Plant Biology and Biogeochemistry Department annual report 1999

Jensen, Arne; Gissel Nielsen, G.; Giese, H.; Nielsen, K.K.; Rasmussen, L.; Rasmussen, S.K.; Østergård, Hanne

Publication date:
2000

Document Version
Publisher's PDF, also known as Version of record

RISØ

Plant Biology and Biogeochemistry Department

Annual Report 1999

Risø National Laboratory

April 2000

Risø-R-1159 (EN)

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 printed version of the annual report from the Plant Biology and Biogeochemistry Department aims to provide information about the progress in our research. Each programme 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.

Hopefully this report will provide a useful introduction to the research and development in the Department, and assist collaborators, companies and other interested groups in connecting to the scientific innovation and results produced in the Department during the year 1999. A more comprehensive and detailed account off results and progress in our research projects can be accessed through the Risø web-site www.Risoe.dk/pbk.

Risø-R-1159(EN)

Plant Biology and Biogeochemistry Department

Annual Report 1999

Edited by A. Jensen, G. Gissel Nielsen, H. Giese, K. K. Nielsen
L. Rasmussen, S. K. Rasmussen, H. Østergård.

Risø National Laboratory
Roskilde · Denmark

April 2000

Contents

Introduction

Arne Jensen, Head of Department

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.

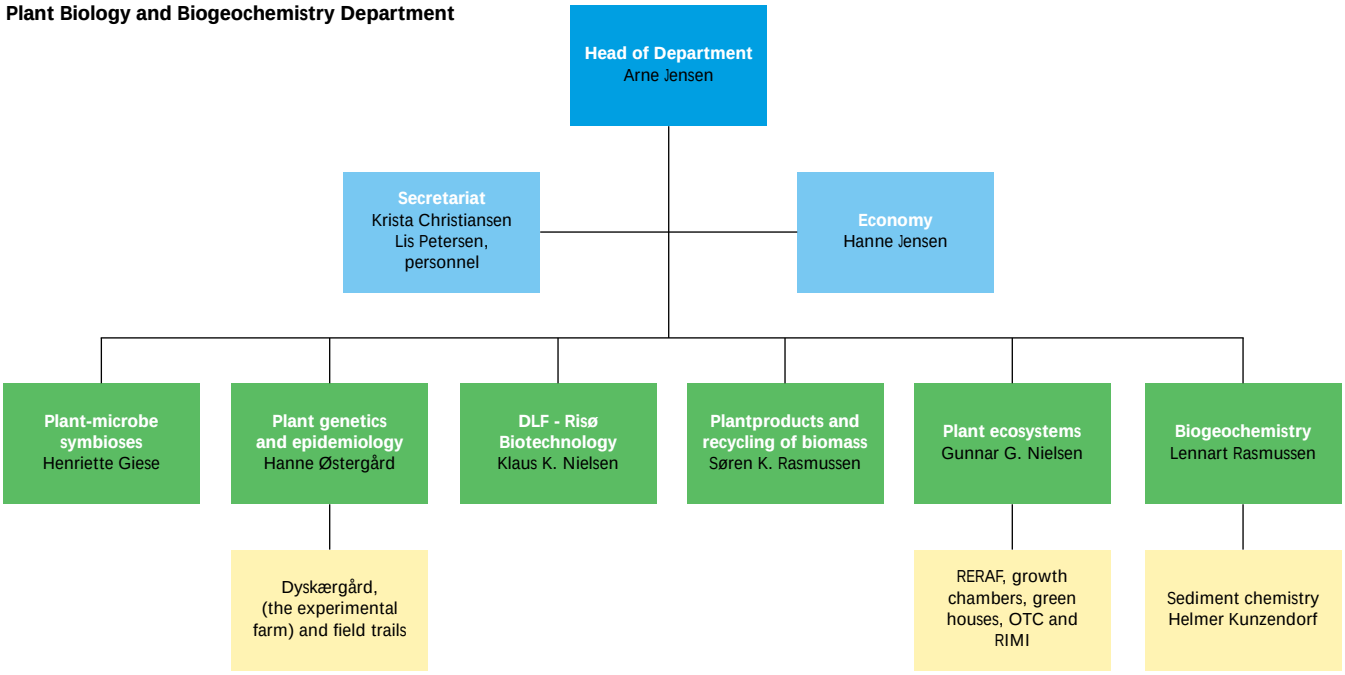
The results of the research and development are disseminated internationally to companies, institutions, organizations and public authorities through scientific publications, research reports, lectures and posters at scientific- and other professional meetings, personal communication with collaborators 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.

Plant Biology and Biogeochemistry Department



Brief Introduction of the Research Programmes

Henriette Giese,
Head of Programme

Plant Microbe Symbioses

The programme seeks to identify and characterize genes and processes involved in the interaction between plants and microbes. The organisms under study include *Rhizobium*/pea, arbuscular mycorrhizal (AM) fungi/tomato and *Medicago truncatula, Blumeria graminis* f.sp. *hordei (Bgh)*/barley, *Erysiphe Bgh/Arabidopsis thaliana, Telletia caries*/wheat and *Phytopthora infestance*/ potato. 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.

Klaus K. Nielsen,
Head of Programme

DLF-Risø Biotechnology

The main research objective of the consortium is to acquire the basic knowledge, required 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.

Søren K. Rasmussen,
Head of Programme

Plant Products and Recycling of Biomass

The programme aims at developing plants with better quality by use of modern 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 carbohydrates potentially available from biomass waste products such as wood chips and agricultural residues or purposely grown crops as chemical raw materials for high value products and fibres are investigated. Identification of natural products (metabolites), which are important for plant development and their potential as primers for production of new high value products, is a new challenge for this programme.

Gunnar Gissel Nielsen,
Head of Programme

Plant Ecosystems and Nutrient Cycling

The research is concentrated on the turnover of nutrients and a number of other important compounds in the soil-plant-atmosphere system. Research is focused on processes and turnover of compounds, the influence of climate changes (carbon dioxide, water, and temperature) on the plant ecosystems, fluxes of volatile compounds, and the genetic background for nutrient uptake.

Hanne Østergård,
Head of Programme

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 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.

Lennart Rasmussen,
Head of Programme

Biogeochemistry

Research in the programme is focused on the occurrence, transport, turnover as well as the effects of trace elements and organic micro contaminants in agricultural and forest ecosystems. The effects of air pollution and global change are studied, both at the plant and at the ecosystem level. 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.

Research Projects

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

Plant-Microbe Symbioses

Research

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, *Erysiphe Bgh/Arabidopsis thaliana, Telletia caries*/wheat and *Phytopthora infestance*/ potato. 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.

