Plant Biology and Biogeochemistry Department annual report 2000

Kossmann, J.; Gissel Nielsen, G.; Nielsen, K.K.; Pilegaard, Kim; Rasmussen, L.; Rasmussen, S.K.; Thordal-Christensen, H.; Østergård, Hanne

Publication date:
2001

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

Plant Biology og Biogeochemistry Department

Annual Report 2000

Risø renewed

In November 2000, Risø's board of Governors adopted a new strategy for the renewed Risø without nuclear facilities following the definite closure of the DR3 research reactor in September 2000.

Mission

To promote an innovative and environmentally sustainable technological development within the areas of energy, industrial technology and bioproduction through research, innovation and advisory services.

Vision

To make significant contributions to the development of new products and to give science based advice that will benefit the environment, the state of health and prosperity.

Results

New knowledge, new technologies, innovative product development and research-based services and training.

Risø's activities in 2000 are reported in the following publications: Risø Annual Report (available in Danish and English), Risø's Annual Performance Report (Danish) and 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, e-mail:

Plant Biology and Biogeochemistry 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 an environmentally benign industrial and agricultural production in the future.

The Department's expertise covers a wide range of areas needed to develop crops that meet the demands to increase agricultural production for a growing population, to produce plants with improved nutritional value, to develop crops that deliver renewable resources to the industry, and to generate plants that are adapted to the future climate.

The projects are organized in six research programmes that are linked to each other through the individual expertise that is delivered by each of the programs to the rest of the department.

Contact:
Jens Kossmann, Head of Department
Phone: 4677 4101; e-mail: jens.kossmann@risoe.dk

Plant-microbe Symbioses Programme

Objectives
The objectives of the research in Plant-Microbe Symbioses Programme is to study how symbiotic plant-microbe interactions are established, maintained and functioning. This is highly relevant to plant production, since symbioses are significant for uptake of nutrients (*e.g. Rhizobium* and *Mycorrhiza* symbioses with plant roots) and development of diseases (*e.g.* powdery mildews). We characterize genes and processes important for these plant-microbe symbioses, and thereby we help providing solutions for sustainable plant production.

Research fields

- The arbuscular mycorrhizal symbiosis
- Rhizobium-legume symbiosis
- The powdery mildew fungus and defence mechanisms in plants
- *Tilletia caries*, the wheat bunt fungus

Contact:
Hans Thordal-Christensen, Head of Programme
Phone: 46774127; e-mail: hans.thordal@risoe.dk

Plant Products and Recycling of Biomass Programme

Objectives
The programme seeks to use modern technology to explore the inherited plasticity of plant organs to produce high value compounds and and to continue the improvement of quality and value of crop plants. Technologies are developed to convert a larger amount of natural products to compounds, which is more applicable for industrial use (waste-to-value). Metabolic engineering will be used to control biosynthesis of secondary compounds and to improve functionality of plant polymers and storage compounds.

Research fields

- Plant respiration
- Localisation
- Bioethanol

Contact:
Søren K. Rasmussen, Head of Programme
Phone 46774121; E-mail: soren.rasmussen@risoe.dk

DLF-Risø Biotechnology Programme

Objectives

A major goal-oriented research programme on the control of flowering is undertaken by the biotechnology consortium among DLF-TRIFOLIUM A/S and Risø National Laboratory.

The overall objective is to engineer grasses incapable of producing stems and flowers during grassland farming. Strategies for the repression of stem and flower development include; *i)* the manipulation of key genes involved in the regulation of the floral transition, and *ii)* ablation of reproductive tissues through the action of toxic genes controlled by tissue and developmental specific promoters.

Research activities include the identification of key genes responsible for the switch from vegetative growth to flowering, tissue and developmental specific promoters as well as gene activation systems allowing control of flowering and seed production. A number of genes involved in flowering have been isolated, and these are now being tested in transgenic grasses.

Research fields

- Flowering gene isolation
- Molecular characterisation of floral transition
- Biological encapsulation
- Conditional expression systems

Contact:
Klaus K. Nielsen, Head of Programme
Phone: 46774281; e-mail: klaus.k.nielsen@risoe.dk

Plant Genetics and Epidemiology Programme

Objectives
The aim of our research is to analyse the agricultural ecosystem with the crop as the main focus. The environment and its biotic (e.g. fungal diseases) and abiotic (e.g. nutrients, pesticides, air pollution, temperature) stress factors influence the expression of inherited traits of crop plants. Our expertise is in analyses of the genetic basis for important agronomic traits and analyses of changes in genetic diversity and biodiversity in time and space when growing crops under different input systems (conventional, organic, biotechnology). We focus on experiments in growth chamber, green house, field and natural sites to measure the considered processes, further we use biometric analyses to relate these measurements to genetic information, e.g. DNA markers, and finally mathematical models to support hypothesis making and predictions. Our high priority plant species are cereals and other grasses as well as oilseed rape and other Brassica species and we study mainly the cereal leaf pathogens *Erysiphe graminis*, *Puccinia hordei* and *Mychosphaerella graminicola.*

Research fields

- Mechanisms of induced resistance
- Crop improvement for developing countries
- Introgression of genes between crops and wild relatives
- Modelling pathogen population dynamics <plg/modelling.htm>

Contact:
Hanne Østergård, Head of Programme
Phone: 46774111; e-mail: hanne.oestergaard@risoe.dk

Biogeochemistry Programme

Objectives
The aim of the research programme is to study the occurrence, transport, turnover and effects of trace elements and organic micro pollutants in agricultural and forest ecosystems and in the human food chain. The research is aimed towards improvement of food quality and food safety. Major emphasis is placed on the development of new methods and processes, which can form the basis for environmental and sustainable plant production.

Research fields

- Organic contaminants - PAC:
- Atmospheric Chemistry:
- Organic contaminants - petroleum hydrocarbons:
- Climate change experiments - CLIMEX:

Contact:
Lennart Rasmussen, Head of Programme
Phone: 4677 4250; e-mail: lennart.rasmussen@risoe.dk

Plant Ecosystems and Nutrient Cycling Programme

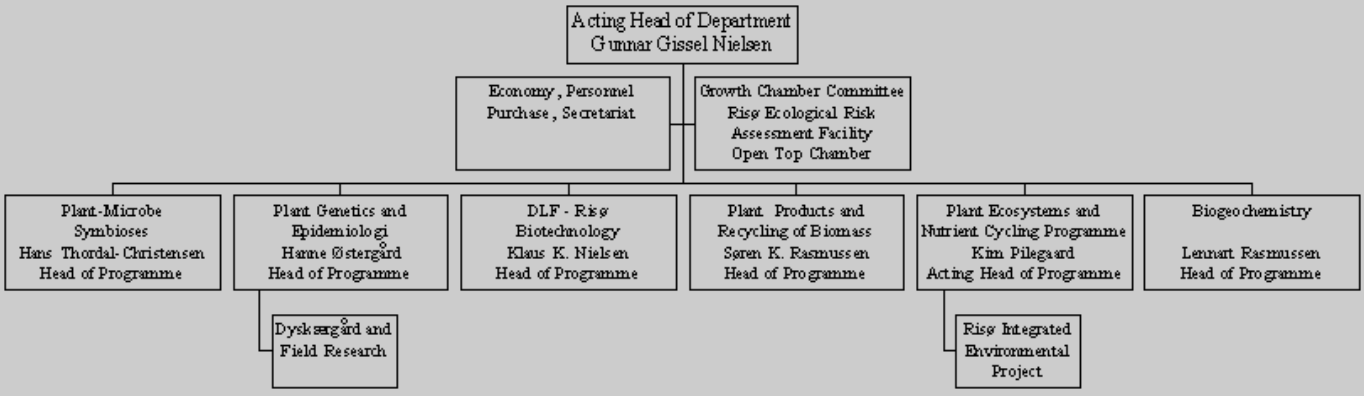
Objectives
The aim of the programme is to study the structure, function, processes, and dynamic of agro- and forest ecosystems, and to develop models to predict the function of the ecosystems in a changing environment.

Research fields

- Effects of sewage sludge application on farmland
- Intercropping in low-input systems:
- Genetic background of P-uptake:
- Precision farming:

Contact:
Kim Pilegaard, Acting Head of Programme
Phone: 4677 4175; e-mail: kim.pilegaard@risoe.dk

Plant Biology and Biogeochemistry Department 2000



Plant-Microbe Symbioses

Interest is taken in how symbiotic plant-microbe interactions are established, maintained and functioning (see figure 1). This is highly relevant to plant production, since symbioses are important for uptake of nutrients (*e.g. Rhizobium* and *Mycorrhiza* symbioses with plant roots) and development of diseases (*e.g.* powdery mildews). In the past year, our research has provided new and exiting data on the physiological and molecular mechanisms of *Mycorrhiza*-assisted phosphate uptake in plant roots, and on the protein composition of the plant derived membrane surrounding the

Rhizobium bacteria.

The pathogenic symbiosis between plants and powdery mildew fungi, in particular the powdery mildews on barley, is a major disease problem for Danish agriculture. This and other pathogens are often controlled using pesticides with high economical and ecological costs, and it is crucial that alternative control means are available. Naturally occurring resistance genes are currently crossed into plant cultivars. However, the resistances provided by these genes, are often circumvented by new races of the pathogens. Therefore, one of our challenges is to clone the plant and pathogen genes involved, in order to understand and exploit these natural resistance mechanisms in advanced ways. In some of these analyses, we take advantage of *Arabidopsis*, a model plant well suited for molecular and genetic studies.

Transgenic barley with improved powdery mildew resistance

Plant pathology has for many years aimed at creating disease resistant plants based on studies of the natural resistance mechanisms present in all plants. These studies have shown that plants react to attack from microbes by activating a complex set of defence components. For example, the plant cell wall is fortified at the site of attack, and proteins inhibitory to fungal growth are produced. This led to the first generation of transgenic resistant plants, which were based on simply over-expressing antifungal proteins in the entire plant at all times.

A second generation strategy
In our attempts to generate barley plants resistant to the powdery mildew fungus, we are taking advantage of the fact that antifungal proteins normally are produced strategically unwisely in relation to the site of powdery mildew fungal growth. The fungus grows exclusively in the outmost cell layer of the leaf, the “epidermis”, while the production of antifungal proteins takes place in the cell layer underneath, the “mesophyll”. Therefore, we aimed at developing resistant transgenic plants, which produce the antifungal proteins in the epidermis, only when the plant is attacked.

A new gene from old parts
Genes are made of a “promoter”, which controls where and when the gene is expressed, and a “coding region”, which determines what the expressed protein looks like. We have a barley promoter, which gives expression in the epidermis, specifically when a powdery mildew attack occurs, and we have coding regions for a number of barley antifungal proteins. A new gene was designed and constructed using the epidermis specific promoter and the coding region for an antifungal “pathogenesis-related” gene no. 5 (PR-5). This new gene (figure 2) was expected to lead to production of the PR-5 proteins in the epidermis when the powdery mildew attacks.

The new gene was introduced into barley plants by the following procedure: The DNA was placed on microscopic gold particles, which were shot into barley embryos taken from developing seeds. In the following period of 3-4 months the barley cells grew unstructured on media, and meanwhile the new DNA was integrated into the chromosomes. Subsequently, a number of “transgenic” barley plants were regenerated from the cells. The transgenic plants were self-pollinated for a couple of generations, and in each generation the progeny was analyzed for presence of the introduced new gene.

The critical test
In order to test whether the transgenic plants in fact are resistant to the powdery mildew fungus, an inoculation experiment was made. As can be seen in Figure 3, the transgenic plants (left) are powdery mildew resistant compared to the control plants (right). Microscopic studies of the resistant leaves indicate that the fungus only develops very small colonies without sporulation compared to susceptible control leaves (figure 4).

We have some exciting analysis to make on these transgenic barley plants. We know that the transgene has been integrated into the genome of several lines. However, only half of these lines show enhanced resistance, and we hope to be able to understand why. Another question is whether the anti-fungal PR-5 protein indeed is produced in the epidermis of the transgenic plants as we expect. The transgenic plants will also be tested for their resistance to other leaf pathogens.

Our results are interesting from a biological point of view. However, there is a potential in practical disease control as well. Our new gene may demonstrate to be a durable alternative to existing resistance genes. Even though the cultivar “Golden Promise” is of very low agronomic value, this has been our experimental plant because of an efficient transformation procedure for this genotype. But it will be interesting to cross the new gene into modern high-yielding barley cultivars, and see how the resistance functions.

