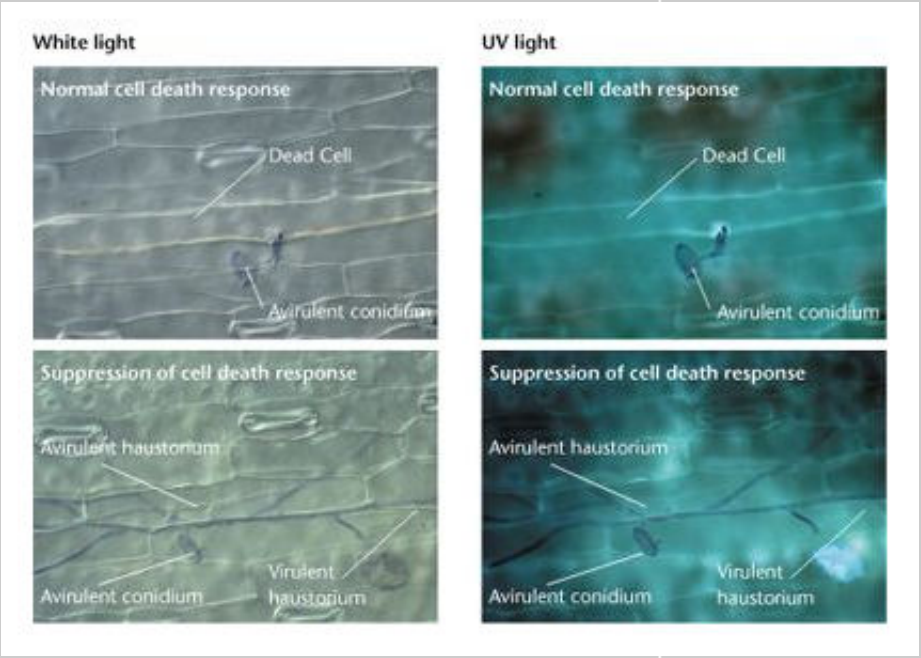

Figure 1: Differential interference contrast micrograph (white light) and incident fluorescence micrograph (UV light) of the same epidermal cells of barley line P-01carrying the *Mla1* resistance gene conferring race-specific cell death response against barley powdery mildew attack.
(Top). Normal cell death response to attack from an avirulent powdery mildew conidium (isolate CC1).
(Bottom). Suppression of the cell death response, due to successful infection (haustorium formation) by a virulent powdery mildew conidium in the adjacent cell. Leaf epidermal cells were first inoculated with virulent conidia (isolate A6), and then incubated for 48 h before removal of superficial fungal structures (conidia, germ tubes, hyphae). The leaf was then challenge inoculated with avirulent conidia (isolate CC1) and incubated for 48 h before fixation.

The durability of resistance to the fungus *Mycosphaerella graminicola,* causing septoria tritici blotch on wheat, has been analysed through disease data covering the last four years from the Danish Observation Plots, kindly supplied by G. Deneken at the Danish Institute of Agricultural Sciences (Hovmøller *et al.* 1999b). Based on this analysis it was not possible to detect any consistent changes in the composition of the pathogen population towards being more virulent on the investigated cultivars. This is encouraging for future resistance breeding. This work was financed by a grant from RVAU.
Also other conclusions have been drawn from analyses of the Danish Observation Plots. For barley leaf blotch caused by the fungus *Rhynchosporium secalis,* (Østergård and Pinnschmidt 1999) concluded that no trend was measurable over years but in some years on specific sites more disease attacks were found in some cultivars. For all the important fungal foliar diseases of barley and wheat the Danish Observation Plots were used for estimating the ranking of Danish grown varieties with respect to disease resistance (Munk *et al.* 1999, Hovmøller *et al.* 1999a). This work was partly financed by the Danish Directorate for Development.

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Population Biology of Fungal Plant Pathogens
(H. Østergård, A. Stockmarr, C. Lett, L. Eriksen)
The occurrence of the sexual stage of the fungus *Mycosphaerella graminicola*, causing septoria tritici blotch on wheat, was monitored over the growing season in a wheat plot untreated with fungicides. The results suggested that in 1999 the sexual stage did not contribute significantly to the epidemic development during the season. The reason was the late appearance of the sexual spores compared to the asexual spores combined with the long latency period of this disease. A simulation model developed in collaboration with M.W. Shaw, The University of Reading, UK supported this conclusion (Eriksen *et al.* 1999a). From our investigations, no reason was found to include air dispersal of sexual spores in the currently used forecasting schemes. Other studies have argued for increasing the emphasis on the airborne sexual spores compared to the asexual spores dispersed by rain splash. The final conclusion will be of importance as the forecasting of fungicide treatments aimed against *M. graminicola* in Denmark at present is made only on the basis of precipitation. The model study did reveal that a considerable proportion of the pathogen population at the end of season would be sexual descendants of the initial population. This might have implications for the durability of disease resistance (Eriksen *et al.* 1999a,b). This work was financed by a grant from RVAU.

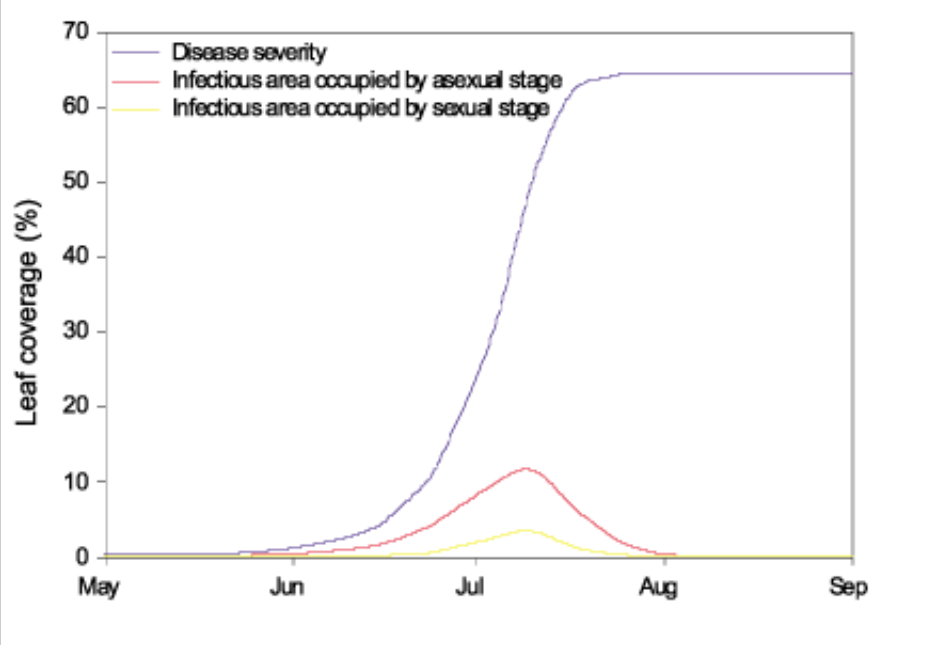

Figure 2: Result of simulation, showing the proportion of diseased leaf area, including post infectious diseased and infectious leaf area occupied by the asexual and sexual stages. The simulation demonstrates the late appearance of the sexual compared to the asexual stage of the fungus. The model simulation is performed in °C days but is here transformed to a time scale according to weather data from Risø 1998/99. Parameter values were: Latent period 300°C

days (20-30 days in summer); Infectious period 75°C days (5-8 days in summer); Multiplication efficiency asexual stage 3 (infected area per infectious area per infectious period); Multiplication efficiency sexual stage 1,2.

A probabilistic model for the dispersion and infection processes for pathogenic fungal spores in a host-pathogen environment has been developed with *Sclerotia sclerotiorum* as a model fungus. The model is designed to avoid the technical problems that may arise during simulations due to the huge amount of spores dispersed from the fruit body of the fungus. This is established by introducing the concept of *effective spores*, *i.e.* spores that survive the dispersion process and are able to infect susceptible plant material. The model includes in addition parameters for infection efficiency, spore production rate and plant fitness whether diseased or healthy. Based on a derivation of a class of distributions of effective spores, a model for the corresponding distribution of infections is formulated in terms of the dispersion distribution for the spores, and criteria for asymptotic independence of the number of infections in disjoint areas are given. A number of simulation studies have been carried out with a negative binomial distributed number of effective spores, where oilseed rape (*Brassica napus*) and its wild relative *B. rapa* are considered as hosts plants (Stockmarr *et al*. in press). This model will be part of a strategic project aiming at investigating the effects on the environment of transgene disease resistant crops (Hauser *et al*. in press). The work is partly financed by the Danish Environmental Research Programme and the Ministry of Environment and Energy.
Another model for the spread of spores from the foliar pathogen of wheat *Puccinia striiformis* f.sp. *tritici*, in a heterogeneous crop is under development. This is financed by the Danish Directorate for Development.

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Molecular Breeding in Cereals

(A. Jahoor, J. Jensen, G. Backes, R.U. Johansen, A. Sabbagh, H. Sayed, A. Schiemann)
In a joint project between Risø National Laboratory and ICARDA (International Center for Agricultural Research in the Dry Areas) agronomic traits important for the dry land agriculture were mapped. A recombinant inbred population of 245 lines (F_6) of the cross between Tadmor and Sel160 was mapped with RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA) and microsatellite markers. An 882 cM linkage map was constructed consisting of 15 individual linkage groups distributed on the seven chromosomes of barley. With this linkage map, QTL (Quantitative Trait Locus) analysis was performed for a number of agronomic and physiological traits as well as disease resistances and straw parameter traits. Due to the variable environment at the two testing sites, ICARDA's research stations in the Northwest of Syria, a number of traits were evaluated only at one location in one year. A major QTL was identified and mapped for barley leaf blotch caused by the fungus *Rynchosporium secalis* on chromosome 4H, and for barley powdery mildew (*Blumeria graminis* f.sp. *hordei*) on chromosome 1H, respectively. A number of QTLs associated with traits related to yield under stress conditions clustered on chromosome 5H. These traits included days to heading, cold damage, grain yield and early growth vigor. A QTL for plant height was located on chromosome 3H, probably in the vicinity of the location of the *denso* gene. This work was partly financed by The German Ministry of Research.

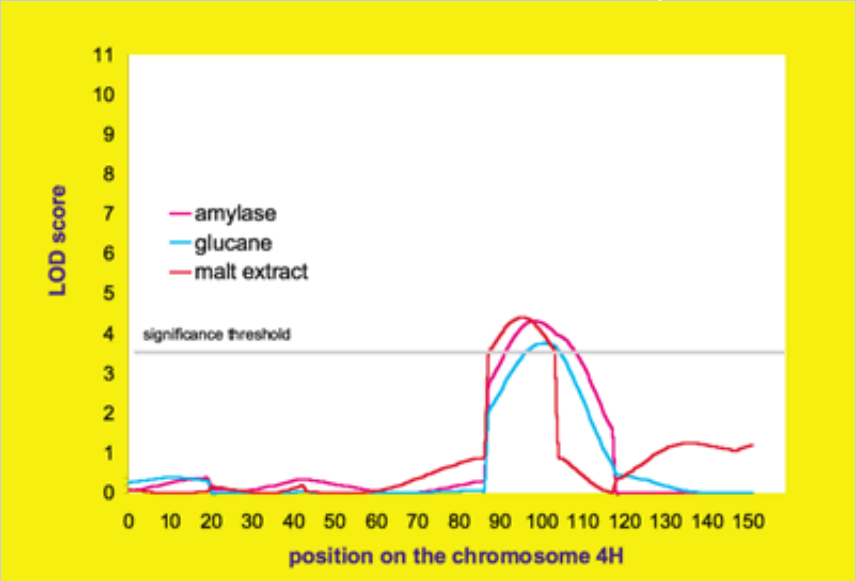

Figure 3: Detection of different traits related to malt quality via interval analysis. The LOD scores show that three important malting traits, amylase, glucane and malt extract, are located in the same region on barley chromosome 4H.

In collaboration between Risø and the three Danish cereal breeding companies, genetics of malting quality traits were studied in double haploid lines from a cross between two European varieties Alexis and Regatta. A genetic linkage map was constructed including 41 RFLP, 22 RAPD, 62 AFLP, 19 SSR (**S**imple **S**equence **R**epeat), 5 STS (**S**eguence **T**agged **S**ite) markers, *mlo* locus enhancing powdery mildew resistance gene, the *denso* locus and nematode resistance. This map was used for localization of QTLs for different malt quality traits. QTLs for different malt quality traits that are related to each other were mapped at the same location using data from the same environment. For the related malting quality traits, a high QTL-environment interaction was observed. Nevertheless, two QTLs for viscosity on 1H and 3H and one QTL for β -glucan content on 1H have been localized at a corresponding position in two environments. QTLs for malt extract and viscosity respectively are mapped near the position of the *denso* gene. Therefore, it is very likely that the *denso* gene also influences these traits. Higher correspondence between results from different environments and within the environments could be obtained by taking into account QTLs with LOD-score lower than the threshold calculated for a 0.05 significance level. The QTL-environment interaction for malting quality traits not only reflects environmental differences at different locations, but also differences in the micro-malting procedures at different breeding stations. Thus by means of molecular markers we could elucidate the complexity of micromalting tests and the need for reliable test environments. Under well-defined test conditions, molecular markers for the malting quality traits could be established. This work is partly financed by the Danish Directorate for Development.
Finally, a third cross population of barley has been studied in collaboration with The Federal Centre for Breeding Research, Aschersleben, Germany (Kickerer *et al*. in press). Here 4 QTL for leaf rust resistance were localized. The QTLs explained 96.1% of the genetic variance. One QTL on chromosome 4H confirmed a position found in another genetic background and one mapped to the same position as the leaf rust resistance gene *Rph16* on chromosome 2H. QTLs influencing heading date, plant length and kernel weight, were also found in the population.

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Risk Analysis of Genetically Modified Crops

(R.B. Jørgensen, M. Johannessen, L. Hansen, M. Pertl, S.I. Shim)
Spread of nuclear and cytoplasmic genes from crops to related wild or weedy plants are

studied in field trials and natural populations with the strategic purpose of evaluating extent and effects of transgene transfer and possible safe insertion sites for transgenes. The outcome of the research is of value to companies in their testing of genetically modified plants (GMPs) and to the competent authorities regulating the release and marketing of GMPs. Oilseed rape (Brassica napus), oat (*Avena sativa*), ryegrass (*Lolium perenne*) and carrot (*Daucus carota*) have been the crop plants used in the model studies (Jørgensen *et al*. 1999; Jørgensen, 1999a,b,c; Snow *et al*. 1999; Tomiuk *et al*. 2000). The research was funded by the Danish Environmental Research Programme and the Ministry of Environment and Energy.
Through cross compatible wild or weedy relatives, transgenes may be transferred from the genetically modified crop to the wider environment. We revealed comprehensive transfer of oilseed rape (*Brassica napus*, AACC=2n=38) genes to the relative *B. rapa* (AA=2n=20) in a large weedy population where oilseed rape and *B. rapa* coexisted for several years. Both nuclear DNA and plastid DNA of the cytoplasm were transferred. As our DNA-markers favoured detection of genes on the C-genome of oilseed rape, the results show that also transgenes on the C-genome will frequently be transferred (Hansen, 2000).
In analogy with this the frequency of transfer of cytoplasmic encoded transgenes is analysed in a field trial over two years. Different proportions and densities of oilseed rape, *B. rapa* and their F1 hybrid are cultivated, and the frequency of hybridization is being analysed with one or the other genotypes as the mother. The preliminary data show that transgenes integrated in the cytoplasm of oilseed rape will be transferred to F1 hybrids all though in a lower frequency than nuclear encoded genes. The effect from plant competition on hybridization and backcrossing has also been estimated in a field trial with oilseed rape, *B. rapa* and the F1 hybrids (Figure 4). The data show that low plant density and high frequency of oilseed rape and F1 hybrids favour hybridization and backcrossing with the wild species as female. Certain genotypes of *B. rapa* are apparently more prone to hybridization than others.