Results

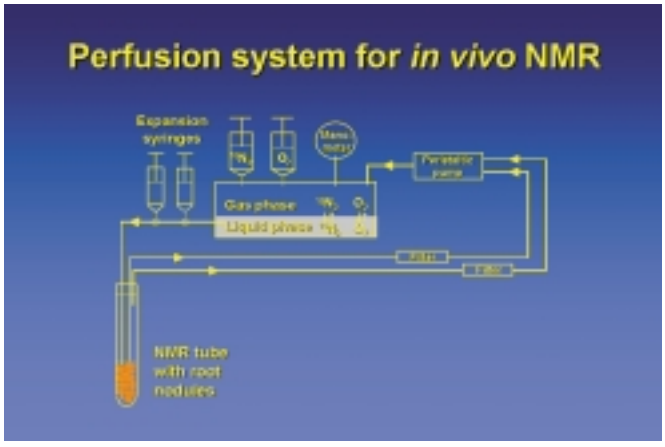
The assimilation of symbiotically fixed nitrogen in the *Rhizobium*/pea system has been studied using $^{15}N_2$ in a gas tight perfusion system for *in vivo* ^{15}N-NMR spectroscopy. The results demonstrate that the first ^{15}N-labelled amino acids to exceed the detection limit are glutamate and asparagine.

A study of proline-rich proteins has shown that these proteins are highly expressed in N_2-fixing nodules and that a tissue-enhanced isoform was located in the interface between the symbionts. Free-living *Rhizobia* have been shown to take up phosphate at a substantially higher rate than that of the symbiotic bacteroid form. There is no indication for the presence of a P-starvation induced high-affinity P-transporter in symbiotic *Rhizobium* bacteroids.

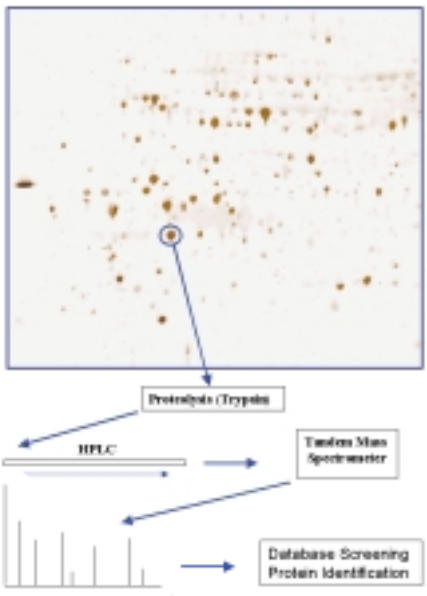
Symbiosomes from the legume-*Rhizobium* symbiosis have been sub-fractionated and analysed by 2D electrophoresis. Analysis of the silver-stained 2D gels using the PDQuest software (Bio-Rad) revealed the presence of hundreds of proteins in the different fractions. Spots from 2D gels were digested and the resulting peptides were analysed by LC-MS using a LCQ mass spectrometer (Finnigan). So far, 13 out of approx. 40 spots could be identified by database search (Sequest).

The effects of AM fungal colonization on the expression of plant P-transporter genes have been found to vary according to the fungal and plant species under study. A zinc transporter gene and a putative nitrate transporter gene from *Medicago truncatula* have been characterized and their expression studied in mycorrhizas.

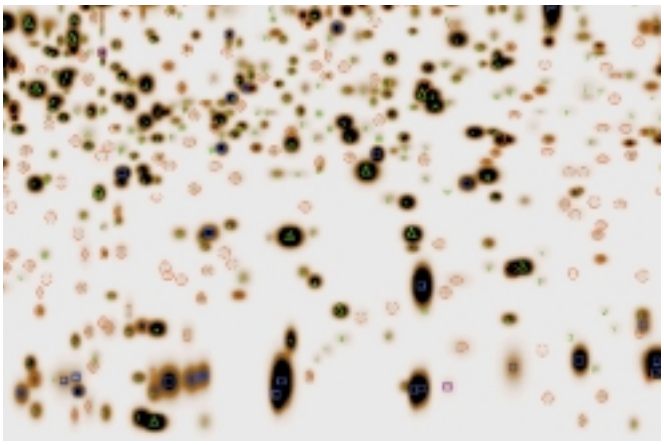
^{31}P-NMR spectroscopy has been used to show that orthophosphate (Pi) taken up by an AM fungus was transformed into polyphosphate (PolyP) with a short chain length. Mycelium excised from the plant roots had the capacity to synthesize PolyP from Pi applied after mycelial excision. Monoxenic cultures of mycorrhizas formed with Ri T-DNA transformed carrot roots were used to show that hyphae of an AM fungus could utilize P from the simple organic P compound adenosine monophosphate (AMP). A study with *Pisum sativum* showed that growth of mycorrhizal plants responds more to elevated at-



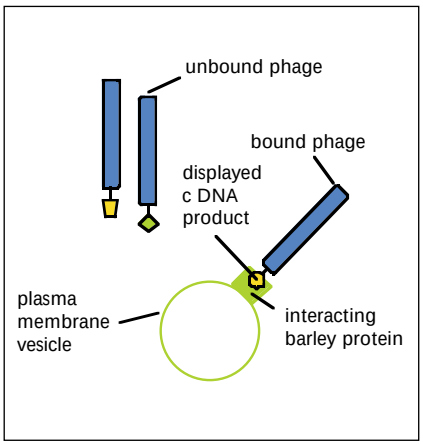
Gas tight perfusion system for *in vivo* ^{15}N-NMR spectroscopy.



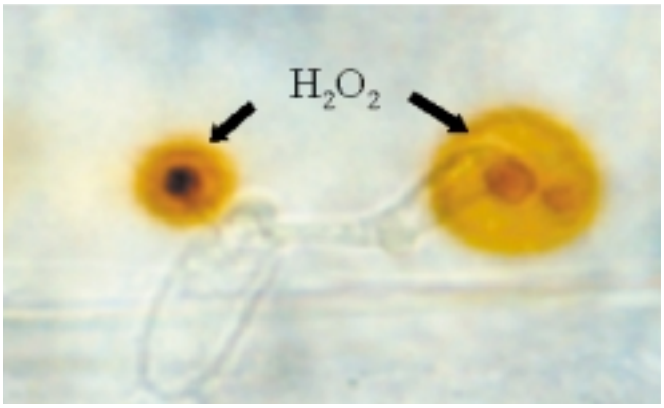
Hundreds to thousands of proteins can be resolved on a 2D gel. The protein spots provide enough material for their identification by mass spectrometry.



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).



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.