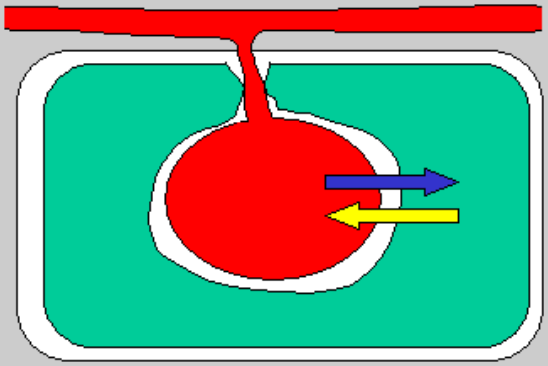


Figure 1. In our three model symbioses, the microbe is partly or entirely place inside the plant cell, where it is surrounded by a plant derived membrane. The symbioses only function when both the microbe and the plant cell are living.

Figure 2. DNA construct with our new resistance gene, which was introduced into barley plants.

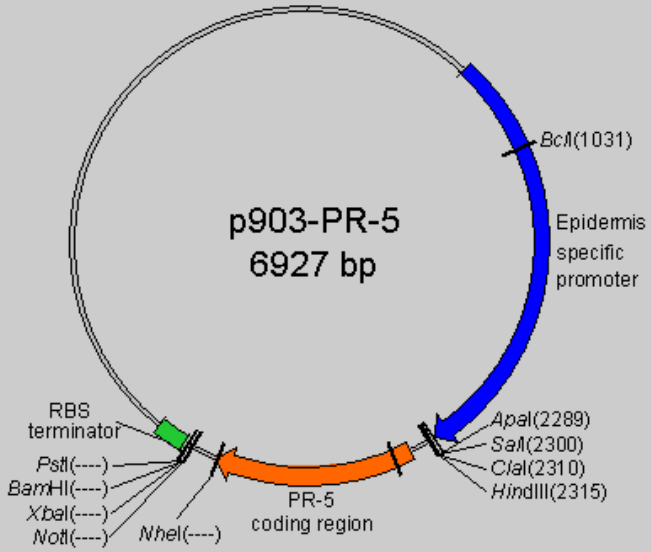


Figure 3. Enhanced powdery mildew resistance in transgenic barley. Transgenic barley with the construct in figure 2 (five plants to the left) and out-segregated control plants (five plants to the right), 5 days after inoculation with the powdery mildew fungus.

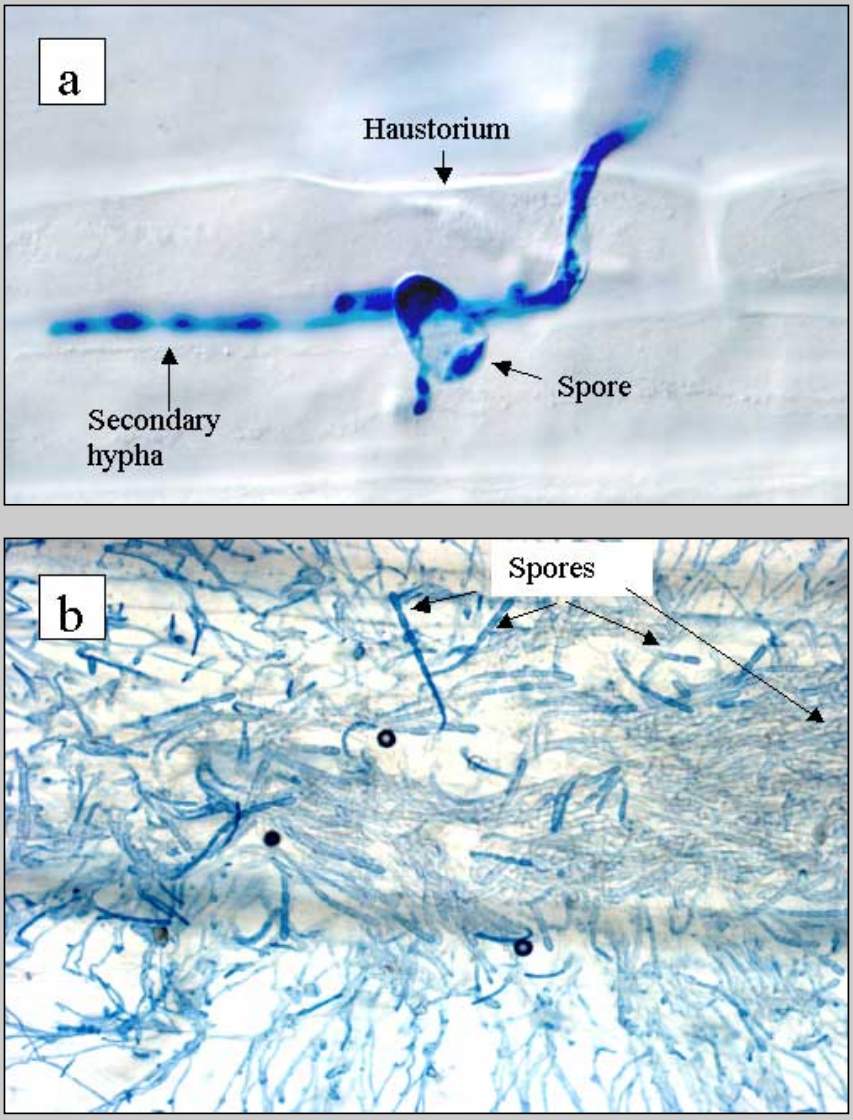


Figure 4. Microscopic studies of barley leaves 7 days after inoculation with the powdery mildew fungus. A, transgenic plant with the construct in figure 2. The fungus has penetrated the epidermis cell, produced a haustorium (feeding-organ) and secondary hyphae. B, non-transformed plant. The fungus has developed vast amounts of secondary hyphae and chains of new spores ready to spread for infections.

Plant Products and Recycling of Biomass

Phytate: impact on nutrition, environment and resources

Phytate is the major storage compound of phosphate in plant seeds, accounting for more than 50% of the total phosphate and even up to 80 % in the main crops barley and wheat used in Danish animal production. Animals such as pigs, hens, chicken and turkey lack enzymes that efficiently can degrade phytate and thus most of the grain phosphate is not utilised. The negatively charged phytate is balanced by cations of magnesium, calcium, iron and zinc, preventing efficient utilisation of these minerals which are needed in the daily feed intake. Thus, phytate is the key nutritional problem regarding phosphate and minerals. When the undigested phytate is released to the fields from animal production, it is deposited as organic-phosphate, which is not readily available for plant uptake. Furthermore, surface leakage and run-off to fresh water lakes and coastal sea creates eutrophication by blooms growth of algae leading to sub-oxygen regimes and ultimately death of aquatic life. The impact on the environment is prone to changes in agricultural practice and variation in soil quality, thus the increasing popularity of pig production out-of-doors creates increasing uncontrolled phosphate load on the environment. Finally many forecasts predict that high quality, bioavailable rock-phosphate is limited resource that will be used within this century. Use of lower qualities with contaminating cadmium will lead to pollution of the environment and cadmium ending up in the food chain. Phytate degradation is not merely a concern in relation to farm animals but also in relation to human nutrition. For people with exclusively grain-based diets as seen in many Asiatic and African countries

the insufficient digestion of phytate is a severe nutritional problem, particularly because it sequesters zinc and iron from uptake. Adding further to the cancer of human health a number of reports on anticancer effects of InsP6 have been published during the last few years. Feeding experiments with rat have demonstrated that labelled InsP6 given in the drinking water is rapidly taken up and distributed in the body. Here, it leads to a 33.5 % reduction in mammary tumour incidences as compared to a control group. The *myo*-inositol moiety of phytate itself, have a similar effect on lung and liver carcinogenesis in mice and it is therefore suspected that a degree of phosphorylation or dephosphorylation of phytate or its inositol-phosphate intermediates compounds must occur in the cells. These reports emphasize the importance of an effective degradation of phytate-aggregates in the food in order to improve the uptake of healthy and necessary food-components.

There are at least five strategies to overcome some the above mentioned problems imposed by inherent phytate content in feed of plant origin. The use of feed enzymes like phytase from the *Aspergillus* fungus has out-competed the simple use of phosphate and minerals, the latter solves the nutritional problem, but not the impact on environment. A second approach is the reduction of phytate content in the grain by mutational breeding. In the year 2000 low-phytate maize varieties were put on the American market confirming the feasibility of this approach. A third route is to produce transgenic barley, wheat, maize or rice that express the fungal phytase genes in the grain. The transgenic strategy may be further improved by modifying the plants own phytase to meet the industry demands for functionality. Finally patent have been filed on transgenic animals, including pigs that express the fungal phytase gene in the digestive tract. Which of these solutions will enter the market depends not only on technical and economical considerations but also on consumer's preferences, e.g. mutational breeding is accepted by organic farmers, while a transgenic approach is not and transgenic animals may even not be legal world wide.

Report on results obtained
We have undertaken to investigate the agronomical potential in mutational breeding of barley and so far more than twenty low-phytate grain mutants have been analysed for growth performance, genetically, and biochemically, studies that bring new information on the biosynthesis of grain phytate.

Mutant phenotype
Nutritionally relevant parameters in barley low-phytate mutant grains were analyzed in order to assess the potential value of these lines for future feeding trials. Phytate (*myo*-inositol 1,2,3,4,5,6-hexakisphosphate) levels in grains from A- and B-type low-phytate mutants corresponded to 25% and 66% of those of the parent line content, respectively. These relative decreases in phytate were accompanied by proportional increases of amounts of inorganic phosphate. Apart from phytate, A-type grains also contained substantial quantities of *myo*-inositol 1,3,4,5-tetrakisphosphate. Phytate levels in straw and root material from mutants were similar to parent line controls, indicating that low-phytate mutations were grain specific. Analysis of K, Mg, Ca and Zn revealed normal or slightly increased mineral cation levels in grains from all low-phytate lines, suggesting that mutationally impaired phytate accumulation did not affect mineral storage capacity. Other nutritionally important parameters such as starch and protein contents were similar to parent line controls. Finally, dynamic changes in the phosphorus composition during kernel development suggested that A-type mutations directly affected phytate synthesis whereas B-type mutations appeared to regulate the synthesis.

Feeding trails with mutants
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. Because grain quantities were limited, rats served as a model for the pig in feeding trial. 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 their zinc content, only rats fed mutant grains had a net absorption and a positive zinc balance indicating an improved availability of this mineral. Rats appear to be a suitable model for P utilisation in pigs and can thus provide plant breeders with a mineral bioavailability assay for use at an early stage.

Mutant accumulate $InsP_4$
Inositol phosphates from barley low-phytate grain mutants and their parent variety were analyzed by metal-dye detection HPLC and NMR. Compound assignment was carried out by comparison of retention times using a chemical hydrolysate of phytate (Ins [1,2,3,4,5,6]P6) as a reference. Co-inciding retention times indicated the presence of phytate, D/L-Ins(1,2,3,4,5)P5, Ins(1,2,3,4,6)P5, D/L-(1,2,4,5,6)P5, D/L-(1,2,3,4)P4, D/L-Ins(1,2,5,6)P4 and D/L-Ins(1,4,5,6)P4 in PLP1B mutants as well as the parent variety. In grain extracts from mutant lines PLP1A, PLP2A and PLP3A unusual accumulations of D/L-Ins(1,3,4,5)P4 were observed whereas phytate and the above mentioned inositol phosphates were present in relatively small amounts. Assignment of D/L-Ins(1,3,4,5)P4 was corroborated by precise co-chromatography with a commercial Ins(1,3,4,5)P4 standard and by NMR spectroscopy. Analysis of inositol phosphates during grain development revealed accumulation of phytate and D/L-Ins(1,3,4,5)P4 which suggested the tetrakisphosphate compound to be an intermediate of phytate synthesis. This assumption was further strengthened by phytate degradation assays showing that D/L-Ins(1,3,4,5)P4 did not belong to the spectrum of degradation products generated by endogenous phytase activity. Metabolic scenarios leading to accumulation of D/L-Ins(1,3,4,5)P4 in barley low-phytate mutants are discussed.

Transgenic wheat and barley with fungal phytase
In a collaborative effort under the Cereal Network, Frame 1 (1995-2001) between RVAU, DIAS and Risø transgenic wheat lines have been produced that overexpress the fungal *Aspergillus* phytase in grains and leaf tissues. The expression of the fungal phytase was under control of the ubiquitin promoter and highest levels of expression of active phytase was achieved with constructs that includes an α-amylase signal peptide. The first lines in barley failed to produce sufficient phytase activity, probably due to co-suppression by using a homologous promotor.