Figure 4: The effect from plant competition on hybridization and backcrossing between oilseed rape and *Brassica rapa* has been analysed in field experiments.

Cultivated oat (*Avena sativa*) and the weedy relative (*A. fatua*) have been grown in mixtures in the field with the purpose of estimating the extent of hybridization between the species (Figure 5). Species with specific AFLP (**A**mplified **F**ragment **L**ength **P**olymorphism) markers have been developed and are presently being converted into SCARs for easy and reliable detection of the interspecific hybrids.
The genetic variation in cultivated (*D. carota ssp. sativus*) and wild carrots (*D. carota ssp. carota*) from Denmark have been estimated using AFLP markers. Cluster analysis separated the carrot collections into wild type, old and new varieties. The presence of markers specific to the cultivated carrot makes it possible to detect introgression from cultivated to wild type (Shim and Jørgensen 2000).
Analysis of possible effects to the environment from cultivating transgenic grasses has been initiated together with the Department of System Analysis (B. Rasmussen) at Risø.

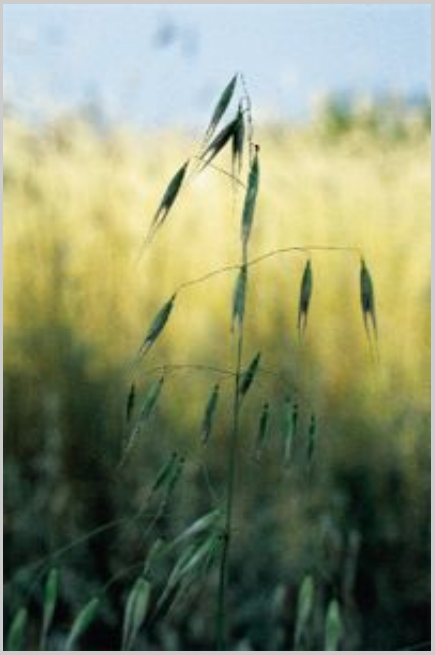

Figure 5: The hybridization between oat and the weedy relative *Avena fatua* (photo) has been analysed in the field.

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Tomiuk, J., Hauser, T.P., Jørgensen, R.B. (1999) A- or C-chromosomes does it matter for the transfer of transgenes from *Brassica napus*? Theor. Appl. Genet. (in press).

Seeds for Educational Purposes

(J. Jensen)

The program offers seed samples of barley and other plant species for teachers in biology. The samples illustrate the Mendelian gene-segregations and the effect of ionizing radiation on various plant species. In 1999, the experiments were distributed to 160 schools.

Special Facilities, Dyskjærgård and Field Experiments

(F. Hasselbalch, P. Gudiksen)

The climate during the winter of 1998/99 was mild and wet, expect for 2 weeks of severe frost in November. The late spring and wet summer resulted in a harvest below normal with respect to yield as well as quality. The winter crops were winter barley (13% of area), winter wheat (25% of area) and winter oilseed rape (8% of area), while the spring crops were spring barley (24% of area) and peas (4% of area). In the herd of Hereford cattle with 21 mother animals, 14 calves were born. In the autumn a new tractor with Fieldstar system and a new sowing machine were bought.

The distribution of crops and 20 field experiments (*e.g.* nitrogen application and utilization, weed and disease control, crop-weed competition, organic plant production, selection for disease resistance, biomass crops on set a side areas) were summarized graphically with a

short description of each experiment in two leaflets "Risø Markplan 1998/1999" and "Risø Markplan 1999".

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Webmaster
16 aug 2000

Annual Project Report 1999

Biogeochemistry

The research in the Biogeochemistry Programme is focused on the occurrence, transport, turnover as well as effects of trace elements and organic micro contaminants in agricultural and forest ecosystems. Trace elements and organic contaminants are followed from the soil or the atmosphere to the crop, and through the human food chain. The effects of air pollution and global change are studied, both at the plant and at the ecosystem level. Major emphasis is placed on the development of new methods and processes, which can form the basis of an environmental, sustainable and food safety plant production. Chemical analyses are also performed for public authorities and private companies on commercial basis.

Trace Elements

Trace Elements in Agricultural and Horticultural Products
(V. Gundersen, S. Stürup, I. Bechmann)
In different countries many studies are conducted on elemental concentrations of potato (*Solanum tuberosum*, Folva) tubers, but very little information is available on the effect of fertilizer and pesticide application on its mineral concentrations, and especially in relation to trace elements, *e.g.* noble and rare earth elements. The objective of the study, which was supported financially by the Danish Food Technology and Development Programme (FØTEK), was to evaluate the elemental concentrations in potatoes affected by application of three levels of N fertilization by pig slurry or calcium ammonium nitrate. The experimental field was located at the Risø National Laboratory Experimental Farm in Roskilde, Denmark. It was cultivated and fertilized for more than 50 years before the experiments were started in the spring of 1995. The field was divided into 6 plots. 3 plots were fertilized with pig slurry and 3 plots were fertilized with calcium ammonium nitrate using the three N-levels: 0 kg, 60 kg, and 120 kg N/ha for both types of fertilizer. From each plot 10 samples (5 tubers from each of 10 plants) were sampled for analysis. Pure pulp from the potato tubers was analysed with every necessary precaution against contamination by multi-elemental HR-ICP-MS analysis. The elements analysed were Ag, Al, Au, Ba, Bi, Ca, Cd, Co, Cr, Cs, Cu, Dy, Er, Fe, Ga, Gd, Ho, In, Ir, La, Lu, Mn, Mo, Nb, Nd, P, Pb, Pd, Pr, Pt, Rb, Re, Rh, Ru, Sb, Sc, Sm, Sn, Sr, Ta, Tb, Th, Ti, Tl, Tm, U, V, Y, Yb, and Zn.
The overall effects of three levels of N-fertilization on elemental concentrations of potato in each field site are evaluated by the use of Discriminant Partial Least Squares Regression (PLS): The data used in the regressions are range normalized. The preliminary results show that to a certain extent, it is possible to see a difference in trace element concentrations in agricultural and horticultural products, grown under ecological (organic farming) and conventional agricultural management practices, respectively. In 1999 the following products were analysed: lettuce, milk, and liver, kidney and meat from pigs.

Figure 1: Pretreatment of samples for trace element analyses.

Isotopic Composition and Isotope Ratios
(S. Stürup)
The potential of a single detector HR-ICP-MS technique for the measurement of isotope ratios in biological and environmental samples has been investigated focusing on the development, optimization and application of specific methods for the measurements of isotope ratios of elements in human nutrition and radionuclides. It was demonstrated that isotope ratios could be measured reliably, using HR-ICP-MS by educated choice of acquisition parameters, scanning mode, mass discrimination correction, and by eliminating the influence of detector dead time. HR-ICP-MS methods were developed for the measurements of isotope ratios of calcium, zinc, molybdenum and iron in human samples (Stürup 1999) and a method for the measurement of plutonium isotope ratios and ultra trace levels of plutonium and neptunium in environmental samples. The figures of merit of these methods demonstrated that isotope ratios could be measured with good precision and accuracy by HR-ICP-MS.

Figure 2: Analysis of isotopic rations applying HR-ICP-MS. Scientist S. Stürup.

Reference
Stürup, S. (1999) Application of HR-ICP-MS for the simultaneous measurement of zinc isotope ratios and total zinc content in human samples. J. Anal. Atom. Spec. (in press).

Sediment Dating and Geochemistry
(H. Kunzendorf)
The work within the project included the management of a national lead-210 dating centre as well as geochemical work on dated and other sediments. The Gamma Dating Centre (GDC) was created in 1995/96 through a funding by the Danish Natural Sciences Research Council. A joint dating centre instrumentation serving 5 Danish research institutes (Universities of Copenhagen and Aarhus, the National Environmental Research Institute, the Geological Survey of Denmark and Greenland, and Risø National Laboratory) was installed at the Risø

laboratory and is presently housed by the Risø Plant Biology and Biogeochemistry Department. GDC has a capacity for dating of about 40 sediment cores (10-15 sediment slices per dating) and has reserved 20 core datings per year to the participant institutes at reduced charges. The centre also serves Danish companies and customers from abroad, and since its operational start, the GDC has carried out analyses of more than 3000 sediment sections. Geochemical work on dated sediments is usually via participation in national and international environmental projects. A large EU Marine Science and Technology (MAST) project BASYS has currently been finished. Through participation within a national project in the arctic environment, geochemical data on dated sediment are used to reconstruct the fate of marine waste after deposition.

Organic Micro Contaminants

Plant-Uptake of Organic Contaminants from Soil Amended with Sewage Sludge
(G.K. Mortensen, F. Laturnus, C. Grøn)
In 1999, a greenhouse study was carried out within the Centre for Sustainable Land Use and Management of Contaminants, Carbon and Nitrogen (project 4: Plant Uptake and Metabolization of Organic Contaminants) under the Danish Strategic Environmental Programme.
Aerobic and anaerobic sewage sludge, compost and pig manure were added to a sandy soil obtained from a Danish experimental station at Lundgaard. Anaerobic sludge was added to the soil corresponding to 10 t dry weight (dw) ha^{-1}, and the additions of the other waste products were then calculated to give the same amounts of carbon (about 2000 kg carbon ha^{-1}). Rape (*Brassica napus*) was sown in pots filled with the mixture of soil and organic waste and harvested after 30 days. The removal and possible plant-uptake of organic contaminants like linear alkylic benzenesulfonates (LAS) and bis(diethylhexyl)phthalates (DEHP) were studied in this experiment. The concentrations of LAS and DEHP in the soil and the different organic waste products are shown in Figure 3.

Figure 3: Concentrations of LAS and DEHP in organic waste products.

organic waste product	LAS mg kg^{-1} dw	DEHP mg kg^{-1} dw
untreated soil	<0.2	<0.1
pig manure	<0.2	0.68
compost	8.50	23.5
aerobic sludge	54.9	89.5
anaerobic sludge	4670	122

The degradation of the water-soluble detergent LAS is very fast (Figure 4). After 30 days, only 8.8% (2.40 mg kg^{-1} dw in soil) was still found in the soil. When rape was grown in the soil, the degradation of LAS increased, but 5% (1,36 mg kg^{-1} dw in soil) still remained in the soil. Although the degradation of LAS was quite high, it was not efficient enough to reach the levels of LAS found in uncontaminated soil (< 0.2 mg kg^{-1} dw).
In contrast, the degradation of the more hydrophobic plasticizer DEHP is less efficient in soil amended with organic waste products (Figure 4). Apparently, DEHP is more recalcitrant in the soil with a degradation of only 19%. Similar to LAS, the growth of rape increased the degradation of DEHP in the treated soil, probably due to either enhanced microbial activity or by aeration of the soil induced by the root growth.

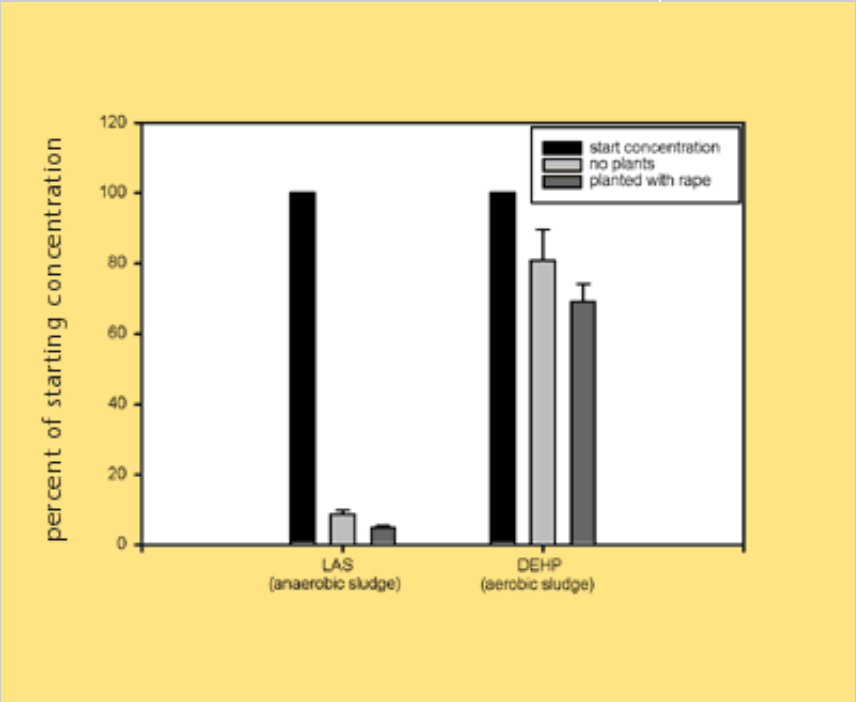

Figure 4: Removal of LAS and DEHP in soil amended with organic waste products and planted with rape.

Plant-Uptake of PAH
(G.K. Mortensen)
In collaboration with different Danish authorities, a project was established to investigate the occurrence of polycyclic aromatic hydrocarbons (PAH) in various fruits and vegetables to assess a possible introduction of these compounds into the human food-chain in relation to the widespread use of PAHs in the environment. The aim of the project is to investigate possible air deposition and plant-uptake of PAHs by crops grown in soil polluted with tar and highly loaded with PAHs. An analytical method was developed using microwave-soxhlet-extraction and solid-phase-extraction clean-up combined with liquid chromatography and fluorescence detection to separate, identify and quantify different PAHs in the crops.

Figure 5: Analysis of organic micro pollutants. Scientist G.K. Mortensen.