Accumulation of H_2O_2 in papillae induced by *Bgh* attempting to invade a barley leaf. This H_2O_2 is potentially generated by *Hv*OxOLP.

mospheric CO_2 than growth of nonmycorrhizal plants. This suggests that the carbon sink activity of the AM fungal symbiont may limit plant growth at ambient CO_2.

Three barley genes, which are up regulated in roots subjected to P starvation, have been cloned and sequenced. One has homology to the nicotianamine synthase gene that is involved in the biosynthesis of phytosiderophores and also induced by Fe-deficiency.

Plant disease resistance is based on a multi-component defence response, mounted upon attack by potential pathogens. In the *Bgh/Arabidopsis* system these defence mechanisms have been studied using *Arabidopsis* mutants lacking penetration resistance towards the powdery mildew fungus. Analysis of the biochemical function of a potential H_2O_2-generator protein (*Hv*OxOLP) from barley, which accumulates during interaction with *Bgh*, has been prepared.

The effect of an increased level of antifungal proteins in transgenic barley plants on infection by *Bgh* has been studied.

In our work on isolating avirulence genes from *Bgh* we have used two approaches: map-based cloning and phage display technology. A detailed genetic map has been constructed and we have isolated BAC clones containing EST-markers closely linked to the a6 avirulence gene. A λ phage display vector has been modified to subclone cDNA libraries constructed from barley epidermis strips infected with *Bgh*.

Pure preparations of epidermis plasma membrane vesicles from barley coleoptiles have been obtained for panning experiments.

A putative *Bgh* phosphate transporter gene and a barley oligopeptide transporter gene have been isolated and characterized to examine nutrient transport in the *Bgh*/barley haustoria. Two different *Bgh* genes encoding putative hydrolases belonging to the serine protease family are characterized to examine the role of hydrolases during Bgh infection of barley.

The expression profile of 16 different *Bgh* genes isolated by differential display has been determined. Two novel genes expressed specifically at the haustorial stage have been characterized in detail. 2D electrophoresis has been used to identify dif-

ferentially expressed genes in different races of *Bgh*. Seven proteins have been extracted from the gels and are now analysed by mass spectrometry for identification.

Bgh isolates, transformed with a vector construct, containing resistance towards Basta have survived several generations of selections. In addition to this, the bioluminiscent marker GFP has been demonstrated in all stages of fungal growth, thus providing a method to study gene expression.

The molecular marker system, AFLP, has been established for genetic analysis of *Tilletia caries*. The results indicate little genetic variation for this species in Denmark.

Cuticle wax of potato 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.

Patent

A transformed obligate plant symbiont. Danish Patent Application No. PA 1999 01011, filed 12 July 1999.

Collaboration at Risø and in Denmark

We have collaborated with Optics and Fluid Dynamics Department, Risø on the visualization of cellular movements in mycorrhizal hyphae.

Antibodies have been raised against *Bgh* proteins in collaboration with J. Hejgaard, DTU.

Identification of barley genes induced by P starvation is carried out in collaboration with N.E. Nielsen, RVAU in the framework "Fremtidens Kulturplanter", Crops for the Future.

Studies of interactions between AM fungi, biocontrol bacteria, and root pathogens have been carried out in collaboration with O. Nybroe, RVAU and J. Larsen, DIAS.

Carbon allocation patterns in relation to AM fungi and climate change have been studied with B.T. Christensen, DIAS.

The genetic analysis of *Tilletia caries* is carried out in collaboration with The Danish Plant Breeders and B. Nielsen, DIAS (Flakkebjerg) in the CERNET centre, the Cereal Network.

International Collaboration

The preliminary results of a collaboration with J. Brown, John Innes Institute, UK, on *Bgh* genetics have shown that our *Bgh* cross shows a recombination rate about five times higher than the comparable English cross using identical markers.

K. Mendgen, Konstanz University, Germany, is trying to perform *in situ* localization using our antibodies against the gene products of two differentially expressed *Bgh* genes, *Egh*7 and *Egh*16.

We are participating in CEREPAT an EU funded collaboration between 7 European universities and 5 Biotech industries.

Funding from the programme "Human Potential – Research Training Networks" under EU 5th Framework has been granted for participation in the network entitled "Research training using *Lotus japonicus*: A model legume for functional genomics".

We collaborate with M.J. Harrison, Noble Foundation, Oklahoma, USA, S.E. Smith and A.F. Smith, The University of Adelaide, South Australia and E. Joner, CRNS, Nancy, France, in our studies on P relationships of arbuscular mycorrhizas.

Collaboration with Companies

A collaborative agreement has been signed for one year with Bayer, Germany.

Competences and new Techniques

Proteome analysis has been successfully established for the study of the *Rhizobium*/pea and *Bgh*/barley symbioses. 2D electrophoresis and mass spectrometric analyses of peptides have been established.

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*. Methods for purification of proteins expressed in *E. coli* have been established to facilitate the production of antibodies.

Relative quantitative reversetranscription PCR techniques have been developed to study the expression of plant nutrient transporters in mycorrhizas and differentially expressed genes in *Bgh*.

An aqueous polymer two-phase partitioning method has been optimized to obtain pure preparations of epidermis plasma membrane vesicles from barley coleoptiles to be used in the phage display system.

A hydroponic system has been established for barley to allow manipulation of P availability. This has been used to identify P-starvation induced genes in barley roots.

Monoxenic cultures of mycorrhizas formed with Ri T-DNA transformed carrot roots have been established for use in molecular studies and P uptake studies of AM fungi.

Education

During 1999 A. Mirza conducted experimental work at Risø which is described in her Master Thesis. She later obtained the Master of Science degree at the Department of Development and Planning, Aalborg University, Denmark.

As a part of the B.Sc. study in chemistry, D.V. Prip has investigated the thermal transformation of phenols related to lignin, aiming at the identification of routes to aromatic hydrocarbons (PAH).

The Centre for Plant-Microbe Symbioses carried out a two-week Nordic Ph.D. course financed by NorFA. Laboratory work was integrated with theoretical discussions and lectures given by internationally leading scientists and by Nordic teachers within the field of plant-microbe symbioses. 15 Ph.D. students enrolled at Nordic universities and representing 11 countries from all over the world attended the course.

A Ph.D. course "Seminars in Plant-Microbe Symbioses" has been carried out in collaboration with RVAU.