Purification of wheat phytase
We have purified a phytase enzyme from wheat bran and amino acid sequence information was obtained enabling identification of related proteins from soybean and other plant species, that have been characterised as purple acid phosphatases but not as phytases. To our knowledge, this is the first time an enzyme purified for its ability to hydrolyse phytate has been assigned to the purple acid phosphatase enzyme-family, but support for this conclusion can be found in the literature. The first and only report of a cloned plant phytase is from maize. The maize phytase has, based on sequence similarity in a 100 base pair segment of the full-length DNA sequence, been suggested to belong to the family of histidine acid phosphatases and shares this feature with most microbial phytases. Our data indicates that even though maize and wheat are both monocots, differences in their storage of phytate and in the absence (maize) or presence (wheat) of phytase activity in the resting grain, may be reflected on the protein level.

DLF-Risø Biotechnology

The *TERMINAL FLOWER1*-like Gene From *Lolium perenne*

DLF-Risø Biotechnology
The establishment of a biotechnology consortium between DLF-Trifolium A/S and the Risø National Laboratory in 1998 was 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 one of the first step is the isolation and characterisation of genes involved in *Lolium perenne* flowering.

The *TERMINAL FLOWER1*-like Gene From L*olium perenne*

Introduction
The life cycle of flowering plants in general can be divided in to three growth phases: vegetative (V), inflorescence (I), and floral (F). In the vegetative phase the shoot apical meristem (SAM) generates leaves that will later ensure the resources necessary to produce fertile offspring. Upon receiving the appropriate environmental (12 weeks 6°C followed by a warm period; in ryegrass) and developmental signals, the plant switches to floral, or reproductive, growth and the SAM enters the inflorescence phase (I_1) and gives rise to an inflorescence with flower primordia. During this phase the fate of the SAM and the secondary shoots that arise in the axils of the leaves is determined by a set of meristem identity genes, some of which prevent and some of which promote the development of floral meristems. Once established, the plant enters the late inflorescence phase (I_2) where the floral organs are produced.

The genes controling these processes have been identified and isolated mainly by the analysis of flowering mutants in dicot plants such as *Arabidopsis, Antirrhinum*, and tobacco.

A mutation in the *TERMINAL FLOWER 1 (TFL1)* gene of *Arabidopsis* results in the conversion of the inflorescence into a terminal flower. In addition to its effect on meristem fate, *TFL1* also extends the V phase of *Arabidopsis.* The TFL1 protein sequence has similarity with mammalian phosphatidylethanolamine-binding proteins (PEBPs). These mammalian proteins were originally named for their ability to bind phospholipids *in vitro*, but have recently been demonstrated to be regulators of the central Raf1/MEK/ERK signaling pathway.

Antagonistically to the *TFL1* gene, another group of genes specify a determinate floral meristem identity. Well characterized genes such as *LEAFY (LFY), APETALA1 (AP1),* and *CAULIFLOWER (CAL)* from *Arabidopsis* belong to this group. Mutations in either *LEAFY* or *AP1* result in replacement of floral meristems by shoot meristems, and in accordance with their role, overexpression of *LFY* or *AP1* in *Arabidopsis* converts shoots into flowers.

Isolation of a *TFL1*-like gene from ryegrass.
Via RT-PCR a DNA fragment with sequence similarity to *Arabidopsis TFL1* was amplified from ryegrass inflorescence mRNA and successfully used to isolate the full length LpTFL1-like cDNA from a ryegrass flower cDNA library . The coding

region of this cDNA shows 87% and 85% DNA sequence identity to two rice TFL1 homologs, *FDR2* and *FDR1,* and 67% identity with *TFL1*, respectively. On the protein level, LpTFL1 shows 91% and 86% identity to the corresponding proteins, FDR2 and FDR1, respectively, and 71% identity to TFL1.

***LpTFL1* delays or prevents flowering in Arabidopsis**

The LpTFL cDNA was fused to the maize ubiquitin promoter and the resulting construct UBI::*LpTFL1* was transformt into *Arabidopsis thaliana, Festuca rubra and Lolium perenne*. Results on the Lolium and Festuca transformants are so far not available, but will be at the end of 2001. However all *Arabidopsis* transformants showed remarkable vegetative characteristics and were much delayed in flowering compared to the wild-type (Figure 1) Whereas wild-type plants bolted 10 days after they were moved from SD to LD photoperiod, even the earliest flowering UBI::*LpTFL1* plants required another month in LD before they bolted. After 3 months, more than half of the plants had not produced a single flower. Overexpression of *LpTFL1* affected both the vegetative and the early inflorescence stage of *Arabidopsis* as observed by the increased number of nodes produced both before and after bolting. Thus, both in terms of time and the number of nodes produced before flowering the majority of the UBI::*LpTFL1* plants appeared to be arrested in the early inflorescence phase. Similar observations were made in *Arabidopsis* plants in which overexpression of *TFL1* was driven by the 35S CaMV promoter. However, the 35S::*TFL1* plants produced only two thirds of the number of rosette leaves and half of the number of cauline leaves compared to the UBI::*LpTFL1* plants, when grown under continuous light. Five UBI::*LpTFL1* plants remained in the early inflorescence stage throughout their life cycle and failed to produce flowers before they senesced and died (after 7 month). In addition to the main SAM, *Arabidopsis* plants transformed with UBI::*LpTFL1* also exhibit abnormal axillary meristem development. The development of coflorescences with developing flowers in the axils of the cauline leaves normally observed in wild-type *Arabidopsis* was rarely seen in the UBI::*LpTFL1* plants. However, in the place of floral organ formation, a `leafy' branch was produced resulting in a highly branched, bushy, and dramatic phenotype (Figure 1). Third-order branching was a common trait among the UBI::*LpTFL1* plants, and in a single plant also fourth-order branching was observed. A reiterative series of leaves was continuously produced from the SAM of the UBI::*LpTFL1* plants, most of them with a high density of trichomes.

***LpTFL1* overexpression in a *tfl1-14* mutant background**

In order to further address the functional similarity between LpTFL1 and *Arabidopsis* TFL1 we asked if LpTFL1 is able to complement the *Arabidopsis tfl1-14* strong mutant allele. In this mutant a C to T mutation leads to a threonine —> isoleucine substitution at position 69. The *tfl1-14* mutant has a short vegetative phase and exhibits reduced plant height with few nodes, increased number of inflorescence arising from the rosette axillary meristems, and a determinate growth pattern. The construct used for transformation of the *Arabidopsis* wild-type was also used for transformation of the *tfl1-14* mutant. More than 100 independent UBI::*LpTFL1-tfl1-14* primary transformants were obtained from each mutant line after selection for the binary plasmid. All the plants displayed a variety of phenotypes from wild-type to the same extended vegetative phenotype seen in the UBI::*LpTFL1* wild-type background. On average (taken only from the first six plants flowering) the UBI::*LpTFL1-tfl1-14* plants produced 15.2 ± 3.5 cauline leaves on the main stem and flowered 33 days later than the *tfl1-14* mutant and 23 days later than the wild-type. All the UBI::*LpTFL1-tfl1-14* plants grew indefinitely and the production of terminal flowers and rosette inflorescence, which is always seen in the *tfl1-14* mutants, was never observed in the transformants. Thus, the *LpTFL1* rescued the *Arabidopsis tfl1-14* mutant in terms of morphology, and the extended vegetative appearance we observe is presumably due to the force of the relatively strong maize ubiquitin promoter.

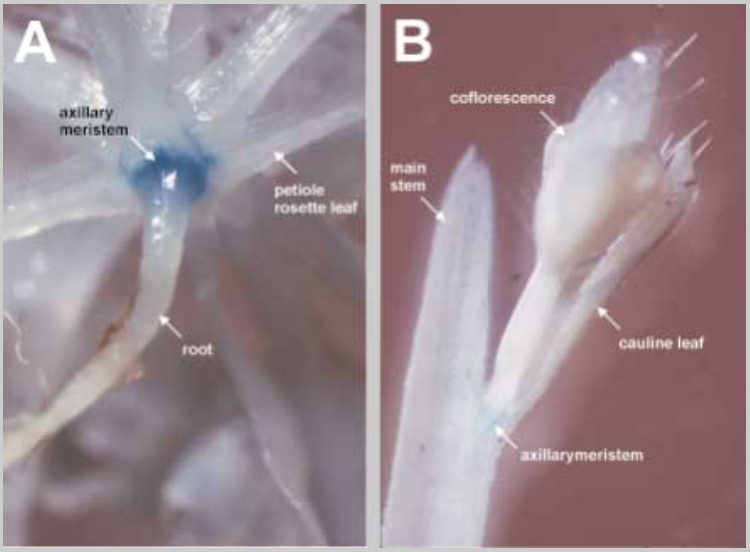
***LpTFL1* is a potential regulator of axillary meristem development.**

To isolate the *LpTFL1* promoter region, we screened a ryegrass genomic library with the full-length *LpTFL1* cDNA clone. In the ~3.6 kb region upstream of the transcription start no likely gene encoding ORFs were found, and therefore we assume this to be the *LpTFL1* promoter.

We examined the properties of the ~3.6 kb putative *LpTFL1* promoter by fusing it to the *Uid*A gene and transformed it into *Arabidopsis*. More than 100 BASTA resistant lines were obtained. Ten seedlings of primary transformants with 6-7 rosette leaves were tested for GUS expression. Three out of the ten transformants showed GUS expression, and in all three plants the expression was confined to a very narrow area in the axillary meristems of the rosette leaves (Figure 2A). Another test for GUS activity was performed after the plants had bolted and had produced 4-5 cauline leaves with flowers in the axils. Fifteen primary transformants were tested, and GUS activity was detected in three of these plants. At this stage GUS activity was still detected in the axillary meristems of the rosette leaves but it was also detected in some of the axillary meristems of the cauline leaves, although weaker (Figure 2B). In this area, however, GUS-activity remained even after the formation of a new flower (Figure 2B). No GUS expression was detected in the apical meristem or in *Arabidopsis* leaves, although *LpTFL1* is expressed in ryegrass leaves. Analysis of GUS positive T_2 lines showed similar results (not shown). Furthermore it showed that GUS expression was restricted to the axillary meristems of rosette leaves and the 3-5 most basal cauline leaves, which were initiated during the vegetative phase. Thus, we find that the activity of the ~3.6kb *LpTFL1* promoter used in this experiment is regulated differently from the expression of *LpTFL1* in ryegrass, and that the GUS expression pattern is similar to *TFL1* expression only in the secondary shoot meristems formed during the vegetative phase.

Figure 1.

Figure 2.



Plant Genetics and Epidemiology

Resistance of cereals towards leaf pathogens – genetics, mechanisms, breeding and management

The most sustainable way to control plant diseases is by means of resistance expressed by the plant itself. Breeding for resistance is a continuous process as the pathogen adapts to the host resistance at a speed depending on the mechanism of the resistance and the corresponding induced selection on the pathogen population. The success of the outcome of this process is founded on the availability of new genes and gene combinations. Characterisation of genes and genetic resources of resistance to different diseases is, therefore, an important prerequisite for breeding programmes. We made this information available for different diseases based on studies of different plant material. We characterize the inheritance of resistance, when simply inherited or inherited by means of several genes with minor effects, as well as the genetic structure of specific resistance gene complexes. In the following, examples of our research published in 2000 is presented. For references see our publication list.

A large collection of spring barley originating from different European countries was tested in field trials with a population of *Pyrenophora teres* f. *maculata* causing net blotch disease. At least three groups of genetically related barley cultivars could be distinguished. The parentage of the three groups of barley cultivars is distantly related and may thus contain three different sources of resistance to the net blotch fungus. In future breeding programmes it would be important to

combine different sources of resistance to achieve durable resistance, e.g., by the use of molecular markers.

Barley cultivars registered in Czechoslovakia/Czech Republic in 1960 – 1999 were analysed to postulate their resistance genes for resistance towards barley powdery mildew caused by the fungus *Blumeria graminis* f.sp. *hordei*. Fifteen known powdery mildew resistance genes and the gene Mla(81), derived from “Nepal 81” and never detected in a cultivar before, were found in these cultivars. Most of the results were confirmed by the pedigree of the cultivars. So also in this material effective resistance sources were identified.

In a cross between a *Hordeum vulgare* accession `HOR 1063' and the cultivar `Krona' the inheritance of partial (quantitative) resistance towards *Puccinia hordei* causing barley leaf rust was studied (figure 1). QTL (Quantitative Trait Locus) analysis for leaf rust resistance was conducted with the help of molecular markers. Four QTLs were localised that explained 96% of the genetic variation in resistance based on AUDPC (Area Under Disease Pressure Curve). Three QTLs were found to be on the barley chromosome 2H whereas one QTL was identified on the barley chromosome 6H (figure 2). All the positive alleles of these QTLs have been detected in a landrace originating from. The molecular markers linked to these QTLs will be important tools for crossing these genes into new breeding lines.