Availability of PAHs and other Hydrocarbons in Soils Highly Polluted with Oil and Petrol
(G.K. Mortensen)

During remediation of soil highly polluted with oil or petrol (*e.g.* soil from former petrol stations, fuel depots), it is of importance to investigate the change in availability of organic compounds occurring in petrol or oil for cleaning treatments. To determine the availability, a method was developed including water extraction and solid phase micro extraction (SPME). The SPME technique is based on the partitioning of the organic compounds between the extraction phase immobilized on a fused silica fibre and the water or air matrix. The technique was used to determine the free-available and the reversible-bound fractions.
Various soils representing different pollution types and degradation steps were investigated. The contents of the organic contaminants in the water extract depended on soil and pollution type. In a sandy soil polluted with petrol, around 40% of the organic contaminants were water-extractable, since petrol contained more polar compounds with higher water solubility. From the total amount of organic compounds in the water extract, approximately 50% were accessible by the SPME-technique. In a soil polluted with tar, the water-extractable fraction was less than 10%, since most of the compounds occurring in tar were more apolar with lower water solubility. Again, approximately 40 to 50% of the total amount of organic compounds in the water phase were available by SPME.
A method was developed to determine the part of the organic compounds in polluted soil, which was immediately available for biodegradation. Desorption experiments were accomplished, where Tenax beads were added to the soil together with the water, and the part of organic contaminants immediately desorbed was measured (Figure 6). The SPME and Tenax methods are sufficient and reliable methods to support the total extraction methods, usually used to investigate petrol and oil polluted soils.

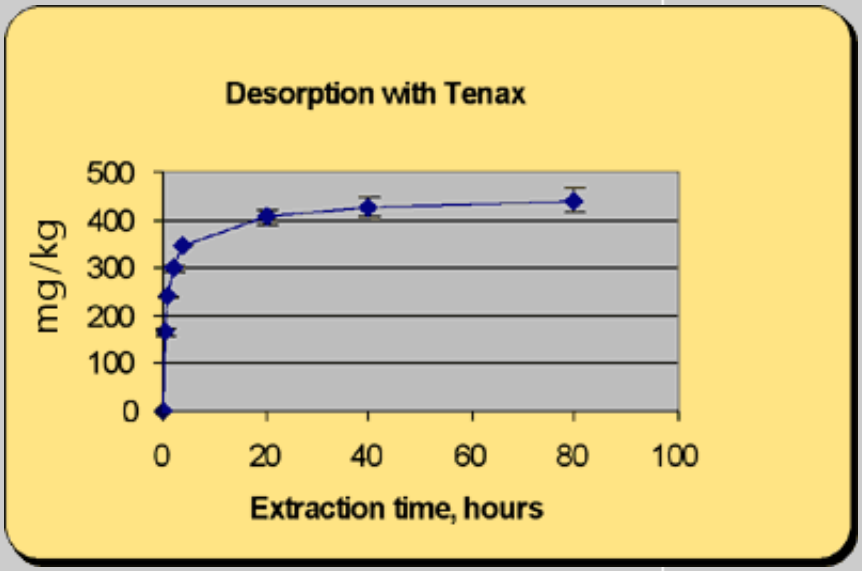

Figure 6: Amount of the total content of organic contaminants adsorbed by Tenax from a soil polluted with tar.

Formation of Chlorinated Solvents in Pristine Forest Environments
(F. Laturnus, C. Grøn)
Due to the widespread use of chlorinated solvents in the environment and their importance for various atmospheric photochemical reactions, we have studied the occurrence of these compounds in groundwater and ambient air of pristine forest ecosystems. Although these locations are remote from industrial areas and free of point sources of soil and groundwater contamination, several chlorinated solvents were detected. In the groundwater, high concentrations of chloroform (up to 1.6 µg L-1) and low concentrations of trichloroethene, tetrachloroethene and 1,1,1-trichloroethane (less than 0.01 µg L-1) were found. Additional investigations of chlorinated solvents in ambient air and soil air at the locations suggested a biogenic production of chloroform in forest soils (Haselmann et al. in press). This hypothesis was supported by field- and laboratory studies of the release of chloroform from forest soils. The other compounds studied probably originated from atmospheric deposition.
From the release, an annual input of chloroform into the atmosphere was calculated for northern temperate regions up to 24,000 tons, which is in the range of the annual industrial

emissions. Furthermore, as concentrations of chloroform measured in groundwater reached levels of public health concern, a natural origin has to be considered when discussing future regulation levels for chloroform in drinking water.

Reference

Haselmann, K.F., Ketola, R.A., Laturnus, F., Lauritsen, F.R., Grøn, C. Occurrence and Formation of Chloroform at Danish Forest Sites. Atmospheric Environment (in press).

Risk Assessment of PAC Polluted Soils

(T. Nielsen)

Our preliminary work in this field within the Centre for Biological Processes in Contaminated Soil and Sediments under the Danish Strategic Environmental Research Programme is described in the following. Previously similar work has been performed to evaluate the carcinogenic risk caused by atmospheric PAH (Polycyclic Aromatic Hydrocarbons) in city air in Denmark. Here the relative potencies for each compound is multiplied by their concentrations in the mixture and summed to estimate the total cancer risk. Evaluation of PAC (Polycyclic Aromatic Compounds) soil pollution is usual based on a comparison of the concentrations of limited number of indicator PAH with criteria levels. A large number of PAC in a tar-polluted soil in Copenhagen and a city background soil has been chemically identified. The biological effect for PAH can be expressed by means of the "carcinogenic potency" of the component (Nielsen *et al.*1996) expressed as benzo(a)pyrene equivalents. For some of the identified compounds, especially the PAH, "carcinogenic potency" data can be found in the literature. A number of basic N-PAC, *e.g.* quinoline, benz(a)acridine and benz(c)acridine, have been tested positive in carcinogenicity tests. But such tests have not yet been performed for a major part of the compounds. For some of these compounds mutagenicity data in short term bioassays are published.

The relative carcinogenic risk (RR) is estimated for 12 indicator PAH and the total amount of PAH in a tar-polluted soil in Copenhagen and a city background soil, for which a large number of carcinogenic data are available (Figure 7).

The relative risk (RR*) has been estimated for N-PAC mainly on the basis of published mutagenicity data. It should be stressed that RR* should be taken with great reservation, because the correlation between mutagenic and carcinogenic potency of these compounds is still not known, and further investigations are required to clarify this effect. It should also be emphasized that the model presumes an additive effect, although the existence of synergistic and antagonistic effects cannot be excluded.

The equation used is:

RR (RR*) = occurrence * bioavailability factor *biological effect

The "occurrence" is the total concentration in the soil of the component. The bioavailability factor is the part of the component, which is free, dissolved in the soil pore water. The factor is proportional with the inverse sorption coefficient for the component, *i.e.* $1/K_{oc}$. The "biological effect" is expressed by means of the "carcinogenic potency" (RR) or "mutagenic potency" (RR*) of the components. For operational reasons the term is expressed in benzo(a)pyrene equivalents both for the carcinogenic and mutagenic effects. The risk factor for a single compound (RF) is the same effect, as RF ppm benzo(a)pyrene would have. Thus if a compound has a RF-value 1, its effect is the same, as it would be, if the soil has contained 1 ppm benzo(a)pyrene and no other mutagenic or carcinogenic components.

The results appear to be remarkable, even though they should only be considered to be preliminary, and as mentioned taken with great reservation. There is fairly good consensus about the applied effect factor for PAH, but not for the other PAC. This is especially important for the basic N-PAC.

Figure 7 suggests:

1) The RF value for a component and its concentrations may deviate strongly from each other
2) The basic N-PAC has a major contribution to RF
3) Conventional PAH (12 indicator-PAH), representing the conventional way to evaluate the occurrence of the pollution and the risk, has only a minor contribution to RF even though their contribution to the total amount of PAC is in the magnitude of 60% and a good indicator for

the total PAC amount.

Figure 7: Survey for the concentrations and the calculated risk factors for different PAC subgroups in a tar polluted soil and a city background soil in the B&W area. Abbreviations: Me-PAH: Methyl substituted PAH, S-PAC: PAC containing a sulphur atom in the aromatic ring system, N-PAC: a nitrogen atom*.

	Tar soil		City background	
	Concentration (ppm / %)	Risk factor (RF) (BaP-equivalents / %)	Concentration (ppm / %)	Risk factor (RF) (BaP-equivalents / %)
PAC – total**	685	670	35	75
12 indicator PAH	401	81	23	4.5
% total PAH	*83*	*14*	*89*	*6.7*
% Me-PAH	*6.4*	*0.9*	*2.3*	*0.2*
S-PAC	*1.5*	*0.2*	*1.2*	*0.1*
N-PAC (basic)	*6.2*	*85*	*4.0*	*93*

*The chemical analyses also include O-PAC, neutral N-PAC and oxidation products of PAH. But so far, the compounds in these subgroups have not been incorporated in the RF calculations because it is necessary with more time to evaluate data on their biological effects.
**PAC – total is the sum of all the compounds, which have been analysed. There will be many PAC, which either require other analytical techniques or are impossible to analyse.

Determination of Sorption Constants for Organic Compounds to Humic Acid
(K. Jonassen, T. Nielsen)
The work in 1999 has been concentrated on the improvement of a method for determination of sorption constants K_{oc} for organic compounds to humic acid. The ratio between organic carbon in the humic acid and the binding material has been essentially improved. We have obtained data, which illustrate to which extent the measurements can be transferred to be valid for the free acids. A procedure is developed, which makes it possible to calculate K_{oc} directly from the obtained data. The use of the method is therefore no longer limited to humic acids from which K_{oc}-values already exist. This also concerns the problem as to which extent the data can be applied to the free acids and consequently to natural conditions.
By changing the way of binding humic acid to the silica surface, binding through carbonyl groups rather than amino groups, we have observed a stronger sorption of polar PAC. In general, we see that the sorption of PAC increases by size and lipophilic substituents and decreases with polar substituents.
We collaborate with M. Hübner, University of Bremen, Germany who has been a guest scientist at Risø since September 1999. In this collaboration, our method is used in studies on humic acid isolated from soils in northern Germany.

Effects of Air Pollution and Global Change

Atmospheric Degradation of Anthropogenic Molecules
(O.J. Nielsen, J. Platz)
To assess the environmental impact of the release of a chemical compound into the atmosphere several issues need to be considered. The first step is to determine its atmospheric lifetime. It determines the geographical extent of the possible direct environmental impact. To calculate the atmospheric lifetime we need information on the kinetics of its reaction with key atmospheric trace species such as OH and NO_3 radicals and O_3, the rate of its photolysis, its solubility and hence the propensity towards wet deposition, and finally its rate of dry deposition. Once the lifetime of the pollutant has been established, the next step is to determine the degradation products and intermediates of its atmospheric oxidation, and to assess whether they pose any environmental threat. The work focussed on three areas: hydrofluoroethers (HFEs), aromatics and oxygenates as alternative fuels and fuel additives.

All the work was done as collaborative studies with Ford Motor Company, USA and several European research institutions. The overall aim was to provide the scientific basis for strategies for emission control.

Formation, Occurrence and Fate of Nitro-PAH
(A. Feilberg, T. Nielsen, M. Poulsen)
Nitro-PAH is a group of potent mutagenic and carcinogenic pollutants, which are emitted by incomplete combustion processes and formed in the atmosphere by chemical reactions. In the current project environmental processes of nitro-PAH have been studied. It has been deduced from field measurements that the formation of nitro-PAH in the atmosphere is predominantly initiated by hydroxyl radicals as opposed to nitrate radicals (see Figure 8), although the latter may dominate under certain conditions at wintertime. The levels of nitro-PAH as well as parent PAH are strongly elevated during episodes of transport of polluted air masses from the European Continent. The mutagenic activity of particle extracts is related to the photochemical age of the particles in a complex manner.
In laboratory experiments it has been demonstrated that the photochemical degradation of particle-associated nitro-PAH is highly dependent on the chemical and physical characteristics of the particles. Light-induced radical chain reactions in the organic phase of combustion particles strongly accelerate the degradation of nitro-PAH. Under certain conditions nitro-PAH may be photo-reduced to their corresponding amines.

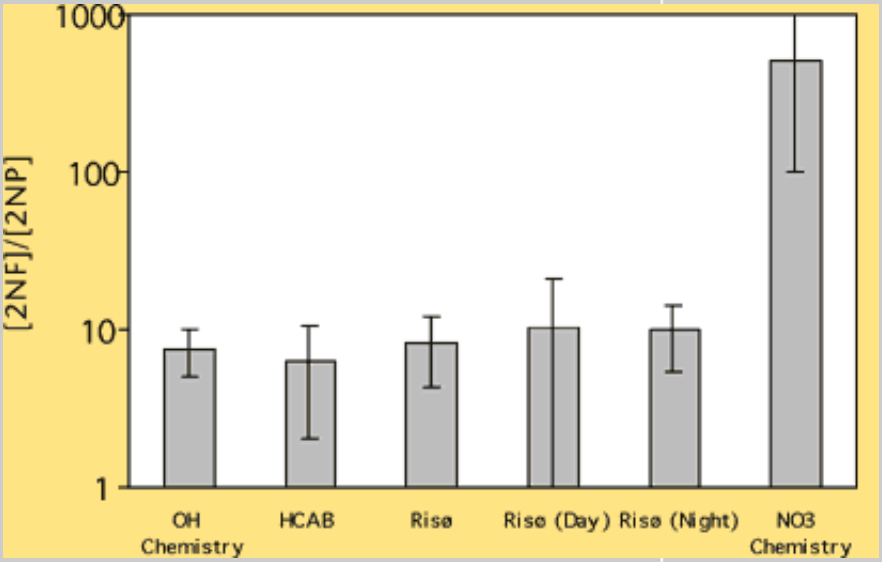

Figure 8: The ratio of 2-nitrofluoranthene (2NF) to 2-nitropyrene (2NP) has been used to evaluate the contribution of OH radical chemistry and NO_3 radical chemistry to the formation of nitro-PAH in the troposphere. The field data from Copenhagen (HCAB) and Risø demonstrate that the OH chemistry dominates.

Effects of Global Change on Forest and Agro Ecosystems
(L. Rasmussen, C. Beier)
Studies on the effects of increased temperature and CO_2 were implemented in an EU project in southern Norway in 1994. The response of an entire catchment to increased CO_2 and temperature was studied by experimental ecosystem manipulation during a 4-year period. The project called CLIMEX (Climate Change Experiment) was conducted in a mountainous pine-birch forest (Pinus silvestris, Betula pubescens) at an elevation of 300 m above sea level at Risdalsheia, Grimstad, Norway. The trees were up to 160 years old with a maximum height of 9 m. The project involved five catchments, two of which were divided in two sub-catchments covered with a big greenhouse or a roof construction, 860 m^2 and 400 m^2, respectively. The total set-up at the experimental site employed multiple treatments and controls. Increased CO_2 and temperature treatments started in 1994. The experimental catchment manipulations were: 1) control, 2) greenhouse control, 3) CO_2 enriched from the ambient level of approx. 360 ppmv to 560 ppmv and air temperature increased 3-5°C above ambient, and 4) soil temperature increased 3-5°C with heating cables.

The recent results showed that increased CO_2 and/or temperature did not significantly influence tree growth - measured as tree ring analyses. Photosynthetic capacity and carbon-nitrogen ratio of new leaves of most plant species did not change and the growing season was prolonged. This has helped to sustain an increase in forest floor plant growth. An increased needle weight and shoot length in all roof and greenhouse covered catchments indicated that the reduced light conditions and shelter effect under the roof and greenhouses overshadowed possible treatment effects. However, soil nitrogen mineralization increased promoting increased nitrate export in stream water. So, the hypothesis that an increase in N-mineralization would be counteracted by a corresponding increase in N uptake, due to the increased CO_2, could not be verified.