Plant Products and Recycling of Biomass

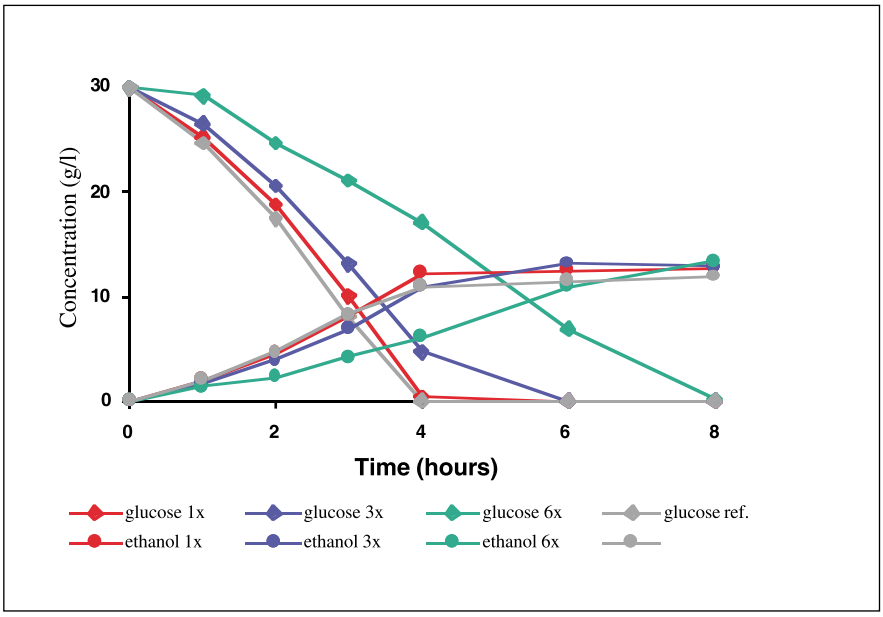
This programme was established to develop plants with better quality by use of modern breeding technology. This included a production of transgenic plants, particularly cereals, in order to create an overproduction of enzymes or to alter specifically the gene expression to modify the nutritional quality (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.

Research

Conversion of Lignocellulose to Bioethanol

The use of bioethanol in fuel is a way to reduce the global CO_2 emission. Together Risø National Laboratory and DTU work with a new concept to produce ethanol from renewable plant resources. Due to a low production of inhibitors, the wet oxidation process was the most promising for the pre-treatment of biomass into fermentable sugars. Different types of biomass have been investigated as substrata for the fermentation of ethanol. For wheat straw, it was found that alkaline wet oxidation was efficient for fractionating the polysaccharides into fermentable sugars. For woods, the alkaline treatment should be avoided; hence wet oxidation without alkaline addition proved the most efficient. The reaction time needed for efficient fractionation was typically 10 minutes with a reaction temperature of 185°C.



Glucose consumption and ethanol production in concentrated wet-oxidized wheat straw (1x, 3x, 6x) with added glucose. Mineral medium was used as a 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.

Plant Fibre Composites

Natural cellulosic plant fibres are potentially among the strongest fibres known. Besides they have a low density, compared to 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. 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 were developed to determine the cellulose chain length and reactivity of cellulosic fibres. It appeared that flax and hemp with the highest cellulose chain length were potentially those with the highest strength. Flax fibre based composites are presently used as inner door panels in BMW- and Mercedes automobiles.

Peroxidases and Plant Stress Metabolites

Plants may contain up to hundred genes coding for peroxidases with 35-100% protein sequence identity, all having similar 3D space filling structure. 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. 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 at-

tempts 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 to indicate that Prx8 interferes with metabolism needed to develop healthy plants. Transient expression assays, in collaboration with the Carlsberg Laboratory, Denmark and the University of Zurich, Switzerland revealed that expression of Prx8 strongly improves the effect on the defensive capabilities of cereal cells against pathogen 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 distinct peroxidases have distinct functions in plant stress metabolism and very likely in general plant metabolism as well.

Low Phytate Barley Mutants: Genetics, Biochemistry and Nutrition

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 in order to locate and estimate the number of structural genes in the biosynthetic pathway from myo-inositol to phytate 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 the publication of a new high-performance thin-layer chromatography method for inositol phosphate analysis.

Animal Feeding Trails with Low-Phytate Barley

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

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

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 a mineral bioavailability test which can be used at an early stage.

Phosphorus Nutrition Evaluated in Pot Cultures

Low-phytate mutants of barley, three of which were 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. The mutants were more sensitive to late addition of P than the wild type.

Patent

A process for solubilizing hemicellulose present in a lignocellulosic material. International Patent Application No. PCT/DK99/00471, filed 7 September 1999.

Collaboration with Companies

Together with the Danish Invention Company Biocrack, the wet oxidation method is refined towards solving a specific problem in the recycling of residual products from cereal crops. A new reactor is under development for demonstrating the wet oxidation process at a larger scale. The continuous flow 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.

Collaboration with Sejet Plant Breeding, Denmark seeks to develop barley lines with reduced phytate content and more free phosphate for animal nutrition, to improve mineral uptake and reduce the agroindustrial P-load on the environment.

Education

To support the training of young scientists, the programme continues to have a high number of Ph.D. students and Master students from several Universities in Denmark. Currently we have four Ph.D. students working on lignin modification by means of *in planta* genetic engineering, identification of fermentation inhibitors from pre-treated ligno- and hemicellulose, natural product formation in biotically and abiotically stressed plants and enzymatic degradation of phytate. During the year one Ph.D. degree was completed and two Master students finished from DTU and RVAU, respectively. Currently three Master students from DTU and one from Aalborg University are working on the utilization of hemp-fibres and the cloning of key enzymes for phosphorylation of inositol. Furthermore we share the training of three Ph.D. students with other research institutes in Denmark.

Research Facilities

Steam explosion, pilot and loop-autoclave, inert-HPLC, GC, FPLC, ABI377 Prism DNA-sequencer, Applied Photophysic stop-flow spectrometry, classified labs and greenhouses for GMOs, PDS1000/He Gengun, Multichannel-mirkotitre spectrometer.

DLF-Risø Biotechnology

Isolation of Key Genes Involved in Stem and Flower Formation

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 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.

Using PCR-based subtractive hybridization techniques, Differential Display Reverse Transcriptase Polymerase Chain Reaction (DDRT-PCR) and Family Specific Domain 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.