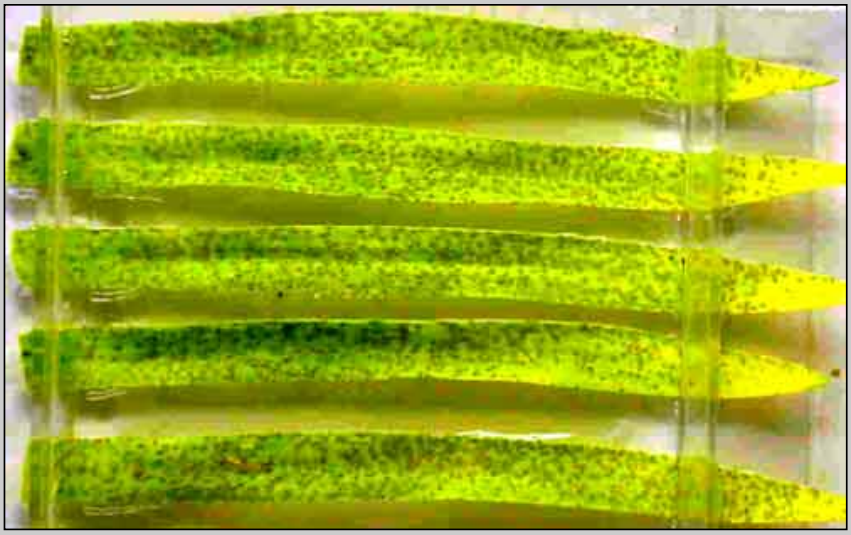


Figure 1. Production of spores of barley leaf rust (*Puccinia hordei*) on barley leaf segments for experimentation.

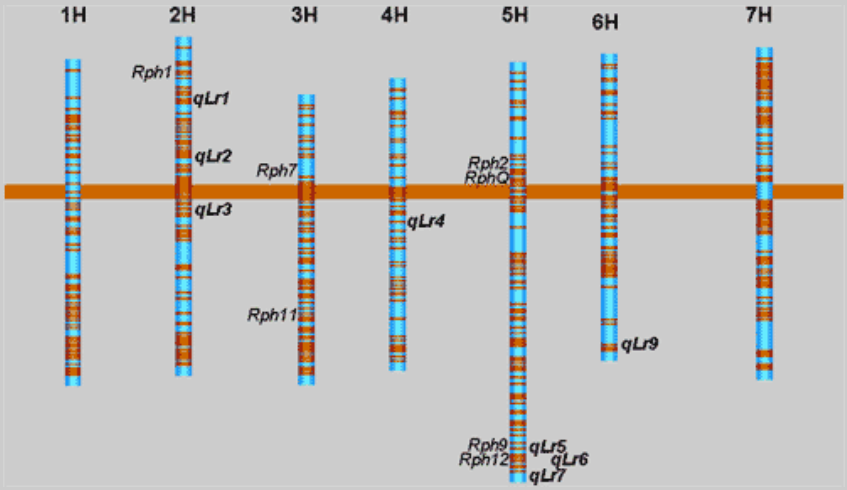


Figure 2. The seven barley chromosomes are shown with specific resistance genes for leaf rust indicated on the left side of the chromosome and partial (quantitative) resistance genes (QTLs) for leaf rust shown on the right side. Each line on the chromosome represents a DNA marker from the barley consensus linkage map. The vertical line denotes the position of the centromer of each chromosome.

The highly polymorphic *Mla* locus for powdery mildew resistance in barley possesses at least 34 different alleles. With a view towards gene isolation, a high-resolution genetic map was constructed for this locus. Three AFLP-markers (Amplified Fragment Length Polymorphisms) were selected as a suitable tool for a chromosome landing strategy. PCR(Poly Chain Reaction)-based screening of approximately 70,000 yeast artificial chromosome (YAC) clones revealed three positive and identical YACs harbouring the *Mla* locus. The presence of NBS (Nucleotide Binding Site)-LRR (Leucin Rich Repeat) regions was found in these YACs. These motives have been detected repeatedly in the DNA sequence of already cloned resistance genes. They presumably play an important role in the recognition of the pathogen.

To find more genes implying efficient and durable resistance, we also study the mechanisms of resistance response during the early phase of infection. This research is mainly focused on host cell responses leading to resistance against

powdery mildew (*Blumeria graminis*) in cereals. When powdery mildew attacks a cereal epidermal cell the fungus tries to force its way into the host cell. To prevent penetration, the attacked cell respond by local reinforcement of the cell wall beneath the site of the penetration attempt. This process is called papilla formation and involves deposition of a callose matrix with induced accumulation of elements like H_2O_2, phenolic autofluorogens and various proteins and glycoproteins with hydrolytic and antifungal properties (reviewed in *Zeyen et al., 2001*). The barley *mlo* gene confers a unique resistant phenotype in spring barley with nearly total efficient papilla resistance. Because of this, *mlo* is now a very much used source for resistance in spring barley in Europe. So far it has remained highly effective. However, *mlo* resistance may be broken down because its efficiency depends on several factors such as host genetic background, environmental conditions and fungal genotype.

The ability of host epidermal cells to prevent powdery mildew penetration is dramatically affected by the outcome of prior attacks by the fungus. This has implications for the field situation where individual cells are confronted by sequential attacks of fungal spores which are deposited continually from the aerial pool of spores. Thus, if an initial attack is successful, the cell shows induced accessibility so that later attacks on that cell will almost invariable succeed. By contrast, failed initial attacks induce 'inaccessibility' within the cell so that subsequent attacks almost invariably fail. While these induced changes are essentially localised phenomena being expressed most strongly in the single cell directly subjected to earlier attack, there is some transmission of effects to immediately adjacent epidermal cells although the effects are often undetectable at two cells distance.

Studies on resistance mechanisms also include studies of infection biology of septoria tritici blotch of wheat caused by the fungus *Mycosphaerella graminicola* (also designated *Septoria tritici* in the asexual stage). Mechanisms involved in resistance to septoria in wheat as well as mechanisms involved in fungal virulence are unknown. Examination of the early phase of infection has not clarified this. However, vegetative spores seem to possess increased growth and development potential compared to pycnidia spores (figure 3).

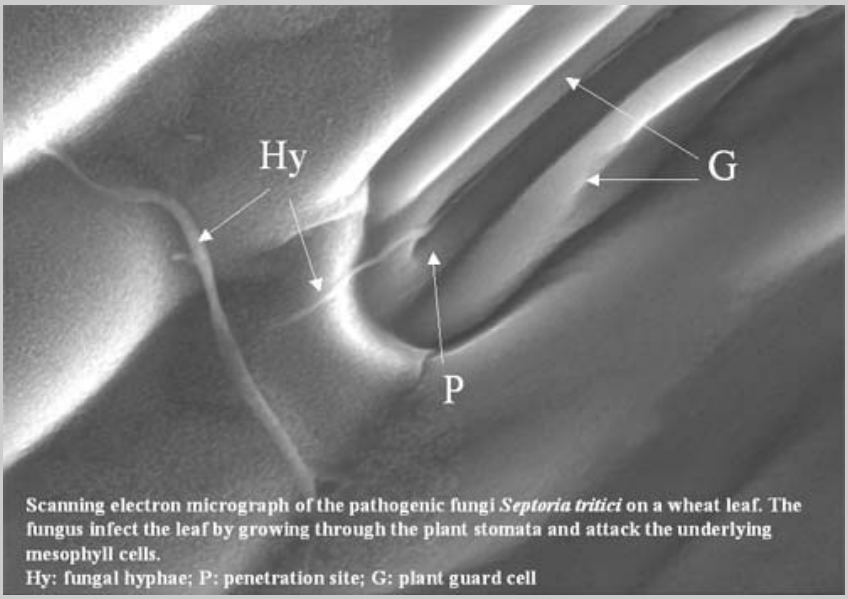


Figure 3. *Septoria tritici* electron microscopy

We contribute also to describing the efficiency of resistance in the cultivars actually grown. This depends very much on the pathogen population at the specific locality. For powdery mildew we have taken part in a description of the situation in France and Denmark in the period 1990 to 1998. The efficiency of specific resistance genes in controlling disease was described by the average fitness of the pathogen population in four different regions. This fitness was high on winter crops but low on spring crops, however, increasing over time from 1990 to 1998. The high efficiency of resistance in spring crops especially in Denmark may be due to a large use of cultivars with *mlo*-resistance here.

The disease septoria tritici blotch has for the past three years been the most important disease on winter wheat in Denmark (figure 4). Data from cultivar trials conducted by The Danish Institute of Agricultural Sciences has been analysed to detect changes in susceptibility of cultivars over years. As rather little is known about this host-pathogen system the data were of much less detail than the data on barley powdery mildew as mentioned above. A trend toward some decrease in resistance was observed over the years 1996-99.

Figure 4. A susceptible wheat cultivar early in the summer 1998 before the epidemic reached and killed the upper leaves.

Biogeochemistry

The SOROFLUX Project

(Effects of land use and organic waste application on carbon and nitrogen fluxes)

The SOROFLUX 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 a collaboration between Risø National Laboratory, University of Copenhagen, Royal Veterinary and Agricultural University and University of Aarhus.

The climate is changing due to the greenhouse effect – the future is expected to be hotter and with more vigorous rainstorms. The single most important greenhouse gas is CO_2, and the overall carbon balance plays an important role in understanding and predicting the positive or negative feedback of CO_2 on the future climatic situation. Simultaneously nitrogen plays an important role in regulating or modifying carbon sequestration. Therefore, it is important to understand and quantify the fluxes of C and N and the net release or uptake of carbon in terrestrial ecosystems.

The main objectives of the SOROFLUX project were to quantify and compare the gaseous and water mediated fluxes of C and N compounds in a European beech *(Fagus silvatica* L.) forest and an agro ecosystem with barley *(Hordeum vulgare* L.). The study included measurements of the fluxes of minor gaseous carbon and nitrogen compounds. In total it provided quantification of the net ecosystem fluxes of NO, NO_2, NH_3, N_2O, CO_2, CH_4, H_2O and water mediated inorganic and organic N and C compounds.

The automated eddy covariance measurements of CO_2 fluxes showed large variations during the day, during the season, during the years and between the forest and agro ecosystems. The net carbon exchange of terrestrial ecosystems is the result of a delicate balance between uptake (photosynthesis) and loss (respiration). Under favourable conditions, the net ecosystem flux was dominated by photosynthesis during daytime, and by respiration at night. In the leafless period of the year respiration also dominated. The fluxes of carbon from the atmosphere to the forest and agro ecosystems were in the range of $11^{-1}2$ t C ha^{-1} yr^{-1}. Since the total ecosystem respiration was in the range of 10 t C ha^{-1} yr^{-1}, of which about 60% was soil respiration, the net ecosystem exchange was in the range of 1-2 t C ha^{-1} yr^{-1}. This is a medium value for C sequestration in European ecosystems, the values ranging from 7 t C ha^{-1} yr^{-1} in southern Europe to zero (or even emission) in northern Europe. The emission or oxidation of CH_4 only influenced the total C flux of the ecosystems with a few kg C ha^{-1} yr^{-1}. Leaching of C in the form of TOC and HCO3- was less than 100 kg C ha^{-1} yr^{-1}.

The inputs of N with rain, throughfall and stemflow to the ecosystems are presented in figure A. In general, the contributions of N deposition from rain and throughfall were in the same range during the year. Stemflow accounted for about 17% and 37% of the total flux of water and nitrogen respectively to the forest floor. The total flux of nitrogen to the forest floor amounted to c. 25 kg N ha^{-1} yr^{-1} and wet deposition to the agricultural fields (and forest) to 16 kg N ha^{-1} yr^{-1}. The difference between the wet deposition and the sum of throughfall and stemflow can be ascribed to dry deposition. However, dry deposition may have been higher than the 9 kg N ha^{-1} yr^{-1}, since nitrogen can be taken up in the canopy, as has been reported for Norway spruce. The presented N deposition values represent medium range values for European ecosystems.

The concentration of NO_3-N in soil water under the root zone was very low in the forest, less than 1 mg L^{-1}. There was no significant difference between samples taken close to the tree trunks and in the middle between tree trunks. In the agricultural field the NO_3-N concentration was higher, 10-50 mg L^{-1}. Using a hydrological ET model, the leaching of NO_3 - N from the agricultural field could be calculated to be in the range of 50-70 kg N ha^{-1} yr^{-1}. This is a relatively high leaching rate compared to general findings for agricultural fields in Denmark. In the forest leaching was only a few kg N ha^{-1} yr^{-1}, which is the general picture for forests in low-medium N deposition areas in Europe. In the summer period there was almost no leaching from the root zone, since both trees and agricultural crops utilised all available soil water. The emission of NO from the forest floor was less than 1 kg N ha^{-1} yr^{-1}. The emission of N_2O was also very small 0.5-3 kg N ha^{-1} yr^{-1}, highest values being obtained from the agricultural field.