Figure 9

The SOROFLUX Project
(L. Rasmussen)
The SOROFLUX project (Effects of land use and organic waste application on carbon and nitrogen fluxes) is located at the field station facility at Lille Bøgeskov, Gyrstinge, Sorø, Denmark. The project was implemented under the Danish Strategic Environmental Research Programme 1997-2000 (Centre for Sustainable Land Use and Management of Contaminants, Carbon and Nitrogen) as an extended national contribution to the EU projects EUROFLUX, EXAMINE and FOREXNOX. The project is collaboration between Risø National Laboratory, University of Copenhagen, Royal Veterinary and Agricultural University and University of Aarhus. The main objectives of the project are to quantify and compare the gaseous and water mediated fluxes of N and C compounds in forest (beech) and agro (barley) ecosystems with and without accelerated input of N and C in the form of sewage sludge. The project provides quantification of the net ecosystem fluxes of NO, NO_2, NH_3, N_2O, HNO_3 (gaseous), CO_2, CH_4, H_2O and water mediated inorganic and organic N and C compounds (Rasmussen *et al.* in press). Measurements of the fluxes of the gaseous compounds are performed with advanced eddy-correlation technique, the gradient technique, the relaxed eddy-accumulation (REA) technique and dynamic chambers. Water mediated fluxes encompass rain, throughfall, stem-flow and leaching from the root zone.
The results of the flux measurements showed that the input-output fluxes of CO_2 to both forest and agro ecosystems led to a net accumulation of 1-2 t carbon per ha/yr for all types of ecosystems. As to the forest, the input was about 11-12 t carbon per ha/yr, and the output from respiration was about 9-10 t carbon per ha/yr. A higher accumulation rate was expected for forest ecosystems based on calculations on tree increment. However, it seems that respiration processes are more important for the carbon balance than anticipated. Sewage sludge application to the agricultural fields had only a short-term effect on the emission of CO_2, CH_4, NO and N_2O during the first 2 months after application. These fluxes only contributed to the total carbon and nitrogen balances with a few kg per ha/yr. However, sewage sludge addition increased biomass production by 20% and grain yield production by 10% the first year after application. No effects of sewage sludge application were observed the second year. The leaching of nitrogen in the form of nitrate was less than 1 kg per ha/yr for the forest ecosystem, but about 100 kg per ha/yr for the agricultural fields, whether they were treated with sewage sludge or not.

From Risø researchers from the Biogeochemistry (L. Rasmussen) and Plant Ecosystem and Nutrient Cycling (C. Beier, K. Pilegaard, P. Ambus) Programmes are involved, together with researchers from the Wind Energy and Atmospheric Physics Department (N.O. Jensen, P. Hummelshøj).

Reference

Rasmussen, L., Beier, C., Pilegaard, K., Ambus, P., Mikkelsen, T., Jensen, N.O., Kjøller, A., Ladekarl, U.L. (2000) Fluxes of NO_3^-, NH_4^+, NO, NO_2, HNO_3^-, N_2O and organic N in an old Danish beech forest. Water, Air, and Soil Pollution (in press).

Figure 10

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Plant Ecosystems and Nutrient Cycling Programme

The research of the Plant Ecosystem and Nutrient Cycling programme is concentrated on the turnover of nutrients and a number of other important compounds in the soil-plant-atmosphere system. It is focused on the processes of the turnover of the compounds, the influence of climate changes (carbon dioxide, water, and temperature) on the plant eco-systems, the fluxes of volatile compounds, and the genetically background for nutrient uptake. The research is carried out in controlled growth facilities and in field experiments at Risø and at field stations in the countryside.

SOROFLUX
(K. Pilegaard)
In the SOROFLUX project we have measured the fluxes of CO_2, H_2O, O_3 and NO_x between the atmosphere and the forest canopy and between the atmosphere and the forest floor of an 85 year old Danish beech forest as well as the fluxes between the atmosphere and a wheat field. Apart from the measurements running continuously (CO_2 and H_2O over forest and field and soil-atmosphere exchange in forest), we have made a special field campaign measuring fluxes of O_3 and NO_x over the forest and field simultaneously. The flux measurements are carried out in collaboration with the Wind Energy and Atmospheric Physics Department, Risø, and are a contribution to the EUROTRAC-2 subproject BIATEX-2.

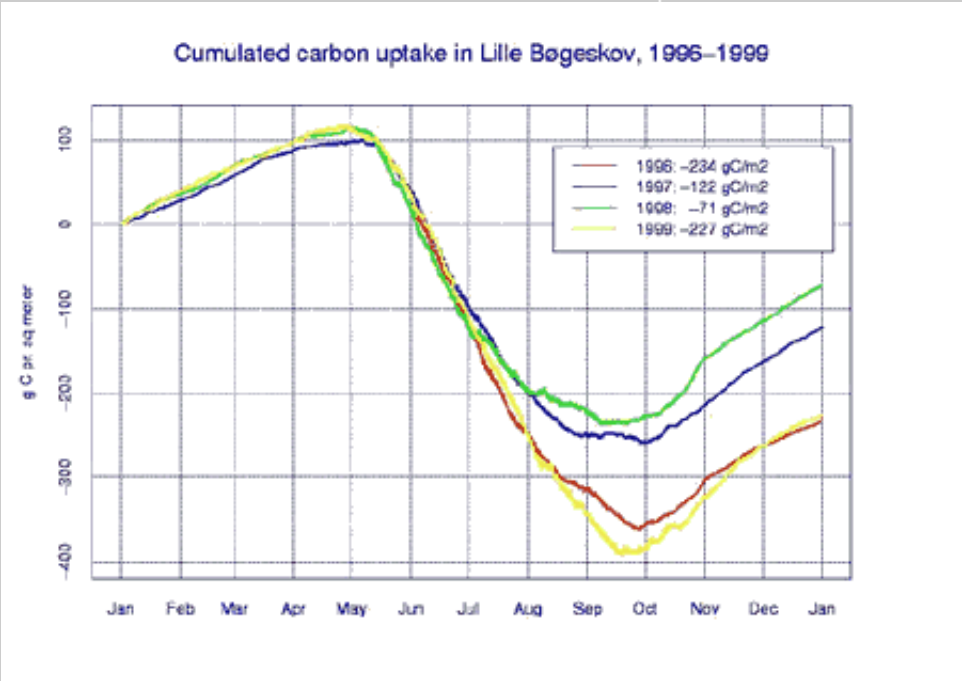

Figure 1: Also water mediated fluxes of C and N have been measured within the SOROFLUX project. The results show that the input of N to the system by wet and dry deposition amounts to approx. 25 kg N/ha/yr equally distributed between NO_3 and NH_4. 25% of the N flux to the

forest floor is transported with water running along the stems (stemflow). The flux of N to the forest floor is larger than to the agricultural field because the dry deposition to the large forest canopy is bigger.

CLIMOOR (Climate Driven Changes in the Functioning of Heath and Moorland Ecosystems)
(C. Beier, A. Tietema, P. Gundersen, B. Emmett, F. Roda, A. Gorissen)
CLIMOOR (http://www.risoe.dk/climoor) is an EU funded research project aimed at investigating the effects of warming and drought on the functioning of heath and moorland ecosystems. The project includes partners from Denmark, the Netherlands, UK and Spain. Within the CLIMOOR project an experimental "climate change" facility has been set-up at Mols Bjerge in Eastern Jutland, Denmark as well as the sites in the Netherlands, UK and Spain. The facility makes it possible to heat the vegetation and to create prolonged drought periods, whereby "climate change" can be applied at field scale and the effects on plants and soil tested. The CLIMOOR facility has operated since March. In the heating plots the temperature has been increased by 1-2ºC. In the drought plots a 2-month drought period has been applied during May-June. Although the manipulations have only run for less than a year, the first results have indicated that both warming and heating affect the plants and the soil.

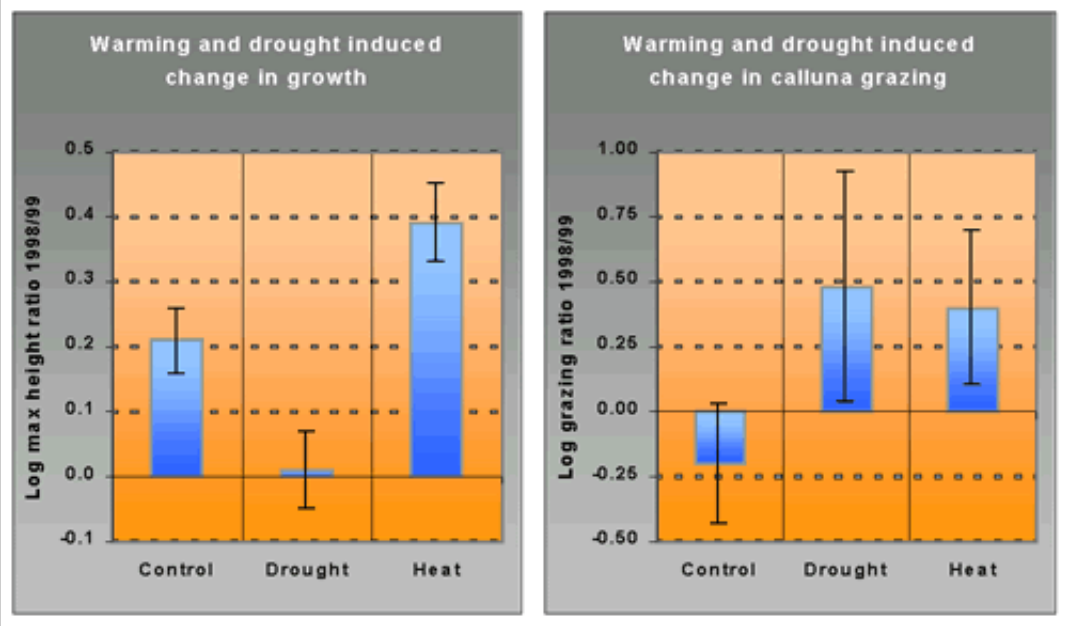

Figure 2: Vegetation changes at Mols (DK) due to "drought" and "warming" compared with untreated "control" plots.
Left: The vertical axis shows the log-transformed ratio between the mean maximal height (M) in 1999 and 1998. A ratio of 0 means unchanged vegetation. A positive ratio indicates an increased M of *Deschampsia flexuosa*. Warming has caused a larger increase of M compared to the control (intermediate) and to drought (near 0). All treatment means are significantly different.
Right: The grazing ratio 99 is the ratio between heather beetle grazed and non-grazed *Calluna* shoots in 1999. A log transformed value of 0 means equal amounts of grazed and non-grazed shoots. Drought or warming cause a more heavy grazing. Drought or warming tends to increase grazing (not significant).

Figure 3

Application of Molecular Biology to Identify and Isolate Genes Important for Phosphorus-Use Efficiency in Barley
(K. Engvild, G. Gissel Nielsen)
The purpose of the project is to obtain the necessary knowledge to enable breeding of barley cultivars with efficient uptake or use of phosphorus so that the need for phosphorus fertilizers is reduced. This would save the use of limited phosphorus resources and contribute to decreased phosphorus runoff from fields to lakes and fjords. The project is collaboration between the Royal Veterinary and Agricultural University (RVAU), Danish Institute of Agricultural Sciences (DIAS), Flakkebjerg and two groups at Risø, financed by "Strukturdirektoratet". RVAU focuses on cultivar differences in root hairs and their importance for phosphate depletion in the rhizosphere; at DIAS barley has been transformed with phosphatase genes, and at Risø several genes induced by phosphate starvation have been isolated, for example a nicotianamine synthetase gene. Differences in root phosphatases in doubled haploid progenies of different cultivars were examined and compared with Quantitative Trait Loci (QTL) for yield.

Isolation and Identification of Natural Chlorine Compounds in Crop Plants
(K. Engvild, P.B. Holst)
It is known that some crop plants such as seeds of pea, broad beans and lentils contain natural chlorine-containing compounds in mg/kg quantities. Other crop plants such as rape, barley, wheat and tomato incorporate radioactive ^{36}Cl into unknown compounds, which move in lipid solvents. Methods are being developed to isolate such compounds, using highly sensitive auto radiography for detecting the radioactive Cl. We have several radioactive organic fractions, but the amounts are still too small to elucidate the chemical structure of the compounds.

Effects of Sewage Sludge and other Organic Wastes on Soil Fertility, Plant Uptake of Organic Contaminants and Soil-Atmosphere Exchange of CH_4
(P. Ambus, L. Kure, E.S. Jensen)
Within the Strategic Environmental Research Programme 1997-2000 a number of activities have been carried out:
Mineralization of N in soils supplied with composted household waste or sewage sludge has been studied in the field, using stable isotope techniques. Gross N mineralization rates were 30% higher in soils supplied with compost compared to control soil or sludge applied soils. However, mineralization in sludge applied soil resulted in a large inorganic N pool during the summer. An additional field study of growing barley in soils supplied with different organic wastes was initiated in 1999 in order to elucidate the bioavailability of N to crops of applied organic wastes.
Sewage sludge not only contains nutrients and organic matter valuable for soil fertility and structure. Many anthropogenic compounds absorb organic matter and are found in large quantities in sewage sludge. The potential risk of bioaccumulation of toxic organic compounds in crop plants was also investigated. We have tested the uptake of ^{14}C-labelled detergents and plasticizers in barley, rape, tomato and carrots grown aseptically in nutrient cultures. Roots of all species accumulated large amounts of these hydrophobic compounds,

but nothing or little was translocated to the green parts of the plants. Apparently roots of rape and carrot can metabolize some of the compounds very quickly, but the analysis of metabolites in plant tissue is not completed. Greenhouse experiments growing carrot, barley and rape in soil supplied with sewage sludge was undertaken in corporation with the programme of Biogeochemistry, Risø, and no uptake of the compounds was seen here either. It has been demonstrated that soil application of sewage sludge for fertility purposes significantly stimulate the soil emission of CH_4, which is important for atmospheric radiative properties. A non-treated agricultural site emitted 0.3 mg CH_4-C m^{-2}, whereas sludge treated soil emitted 7.7 mg C m^{-2}. The emissions of CH_4 occurred mainly in a short transient period following applications (Figure 4). The study has also verified that forest soils, regardless of drainage status, possess a strong capability for CH_4 oxidation. The CH_4 oxidation is markedly depressed when the soil receives an N-fertilizer.