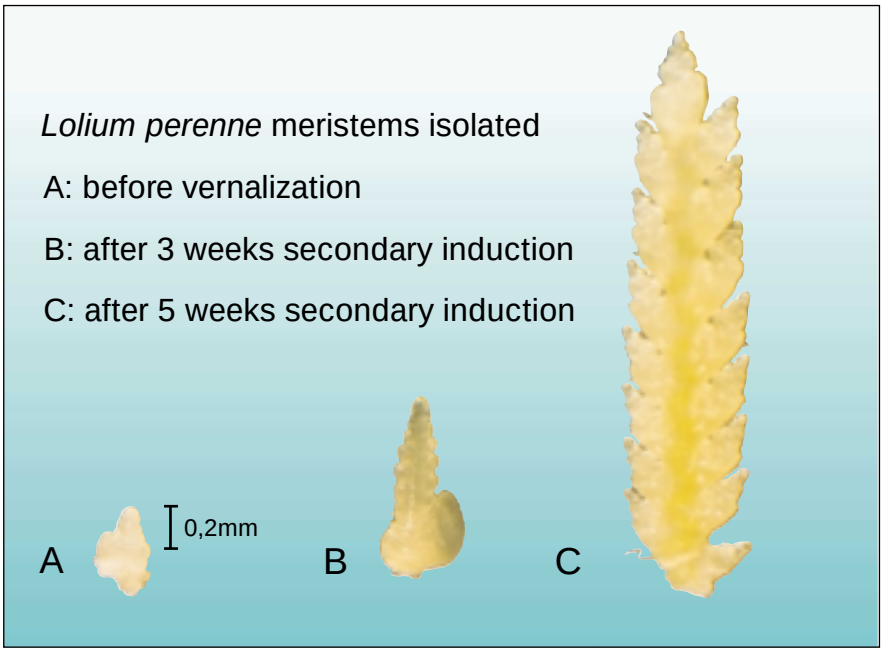
Using the genetic information gained on the characterization of flowering genes from other plant systems (*Arabidopsis*, Rice etc.) we succeeded in cloning several orthologues to flowering genes from other plant species like the orthologues to LEAFY, TFL and GA-MYB from *Arabidopsis*.

In a project the DLF/Risø consortium 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.

Characterization of *Lolium* Flowering Genes

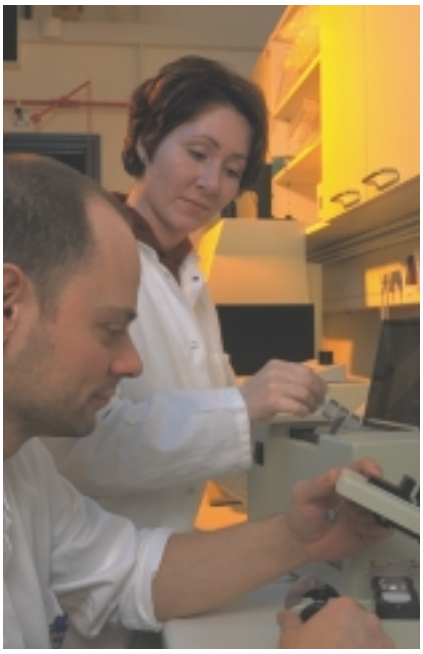
Once the differentially expressed genes are identified they are investigated using northern analysis, southern analysis and *in situ* hybridization or *in situ* PCR. For low expressed genes we use RNase Protection Assays or semi-quantitative RT-PCR to verify the differential expression pattern. As a method to understand 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.

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.



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

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.



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

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

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 point in the plant development or even only in a specific generation or progeny of the plant. For this project we therefore work on the development of conditional expression systems in plants in general and in ryegrass in particular. One strategy is the isolation of 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. For the use in ryegrass, we are currently adapting an ethanol inducible expression system, which proved efficient in *Arabidopsis* and Tabacco. By means of 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 thus favours this system, compared to systems using hormones or environmentally harmful chemicals.

In an additional strategy we investigated 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 Model Plant

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.

Education

In 1999 two Master students finished their Master Theses and a new Master student is starting his thesis in February 2000. Two Ph.D. students worked in 1999 on the isolation and characterization of *Lolium* flowering genes and an additional new Ph.D. student starts February 2000 with implementation of *Brachypodium* as a model grass plant.

Plant Genetics and Epidemiology

Research

In the study of disease resistance mechanisms and evolution of pathogen populations we used a newly established fluorescence microscope facility with digital camera. We extended our expertise in cellular resistance mechanisms and in epidemiological modelling.

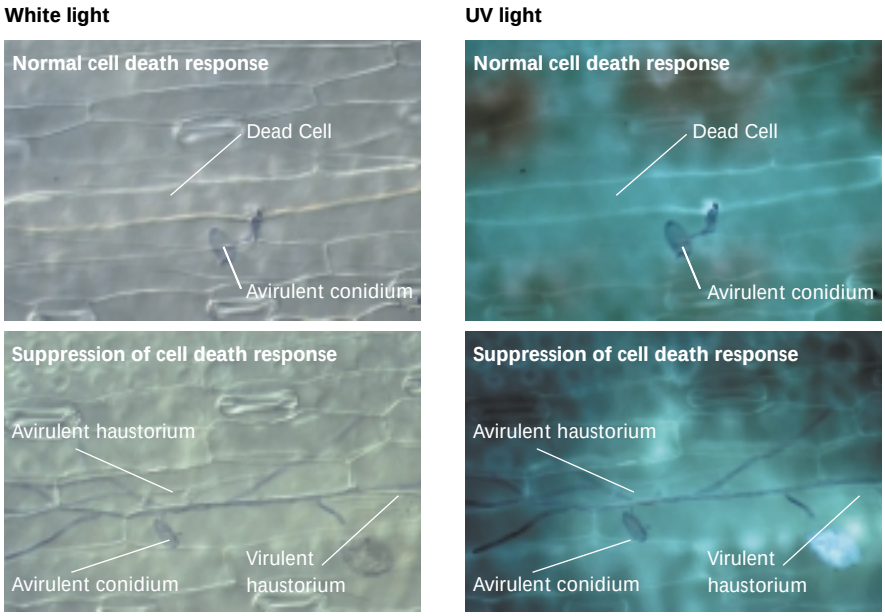
In collaboration with IGER Wales, UK the induction of cellular resistance mechanisms was studied in two sets of isogenic barley lines with and without the race-specific powdery mildew resistance gene *Mla1*. Here we found that the *Mla1* resistance gene suppressed the ability in attacked host cells to initiate the resistance mechanisms. This was demonstrated through an infection (haustorium formation) by a virulent powdery mildew isolate of barley.

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 winter wheat plot not treated 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 sexual spores compared to asexual spores combined with the long latency period of this disease. A simulation model, which was developed in collaboration with The University of Reading, UK, supported this conclusion.

In several studies we used the techniques of older well-established DNA markers as well as new ones, such as (RFLP, AFLP, SSR, Inter-SSR etc.) and extended our expertise in QTL analysis.

In a joint project between Risø and ICARDA (International Centre for Agricultural Research in the Dry Areas), agronomic traits important for the dry land agriculture were mapped. 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.