Taking the atmospheric deposition in consideration and the internationally used “critical load” criteria, the critical load for N seems to be exceeded by 5 kg N ha^{-1} yr^{-1}.

From the project it can be concluded that the net carbon ecosystem exchange is relatively modest in both the examined forest and agro ecosystems. However, scaling up the examined area to the total area of Europe, carbon sequestration in forest might amount to be about 20-40 % of the CO_2 emission from fossil fuels. But taking into consideration that a part of the produced biomass from forest and agro ecosystems is burned or decomposes quickly, the long-term carbon sequestration in terrestrial ecosystems may be reduced to 2-3 % of the CO_2 emission. Anticipating that dry deposition of N in agricultural fields is relatively low, the atmospheric deposition of N is about 40% higher in forests than in agricultural fields. Comparing forest and agro ecosystems, afforestation will undoubtedly improve the groundwater quality with respect to NO_3-content, although the amount of formed groundwater might be less due to the higher evapotranspiration in forests.

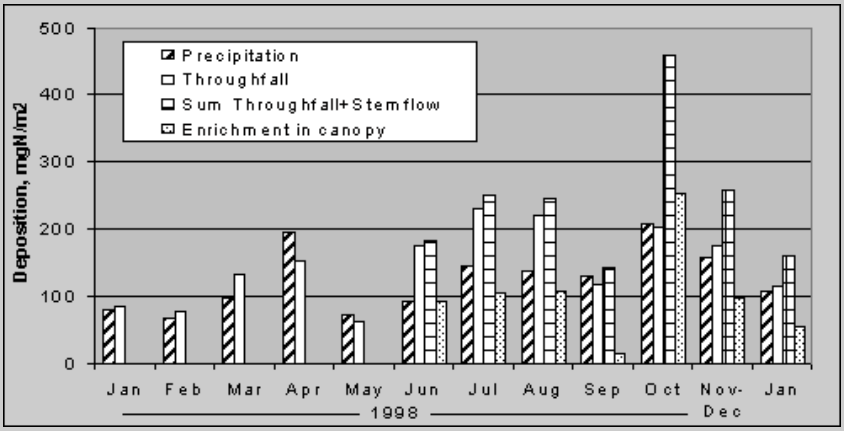


Figure A. N deposition with precipitation, throughfall and stemflow.

Comparative Investigation of Concentrations of Major and Trace Elements in Organically and Conventionally Cultivated Onions and Peas

Elements are important constituents in human and animal nutrients. Which elements we name essential neutral or toxic is still changing which among other things is caused by evolution of analytical techniques with lower detection limits. Compared with the fact that the uptake of some elements via human food may be subject to competitive uptake of other elements, it is necessary not only to look at few elements when nutritional aspects of organic crops are compared with conventional crops.

In a superior consideration the elemental uptake of a specific crop is a function of the environment and the genetics. The environment is affected by the character of the soil, the climate, the supply of agricultural chemicals and manure, the microbiological activity in the soil, and the biological attacks on the plant from fungi, insect etc. In the same geographical area there is only little variation in the climate but the variation in soil character from site to site may be considerable but not necessarily the most important parameter in determining the uptake of elements by plants. Other parameters affecting plant uptake are related to cultivation practices. In conventional farming with an intensive use of fertilisers and pesticides the biological activity in the soil and the biological attacks from fungi and insects may be very different from that in the organic farming with the use of farmyard manure and frequent rotation of crops but no use of pesticides. In the light of these facts it will be of great importance to verify if it is possible by multivariate analyses of elemental concentration profiles to separate crops, of uniform genetics (same sort) cultivated organically and conventionally in the same geographic area, by the cultivation practices.

The Danish Food Technology and Development Program (FØTEK) financed this study as a part of a comprehensive survey of the influence of different cultivation practices on the elemental concentration profiles in different types of vegetables (leafy, root, legume) and various grains. The different cultivation methods are organic cultivation in accordance with the Danish law (1987) and EEC Council Regulation (1991) and conventional cultivation with use of fertilisers and pesticides. In this study the investigated vegetables are onions and peas which both are important ingredients in Danish human diets.

Site Selection. Onion samples were collected from 11 conventional and 10 organic production areas. Pea samples were collected from 10 conventional and 9 organic production areas. All production areas for both crops are located on Funen and Jutland in Denmark and the six most widespread soil types are represented in each cultivation practice as evenly as possible. The cultivation and harvest were performed in 1995.

Sampling. Ten undamaged, healthy, average sized and normal shaped onions were sampled evenly across each site. A total of 20 undamaged closed pea pods from ten healthy pea plants (2 pea pods per plant) were nipped evenly across each site. To avoid decomposition the peas were refrigerated in the laboratory until sample preparation.

Sample Preparation. All sample preparations were performed under controlled condition in three rooms with lock-gate connection. The rooms are classified as R1 (ordinary condition), R2 (fairly clean), and R3 (clean, class 1000 room).

All sample preparations after the cleaning procedures were carried out in the class 1000 environment (R3). The samples were homogenised and digested with redistilled nitric acid in a microwave oven.

Multielement Determination. The sample solutions were diluted with double-deionized water, and HR-ICP-MS was used to determine as many elements in the sample solutions and blanks that we found possible in a routine HR-ICP-MS method. In the onion samples 63 elements (Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Hg, Ho, In, Ir, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, P, Pb, Pr, Pt, Rb, Re, Ru, S, Sb, Sc, Si, Sm, Sn, Sr, Tb, Te, Th, Ti, Tl, Tm, U, V, W, Y, Yb, Zn, and Zr) and in the pea samples 55 elements (Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Ho, Ir, La, Lu, Mn, Mo, Nb, Nd, P, Pb, Pd, Pr, Pt, Re, Rh, Sb, Sc, Se, Si, Sm, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, Y, Yb, Zn, and Zr) were determined.

Comparative statistical tests. The effect of the two farming methods on the elemental content in the crops was examined by comparison of the mean values for each farmer of each of the elements for the two farming methods. The means are compared by Students t-test, testing the hypothesis H0: 1 = 2 against the alternative H0: 1 2. For each element an F-test is performed to test for equality of the variances of the groups. The results of the test of equality of variances show that for part of the elements the variances of the two populations are unequal at 5% level. For these elements the t-test is based on an approximative test.

It appears that the levels of Ca, Mg, B, Bi, Dy, Eu, Gd, Lu, Rb, Sb, Se, Sr, Ti, U, and Y differ significantly ($p < 0.05$) between the organically and conventionally grown onions. Only the levels of P, Gd, and Ti were found to differ significantly ($p < 0.05$) between the organically and conventionally grown peas. Compared to onions the difference between the two farming methods for peas is thus less evident based on the t-test.

Principal component analysis. Principal component analysis (PCA) was applied to the 63 elements measured in the individual onion samples from the 21 farmers to investigate the relevant and interpretable structure in the data. The variables were weighted with the inverse of the standard deviation of all objects before the PCA. This was done to compensate for the different scales of the variables. It was found that three principal components (PCs) explained only 29% of the variation in the data set (PC1: 13%, PC2: 9%, and PC3: 7%). However, as it appears from Figure 1, the onion samples split up into groups according to the farming method when the scores of the first and third principal components (PCs) are plotted against each other. In Figure 1 the samples with second letter E represent organically grown samples (marked with green) and the samples with second letter C represent conventionally grown samples (marked with red).

The model is based on individual samples from each farmer and thus includes the variations between the individual samples from each farmer. In case of authority control of onions, this would be an advantage because it would be possible to identify even a few conventionally grown onions in a batch of organically grown onion samples.

A PCA model for peas based on 190 samples and 55 elements was made. The variables were weighted with the inverse of the standard deviation of all objects. This model showed only a weak tendency to separate organically grown peas from conventionally grown peas. However, when the variations between the individual samples from each farmer are eliminated and the model is based on mean values for each farmer the separation of the two farming methods is improved.

The first four principal components for the PCA model on mean data (19 farmers and 55 elements) explained 56% of the variation in the data set (PC1: 18%, PC2: 15%, PC3: 8% and PC4: 11%). Particularly the variation explained by the third and fourth principal components is found to be related to the farming method. The scores for the third and fourth component of the PCA model are given in Figure 2.

The samples with second letter E represent organically grown samples (marked with green) and the samples with second letter C represent conventionally grown samples (marked with red).

It appears that the pea samples split up into groups according to the farming method. However, the conventional farmer marked PC is located together with the organic farmers. This farmer has used cattle slurry and is therefore more alike the organic farmers than the other conventional farmers who use fertilisers (RC, SC, TC, and UC) or nothing (QC, XC, YC, and ZC).

Comparative statistical tests of element concentrations in organically and conventionally cultivated onions and peas have shown that the cultivation method affects the concentration of some elements in the crops. Principal component analysis (PCA) of the analytical data has convincingly demonstrated that the elemental concentration profiles are different for organically and conventionally grown onions and peas and that it is possible by multivariate analysis of multi-element concentration data for onions and peas to separate a crop after the cultivation method. The methodology used in this study may be developed to authenticity control for organic cultivation, but further comprehensive investigations are needed.

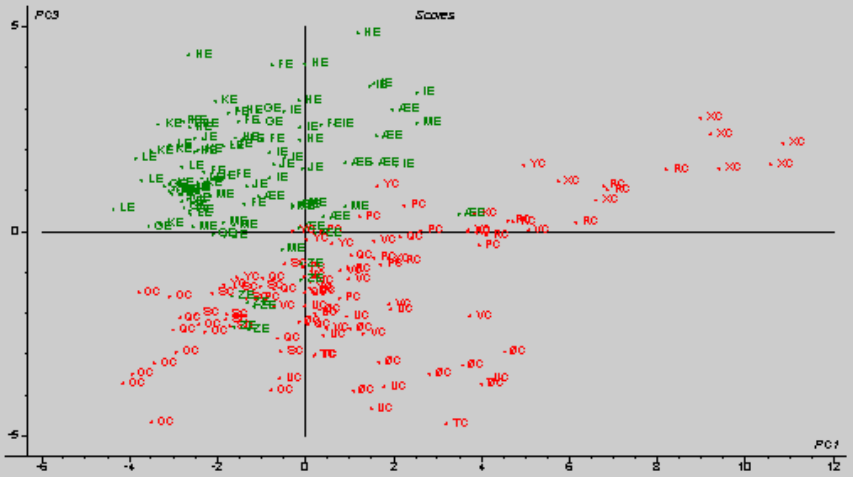


Figure 1. Scores plot for the first and third principal component of the PCA model for individual onion samples. Sites with second letter E are organic and sites with second letter C are conventional.

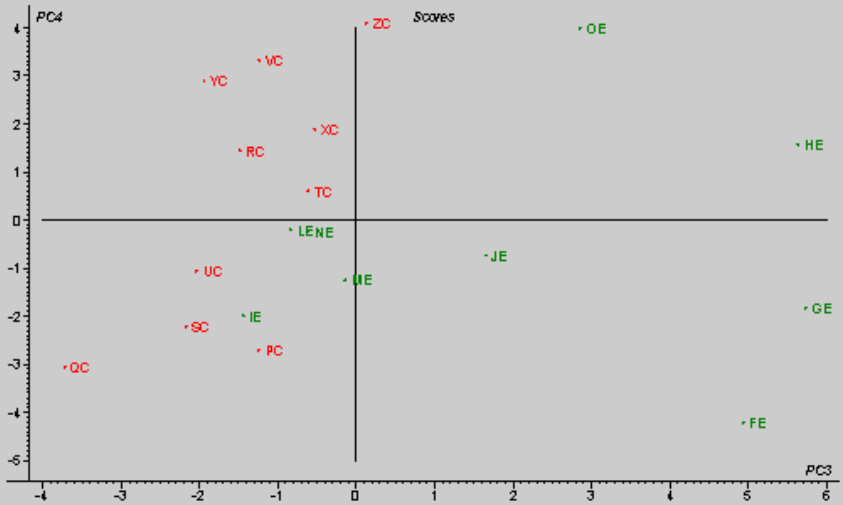


Figure 2. Scores plot for the third and fourth principal component of the PCA model for peas. The model is based on mean values from each site. Sites with second letter E are organic and sites with second letter C are conventional.

Plant Ecosystems and Nutrient Cycling Programme

See also SOROFLUX

CLIMOOR

- a research project designed to investigate the effects of a changing climate on our natural ecosystems

The climate is under change. In a relatively near future the climate will get warmer and the distribution of precipitation over the year will change considerably. This is among the conclusions recently drawn by the Intergovernmental Panel on Climate Change (IPCC). IPCC's conclusions are based on present knowledge of emissions of green house gasses and climate formation and on observations of the change in climatic conditions until now.