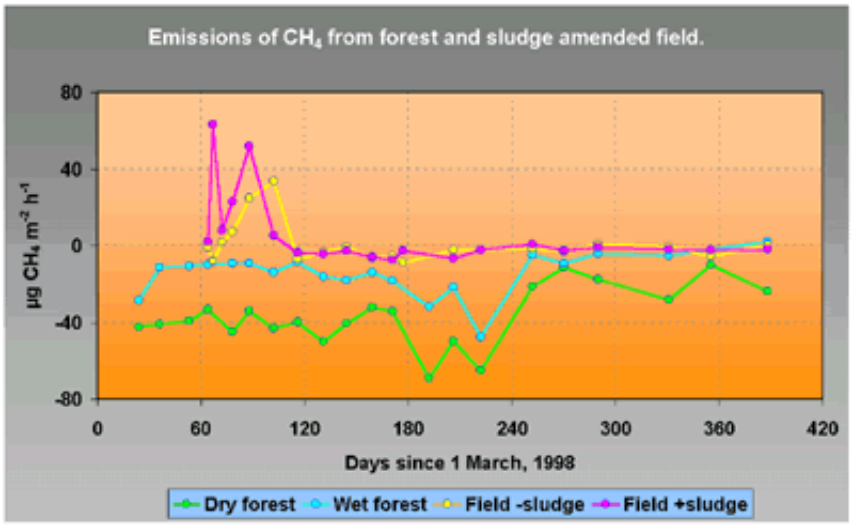

Figure 4

Competitive Interactions, Resource Use and Nitrogen Dynamics in Annual Intercrops in Low-Input Cropping Systems
(P. Ambus, H. Hauggaard-Nielsen, E.S. Jensen)
Pea-barley interspecific competition decreases early pea growth, possibly due to a reduced root nodule establishment. Present fieldwork illustrates how pea root distribution (^{32}P technique) concentrates in the 0-25 cm soil layer. Barley is shown to have a faster spatial root development causing a rapid and efficient utilization of soil N resources. Studies in controlled environments (RERAF) indicate that a 7-day sowing delay of barley in the intercrop results in up to 50% improved pea growth compared to simultaneous sowing. Relay intercropping strategies thus seem promising when trying to increase symbiotically fixed N input to the agroecosystem without reducing the barley N-uptake significantly.

Precision Farming
(G. Gissel Nielsen, R. Jørgensen, P. Møller Hansen, V. Haahr)
Within the project of precision farming we have established a frame experiment set-up for the testing of sensors. This is fundamental for improving the methods and techniques in sensor based evaluation of the nutrient status of plants. A preliminary experiment has shown good relations between sensor measurements and the N status of the plants. We have developed a device placed on the harvester for site-specific sampling of grain for analyses. We have participated in field tests of the Norsk Hydro sensor for site specific nitrogen fertiliztion.

Facilities

RERAF (Risø Environmental Risk Assessment Facility, http://www.risoe.dk/pbk/reraf)
(T. Mikkelsen)
RERAF is a unique plant growth facility belonging to a new generation of phytotrons. It

comprises a row of physically and electronically separated environmental chambers with separate top and root compartments. Environmental risk assessment experiments can be carried out under fully controlled conditions.
In 1999 several experiments related to atmospheric changes or transgenic plants have been conducted, but also experiments with special demand *e.g.* high light intensities or controlled soil temperature have been carried out.
Enhanced levels of atmospheric CO_2 stimulate growth in C3-Plants, but the growth varies between species and varieties. A growth response experiment with several oat varieties (*Avena sativa*) is conducted. A hydroculture study followed the growth of two species of milfoil (*Achilléa*) and plantain (*Plantágo*), a fast and a slow growing species. Exploitation of CO_2 is also depending on root development and interaction with symbionts (*e.g.* mycorrhizal fungi). Wheat (*Triticum*) and pea (*Pisum*) are examined during several experiments. Plants with mycorrhizal fungi can assimilate and utilize CO_2 better than plants without mycorrhizal fungi. To study the frequencies of gene-distribution from rape (*Brassica napus*) to navew (*Brassica rapa*) experiments with field grown plants and RERAF grown plants have been conducted.

Figure 5

CONFIRM (Centre for Continuous Flow Isotope Ratio Mass Spectrometry)
(P. Ambus)
CONFIRM encompasses state-of-the-art instrumentation for analysis of ^{13}C and ^{15}N in various samples of soil, biological or atmospheric origin. CONFIRM is supported by the National Research Councils Instrument Centre Programme and Risø has provided the basis for collaborative work with scientists at the University of Copenhagen, the Technical University of Denmark (DTU) and Danish Institute of Agricultural Sciences (DIAS) in which various disciplines of stable isotope techniques have been implemented. *E.g.* at DTU a masters project has focused on the development of a method for measuring $^{13}CH_4$ in the soil profile in order to describe the soil-atmosphere exchange of CH_4 mathematically. The instrumentation and analytical routines of CONFIRM were characterized by very high analytical precision and accuracy when participating in an inter comparison on ^{13}C and ^{18}O determinations in atmospheric CO_2, organized by the European Joint Research Centre, Institute for Reference Materials and Measurements.

Sorø
(K. Pilegaard)
Two field stations are located near Sorø, Denmark. One is in an 85 year old beech forest and the other is on an agricultural field. Both stations have an instrument hut, and carry mains

power and telephone lines. The forest station has a 24 m high scaffolding tower and a 57 m high mast. The agricultural field station has a 10 m high mast. Both field stations are used for flux measurements of gaseous and water mediated carbon and nitrogen compounds. The scientific activities are described under the SOROFLUX project. The carbon dioxide flux measurements are utilized as a Danish contribution to the international EUROFLUX and FLUXNET projects. The stations are run in collaboration with the Wind Energy and Atmospheric Physics Department, Risø.

Mols Bjerge
(C. Beier)
A field station to test the climate change effects on the heath ecosystem. The facility consists of 9 plots: 3 warming plots, 3 drought plots and 3 untreated control plots. Heating is performed by automatically covering the vegetation at night by reflective curtains. Drought is performed by covering the vegetation during rain events for 2 months in the summer. The facility is open to researchers and students wanting to study specific processes in relation to climate change.

Figure 6

Figure 7

OTC (Open Top Chamber Facility)
The open top chamber facility consists of 33 chambers some with roofs and some with lysimeters. The facility can be used for fumigation experiments with compounds such as ozone, nitrogen oixides and carbon dioxide.

Lille Valby (**RIMI** – Risø Integrated Environmental Initiative)
(K. Pilegaard)
The field station in Lille Valby close to Risø is placed on a permanent grass field. It consists of a hut for instruments and a 10 m meteorological mast. The field station has mains power and telephone lines. The station is used for climate measurements, eddy correlation flux measurements of CO_2 and H_2O and concentrations of air pollutants (SO_2, NO_x, O_3 and

particles). The National Environmental Research Institute carries out the air pollution measurements. The station is run in collaboration with the Wind Energy and Atmospheric Physics Department, Risø.

Figure 8

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Scientific Results and Finances
Plantbiology og Biogeochemistry Department

	Results 1997	Results 1998	Results 1999
INDICATORS			
Basic research and development (man-month)	361	297	309
Research programmes (man-month)	781	877	957
Commercial contracts (man-month)	77	38	57
Technical support for research (man-month)	66	19	58
Management (man-month)	118	69	134
Total	1403	1381	1515
DISSEMINATION OF RESULTS			
Papers in international journals and books	84	97	73
Papers in Danish journals and books	17	11	12
Risø reports, R , M and I	6	7	9
Danish books and reports	11	7	17
International books and reports	4	5	4
Other publications	6	8	5
Papers in conference proceedings	30	16	15
Other international conference contributions	90	95	110
Other Danish conference contributions	30	44	56
Patent proposals	0	1	3
NETWORKING AND COLLABORATION			
Ph.D. students (number)	21	29	29
Ph.D. degrees (number)	5	6	6
Risø leave of abcence (man-month)	21	25	19
Visiting scientists at Risø (man-month)	29	37	57
Scientific papers reviewed (number)	162	93	135
"Committees for Ph.D.thesis, promotion of scientists, senior scientists, professors (number) "	17	28	16
Committee memberships (number)	36	31	32
Collaboration with companies (man-month)	172	258	69
Contribution from companies in projects (man-month)		166	172
Collaboration with research institutes (man-month)	462	368	470
Contribution from institutes in collaboration (man-month)		662	445
Collaboration with public authorities (man-month)	7	3	4

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Acronyms

2D	Two-Dimensional
AFLP	Amplified Fragment Length Polymorphism
AM	Arbuscular Mycorrhiza
AMF	Arbuscular Mycorrhizal Fungi
AMP	Adenosine Mono Phosphate
BAC	Bacterial Artificial Chromosome
BASYS	Baltic Sea System Study
Bgh	*Blumeria graminis* f.sp. *hordei*
BIATEX	Biosphere Atmosphere Experiment
CLIMEX	Climate Change Experiment
CLIMOOR	Climate driven changes in the functioning of heath and Moorland ecosystems
CONFIRM	Centre for Continuos Flow Isotope Ratio Mass spectrometry
DDRT-PCR	Differential Display Reverse Transcriptase Polymerase Chain Reaction
DEHP	Di-ethyl-hexyl-phthalate
DIAS	Danish Institute of Agricultural Sciences
DLF	Danske Landboforeningers Frøforsyning
DMT	Dimethyl Ether
DTU	Technical University of Denmark
EMS	Ethyl Methan Sulphonat
EST	Expressed Sequence Tag
EUROFLUX	Effects of CO_2 exchange over European forests
EUROTRAC	European Experiment on Transport and Transformation of Environmental Relevant Trace Constituents of Anthropogenic and Natural Origin
EXAMINE	Exchange of Atmospheric Ammonia with European ecosystems
FOREXNOX	Effectc of nitrogen oxides on European forests
FSD	Family Specific Domain
FTIR	Fourier Transform Infra-Red
FØTEK	Danish Food Technology and Development Programme
GC-MS-MS	Gas Chromatography/Mass Spectrometry
GDC	Gamma Dating Centre
GFP	Green Fluorescent Protein
GMP	Genetically Modified Plants
HFE	HydroFluoroEther
HPLC	High Pressure Liquid Chromatography
HR	Hypersensitive Response

HR-ICP-MS	High Resolution Inductively Coupled Plasma Mass Spectrometry
ICARDA	International Centre for Agricultural Research in Dry Areas
IGER	Institute of Grassland and Environmental Research
LAS	Linear Alkyl benzene Sulfonates
LC-MS	Liquid Chromatography Mass Spectrometry
LOD-score	Log of the odds ratio score
MAST	Marine Science and Technology Programme
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
OTC	Open Top Chamber
PAC	Polycyclic Aromatic Compounds
PAH	Polycyclic Aromatic Hydrocarbons
PBM	PeriBacteroid Membrane
PBS	PeriBacteroid Space
PCR	Polymerase Chain Reaction
PEG	PolyEthylen Glycol
PLFA	PhosphoLipid Fatty Acid
PI	Phytophthoras infestance
PLA	Polylactic Acid
PLS	Discriminant Partial Least Squares regression
PR	Pathogenesis-Related
PRP	Proline-Rich Protein
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA Technique
REA	Relaxed Eddy-Accumulation
RERAF	Risø Ecological Risk Assessment Facility
RF	Risk Factor
RFLP	Restriction Fragment Length Polymorphism
RIMI	Risø Integrated Environmental Project
RR	Relative Carcinogenic Risk
RR*	Relative Mutagenic Risk
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
RVAU	The Royal Veterinary and Agricultural University
SOROFLUX	Effects of land use and organic waste application on carbon and nitrogen fluxes
SPME	Solid Phase Micro Extraction
SSR	Simple Sequence Repeat
STS	Sequence Tagged Site
TLC	Thin Layer Chromatography
YAC	Yeast Artificial Chromosome

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Refereed Journals

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Backes, G., Jensen, J., Jahoor, A. Development of markers for breeding of barley with high malting quality and disease resistance. 3th Annual Meeting of Danish Cereal Network, Slagelse, Denmark 3-4 November.
Beier, C. En dansk forskers erfaringer fra EU-ansøgninger/projekter. Informationsseminar "Globale ændringer, klima og biodiversitet" og "Fremtidens by og kulturarven", EU 5th Rammeprogram "Energi, miljø og bæredygtig udvikling", Forskningsministeriet, Copenhagen 6 May (oral - in Danish).
Bousset, L., Østergård, H., Hovmøller, M., Caffier, V. Population dynamics in barley powdery mildew. COST 817 Workshop on Disease Resistance and Cereal Leaf Pathogens beyond the Year 2000. Martina Franca, Italy 11-12 November.
Beier, C., Rasmussen, L., Pilegaard, K., Ambus, P., Mikkelsen, T., Jensen, N.O., Kjøller, A., Ladekarl, U.L. Fluxes of NO_3^-, NH_4^+, NO, NO_2, and N_2O in an old Danish beech forest. "Conference on Critical Loads", Copenhagen 21-25 November. UN-ECE Convention on Long-Range Transboundary Air Pollution (poster).
Christiansen, S.K. Transformation of obligate biotropic fungi. NorFA-course on Communication in Plant-Microbe Symbioses, Risø, Roskilde, Denmark 15-25 August (unpublished).
Christiansen, S.K. Transformation of the barley powdery mildew fungus. RVAU, Copenhagen 26 January (unpublished).
Egsgaard, H. Investigation of low molar mass polymers by use of electrospray ionization and mass spectrometry. Danish Society for Polymer Technology (PTS) thematic meeting, Risø, Roskilde, Denmark 5 October (unpublished) (abstract available).
Eriksen, L. Det perfekte stadium af *Septoria tritici*, Plantepatologisk Selskabs møde 18 January.
Eriksen, L. Epidemiology and partial resistance, RVAU, Copenhagen, Septoria meeting 25-26 November.
Eriksen, L. Population dynamics of the wheat pathogen *Mycosphaerella graminicola*, Institute of Plantbiologi, RVAU, *Copenhagen* 18 June.
Eriksen, L., Munk, L., Shaw, M.W., Østergård, H. Does the teleomorph stage, *Mycosphaerella graminicola*, matter to epidemics of septoria tritici blotch? Workshop on Disease Resistance and Cereal Leaf Pathogens beyond the Year 2000, Martina Franca, Italy 11-12 November.
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Feilberg, A. Atmosfærekemi af nitro-polycycliske aromatiske kulbrinter. Annual meeting of the Danish Chemical Society. University of Southern Denmark, Odense, Denmark 10 June.
Feilberg, A. Atmospheric Chemistry of Nitro-polycyclic Aromatic Hydrocarbons. Colloquium. Joint Research Centre Ispra, Italy 30 September.
Feilberg, A. Objectives and outline of the Danish GLOCONA project. Meeting at Silwood Park, Ascot, UK 8 February.
Feilberg, A. Occurrence and fate of nitro-polycyclic aromatic hydrocarbons in the atmosphere. 1st DSAR conference. Copenhagen, Denmark 14-15 October.
Frederiksen, H.B., Gavito, M.E., Jensen, B., Petersen, B.M. Plant-soil interactions at elevated

CO_2: Effect on wheat growth, rhizodeposition and response of the soil microflora and fauna in FACE experiment. New Phytologist and GCTE Symposium: Root dynamics and Global Change. Townsend, Tennessee, USA 19-22 October (poster).

Frøsig, L., Egsgaard, H. Identification of flame ions derived from methoxycarbenes in premixed flames of oxygenated fuels. Bi-annual Ph.D.-seminar, Department of Chemistry, University of Copenhagen 4 June.