Spread of nuclear and cytoplasmic genes from oilseed rape to natural populations of *B. rapa* were studied in natural populations with the strategic purpose of monitoring the extent of gene transfer in nature and evaluating the possible safe insertion sites for transgenes. Through cross compatible wild or weedy relatives transgenes may be transferred from the genetically modified crop to the wider environment. We revealed a comprehensive



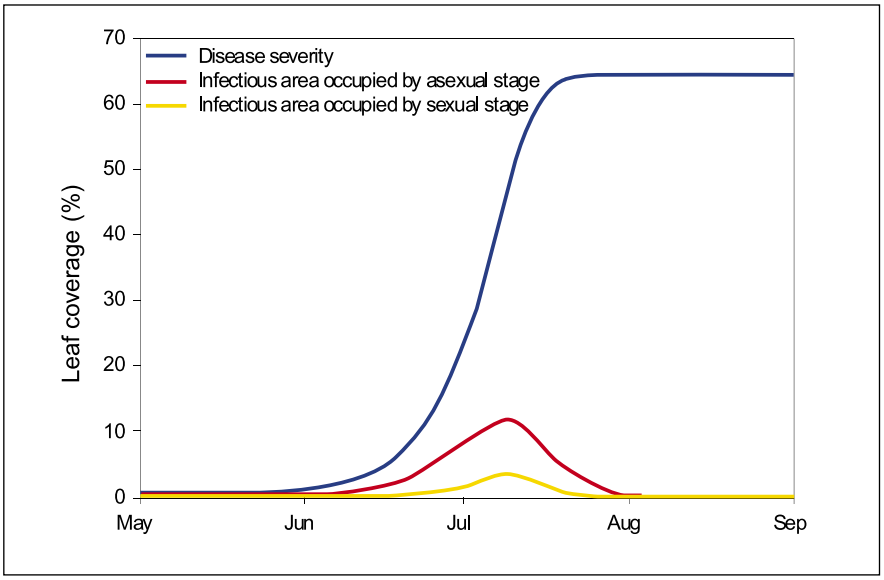
Differential interference contrast micrograph (white light) and incident fluorescence micrograph (UV light) of the same epidermal cells of barley line P-01 carrying the *Mla1* resistance gene conferring race-specific cell death response against barley powdery mildew attack.
(Top). Normal cell death response 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 effect from plant competition on hybridization and backcrossing between oilseed rape and *Brassica rapa* has been analysed in field experiments.

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* co-existed for several years. The results indicated that both nuclear and plastid encoded oilseed rape genes were transferred to the wild relative.

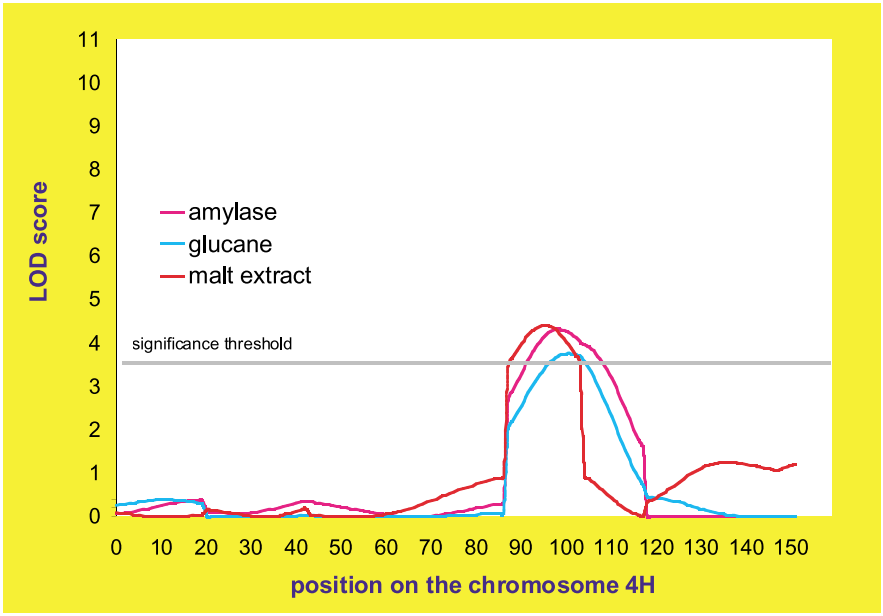
Collaboration with Companies

In a 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. Markers for some of the traits were located on a linkage map, however, with some variation in location for data from different environments. Thus by means of molecular markers we could elucidate the complexity of micromalting tests and the need for reliable test envi-



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. Parametre 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.

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



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.

ronments. Under well-defined test conditions molecular markers for the malting quality traits could be established.

Education

In 1999 we have supervised three Master students and five Ph.D. students (for three of them as main supervisor) coming from RVAU, University of Copenhagen and University of Aarhus, respectively.

Risø is part of DINA (The Danish Information Technology Network in Agriculture) an education and research network where we are responsible for the following disciplines: quantitative genetics and bioinformatics within plant science.

Every year we hold a 2-week Ph.D. seminar on molecular markers in plant breeding with participants from the Nordic countries. During the seminar the students receive basic knowledge about molecular markers and their application in plant genetics and breeding.

Consultancy for the Authorities

Research activities are offered in relation to the possible consequences of cultivating genetically modified plants (GMPs). Within this topic, Risø has collaborative projects with competent Danish authorities. Some projects are funded directly by the Ministry of Environment and Energy: *e.g.* a cost analysis of expressing transgenes in wild recipient species has been carried out, and at present the vegetative propagation of non-flowering grasses is evaluated. The authorities are also represented in projects funded by others (*e.g.* The Danish Environmental Research Programme and the National Research Councils) through the board of clients interested in effect analysis in relation to GMPs.

Research Facilities

The programme runs an ABI Prism 377 DNA analysis system. With this system fast and reliable sequencing as well as fragment analysis are possible.

The programme runs a histopathological laboratory equipped with advanced light and fluorescence microscope facility, CCD camera and analytic bioimaging software.

The programme runs a 125 ha farm with experimental fields where our information of how the fields have been run goes back 40 years. We have specialized in miniplot experiments, but in recent years large scale plots have been conducted using GPS technology. The field expertise includes nitrogen application and utilization, weed and disease control, crop-weed competition, organic plant production and selection for disease resistance.

Biogeochemistry

Research

The research in the Biogeochemistry Programme is focused on the occurrence, transport, turnover as well as the 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 a commercial basis.

Plant-Uptake of Organic Contaminants from Soil Amended with Sewage Sludge

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 degradation of the water-soluble detergent LAS is very fast. 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 fast, 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. Apparently, DEHP is more recalcitrant in the soil with 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.