A change in the climatic conditions will potentially affect the functioning of ecosystems. In terrestrial ecosystems a warmer climate is likely to increase the availability of nutrients for plant growth because of increased rates of decomposition. Periods of severe drought are likely to work in the opposite direction. The resulting changes in nutrient availability will change the growth conditions for plants and affect the competition among different plant species. Together with the simultaneous change in the CO_2 concentration in the atmosphere this can lead to strong changes in ecosystems. However, the processes involved in these changes form a very complex puzzle and the resulting effects are generally difficult to predict and in many cases unknown.

In order to improve our understanding of the functioning of the natural ecosystems and thereby to assess the impacts of climate change on them, a large number of research projects have been initiated during the past decade - in most cases initiated by EU research programmes. RISØ participates in a number of such projects - CLIMOOR being one of them.

CLIMOOR was started in 1998 and was specifically designed to investigate the effects of warming and drought on the functioning of heath and moorland ecosystems in Europe. The project involves 6 European partner institutions from Denmark, UK, the Netherlands and Spain and RISØ co-ordinates the project. From Denmark the Danish Forest and Landscape Research Institute also participates in the project.

How to manipulate the ecosystems?
In CLIMOOR we "manipulate" the ecosystems. This means that we try experimentally to create the conditions in our ecosystems that has been forcasted for the future. We apply warming and drought in the field by small (20 m^2) automatically operated curtains that create warming by reducing the heat loss at night or create drought by removing the precipitation. The critical points in this type of field scale experiments are that the manipulations have to be realistic in relation to the impacts you want to study and they must only manipulate the factors you want to study, i.e they must not create artefacts. For the CLIMOOR project, RISØ developed a new experimental set-up in a colaboration with the other partners and 3 Danish companies with expertise on tailoring technical field scale solutions for greenhouse industries and environmental research.

Figure 1
Heating roof at Mols Bjerge. The curtain is automatically drawn over the vegetation at night to reduce the heat loss and thereby increase the temperature. Next to the heating roof are the scaffoldings to host the plots for drought and control.

The manipulations are warming, drought and control:

Warming - is created by covering the ecosystem with IR reflective curtains. The curtains are automatically drawn over the vegetation when the sun sets. Hereby the loss of IR radiation at night is prevented and the temperature in the air and the soil increases. In the morning the curtain will be removed when the sun rises, which means that the light conditions for the plants are not altered. Furthermore the curtains are automatically removed during rain events in order to avoid disturbance of the hydrology. Finally the curtain design with open sides mean that the wind conditions are only affected to an absolute minimum.

Drought - is created by a similar curtain system, except that the curtain material is a regular water tight plastic. Drought is applied for 2 months in the spring and early summer. Whenever it rains during the drought period the curtains are drawn over the vegetation to remove the water and when the rain stops the curtains are removed. Also here this means that the light conditions are only altered very little.

Control - In order to assess the effects of the manipulations the results from these experimental plots are compared to untreated control plots. In all plots the measurements focus on changes in soil and plant processes.

Combining manipulations and natural differences.
If we look at Europe we all know that the climate differs among areas and countries - in general the temperature increases when we go south and in many cases the precipitation increases when we go west. This means that there is a natural difference or gradient with respect to both temperature and precipitation - a difference that has persisted for centuries. We can take advantage of these natural differences in climatic conditions, because they may in fact help us answering questions about the long term effects of climate change. In CLIMOOR we have tried to combine the two approaches – the experimental manipulations and the natural gradient by setting up our experimental studies in Denmark, the Netherlands, Wales and Spain. Together these sites span gradients in precipitation and temperature - the same climatic factors that we manipulate at each site. Hopefully the combination will help us to gain insight into the long term climatic effects on terrestrial ecosystems as well as the short term and transitional effects.

Figure 2
Climoor roof setup at Garraf in NE Spain. The scaffolding carries the heating curtain, which is automatically drawn over the vegetation at night. The ecosystem at the Spanish site is dominated by ericaceous shrubs, which is typical for the region.

Warming the ecosystem by 1 °C alters plant competition
The experiments have now been running for 2 years. The experimental setup increases the temperature by c. 1 °C with some marked differences between the countries. The biggest temperature increase (c. 2 °C) occurs in Spain, which has the largest energy input during the day. The smallest increase is in Wales that has the lowest energy input and the largest water input, which creates an additional energy-pathway.

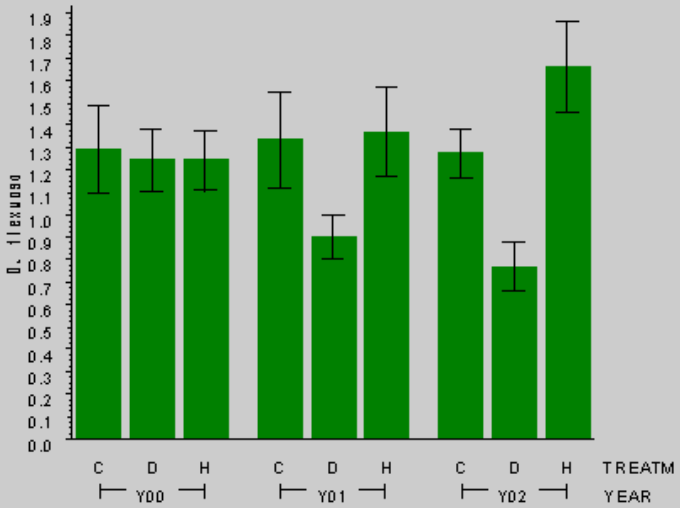


Figure 3
Effects of heating and drought on the growth of grasses at Mols. The growth of the different plant species are measured every year by measuring the number of times you hit the different plant species if you point with a pin at at a large number of points in each plot. The average number of times can exceed 1 if there are more than one plant at each point. The treatments are C: Control, D: Drought and H: Heat and the years are Y00: year before treatment started, Y01 and Y02: First and second treatment years. The of number of grass plants per hit has increased significantly by the temperature increase and has gone down by the drought The growth measurements are conducted by the Danish Forest and Landscape Research Institute.

One degree is not much. In fact it is within the natural variation that may occur between two successional years at anyone site. Therefore the effects are expected to be correspondingly small. If we look at the decomposition and mineralisation of the organic material in the soil, which we expected to be the most sensitive processes to changes in both temperature and water, the manipulations has not caused any significant changes. Correspondingly, if we look at the soil water that supplies water and nutrients to the plants, this has not changed either. However, if we look at the plant growth something has surprisingly changed - at least at some of the sites. In both Denmark and Wales the measurements have shown, that the growth rate of the different plants in the ecosystem are affected differently e.g. in the Danish site at Mols, the grasses grow better in the treatment with increased temperature, which is not the case for calluna (figure 3). This means that the grasses get an advantage compared to calluna plants if the temperature increases, which means that warming may potentially cause a shift in the balance between calluna and grasses which in the long run can be a threat to the calluna heathland. Under todays atmospheric input of nitrogen from agricultural emissions the calluna heathland is already under severe pressure of being transformed into grassland. It seems that climate change may increase this threat even further, even under moderate temperature increases.

VULCAN - a follow up on CLIMOOR
It has been surprising that the modest temperature increase has caused such a marked effect, especially concidering that none of the drivers in the cosystem, especially nutrients and water, seem to have changed. The results from CLIMOOR cannot answer why this happens. In order to help the European decision makers in the international political negotiations on green house gas reductions and in order to help and advise land owners and managers on how to manage these seminatural shrublands it is important to gain more insight into the reasons for the observed changes. Therefore we have initiated a new project called VULCAN - that will look more closely at the plant part of the responses, especially the biodiversity issue of the problem during the next four years.

Personnel

Scientific staff

Ambus, Per
Andersen, Claus H.
Aubert, Dominique
Backes, Gunter
Baunsgaard, Lone (until 30.11.00)
Bechmann, Iben Ellegaard

Beier, Claus
Bjerre, Karsten
Burleigh, Steven
Christensen, Anders B.
Christiansen, Solveig Krogh
Didion, Thomas
Egsgaard, Helge
Engvild, Kjeld C.
Feilberg, Anders
Gavito, Mayra (until 31.12.00)
Giese, Henriette (until 31.01.00)
Gissel Nielsen, Gunnar
Gjetting, Torben
Gundersen, Vagn
Hansen, Torben Michael
Hatzack, Frank (until 31.05.00)
Hauser, Thure
Jahoor, Ahmed
Jakobsen, Iver
Jensen, Arne (until 30.04.00)
Jensen, Erik Steen
Jensen, Jens
Jensen, Lisbeth Gath
Johansen, Katja Salomon
Jørgensen, Rikke Bagger
Kristensen, Brian
Kunzendorf, Helmar (until 31.08.00)
Kure, Liv
Lange, Sabine (until 30.11.00)
Larsen, Elfinn (until 31.01.00)
Laturnus, Frank (until 30.09.00)
Lett, Christophe (until 14.08.00)
Levy, Yaron
Lynggård, Bent
Lyngkjær, Michael
Mikkelsen, Teis Nørgaard
Mortensen, Gerda Krog
Mouritzen, Peter (until 31.10.00)
Møller, Ian Max
Nielsen, Klaus K.
Nielsen, Torben
Olesen, Karen Lambæk
Pedersen, Carsten
Pilegaard, Kim
Rasmussen, Lennart
Rasmussen, Søren Kjærsgård
Richter, Hannes (until 31.03.00)
Rosendahl, Lis (until 31.07.00)
Salchert, Klaus-Dieter
Schmidt, Anette Skammelsen
Schou, Christian
Stockmarr, Anders (until 31.12.00)
Storgaard, Morten
Stürup, Stefan (until 31.10.00)
Sørensen, Mikael Blom
Saalbach, Gerhard
Thomsen, Anne Belinda
Thordal-Christensen, Hans
Wienkoop, Stefanie
Woidemann, Anders (until 13.02.00)
Wu, Boqian
Østergård, Hanne

Technical Staff

Andersen, Bente

Andersen, Margit Elm
Arildsen, Lene
Bonde, Rikke
Brandt, Lis
Brink-Jensen, Merete
Djurdjevic, Stanko
Fernqvist, Tomas
Fosskov Jensen, Jette (until 31.05.00)
Gudiksen, Peter (until 30.11.00)
Hansen, Ina
Hasselbalch, Finn
Ibsen, Elly
Jensen, Birgit (until 31.01.00)
Jensen, Ellen Møller
Jensen, Linette Munksgaard
Kock, Gertrud
Koutras, Charlotte (until 30.04.00)
Larsen, Inge Merete
Larsen, Ingelis
Larsen, Tina Bøgeskov (until 31.08.00)
Meltofte, Liselotte
Nielsen, Anja Christina
Nielsen, Jette Bruun
Nielsen, Vagn Aage
Olsen, Anette
Olsen, Anne
Olsen, Inge
Petersen, René
Sillesen, Anerikke
Storm Petersen, Anne-Mette (until 30.04.00)
Sørensen, Poul
Tung, Tran Duc Tuan
Vinther Kristensen, Lis
Walther Jensen, Lise
Wojtaszewski, Hanne

Administrative Staff

Bay, Kirsten (until 30.09.00)
Borring Sørensen, Marit
Christiansen, Krista
Frandsen, Anette
Hjorth, Aase
Jensen, Hanne
Krogh, Helle
Lilholt, Ulla
Løje, Søren
Petersen, Lis

Ph.D. Students

Ammitzbøll, Henriette
Bruhn, Dan
Burhenne, Kim
Christiansen, Pernille
Erik, Pinar
Frøsig, Lars
Gavnholt, Britta
Grell, Morten (until 05.04.00)
Hansen, Poul Møller
Hauggaard-Nielsen, Henrik
Holm, Kirsten Bagge
Holst, Pia Bachmann (until 31.08.00)
Jensen, Christian Sig
Johannessen, Marina
Johansen, Runa Ulsøe

Jonassen, Kristoffer
Jørgensen, Rasmus Nyholm
Klinke, Helene B.
Nielsen, Jock
Petersen, Klaus
Poulsen, Tina Tandrup
Rasmussen, Nanna
Scharff, Anne Marie
Tranekjær, Michael

M.Sc. and B.Sc. Students

Agerskov, Henrik
Arp, Thomas
Bohn, Vibeke
Christophersen, Helle
Fast, Helene
Fischer, Pernille Hertz
Hansen, Lise
Holm, Kirsten Bagge
Holmegaard Nielsen, Anne
Hvolbæk, Trine
Jacobsen, Annemarie
Jensen, Karen Dahl
Josefsen, Lone
Jørgensen, Anne Charlotte
Lange, Mette
Møller, Annette
Mønster, Henrik Ringgaard
Nielsen, Kristina Vad
Poulsen, Morten
Ringgård, Trine
Schultz, Peter Hjort
Stenby, Charlotte
Thomsen, Karen
Thygesen, Anders
Thyme, Mette