Gavito, M.E., Bruhn, D., Jakobsen, I. Pea (*Pisum sativum* L.) root and mycorrhiza responses to elevated carbon dioxide. New Phytologist and GCTE Symposium: Root dynamics and Global Change. Townsend, Tennessee, USA 19-22 October (poster).

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Gavnholt, B., Larsen, K., Rasmussen, S.K. Kloning af rajgræs gener til modificering af lignin biosynteser. Fremtidens kulturplanter, midtvejsmøde, RVAU, Copenhagen 21 October.

Giese, H. Molecular approaches to identify genes involved in the interaction between *Blumeria graminis* and barley. Seminar at Kiel University, Germany 23 November (unpublished).

Giese, H. Molecular studies of the biotrophic fungus *Blumeria graminis* f.sp. *hordei*. Seminar at Bayer AG, Leverkusen, Germany 26 April (unpublished).

Giese, H., Pedersen, C., Justesen, F.A., Christiansen, S.K. Nuclear and extranuclear genetics of *Blumeria graminis* f.sp. *hordei*. 1st International powdery mildew conference, Palais des Papes, Avignon, France 29 August-3 September (unpublished).

Gissel-Nielsen, G. Bioteknologi og kvælstofforbrug i fremtidens landbrug. 12-mandsforening i Østjylland, Odder, Denmark 15 November.

Gissel-Nielsen, G. Current legislation on nutrient management in Denmark EU-Meeting, Gent, Belgium (oral).

Grell, M.N. How is the establishment of symbiotic structures in plant tissues regulated? NorFA-course on Molecular Communication in Plant-Microbe Symbioses, Risø, Roskilde, Denmark 14-25 August.

Grell, M.N., Lange, S., Mouritzen, P., Giese, H. Differential expression of fungal genes in the barley-*Blumeria graminis* f.sp. *hordei* symbiosis. 1st International Powdery Mildew Conference, Palais des Papes, Avignon, France 29 August-3 September (abstract).

Grøn, C. Organisk affald - uudnyttet ressource eller uløst affaldsproblem? Temamøde om recirkulering af næringsstoffer fra by til land. RVAU, Copenhagen, 5 May (in Danish).

Grøn, C. Organiske stoffer i jord-plantesystemer. RVAU, Copenhagen, 6 May (in Danish).

Grøn, C. Research in the area of sustainable land use and recycling of waste in agriculture. SPT work shop on LAS risk assessment for sludge-amended soils, Eigtveds Pakhus, Copenhagen 19-20 April.

Hasager, C.B., Pilegaard, K., Jensen, N.O., Mikkelsen, T.N. CO_2 balance målinger og modellering for danske landområder. DMU 10 år. Konferencen Dansk Miljøforskning 1999, 19-20 August, Copenhagen (poster).

Hatzack, F. Barley mutants with a low content of phytic acid: Metabolic and nutritional aspects. Seminar at Department. of Biochemistry, Lund University, Sweden 15 October.

Hatzack, F., Johansen, K.S., Nylev, P., Rasmussen, S.K. Low-Phytate barley mutants: biochemical and nutritional aspects. 27th Nordic Cerealist Conference, LO-skolen, Elsinore, Denmark 9-11 May.

Hatzack, F., Johansen, K.S., Nylev, P., Rasmussen, S.K. Strategies towards an improved nutritional availability of grain phosphorus. Plant Biology '99, Annual Meeting of American Society of Plant Physiology, Baltimore, MD, USA 24-28 July.

Haugaard, H., Collinge, D.B., Lyngkjær, M.F. How three Fungal Mycelia extracts protects barley against powdery mildew. 1st International Powdery Mildew Conference, Palais des Papes, Avignon, France 29 August—2 September.

Jahoor, A. Is breeding against weed possible? DIAS, Flakkebjerg, Denmark 6 October.

Jahoor, A. Localisation of QTL for malting quality and disease resistance in barley. Prague, Czech Republic 21-27 August.

Jahoor, A. Marker assisted selection in cereals. 27th Nordic Cerealist Conference, LO-skolen,

Elsinore, Denmark 9-11 May.
Jahoor, A. Screening of genetic diversity for mildew resistance in *H. spontaneum* collected in Israel. Quedlinburg, Germany 26-28 April.
Jahoor, A. Use of molecular markers in plant breeding. ICARDA, Syria 18-23 September.
Jahoor, A., Herz, M. Analysis of QTL´s including malting quality, agronomic important characters and disease resistance with molecular markers. Weihenstephan, Germany 23-25 April.
Jakobsen, I. The role of arbuscular mycorrhizal fungi in plant nutrition and vegetation processes. Applied Soil Ecology - From rhizosphere to landscape level. Wageningen, The Netherlands 24-29 October.
Jakobsen, I. Gennemgang af hovedresultater fra PEASE- projektet. Afslutningsworkshop for PEASE (Pesticiders indflydelse på nedbrydnings- og opbygningsprocesser i rodzonen) Gentofte, Denmark 7-8 October.
Jakobsen, I. The role of mycorrhizal fungi in phosphorus supply to plants. NorFA-course on Molecular Communications in Plant-Microbe Symbioses. Risø, Roskilde, Denmark 14-25 August.
Jensen, L.G., Christiansen, S.K., Giese, H. Genetic transformation of *Blumeria graminis* f. sp. *hordei*. 1st International Powdery Mildew Conference, Avignon, France 29 August-3 September (unpublished).
Johansen, K.S., Kristensen, B.K., Hatzack, F., Tung, D.T., Koutras, C., Rasmussen, S.K. Phosphat i kornsorter, Roskilde Dyrskue 11—13 June.
Johansen, K.S., Kristensen, B.K., Hatzack, F., Tung, D.T., Koutras, C., Rasmussen, S.K. Genmodificerede planter — til gavn for miljøet. Roskilde Dyrskue 11—13 June.
Johansen, K.S., Kristensen, B.K., Hatzack, F., Tung, D.T., Koutras, C., Rasmussen, S.K. Genmodificerede planter — til gavn for miljøet. Folkemøde om gensplejsning, Odense Congress Center 16 November.
Johansen, K.S., Kristensen, B.K., Hatzack, F., Tung, D.T., Koutras, C., Rasmussen, S.K. Fosfat i kornsorter. Folkemøde om gensplejsning, Odense Congress Center 16 November.
Jørgensen, R.B. Debataften om gensplejsede afgrøder, RVAU, Copenhagen 26 April.
Jørgensen, R.B. Vurdering af effekter på miljøet ved dyrkning af gensplejsede afgrøder. Hvad vurderer man? er det godt nok?. Det Kgl. Danske Landhusholdningsselskab vintermøde: Gensplejsede eller almindelige fødevarer. Hvad er forskellen? Copenhagen 9 March.
Jørgensen, R.B. Bedre mad på bordet — hvordan? — folkemøde om gensplejsning, Odense Congress Center 16 November.
Jørgensen, R.B. Gensplejsede organismer. Ved vi nok til at kunne vurdere sikkerheden for miljø og mennesker? Ingeniørforeningen i Danmark, Ingeniørhuset 28 September.
Jørgensen, R.B. Gensplejsning i menneskets tjeneste og i naturens? Dansk Naturhistorisk Forening, August Krogh Instituttet, Copenhagen 18 November.
Jørgensen, R.B. Muligheder med plante-DNA. Kriminalteknisk Seminar, Avnø, Denmark 6 May.
Jørgensen, R.B. Risiko for spredning af genmodificerede planter i naturen. Steins Laboratorium A/S, Radisson SAS Scandinavia Hotel, Copenhagen 23 August.
Jørgensen, R.B. Spridning av resistensgener från gröda (GMO) till ogräs — risker och konsekvenser. Nationell Ogräsdag, Uppsala, Sweden 15 December.
Kellmann, J.-W., von Bargen, S., Salchert, K., Bauer, C., Koncz, C., Schreier, P.H., Piechulla, B. Systemische Ausbreitung des Tomatenbronzefleckenvirus (TSWV) in *Arabidopsis thaliana*: Determinierung möglicher Wirtsfaktoren mit Hilfe des Hefe-Zweihybrid Systems. 12. Tagung "Molekularbiologie der Pflanzen", P/JWK, Dabringhausen, Germany 3-6 March.
Kjærsgård, I.V.H., Rasmussen, S.K., Welinde, K.G. Expression of *Arabidopsis* peroxidase ATP1 and ATP2 in *E. coli,* and contruction of transgenic tobacco plants overexpressing ATP 1. Peroxidase '99, Columbus, Ohio, USA 17-21 July.
Kristensen, B., Rasmussen, S.K. Defence response of barley after infection with powdery mildew. M.Sc. course on Resistance Genetics and Breeding. Risø, Roskilde, Denmark 25-26 February (lecture).
Kure, L.K., Ambus, P., Jensen, E.S. Uptake of organic contaminants in plants. 9th Annual meeting of SETAC-Europe, Leipzig, Germany 25-29 May (poster).
Larsen, E. Kemi i relation til tjærestoffer fra forgassere. Kollokvium på Institut for

Energiteknik, Technical University of Denmark, Lyngby, Denmark 1 December.
Larsen, E. Tjære- og Kemianalyser. Seminar om temaet "Elreformen" under opfølgningsprogrammet for decentrale kraftvarmeværker. Middelfart, Denmark 18-19 November (abstract).
Laturnus, F. Volatile Halocarbons from Marine Macroalgae. 14th Hanse-meeting, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany 14 October.
Lyngkjær, M.F, Carver, T.W.L. Potentiation of cellular resistance against powdery mildew in cereals. 13th John Innes Symposium, John Innes Centre, Norwich, Norfolk, UK 20-23 July.
Lyngkjær, M.F. Induced accessibility and inaccessibility to powdery mildew in barley and oat epidermal cells. Carlsberg, Copenhagen 19 May.
Lyngkjær, M.F. Induced cellular accessibility in susceptible and *mlo*-resistant barley. Powdery mildew interactions, Somerville College, Oxford, UK 16 April.
Lyngkjær, M.F. Microscopy of the infection process, RVAU, Copenhagen, Septoria meeting 25-26 November.
Lyngkjær, M.F. MLO resistance and virulence in the Barley-powdery mildew system. COST 817 Workshop on Disease Resistance and Cereal Leaf Pathogens beyond the Year 2000, Martina Franca, Italy 11-12 November.
Lyngkjær, M.F., Carver, T. Induced accessibility and inaccessibility to *Blumeria graminis* in cereal epidermal cells. 1st International Powdery Mildew Conference, Palais des Papes, Avignon, France 29 August—2 September.
Marttila, S., Roberts, T.H., Rasmussen, S.K., Hejgaard, J. Immunolocalization studies of barley serpins. 2nd European Symposium on Enzymes in Grain Processing ESEGP-2, Helsinki, Finland 8-10 December.
Mikkelsen, T.N., Ro-Poulsen, H. Kulstof- og vandbalance gennem fire år i et vestjydsk skovøkosystem. University of Copenhagen, Botanical Institute, Copenhagen 15 November (oral - in Danish).
Mikkelsen, T.N., Ro-Poulsen, H., Jensen, N.O., Bille-Hansen, J., Hummelshøj, P. Long term measurements of carbon dioxide exchange over a mixed temperate forest in relation to growth increment and variations in climate. Canopy Dynamics and Forest Management — A Missing Link? Joint workshop organized by the IUFRO working groups for Canopy Processes (2.01.12) and Whole Plant Physiology (2.01.15) and IGBP/GCTE's Activity Managed Forests (A 3.5), Estonia, Finland and Sweden. Joensuu, Finland 1-11 August (poster).
Mortensen, G.K. Nedbrydning af LAS i jord og optag i planter - Resultater fra væksthusforsøg. Aalborg University, Denmark 18 August.
Mortensen, G.K. Planteoptag af organiske forbindelser. VKI, Copenhagen, Denmark 27 October.
Mouritzen, P. Phage display technology to study protein-protein interaction in plant-microbe symbioses. NorFA-course on Molecular Communication in Plant-Microbe Symbioses, Risø, Roskilde, Denmark 14-25 August.
Mouritzen, P., Mirza, A., Christensen, S.K., Hejgaard, J., Giese, H. Phage display cloning of mildew proteins interacting with barley proteins on the plasma membrane. 1st International Powdery Mildew Conference, Palais des Papes, Avignon, France 29 August-3 September (abstract).
Møller, M.G., Rasmussen, S.K., Holm, P.B. Molecular cloning and characterization of asparagine synthetase from barley (*Hordeum vulgare* L.). International Conference on Assimilate Transport and Partitioning, Newcastle, Australia 15-20 August.
Nielsen, B.J., O´Hara, R.B., Østergård, H. The effect of fungicide dose on population structure of Barley powdery mildew (*Erysiphe Graminis* f.sp. *hordei*). COST 817 Workshop on Disease Resistance and Cereal Leaf Pathogens beyond the Year 2000. Martina Franca, Italy 11-12 November.
Olsson, L., Ahring, B.K., Thomsen, A.B., Klinke, H.B. Influence of inhibitors from wet-oxidized wheat straw on *Saccharomyces cerevisiae*. The International Energy Agency Workshop on Conversion of Lignocellulosic Material, Itala Game Reserve, KwaZulu-Natal, South Africa 22-26 August.
Pedersen, C. Isolation of genes by map based cloning. NorFA-course on Molecular Communication in Plant-Microbe Symbioses, Risø, Roskilde, Denmark 14-25 August (oral).
Pedersen, C. Molecular genetics of the powdery mildew fungus. RVAU, Copenhagen 26