Risk Assessment of PAC Polluted Soils

Evaluation of PAC (Polycyclic Aromatic Compounds) soil pollution in relation to cancer risk is usually based on a comparison of the concentrations of a limited number of indicator PAH (Polycyclic Aromatic Hydrocarbons) with criteria levels. The relative carcinogenic risk was 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 were available. The relative risk has been estimated for N-PAC mainly on the basis of published mutagenicity data. The preliminary results show that conventional PAH (12 indicator-PAH) only contribute to a minor degree to the relative risk, 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. The basic N-PAC were the major

Analysis of Organic Pollutants. Scientist G.K. Mortensen.

risk contaminants, despite a contribution to the total PAC of only 5%.

Formation, Occurrence and Fate of Nitro-PAH

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, 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 airmasses 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.

Effects of Global Change on Forest and Agro Ecosystems

Studies of 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, 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. The 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 in the new leaves of most plant species did not change. 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.

Sediment Dating and Geochemistry

The EU MAST project BASYS terminated with the presentation of results at the 3rd BASYS Science meeting in Warnemünde, Germany. As a result of the Risø geochemical work in particular, salt-water inflow into the central Baltic has been traced through the Mn(Ca) carbonate mineral kuthnohorite. A periodicity has been deduced from the analytical data resulting in 300-year cycles of enhanced salt-water inflow activity followed by a reduced inflow of similar duration. These patterns can be coupled to cyclic sea level variations, which in turn are coupled to solar flux variations. The trace element molybdenum, which is found in mainly laminated sediments, has been coupled to primary productivity in the surface water in that Mo is an essential micro nutrient in the N_2 fixing process of cyanobacterial blooms. Blooming remains are thought to transport the Mo into the sediments. Systematic measurements show that the Baltic has also been opposed to such blooming between 4000 and 6500 and between 7000 and 8000 before the present time. A sedimentation model accounting for occurrences

The SOROFLUX project. Automatic stem-flow collection and bags for litter collection. In the background white boxes for measurement of NO and CO_2 emission from the soil.

Analysis of Isotopic Ratios Applying HR-ICP-MS. Scientist S. Stürup.

of laminated and homogeneous sediments during the past 8000 years has also been proposed.

The SOROFLUX Project

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 results of the flux measurements show 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. For 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 of 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, the first year after application, sewage sludge addition increased biomass production by 20 % and grain yield production by 10 %. 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.

Patent

A Method for a Determination that a Product has been Organically Produced. Danish Patent Application No. PA 1999 01725, filed December 1999.

Collaboration at Risø and in Denmark

Close collaboration at Risø with the research Programme for Plant Ecosystems and Nutrient Cycling, the Nuclear Safety Research and Facilities Department and the Wind Energy and Atmospheric Physics Department. Other collaborating partners in Denmark are The Royal Veterinary and Agricultural University, The University of Copenhagen, University of Aarhus, Aalborg University, Roskilde University Centre, University of Southern Denmark, Danish Institute of Agricultural Sciences, The National Environmental Research Institute, The Geological Survey of Denmark and Greenland, Danish Forest and Landscape Research Institute, Institute for Water Environment, The Environmental Protection Agency, The National Forest and Nature Agency, Danish Agency for Trade and Industry, Danish Bacon and Meat Council, MD Foods and The Danish Veterinary and Food Administration.

International Collaboration

University of California, Irvine, CA, USA; University of North Carolina, Chapel Hill, NC, USA; University of London, Imperial College, UK; University of Bremen, Germany; ISPRA, Italy; University of Tartu, Finland; Institute of Chemical Physics and Biophysics, Tallinn, Estonia; GEOMAR, Kiel,

Germany; IOW, Rostock, Germany; University of Sheffield, UK; University of Stockholm, Sweden; University of Bergen, Norway; University of Tromsø, Norway; Geological Survey of Finland, Espoo, Finland; Geological Survey of Norway, Trondheim, Norway; Utrecht University, The Netherlands; University of Göttingen, Germany; Agricultural University of Wageningen, The Netherlands; Norwegian Institute for Water Research (NIVA), Oslo, Norway; Institute of Hydrology, Wallingford, UK; Swedish Environmental Research Institute (IVL), Gøteborg, Sweden; University of Lund, Sweden.

Collaboration with Companies

Condea, Milano, Italy; Petresa, San Roque, Spain; Ford Motor Company, USA; HOH Vand og Miljø A/S, Denmark; Haldor Topsøe A/S, Denmark; Rockwool, Denmark; Rexam Closures and Containers A/S, Denmark; LK-Engineering A/S, Denmark; Dansk Ædelmetal, Denmark.

Competence Building

In 1999 the process of accreditation of the laboratory of the Biogeochemistry Programme and the Plant Biology and Biogeochemistry Department in general was implemented.

New Methods and Techniques

Isotopic Composition and Isotope Ratios

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 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.

Availability of PAHs and other Hydrocarbons in Soils Highly Polluted with Oil and Petrol

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.

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. 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.

Plant-Uptake of PAH

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. Thus, 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.

Research Facilities

HR-ICP-MS, ICP-OES, GC, GC-MS, LC, the Gamma Dating Centre (GDC) joint dating centre of 5 national institutes initially funded by the Danish Natural Sciences Research Council, and Ecosystem field station at Gyrstinge, Sorø, Denmark.

Plant Ecosystems and Nutrient Cycling Programme

Research

In our project on organic farming field experiments have illustrated how pea root distribution (32_P technique) concentrates in the 0-25 cm soil layer, while barley was 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 intercropping with pea results in up to 50% improved pea growth compared to simultaneous sowing.

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}.

Mineralization of N in soils with application of 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 supplied soils. However, mineralization in sludge supplied soil resulted in a large inorganic N pool during the summer.

The purpose of the project "Application of molecular biology to identify and isolate genes important for phosphorus-use efficiency in barley" 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. The work is concentrated on improving methods for tracing barley lines with high ability for P uptake.

Within the project "Precision farming" we have established an experimental set-up with iron frames for 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 with nitrogen and water as the experimental factors proved the reliability of the system in testing the nitrogen status of wheat plants with optical sensors. This system will be further improved and more sensors will be tested.

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 field campaign measuring fluxes of O_3 and NO_X over the forest and field simultaneously. The flux measurements are a contribution to the EUROTRAC-2 subproject BIATEX-2. Using portable gas exchange equipment the CO_2 and H_2O fluxes between photosynthetic leaves and non-photosynthetic plant parts have been measured weekly over the growth season. Also water-mediated fluxes of C and N have been measured within SOROFLUX. 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.

Within the CLIMOOR project an exper-

Field station Sorø

Field station Mols Bjerge

imental "climate change" facility has been set-up at Mols Bjerge in Eastern Jutland, Denmark. 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 been operated since March 1999. In the heating plots the temperature has been increased by approx. 1°C. In the drought plots a 2-month drought period has been applied during May-June. Although the manipulations have only run since March, the first results have already indicated that both warming and drought affect the plants and the soil. Similar manipulation experiments are conducted in the UK, The Netherlands and Spain.