Apprentices

Andersen, Sophie Rud
Berg, Suanne
Bonde, Laura Hesel
Dyrberg, Mette
Ebbesen, Jacob
Hasselsteen, Pia
Jakobsen, Karen
Jensen, Thomas
Johansen, Hanne
Niebuhr, Lene
Pedersen, Hanne
Rasmussen, Winnie

Visiting scientists

Abbott, Lynette, Soil Science and Plant Nutrition, The University of Western Australia (1 month)
Carlsson, Malin, Swedish University of Agricultural Sciences, Svalöv, Sweden (4 months)
Elisabeth Anne Drew, The University of Adelaide, Australia (2 months)
Eriksen, Lars B., Sejet Plant Breeding, Denmark (3 months)
Hübner, Matthias, University of Göttingen, Germany (3 months)
Jacobsen, Frank, HOH Water Tech.nology, Denmark (4 months).
Kirby, Kent, Elmhurst College, Illinois, USA (3 months)
Kvaløy, Kirsti, Norwegian Institute for Nature Research, Trondheim, Norway (3 weeks)
Linde, Marcus, Institute for Ornamental Plant Breeding, Ahrensburg, Germany (1 week)
Molbek, Hamid (1 week) marts
Naghavi, Reza, Tehran University, Iran (4 months)

Ohura, Mariko, University of Shizuoka, Japan (2 months)
Ohura, Takeo, University of Shizuoka, Japan (2 months)
Palonen, Hetti, VTT Biotechnology and Food Research, Espoo, Finaldn (3 months)
Sayed, Haithan, International Centre for Agriculture, Research in the Dry Areas, Aleppo, Syria (11 months)
Schiemann, Andrea, Pajbjerg Foundation, Denmark (1 year)

Articles in international journals, books and reports

Andrén, E.; Andrén, T.; Kunzendorf, H., Holocene history of the Baltic Sea as a background for assessing records of human impact in the sediments of the Gotland Basin. *Holocene* (2000) v. 10 p. 687-702

Aubinet, M.; Grelle, A.; Ibrom, A.; Rannik, U.; Noncrieff, J.; Foken, T.; Kowalski, A.S.; Martin, P.H.; Berbigier, P.; Bernhofer, C.; Clement, R.; Elbers, J.; Granier, A.; Grunwald, T.; Morgenstern, K.; Pilegaard, K.; Rebmann, C.; Snijders, W.; Valentini, R.; Vesala, T., Estimates of the annual net carbon and water exchange of forests: The EUROFLUX methodology. *Adv. Ecol. Res.* (2000) v. 30 p. 113-175

Bechmann, I.E.; Stürup, S.; Kristensen, L.V., High resolution inductively coupled plasma mass spectrometry (HR-ICPMS) determination and multivariate evaluation of 10 trace elements in mussels from 7 sites in Limfjorden, Denmark. *Fresenius J. Anal. Chem.* (2000) v. 368 p. 708-714

Berczi, A.; Møller, I.M., Redox enzymes in the plant plasma membrane and their possible roles. *Plant Cell Environ.* (2000) v. 23 p. 1287-1302

Bothmer, R. von; Salomon, B.; Linde-Laursen, I., Chromosome pairing patterns in interspecific *Hordeum lechleri x H. vulgare* (cultivated barley) hybrids with 2n=21-29. *Hereditas* (1999) v. 131 p. 109-120

Bousset, L.; Østergård, H.; Hovmøller, M.S.; Caffier, V.; Vallavieille-Pope, C. de, Effectiveness of powdery mildew resistance genes in autumn-sown and spring-sown barley crops. *Acta Phytopathol. Entomol. Hung.* (2000) v. 35 p. 153-159

Brandt, P.; Larsen, E.; Henriksen, U., High tar reduction in a two-stage gasifier. *Energy Fuels* (2000) v. 14 p. 816-819

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. Breed.* (2000) v. 6 p. 195-206

Bruhn, D.; Leverenz, J.W.; Saxe, H., Effects of tree size and temperature on relative growth rate and its components of *Fagus sylvatica* seedlings exposed to two partial pressures of atmospheric [CO2]. *New Phytol.* (2000) v. 146 p. 415-425

Burhenne, K.; Gregersen, P.L., Up-regulation of the ascorbate-dependent antioxidative system in barley leaves during powdery mildew infection. *Mol. Plant Pathol.* (2000) v. 1 p. 303-314

Burleigh, S.H., Cloning arbuscule-related genes from mycorrhizas. *Plant Soil* (2000) v. 226 p. 287-292

Christensen, C.S.; Hummelshøj, P.; Jensen, N.O.; Larsen, B.; Lohse, C.; Pilegaard, K.; Skov, H., Determination of the terpene flux from orange species and Norway spruce by relaxed eddy accumulation. *Atmos. Environ.* (2000) v. 34 p. 3057-3067

Christensen, C.S.; Skov, H.; Nielsen, T.; Lohse, C., Temporal variation of carbonyl compound concentrations at a semirural site in Denmark. *Atmos. Environ.* (2000) v. 34 p. 287-296

Dreiseitl, A.; Jørgensen, J. Helms, Powdery mildew resistance in Czech and Slovak barley cultivars. *Plant Breed.* (2000) v. 119 p. 203-209

Emeis, K.C.; Struck, U.; Leipe, T.; Pollehne, F.; Kunzendorf, H.; Christiansen, C., Changes in the C, N, P burial rates in some Baltic Sea sediments over the last 150 years - relevance to P regeneration rates and the phosphorus cycle. *Mar. Geol.* (2000) v. 167 p. 43-59

Fauser, P.; Tjell, J.C.; Mosbæk, H.; Pilegaard, K., Quantification of bitumen particles in aerosol and soil samples using HP-GPC. *Pet. Sci. Technol.* (2000) v. 18 p. 989-1007

Fauser, P.; Tjell, J.C.; Mosbæk, H.; Pilegaard, K., Quantification of tire-thread particles using extractable organic zinc as a tracer. *Rubber Chem. Technol.* (1999) v. 72 p. 969-977

Feilberg, A.; Holcman, J.; Nielsen, T.; Sehested, K., Atmospheric oxidation of N-PAC and nitro substituted N-PAC in water droplets. *Polycyclic Aromat. Compound.* (1999) v. 14 p. 137-150

Feilberg, A.; Kamens, R.M.; Strommen, M.R.; Nielsen, T., Photochemistry and partitioning of semivolatile nitro-PAH in the atmosphere. *Polycyclic Aromat. Compound.* (1999) v. 14 p. 151-160

Feilberg, A.; Nielsen, T., Effect of aerosol chemical composition on the photodegradation of nitro-polycyclic aromatic hydrocarbons. *Environ. Sci. Technol.* (2000) v. 34 p. 789-797

Feilberg, A.; Poulsen, M.W.B.; Nielsen, T.; Skov, H., Occurrence and sources of particulate nitro-polycyclic aromatic hydrocarbons in ambient air in Denmark. *Atmos. Environ.* (2001) v. 35 p. 353-366

Gahoonia, T.S.; Asmar, F.; Giese, H.; Gissel Nielsen, G.; Nielsen, N.E., Root-released organic acids and phosphorus uptake of two barley cultivars in laboratory and field experiments. *Eur. J. Agron.* (2000) v. 12 p. 281-289

Gallagher, M.W.; Clayborough, R.; Beswick, K.M.; Hewitt, C.N.; Owen, S.; Moncrieff, J.; Pilegaard, K., Assessment of a relaxed eddy accumulation for measurements of fluxes of biogenic volatile organic compounds: Study over arable crops and a mature beech forest. *Atmos. Environ.* (2000) v. 34 p. 2887-2899

Gavito, M.E.; Curtis, P.S.; Mikkelsen, T.N.; Jakobsen, I., Atmospheric CO2 and mycorrhiza effects on biomass allocation and nutrient uptake of nodulated pea (*Pisum sativum* L.) plants. *J. Exp. Bot.* (2000) v. 51 p. 1931-1938

Geppert, W.D.; Getoff, N.; Sehested, K.; Holcman, J., One electron reduction and oxidation of 2-, 3- and 4-chlorobenzonitrile in aqueous solution: A pulse radiolysis study. *Radiat. Phys. Chem.* (2000) v. 59 p. 39-47

Gundersen, V.; Bechmann, I.E.; Behrens, A.; Stürup, S., Comparative investigation of concentrations of major and trace elements in organic and conventional Danish agricultural crops. 1. Onions (*Allium cepa* Hysam) and peas (*Pisum sativum* Ping Pong). *J. Agric. Food. Chem.* (2000) v. 48 p. 6094-6102

Haselmann, K.F.; Ketola, R.A.; Laturnus, F.; Lauritsen, F.R.; Grøn, C., Occurrence and formation of chloroform at Danish forest sites. *Atmos. Environ.* (2000) v. 34 p. 187-193

Haselmann, K.F.; Laturnus, F.; Svensmark, B.; Grøn, C., Formation of chloroform in spruce forest soil - results from laboratory incubation studies. *Chemosphere* (2000) v. 41 p. 1769-1774

Hatzack, F.; Johansen, K.S.; Rasmussen, S.K., Nutritionally relevant parameters in low-phytate barley (*Hordeum vulgare* L.) grain mutants. *J. Agric. Food. Chem.* (2000) v. 48 p. 6074-6080

Jahoor, A.; Backes, G., Molecular mapping of powdery mildew and leaf rust resistance genes by means of molecular markers in barley. *Acta Phytopathol. Entomol. Hung.* (2000) v. 35 p. 103-104

Jensen, E.S.; Ambus, P., Prospects for manipulating crop residues to control nitrogen mineralisation-immobilisation in soil. *Kungl. Skogs- Lantbruksakad. Tidskr.* (2000) (no.8) p. 25-42

Jensen, N.O.; Hasager, C.B.; Hummelshøj, P.; Pilegaard, K.; Barthelmie, R.J., Surface flux variability in relation to the mesoscale. In: Exchange and transport of air pollutants over complex terrain and the sea. Larsen, S.E.; Fiedler, F.; Borrell, P. (eds.), (Springer-Verlag, Berlin, 2000) (Transport and chemical transformation of pollutants in the troposphere, v. 9) p. 322-328

Johansen, K.S.; Svendsen, I.; Rasmussen, S.K., Purification and cloning of the two domain glyoxalase I from wheat bran. *Plant Sci.* (2000) v. 155 p. 11-20

Joner, E.J.; Johansen, A., Phosphatase activity of external hyphae of two arbuscular mycorrhizal fungi. *Mycol. Res.* (2000) v. 104 p. 81-86

Joner, E.J.; Ravnskov, S.; Jakobsen, I., Arbuscular mycorrhizal phosphate transport under monoxenic conditions using radio-labelled inorganic and organic phosphate. *Biotechnol. Lett.* (2000) v. 22 p. 1705-1708

Jørgensen, J. Helms; Bech, C.; Jensen, J., Reaction of European spring barley varieties to a population of the net blotch fungus. *Plant Breed.* (2000) v. 119 p. 43-46

Kerovuo, J.; Rouvinen, J.; Hatzack, F., Analysis of *myo*-inositol hexakisphosphate hydrolysis by *Bacillus* phytase:

Indication of a novel reaction mechanism. *Biochem. J.* (2000) v. 352 p. 623-628

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.* (2000) v. 100 p. 881-888

Kleinow, T.; Bhalerao, R.; Breuer, F.; Umeda, M.; Salchert, K.; Koncz, C., Functional identification of an Arabidopsis snf4 ortholog by screening for heterologous multicopy suppressors of snf4 deficiency in yeast. *Plant J.* (2000) v. 23 p. 115-122

Kollist, H.; Moldau, H.; Mortensen, L.; Rasmussen, S.K.; Bolt-Jørgensen, L., Ozone flux to plasmalemma in barley and wheat is controlled by stomata rather than by direct reaction of ozone with cell wall ascorbate. *J. Plant. Physiol.* (2000) v. 156 p. 645-651

Kristensen, B.K.; Bloch, H.; Tran Duc, T.; Rasmussen, S.K., Characterization and functional analysis of two peroxidases from barley induced by the powdery mildew fungus. *Plant Peroxidase Newslett.* (2000) (no.14) p. 111-115

Kuijpers, A.; Abrahamsen, N.; Hoffmann, G.; Hühnerbach, V.; Konradi, P.; Kunzendorf, H.; Mikkelsen, N.; Thiede, J.; Weinrebe, W., Climate change and the Viking-age fjord environment of the Eastern Settlement, South Greenland. *Geol. Greenland Surv. Bull.* (1999) (no.183) p. 61-67

Kyvsgaard, P.; Sørensen, P.; Møller, E.; Magid, J., Nitrogen mineralization from sheep faeces can be predicted from the apparent digestibility of the feed. *Nutr. Cycl. Agroecosyst.* (2000) v. 57 p. 207-214