January (unpublished).
Pedersen, C. Towards map-based cloning of avirulence genes in the barley powdery mildew fungus. 1st International Powdery Mildew Conference, Palais des Papes, Avignon, France 29 August-3 September (poster).
Pilegaard, K. Exchange of NO_x and O_3 at the soil surface. 1st DSAR conference, Eigtveds Pakhus, Copenhagen 14-15 October (oral).
Pilegaard, K. Udveksling af ozon mellem luft og overflader samt ozons effekter på planter. DaMS - Dansk Meteorologisk Selskab, Møde om OZON, H.C. Ørsted Instituttet, Copenhagen 30 September (oral - in Danish).
Rasmussen, N. In vivo ^{31}P NMR studies of P metabolism and translocation in living Arbuscular Mycorrhizal mycelia. 20th Danish NMR-Meeting, Sorø, Denmark 28-29 January (oral).
Rasmussen, S.K. Barley peroxidase gene expression. Peroxidase '99, Columbus, Ohio, USA 17-21 July.
Rasmussen, S.K. Developing barley lines with reduced grain phytate content. Annual Meeting Danish Cereal Network, Slagelse, Denmark 3-4 November.
Rasmussen, S.K. Modulation of the phosphor-availabillity in cereal grains. Biogemma, Limagrain, Aubiere, France 30 April.
Rasmussen, S.K. Strategies to improve availability of phosphate and minerals in cereals for food and feed. 2nd European Symposium on Enzymes in Grain Processing ESEGP-2, Helsinki, Finland 8-10 December.
Rasmussen, S.K., Roberts, T.H., Jensen, H.Ø., Hellgren, L., Hejgaard, J. Expression of Barley serpin genes, cloning of wheat serpins, and a loop deletion in *Arabidopsis* serpin sequences. 2nd International Symposium on the Structure and Biology of Serpins, Queens College, UK 27 June—1 July.
Rosendahl, L. Transport of nitrogen across the symbiotic interface in legume/Rhizobium symbioses. NorFA-course on Molecular Communication in Plant-Microbe Symbioses, Risø, Roskilde, Denmark 14-25 August (oral).
Rosendahl, L., Scharff, A.M., Rasmussen, N., Hansen, P.E., Jakobsen, I. Sustainable plant production using plant-microbe symbioses. 1st Environmental Science Day, Roskilde University Centre, Roskilde, Denmark 15 November (oral).
Rønn, R., Gavito, M.E., Jakobsen, I., Frederiksen, H.B., Christensen, S. Competition for carbon between symbionts and free-living organisms in the rhizosphere. New Phytologist and GCTE Symposium: Root dynamics and Global Change. Townsend, Tennessee, USA 19-22 October (poster).
Schou, C. Anvendelse af ESI-LCQ ved analyse af peptider. Møde arrangeret af Dansk Selskab for Massespektrometri, Odense, Denmark 21 October (unpublished).
Schou, C. Naturlig plantebeskyttelse mod patogene skimmelsvampe. Workshop on detection and control of diseases in potato, DIAS, Flakkebjerg, Denmark 10 November (unpublished).
Stockmarr, A. DNA-fingeraftryk i kriminalsager — kan man dømme efter store tals lov? Institut for Folkesundhedsvidenskab 11 May.
Saalbach, G. Die 14-3-3 Protein-Familie: Modulatoren von Protein-Wechselwirkung und -Aktivität. Lecture of Probation for University Teaching. Biologisch-Pharmazeutische Fakultät, Friedrich-Schiller-Universität Jena, Germany 3 May.
Saalbach, G. Klonierung, gentechnische Modifikation und intrazellulärer Transport von pflanzlichen Speicherproteinen. Defense of Thesis of Habilitation.
Biologisch-Pharmazeutische Fakultät, Friedrich-Schiller-Universität Jena, Germany 6 April.
Saalbach, G. Proteom Analyse von Symbiosomen aus *Rhizobium*-induzierten Wurzelknöllchen der Erbse. Seminar, Lehrstuhl für Genetik, Universität Bielefeld, Germany 6 December.
Poulsen, T.T. Transgen byg med forbedret sygdomsresistens. Annual Meeting Danish Cereal Network, Slagelse, Denmark 3-4 November (oral) (abstract in Danish).
Poulsen T.T., Gjetting, S., Folling, L., Gregersen, G., Bryngelsson, T., Olesen, A., Nielsen, K., Collinge, D.B., Oliver, R., Thordal-Christensen, H. Towards transgenic barley with enhanced resistance against the powdery mildew fungus. 1st International Powdery Mildew Conference, Palais des Papes, Avignon, France 29 August-3 September (poster).

Thordal-Christensen, H., Fischer, P.H., Somerville, S.C. Arabidopsis mutants with reduced resistance against the barley powdery mildew fungus. 9th International Congress in Molecular Plant-Microbe Interactions. Amsterdam, The Netherlands 25-30 July (poster).
Thordal-Christensen, H., Fischer, P.H., Somerville, S.C. Arabidopsis mutants with reduced resistance against the barley powdery mildew fungus. 1st International Powdery Mildew Conference, Palais des Papes, Avignon, France 29 August-3 September (poster).
Thordal-Christensen, H., Zhou, F., Burhenne, K., Collinge, D.B., Ehrhardt, D. Model for activation of epidermal single cell HR in the barley/powdery mildew interaction. 1st International Powdery Mildew Conference, Palais des Papes, Avignon, France 29 August-3 September (oral).

Papers Accepted for Publication

Brinch-Pedersen, H., Olesen, A., Rasmussen, S.K., Holm, P.B. Generation of transgenic wheat (*Triticum aestivum* L.) for constitutive accumulation of an *Aspergillus* phytase. Mol. Breeding.
Burleigh, S. Cloning arbuscular-related genes from mycorrhizas. Plant and Soil.
Christensen, C.S., Skov, H., Nielsen, T., Lohse, C. The influence of photochemical and transport processes on the occurrence of carbonyl compounds at northern latitudes: Lille Valby, Denmark (55ºN). Atmos. Environ.
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Feilberg, A., Holcman, J., Lohse, C., Nielsen, T., Sehested, K. Atmospheric oxidation of N-PAC and nitro substituted N-PAC in water droplets. Polycyclic Aromatic Compounds.
Feilberg, A., Kamens, R.M., Strommen, M.R., Nielsen, T. Photochemistry and partitioning of semivolatile nitro-PAH in the atmosphere. Polycyclic Aromatic Compounds.
Feilberg, A., Nielsen, T. Effect of Aerosol Chemical Composition on the Photodegradation of Nitro-Polycyclic Aromatic Hydrocarbons. Environ. Sci. Technol.
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Gissel-Nielsen, G., Gupta, U.C. Agronomic approaches to increase selenium concentration in livestock feed and food crops. In: Impact of agriculture on human health and nutrition. UNESCO.
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Grøn, C., Christensen, J.B., Jensen, D.L., Kjeldsen, P., Ostefeldt, P. Organic halogens in landfill leachates. Water, Air and Soil Pollut.
Hagen, A.R., Giese, H., Brochmann, C. (1999) Trans-atlantic dispersal and phylogeography of *Cerastium arcticum (Caryophyllaceae)* inferred from RAPD and Scar markers. Amer. J. Bot. (in press).
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Holst, P.B., Engvild, K.C. (2000) Naturlige chlorerede forbindelser. 17. Danske Planteværnskonference. DJF rapport (in press).
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Johansen, K.S., Svendsen, I., Rasmussen, S.K. Purification and cloning of the two domain glyoxalase I from wheat bran. Plant Science.
Jørgensen, J.H., Bech, C., Jensen, J. Reaction of European spring barley varieties to a population of the net blotch fungus. Plant Breeding.
Kicherer, S., Backes, G., Walther, U., Jahoor, A. Localising QTLs for leaf rust resistance and agronomic traits in barley (*Hordeum vulgare* L.) Theor Appl. Genet.
Kollist, H., Moldau, H., Mortensen, L., Rasmussen, S.K., Bolt-Jørgensen, L. Protective capacities of cell wall ascorbate against ozone in young leaves of barley (*Hordeum vulgare* L.) and wheat *(Triticum aestivum* L.). J. Plant Physiol.

Lee, S.B., Rasmussen, S.K. Molecular markers in some medicinal plants of the *Apiaceae* family. Euphytica.
Lyngkjær, M.F., Carver, T.L.W. Conditioning of cereal leaf cellular defence responses to powdery mildew by prior attack. Mol. Plant Pathol.
Madriz-Ordenaña, K., Montero, R.R., Lundsgaard, T., Ramírez, P., Thordal-Christensen, H., Collinge, D.B. (1999) Mechanical transmission of maize rayado fino marafivirus (MRFV) to maize and barley by means of the vascular puncture technique. Plant Pathol. (in press).
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Rasmussen, L. Transport og omsætning af kulstof- og kvælstofforbindelser i skov og på landbrugsarealer - SOROFLUX - et SMP projekt. In: Resume af foredrag og posters (eds. K. Secher, L.W. Brogaard, L. Thorsted). Konferencen dansk miljøforskning 1999, Copenhagen 19-20 August, DMU, Roskilde, p. 48 (abstract).
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Rasmussen, N., Lloyd, D., Ratcliffe, R.G., Hansen, P.E., Jakobsen, I. ^{31}P NMR for the study of P metabolism and translocation in arbuscular mycorrhizal fungi. Plant and Soil.
Ravnskov, S. Effects of Pseudomonas fluorescens DF57 on growth and uptake of two arbuscular mycorrhizal fungi in symbiosis with cucumber. Mycorrhiza, Springer Verlag.
Rosendahl, L., Mouritzen, P., Rudbeck, A. Nitrogen transfer in the interface between the symbionts in pea root nodules. Plant and Soil.
Rosendahl, L., Rudbeck, A., Scharff, A.M., Mouritzen, P. Assimilation of fixed nitrogen studies by membrane vesicle transport and *in vivo* ^{15}N-NMR spectroscopy in pea. In: Nitrogen Fixation: From Molecules to Crop Productivity (eds. F.O. Pedrosa, M. Hungria, M.G. Yates, W.E. Newton) Proceedings 12th International Congress on Nitrogen Fixation, Foz do Iguazu, Brazil 12-17 September, Kluwer Acad. Publ.
Schweiger, P.F., Jakobsen, I. Laboratory and field methods for measurement of hyphal uptake of nutrients from soil. Plant and Soil.
Shim, S.I., Jørgensen, R.B. Genetic structure in cultivated and wild carrots (*Daucus carota* L.) revealed by AFLP analysis. Theor. Appl. Genet.
Struck, U., Emeis, K.C., Voss, M., Christiansen, C., Kunzendorf, H. Records of southern and central Baltic Sea eutrophication in $\delta\,^{13}C$ and $\delta\,^{15}N$ of sedimentary organic matter. Mar. Geol.
Poulsen, T.T., Bryngelsson, T., Collinge, D.B., Nielsen, K., Oliver, R., Thordal-Christensen, H. Towards Transgenic Barley With Enhanced Disease Resistance. Proceedings 3rd Annual Meeting of Danish Cereal Network, Slagelse, Denmark 3-4 November.
Thingstrup, I., Kahiluoto, H., Jakobsen, I. Phosphate transport by *hyphae* of field communities of arbuscular mycorrhizal fungi at two levels of P fertilisation. Plant and Soil.
Tomiuk, J., Hauser, T.P., Jørgensen, R.B. A- or C-chromosomes, does it matter for the transfer of transgenes from *Brassica napus*? Theor. Appl. Genet.
Zeyen, R.J., Carver, T.L.W., Lyngkjær, M.F. The Formation and Role of Papillae. In: The Powery Mildews: A Comprehensive Treatise. Book chapter.

Education

Ph.D. Theses

Feilberg, A. Polycyclic Aromatic Compounds, particularly nitro-PAH-sources and atmospheric chemical processes. Risø National Laboratory and University of Southern Denmark, Odense. November 1999.
Platz, J. Atmospheric chemistry of traffic related compounds. Oxygenates and aromatics. Risø National Laboratory and University of Southern Denmark, Odense. February 1999.
Stürup, S. Development, optimisation, and application of ICP-SFMS methods for the measurement of isotope ratios. Risø National Laboratory and Technical University of Denmark. November 1999.

Thomsen, A.B. Combined Wet Oxidation and Biological Treatment of Creosote Compounds in Soil with Special Attention to Quinoline. Risø National Laboratory and Aalborg University.

M.Sc. Theses

Bondo-Larsen, L. Use of exotic material and AFLP markers in oilseed rape (*Brassica napus* L.) breeding. Risø National Laboratory and The Royal Veterinary and Agricultural University, Copenhagen, Denmark.
Borch, T. Nedbrydning af flygtige chlorerede alifater i reducerede mikromiljøer i ikke vandmættede organiske jorde. Risø National Laboratory and University of Copenhagen, Denmark.
Broeng, S. MADS box genes in *Lolium perenne* L. Risø National Laboratory and The Royal Veterinary and Agricultural University, Copenhagen, Denmark.
Christensen, H.D. Hastighedskonstanter for udeuteret og deuteret acetaldehyds reaktion med F, Cl og OH bestemt ved pulsradiolyse af UV detektion. Risø National Laboratory and University of Copenhagen, Denmark.
Dræby, I. Flower initiating *LEAFY* and *APETALA1* homologues isolated from *Lolium perenne* L. Risø National Laboratory and The Royal Veterinary and Agricultural University, Copenhagen, Denmark.
Hansen, K.A. Isolering og karakterisering af GaMyb i *Lolium perenne L.*, samt gibberellin induktion af vegetative planter af samme art. Industribachelor projekt. Risø National Laboratory and University of Copenhagen, Denmark.
Hansen, L. Introgression between oilseed rape (*Brassica napus*) and *Brassica rapa* in a natural population. Risø National Laboratory and University of Copenhagen, Denmark.
Haselmann, K.F. Chloroform og andre flygtige chlorerede organiske forbindelser i det terrestriske miljø. Risø National Laboratory and University of Copenhagen, Denmark.
Jensen, H.Ø. Wheat Serpins. Identification, cloning, expression and characterization. Risø National Laboratory and Technical University of Denmark.
Mirza, A. Gene technology or not. Purification of plasma membrane from coleoptiles of barley seedlings. Department of Development and Planning, Aalborg University, Denmark.
Nylev, P. Molekylærbiologisk undersøgelse af inositol-1,2,3-triphosphat-5/6-kinase-gen fra byg. Risø National Laboratory and The Royal Veterinary and Agricultural University, Copenhagen, Denmark.
Poulsen, M. Forekomsten af 6 partikel-associerede, mutagene nitro-PAH i troposfæren over Danmark. Risø National Laboratory and University of Copenhagen, Denmark.
Prip, D.V. Hovedrapport — Ingeniør Praktik F99. Risø National Laboratory and Technical University of Denmark.

External Examiners

Gissel Nielsen, G. Censor in plant nutrition and crop physiology at The Royal Veterinary- and Agricultural University, Copenhagen.
Gundersen, V. Censor in chemical analysis at Technical University of Denmark.
Jakobsen, I. Censor at University of Copenhagen and University of Aarhus.
Jensen, A. Censor in biology at all Danish Universities.
Jørgensen, R.B. Censor in molecular biology at University of Aarhus.
Nielsen, O.J. Censor in chemistry at University of Copenhagen and University of Southern Denmark, Odense.
Pedersen, C. Censor in biotechnology at Slagteriskolen, Roskilde.
Pilegaard, K. Censor in ecology at University of Copenhagen.
Censor in air pollution at Technical University of Denmark.
Rasmussen, L. Censor in ecology at University of Copenhagen.
Censor in environmental sciences at Technical University of Denmark.
Rasmussen, S.K. Censor at The Royal Veterinary and Agricultural University, Copenhagen.
Rosendahl, L. Censor in biology at all Danish Universities.
Østergård, H. Censor in biology at University of Aarhus and University of Copenhagen.

External Teaching and Lectures

Jakobsen, I. Jordbundsbiologi - temadag, KU. Titel: Betydningen af arbuskulær mykorrhiza

for planters næringsoptagelse, University of Copenhagen, Denmark, 28 August.
Jakobsen, I. Plantebiokemi, KVL. Titel for dobbeltforelæsning: Planters fosfatoptagelse, The Royal Veterinary and Agricultural University, Copenhagen, Denmark, 6 November.
Rasmussen, S.K. Lecture in Plant Biochemistry at The Royal Veterinary and Agricultural University, Copenhagen, Denmark, 19 November.