Collaboration with Companies

During 1999 a close collaboration has been established with the VEGA company, who produced the experimental facility for the CLIMOOR project at Mols, Jutland, Denmark. Risø and VEGA have agreed to continue the collaboration in order to improve and hopefully sell more equipment for similar projects elsewhere.

The Norsk Hydro sensor used in our research on precision farming was tested in the field in a co-operation with Hydro Agri. Our work on precision farming is carried out in collaboration with The Danish Agricultural Advisory Centre, private farmers, and agricultural machine companies.

Education

Two Ph.D. students and one Bachelor student are involved in the precision farming project. One of the Ph.D. students lectures on the subject at the Royal Veterinary and Agricultural University, RVAU, Copenhagen. The head of the programme is attached to the RVAU as a teacher in trace elements in plant nutrition.

Field station Lille Valby

One Ph.D. student is studying gas fluxes to and from leaves under controlled conditions and in the forest at our research area in Sorø, Denmark. His supervisor at Risø lectures on plant physiology at the University of Copenhagen.

One Ph.D. student studies the content of organic Cl compounds in plants and the function of these compounds. One Ph.D. student is involved in the project on intercropping in low-input farming systems.

Consultancy for the Authorities

The senior scientists of the programme have acted as experts in the evaluation of research proposals to the EU 5th framework programme as well as to German and British national research programmes.

Research Facilities

CONFIRM. Centre for Continuous Flow Isotope Ratio Mass Spectrometry is a forum for interdisciplinary research based on advanced analytical equipment for studies of stable isotopes in elemental cycling.

RERAF. Risø Environmental Risk Assessment Facility is a system of highly controlled growth chambers. The closed system makes it possible to study changes in the atmospheric content of gasses, GMO, and use of isotopes.

OTC. 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 oxides and carbon dioxide.

Sorø experimental area is a field station equipped to study fluxes of gaseous and water-mediated compounds in a beech forest and farmland.

Mols Bjerge – a field station to test climate change effects on the heath ecosystem. The facility consists of 9 plots: 3 heated 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.

Lille Valby field station is part of the RIMI project (Risø Integrated Environmental Initiative). Fluxes and concentrations of gaseous compounds in the atmosphere have been measured with a range of techniques in collaboration with the National Environmental Research Institute.

Education

Ph.D. Theses

Feilberg, A. Polycyclic Aromatic Compounds, particularly nitro-PAHsources 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, Denmark.

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.

Personnel

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
Brandt, Lis
BrinkJensen, Merete
Carlsen, Merete
Christensen, Gertrud
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
Koutras, Charlotte
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).

Finances

The activities of the Department are supported by a combination of basic government funding, Project funds from national and international research programmes and fully commercial industrial contracts.

		7,6648
1999	DKK 1000	USD 1000
Income		
Basic funding	37.738	4.924
Programmes and contracts	36.120	4.712
Durable equipment	1.495	195
Total	75.353	9.831
Expenditure		
Salaries	59.583	7.774
Operating expenses	14.243	1.858
Durable equipment	4.447	580
Total	78.273	10.212

Additional funding has been obtained for Ph.D. grants, Post. doc. fellowships, apprentices and trainees.

Acronyms

2D	Two-Dimensional
AFLP	Amplified Fragment Length Polymorphism
AM	Arbuscular Mycorrhiza
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
DINA	Danish Information Technology Network in Agriculture
DLF	Danske Landboforeningers Frøforsyning
DTU	Technical University of Denmark
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	Effects of nitrogen oxides on European forests
FP-LC	Fast Protein Liquid Chromatography
FSD	Family Specific Domain
GC-MS-MS	Gas Chromatography/Mass Spectrometry
GDC	Gamma Dating Centre
GFP	Green Fluorescent Protein
GMO	Genetically Modified Organisms
GMP	Genetically Modified Plants
GPS	Global Positioning System
HP-LC	High Pressure Liquid Chromatography
HR-ICP-MS	High Resolution Inductively Coupled Plasma Mass Spectrometry
ICARDA	International Centre for Agricultural Research in Dry Areas
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IGER	Institute of Grassland and Environmental Research
LAS	Linear Alkyl benzene Sulfonates
LC-MS	Liquid Chromatography Mass Spectrometry
MAST	Marine Science and Technology Programme
NMR	Nuclear Magnetic Resonance
NorFA	Nordisk Forskerutdanningsakademi
OTC	Open Top Chamber
PAC	Polycyclic Aromatic Compounds
PAH	Polycyclic Aromatic Hydrocarbons
PCR	Polymerase Chain Reaction
PEG	PolyEthylen Glycol
RERAF	Risø Ecological Risk Assessment Facility
RIMI	Risø Integrated Environmental Project
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 Seguence Repeat

Bibliographic Data Sheet

Risø-R-1159 (EN)

Groups own reg. number(s)
Project/contract No(s)

Title and authors

Plant Biology and Biogeochemistry Department
Annual Report 1999

Pages 28

Illustrations 29

A. Jensen, G. Gissel Nielsen,
H. Giese, K. K. Nielsen, L. Rasmussen,
S. K. Rasmussen, H. Østergård

ISBN 87-550-2653-2
ISBN 87-550-2654-0 (internet)
ISSN 0106-2840
ISSN 1397-8977

Department or group

Plant Biology and Biogeochemistry Department

Date: April 2000

Risø is a national laboratory under the Ministry of Research and Information Technology with its own board.

Risø carries out scientific and technological research in order to create new technological development. The results of Risø's research are used by industry, authorities and international organisations. Risø contributes to the education of scientists by offering special projects and by participating in Ph.D. and post-doctoral programmes.

Risø is a government advisor on nuclear issues. Risø operates large-scale research facilities for the benefit of Danish and international research.

Risø's activities in 1999 are reported in the following publications: Risø Annual Report (available in Danish and English), Risø's Annual Performance Report (Danish), Risø's Publication Activities (Danish/English), as well as the annual progress reports of the seven research departments (English). All publications and further information can be obtained from Risø's webserver www.risoe.dk. Printed publications are available from the Information Service Department, tel. +45 4677 4004, email risoe@risoe.dk, fax +45 4677 4013.

Design: Grafikerne.dk
Printing: Nordgraf A/S

ISBN 87-550-2653-2
ISBN 87-550-2654-0 (Internet)
ISSN 0106-2840
ISSN 1397-8977

The Department of Plant Biology and Biogeochemistry
Risø National Laboratory
April 2000