Guest Lectures

Hansen, P.E. Roskilde University Centre, Denmark:
In vivo NMR — Hvad kan man og hvad kan det bruges til. 18 March.
Grogan, P. University of Copenhagen, Denmark*:*
Controls on ecosystem net carbon balance in the Arctic. 8 April.
Rasmusson, A. University of Lund, Sweden*:*
Towards a molecular understanding of respiratory NAD(P)H dehyrogenases in plants. 15 April.
Larsen, J. Research Centre Flakkebjerg, Denmark:
Mycelial interactions between arbuscular mycorrhizal fungi and saprotrophic fungi in soil. 22 April.
Abbott, L. The University of Western Australia:
How do scientists explain sort biological fertility to land managers. 20 May.
Torry-Smith, M. Technical University of Denmark:
Anaerob rensning af procesvand til bioethanol-produktion. 17 June.
Harrison, M.J. Samuel Roberts Noble Foundation, Oklahoma, USA:
Characterization of phosphate transporter expression in mycorrhizas. 15 August.
Joner, E. CNRS Vandoevre-les-Nancy, France:
Compartmentation techniques for *in vivo* transport studies in symbioses. 16 August.
Somerville, S. Stanford University, California, USA:
The use of microarray tools to characterize the interaction between micro-organisms and plants. 18 August.
Raudaskoski, M. University of Helsinki, Finland:
Role of cytoskeletons in membrane transport processes. 18 August.
Bergman, B. University of Stockholm, Sweden:
Chemical signalling in Gunnera-Nostoc symbioses. 20 August.
Day, D.A. Australian National University, Canberra, Australia:
Metabolite transport through symbiotic membranes. 20 August.
Hansen, P.E. Roskilde University Centre, Denmark:
In vivo NMR as a tool to study nutrient transport and assimilation in plants. 24 August.
Rasmussen, S.W. Carlsberg Research Laboratory, Denmark:
Large scale cDNA sequencing — EST databases. 24 August.
Harrison, M.J. Samuel Roberts Noble Foundation, Oklahoma, USA:
Molecular and genetic approaches to analyse the development and functioning of an arbuscular mycorrhizal symbiosis. 16 September.
Mäck, G. Universität Göttingen, Germany:
Glutamine Synthetase in Sugarbeet. 28 Oktober.
Schweizer, P. University of Zürich, Switzerland:
Functional transscriptosome analysis in wheat-prospects for cereal biotechnology. 29 October.
Platz, J. Danish National Environmental Research Institute:
Alternative brændsler til biler - ether og aromater. 4 November.
Ferstad, H-G. Norges Landbruks Høgskole, Ås, Norway:
Isolation of important promoter elements for aleurone specific expression of the barley. 11 November.
Haugaard, H. The Royal Veterinary and Agricultural University, Copenhagen, Denmark:
The ability of mycelial extracts to protect barley against powdery mildew. 18 November.
Gregersen, P. The Royal Veterinary and Agricultural University, Copenhagen, Denmark:
Gene expression in the barley powdery mildew pathosystem. 25 November.
Boiffin, V. Philipps Universität Marburg, Germany:
Symbioses and defence in the interaction of plant and micro-organism. 16 December.

Committee Membership

National

Giese, H. OECD Committee for Biotechnology.
Danish Society for the Conservation of Nature, Committee for Environmental Issues.
Gissel-Nielsen, G. Danish Academy of Technical Sciences.
Research Centre for Organic Farming.
Danish Society for the Conservation of Nature, Committee for Environmental Issues.
Board member of University Extension Service.
Jahoor, A. Member of Danish Gene Bank Committee.
Management Committee for EU-Project (Genres CT98.104).
Jensen, A. Member of the board of directors for Pajbjerg Foundation, Denmark.
Member of The Danish Academy of Technical Sciences.
Jørgensen, R.B. Environmental Appeal Board, The Ministry of Environment and Energy.
Board member, The Danish Environmental Research Programme. "Centre for Effects and Risks of Biotechnology in Agriculture".
Larsen, E. Danish Society of Mass Spectrometry.
The Danish National Committee for Chemistry.
Nielsen, T. Board member of the Centre of Biological Processes in Contaminated Soil and Sediment.
Rasmussen, L. Board member of the Centre for Sustainable Land Use and Management of Contaminants, Carbon and Nitrogen.
Rasmussen, S.K. Society of Danish Engineers, Board of Chemistry Section.
Board of Danish Cereal Association.
Østergård, H. Ph.D. Committee for Education, The Royal Veterinary and Agricultural University, Copenhagen.
Co-ordination group for resistance and virulence in cereals and cereal pathogens in Denmark.
Board of Centre for Effects and Risks of Biotechnology in Agriculture (The Danish Environmental Research Programme).
Head of Board of Cereal Network Group "Cereals without Pesticides".

International

Ambus, P. U.S. Trace Gas Network (TRAGNET), working group.
Giese, H. Editorial board, Plant Pathology on line.
OECD committee for Biotechnology.
Jakobsen, I. Member of Advisory Board for New Phytologist.
Member of Management Committee of "COST 838 Managing arbuscular mycorrhizal fungi for improving soil quality and plant health in agriculture".
Jørgensen, R.B. Member of Panel of Experts, International Biosafety Forum, Third World Network.
Board member, European Science Foundation Programme "Assessing the Impacts of genetically Modified Plants (AIGM)".
Kunzendorf, H. Editorial board, Marine Georesources and Geotechnology.
Larsen, E. European Commission, Access to Large-Scale Facilities, Technical Audit of the HCM-LSF contracts.
Pilegaard, K. Member of steering committee for BIATEX-2 under EUROTRAC-2.
Rasmussen, L. Member of the Swedish Research Council for Forestry and Agriculture, Section for Biogeochemistry.
Member of Editorial Board for Forest Ecology and Management.
Thomsen, A.B. IEA - Bioenergy agreement Task XIII. Bioconversion activity. Alternative member of committee.
Østergård, H. Chairperson for COST 817 Management Committee on Population Studies of Airborne Pathogens on Cereals as a Mean of Improving Strategies for Disease Control.
Editorial board of Agronomie.

Seminars and Courses Organized

NorFA-course on Molecular Communication in Plant-Microbe Symbioses, Risø National Laboratory 14-25 August 1999 (L. Rosendahl).
Pesticide effects on the agricultural soil ecosystem — Final workshop, Gentofte, Denmark 7-8 October 1999 (I. Jakobsen).
Resistance Genetics, M.Sc. Course, Risø National Laboratory, 22-25 February 1999 (A. Jahoor).
DNA markers in Plant Breeding, Ph.D. Course, Risø National Laboratory 14-16 June 1999 (A. Jahoor, G. Backes).
10th Nitrogen Workshop, The Royal Veterinary and Agricultural University, Copenhagen, Denmark 23-26 August 1999. (E.S. Jensen, K. Pilegaard).

Personnel

The research activities are organized into 6 research programmes and supported by special facility units.
The Department includes 74 full time scientific staff members and 42 full time technical staff members.
The list also includes short-term employees.

Head of Department
Arne Jensen

Research Programmes

Plant-Microbe Symbiosis
Head: Henriette Giese

Plant Products and Recycling of Biomass
Head: Søren K. Rasmussen

DLF-Risø Biotechnology
Head: Klaus K. Nielsen

Plant Genetics and Epidemiology
Head: Hanne Østergård

Biogeochemistry
Head: Lennart Rasmussen

Plant Ecosystems and Nutrient Cycling
Head: Gunnar Gissel Nielsen

Special Facility Units

Risø Integrated Environmental Facility (RIMI)
Head: Kim Pilegaard

Risø Environmental Risk Assessment Facility (RERAF)
Head: Teis Mikkelsen

Growth chambers, greenhouses and the experimental farm, Dyskærgaard.

Scientific Staff

Ambus, Per
Andersen, Claus H.
Aubert, Dominique
Backes, Gunter
Baunsgaard, Lone
Bechmann, Iben Ellegaard
Beier, Claus
Bjergbakke, Erling (until 31.03.99)

Burleigh, Steven
Christensen, Anders B.
Christensen, Lene Krogh (until 31.08.99)
Christiansen, Solveig Krogh
Didion, Thomas
Egsgaard, Helge
Engvild, Kjeld C.
Feilberg, Anders
Gavito, Mayra
Giese, Henriette
Gissel Nielsen, Gunnar
Grøn, Christian (until 31.07.99)
Gundersen, Vagn
Hatzack, Frank
Holcman, Jerzy (until 30.04.99)
Jahoor, Ahmed
Jakobsen, Iver
Jensen, Erik Steen
Jensen, Jens
Jensen, Lisbeth Gath
Jørgensen, Rikke Bagger
Kristensen, Brian
Kunzendorf, Helmar
Kure, Liv
Lange, Sabine
Larsen, Elfinn
Laturnus, Frank
Lett, Christophe
Lynggård, Bent
Lyngkjær, Michael
Mikkelsen, Teis Nørgaard
Mortensen, Gerda Krog
Mouritzen, Peter
Nielsen, Klaus K.
Nielsen, Ole John (until 30.09.99)
Nielsen, Torben
Nilsson, Karen (until 31.07.99)
Pagsberg, Palle (until 30.11.99)
Pedersen, Carsten
Pilegaard, Kim
Rasmussen, Lennart
Rasmussen, Søren Kjærsgård
Ravnskov, Sabine (until 31.08.99)
Richter, Hannes
Rosendahl, Lis
Salchert, Klaus-Dieter
Schmidt, Anette Skammelsen
Schou, Christian
Sehested, Jens (until 28.02.99)
Sehested, Knud (until 31.12.99)
Stockmarr, Anders
Storgaard, Morten
Stürup, Stefan
Saalbach, Gerhard
Thomsen, Anne Belinda
Thordal-Christensen, Hans
Woidemann, Anders

Wu, Boqian
Østergård, Hanne

Technical Staff

Andersen, Bente
Andersen, Margit Elm
Bonde, Rikke
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Carlsen, Merete
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Djurdjevic, Stanko
Fernqvist, Tomas
Fosskov Jensen, Jette
Gudiksen, Peter
Hansen, Ina
Hansen, Ivan (until 31.08.99)
Hasselbalch, Finn
Ibsen, Elly
Jensen, Birgit
Jensen, Ellen Møller
Jensen, Linette Munksgaard
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Larsen, Inge Merete
Larsen, Ingelis
Larsen, Tina Bøgeskov
Meltofte, Liselotte
Møller, Anette (until 30.04.99)
Møller, Trine (until 31.07.99)
Nielsen, Anja Christina
Nielsen, Jette Bruun
Nielsen, Vagn Aage
Olsen, Anette
Olsen, Anne
Olsen, Inge
Petersen, René
Sillesen, Anerikke
Storm Petersen, Anne-Mette
Sørensen, Poul
Tung, Tran Duc Tuan
Vestesen, Hans (until 31.12.99)
Vinther Kristensen, Lis
Wojtaszewski, Hanne

Administrative Staff

Bay, Kirsten
Borring Sørensen, Marit
Christiansen, Krista
Frandsen, Anette
Hjorth, Aase
Jensen, Hanne
Krogh, Helle
Lilholt, Ulla
Løje, Søren
Petersen, Lis

Ph.D. Students

Bruhn, Dan
Burhenne, Kim
Erik, Pinar
Eriksen, Lars B.
Feilberg, Anders (until 30.06.99)
Frøsig, Lars
Gavnholt, Britta
Grell, Morten
Hansen, Poul Møller
Hauggaard-Nielsen, Henrik
Holst, Pia Bachmann
Jensen, Christian Sig
Johannessen, Marina
Johansen, Katja Salomon
Johansen, Runa Ulsøe
Jonassen, Kristoffer
Jørgensen, Rasmus Nyholm
Klinke, Helene B.
Møller, Marianne Gellert
Nielsen, Jock
Petersen, Klaus
Platz, Jesper (until 15.02.99)
Poulsen, Tina Tandrup
Rasmussen, Nanna
Scharff, Anne Marie
Storgaard, Morten (until 31.07.99)
Stürup, Stefan

M.Sc. and B.Sc. Students

Arp, Thomas
Asser Hansen, Kirsten
Bohn, Vibeke
Bondo-Larsen, Louise
Borch, Thomas
Broeng, Stine
Christensen, Hasse Dyhr
Christophersen, Helle
Dræby, Ingrid
Fischer, Pernille Hertz
Hansen, Lise
Haselmann, Kim
Haugaard, Helle
Holmegaard Nielsen, Anne
Jensen, Henrik Østergaard
Milandt, Jan
Mirza, Almas
Mønster, Henrik Ringgaard
Nielsen, Kristina Vad
Nylev, Peter
Pertl, Maria
Poulsen, Morten
Prip, Dorthe Vinkel
Ringgård, Trine
Stein, Thomas N.N.
Thim, Per

Apprentices

Abdellahi, Ebtisan
Carlsen. Merete
Dyrberg, Mette
Hansen, Helle
Hansen, Lisbeth
Hasselsteen, Pia
Heinvig, Tania
Jensen, Brian Arnt
Niebuhr, Lene
Nielsen, Thomas
Rasmussen, Winnie
Thomsen, Anders K.
Udbjørg, Charlotte

Visiting Scientists

Abbott, L. Soil Science and Plant Nutrition, The University of Western Australia (2 months).
Aveline, A. Ecole Supérieure d'Agriculutre, Angers, France (2 months).
Bergman, B. University of Stockholm, Sweden (1 week).
Blaskova, V. Research Institute of Crop Production, Ruzyne Prague, Czech Republic (3 weeks).
Bousset, L. INRA, Grignon, France (2 weeks).
Day, D.A. Australian National University, Canberra, Australia (2 weeks).
Ferstad, H-G. Norges Landbruks Høgskole, Ås, Norway (3 weeks).
Harrison, M.J. Plant Biology Division, Samuel Roberts Noble Foundation, Oklahoma, USA (2 months).
Herz, M. Technical University of Munich, Germany (2 weeks).
Hübner, M. University of Göttingen, Germany (3 months).
Jacobsen, F. HOH Water Technology, Denmark (1 year).
Joner, E. CNRS Vandoevre-les-Nancy, France (2 weeks).
Lundström, T. Lindköping Institute of Technology, Sweden (3 months).
Mouritzen, P. Technical University of Denmark (7 months).
Mulder, L. Cereal Research, John Innes Centre, UK (5 weeks).
Olsson, P.A. University of Lund, Sweden (8 months).
Ovesna, J. Research Institute for Crop Production, Ruzyne Prague, Czech Republic (5 weeks).
Raudaskoski, M. University of Helsinki, Finland (1 week).
Sabbagh, A. International Centre for Agriculture, Research in the Dry Areas, Aleppo, Syria (4 months).
Sayed, H. International Centre for Agriculture, Research in the Dry Areas, Aleppo, Syria (2 months).
Shim, S.I. Department of Agronomy, College of Natural Resources, Korea University, Seoul, Korea (6 months).
Sip, V. Research Institute for Crop Production, Ruzyne Prague, Czech Republic (1 week).
Somerville, S. Carnegie Inst. of Washington, Stanford University, California, USA (2 weeks).
Zeuthen, J. University of Copenhagen, Denmark (3 months).

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Title and authors

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Department or group

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