Laturnus, F.; Giese, B.; Wiencke, C.; Adams, F.C., Low-molecular-weight organoiodine and organobromine compounds released by polar macroalgae - The influence of abiotic factors. *Fresenius J. Anal. Chem.* (2000) v. 368 p. 297-302

Laturnus, F.; Lauritsen, F.R.; Grøn, C., Chloroform in a pristine aquifer system: Toward an evidence of biogenic origin. *Water Resour. Res.* (2000) v. 36 p. 2999-3009

Lee, S.B.; Rasmussen, S.K., Molecular markers in some medicinal plants of the Apiaceae family. *Euphytica* (2000) v. 114 p. 87-91

Lett, C.; Østergård, H., A stochastic model simulating the spatiotemporal dynamics of yellow rust on wheat. *Acta Phytopathol. Entomol. Hung.* (2000) v. 35 p. 287-293

Lyngkjær, M.F.; Carver, T.L.W., Conditioning of cellular defence responses to powdery mildew in cereal leaves by prior attack. *Mol. Plant Pathol.* (2000) v. 1 p. 41-49

Lyngkjær, M.F.; Newton, A.C.; Atzema, J.L.; Baker, S.J., The barley *mlo*-gene: An important powdery mildew resistance source. *Agronomie* (2000) v. 20 p. 745-756

Mikkelsen, T.N.; Ro-Poulsen, H.; Pilegaard, K.; Hovmand, M.F.; Jensen, N.O.; Christensen, C.S.; Hummelshøj, P., Ozone uptake by an evergreen forest canopy - temporal variation and possible mechanisms. *Environ. Pollut.* (2000) v. 109 p. 423-429

Mølgaard, C.; Hansen, M.; Stürup, S.; Michaelsen, K.F., Calcium absorption rate in young girls is not related to calcium intake. *J. Bone Miner. Res.* (2000) v. 15 p. SU011

Nath, B.N.; Kunzendorf, H.; Plüger, W.L., Influence of provenance, weathering, and sedimentary processes on the elemental ratios of the fine-grained fraction of the bedload sediments from the Vembanad Lake and the adjoining continental shelf, southwest coast of India. *J. Sediment. Res.* (2000) v. 70 p. 1081-1094

O'Hara, R.B.; Nielsen, B.J.; Østergård, H., The effect of fungicide dose on the composition of laboratory populations of barley powdery mildew. *Plant Pathol.* (2000) v. 49 p. 558-566

Olsson, P.A.; Johansen, A., Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. *Mycol. Res.* (2000) v. 104 p. 429-434

Rasmussen, N.; Lloyd, D.C.; Ratcliffe, R.G.; Hansen, P.E.; Jakobsen, I., 31P NMR for the study of P metabolism and translocation in arbuscular mycorrhizal fungi. *Plant Soil* (2000) v. 226 p. 245-253

Schwarz, G.; Herz, M.; Huang, X.Q.; Michalek, W.; Jahoor, A.; Wenzel, G.; Mohler, V., Application of fluorescence-based semi-automated AFLP analysis in barley and wheat. *Theor. Appl. Genet.* (2000) v. 100 p. 545-551

Schweiger, P.F.; Jakobsen, I., Direct measurement of arbuscular mycorrhizal phosphorus uptake into field-grown winter wheat. *Agron. J.* (1999) v. 91 p. 998-1002

Schweiger, P.F.; Jakobsen, I., Laboratory and field methods for measurement of hyphal uptake of nutrients in soil. *Plant Soil* (2000) v. 226 p. 237-244

Shim, S.I.; Jørgensen, R.B., Genetic structure in cultivated and wild carrots (*Daucus carota* L.) revealed by AFLP analysis. *Theor. Appl. Genet.* (2000) v. 101 p. 227-233

Smith, F.A.; Jakobsen, I.; Smith, S.E., Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. *New Phytol.* (2000) v. 147 p. 357-366

Stockmarr, A., The choice of hypotheses in the evaluation of DNA profile evidence. In: Statistical science in the courtroom. Gastwirth, J. (ed.), (Springer, Berlin, 2000) p. 143-159

Struck, U.; Emeis, K.C.; Voss, M.; Christiansen, C.; Kunzendorf, H., Records of southern and central Baltic Sea eutrophication in C-13 and N-15 of sedimentary organic matter. *Mar. Geol.* (2000) v. 164 p. 157-171

Stürup, S., Application of HR-ICP-MS for the simultaneous measurement of zinc isotope ratios and total zinc content in human samples. *J. Anal. Atom. Spectrom.* (2000) v. 15 p. 315-321

Thingstrup, I.; Kahiluoto, H.; Jakobsen, I., Phosphate transport by hyphae of field communities of arbuscular mycorrhizal fungi at two levels of P fertilization. *Plant Soil* (2000) v. 221 p. 181-187

Thordal-Christensen, H.; Gregersen, P.L.; Collinge, D.B., The barley /*Blumeria* (syn. *Erysiphe*) *graminis* interaction. In: Mechanism s of resistance to plant diseases. Slusarenko, A.J.; Fraser, R.S.S.; Loon, L.C. van (eds.), (Kluwer Academic Publishers, Dordrecht, 2000) p. 77-100

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.* (2000) v. 100 p. 750-754

Trieu, A.T.; Burleigh, S.H.; Kardailsky, I.V.; Maldonado-Mendoza, I.E.; Versaw, W.K.; Blaylock, L.A.; Shin, H.; Chiou, T.-J.; Katagi, H.; Dewbre, G.R.; Weigel, D.; Harrison, M.J., Transformation of *Medicago truncatula* via infiltration seedlings or flowering plants with *Agrobacterium*. *Plant J.* (2000) v. 22 p. 531-541

Valentini, R.; Matteucci, G.; Dolman, A.J.; Schulze, E.-D.; Rebmann, C.; Moors, E.J.; Granier, A.; Gross, P.; Jensen, N.O.; Pilegaard, K.; Lindroth, A.; Grelle, A.; Bernhofer, C.; Grünwald, T.; Aubinet, M.; Ceulemans, R.; Kowalski, A.S.; Vesala, T.; Rannik, Ü.; Berbigier, P.; Loustau, D.; Gudmundsson, J.; Thorgeirsson, H.; Ibrom, A.; Morgenstern, K.; Clement, R.; Moncrieff, J.; Montagnani, L.; Minerbi, S.; Jarvis, P.G., Respiration as the main determinant of carbon balance in European forests. *Nature* (2000) v. 404 p. 861-865

Vallavieille-Pope, C. de; Giosue, S.; Munk, L.; Newton, A.C.; Niks, R.E.; Østergård, H.; Pons-Kühnemann, J.; Rossi, V.; Sache, I., Assessment of epidemiological parameters and their use in epidemiological and forecasting models of cereal airborne diseases. *Agronomie* (2000) v. 20 p. 715-727

Vinther, F.P.; Jensen, E.S., Estimating legume N-2 fixation in grass-clover mixtures of a grazed organic cropping system using two N-15 methods. *Agric. Ecosyst. Environ.* (2000) v. 78 p. 139-147

Zhou, F.S.; Andersen, C.H.; Burhenne, K.; Fischer, P.H.; Collinge, D.B.; Thordal-Christensen, H., Proton extrusion is an essential signalling component in the HR of epidermal single cells in the barley-powdery mildew interaction. *Plant J.* (2000) v. 23 p. 245-254

Østergaard, H.; Rasmussen, S.K.; Roberts, T.H.; Hejgaard, J., Inhibitory serpins from wheat grain with reactive centers resembling glutamine-rich repeats of prolamin storage proteins. *J. Biol. Chem.* (2000) v. 275 p. 33272-33279

International books and reports

Kling, M.; Graham, J.; Jakobsen, I.; Miller, M. (eds.), Proceedings. Workshop on methods for study of the hyphae of AMF, Uppsala (SE), 1-4 Jul 1998. (Kluwer, Dordrecht, 2000) (Plant and Soil, v. 226, no. 2) 178 p.

Markert, F.; Egsgaard, H., Research and development of technologies for safe and environmentally optimal recovery and disposal of explosive wastes. Task 8: Chemical analysis of the closed detonation products. (2000) vp.

Østergård, H.; Coudert, F. (eds.), COST action 817. Airborne pathogens on cereals. Population studies of airborne pathogens on cereals as a means of improving strategies for disease control. Annual report 1998. EUR-19222 (2000) 192 p.

Proceedings

Ambus, P.; Jensen, E.S.; Robertson, G.P., Nitrous oxide and water mediated N-losses from agricultural soil: Influence of

crop residue particle size, quality and placement. In: Papers. Internet-conference on nitrogen emissions from soil, 8 Sep - 31 Dec 2000. vp.
www.nitro-soil.at/

Backes, G.; Jensen, J.; Jahoor, J., Localising QTLs for malting quality traits in barley. In: Barley genetics 8. Proceedings. Vol. 2 - Contributed papers. 8. International barley genetics symposium, Adelaide (AU), 22-27 Oct 2000. Logue, S. (ed.), (Adelaide University, Adelaide, 2000) p. 217-219

Borch, T.; Ambus, P.; Laturnus, F.; Svensmark, B.; Grøn, C., Degradation of chlorinated solvents in unsaturated soils. In: Contaminated soil 2000. Proceedings. Vol. 2. 7. International FZK/TNO conference on contaminated soil, Leipzig (DE), 18-22 Sep 2000. (Thomas Telford, London, 2000) p. 767-768

Brinch-Pedersen, H.; Olesen, A.; Hatzack, F.; Rasmussen, S.K.; Holm, P.B., Transformation of wheat (*Triticum aestivum* L.) for improved phosphate bioavailability. In: Proceedings. 3. Annual meeting in the Danish Cereal Network, Slagelse (DK), 3-4 Nov 1999. (Danish Cereal Network, Slagelse, 2000) p. 50-64

Garde, A.; Schmidt, A.S.; Jonsson, G.; Andersen, M.; Thomsen, A.B.; Ahring, B.K.; Kiel, P., Agricultural crops and residuals as a basis for polylactate production in Denmark. In: Conference proceeedings. Food BioPack conference, Copenhagen (DK), 27-29 Aug 2000. Weber, C.J. (ed.), (Royal Veterinary and Agricultural University, Frederiksberg, 2000) p. 50-55
www.mli.kvl.dk/foodchem/special/biopack/html/Proceedings_biopack0807.pdf

Grando, S.; Backes, G.; Ceccarelli, S.; Sabbagh, A.; Jahoor, A.; Baum, M., Quantitative trait loci (QTL) analysis for agronomic traits in recombinant inbred lines of the cross arta x *H. spontaneum* 41-1. In: Barley genetics 8. Proceedings. Vol. 3 - Contributed papers. 8. International barley genetics symposium, Adelaide (AU), 22-27 Oct 2000. Logue, S. (ed.), (Adelaide University, Adelaide, 2000) p. 61-63

Hovmøller, M.S.; Pinnschmidt, H.; Eriksen, L.; Østergård, H., Resistance to foliar pathogens on cereals. In: Proceedings. 3. Annual meeting in the Danish Cereal Network, Slagelse (DK), 3-4 Nov 1999. (Danish Cereal Network, Slagelse, 2000) p. 1-7

Jahoor, A.; Backes, G.; Jensen, J.; Baum, M.; Walther, U., Are quantitative resistance genes different than major race-specific resistance genes?. In: Barley genetics 8. Proceedings. Vol. 1 - Invited papers. 8. International barley genetics symposium, Adelaide (AU), 22-27 Oct 2000. Logue, S. (ed.), (Adelaide University, Adelaide, 2000) p. 53-55

Jensen, J., An easy method to establish linkage groups. In: Barley genetics 8. Proceedings. Vol. 3 - Contributed papers. 8. International barley genetics symposium, Adelaide (AU), 22-27 Oct 2000. Logue, S. (ed.), (Adelaide University, Adelaide, 2000) p. 24-26

Johansen, K.S.; Hatzack, F.; Mønster, H.; Engvild, K.C.; Rasmussen, S.K., Improved bioavailability of phosphorus in barley. In: Barley genetics 8. Proceedings. Vol. 2 - Contributed papers. 8. International barley genetics symposium, Adelaide (AU), 22-27 Oct 2000. Logue, S. (ed.), (Adelaide University, Adelaide, 2000) p. 291-293

Johansen, R.U.; Andersen, S.B.; Jahoor, A., Molecular diversity and phylogeny of the genus *Hordeum* elucidated using microsatellite markers. In: Barley genetics 8. Proceedings. Vol. 2 - Contributed papers. 8. International barley genetics symposium, Adelaide (AU), 22-27 Oct 2000. Logue, S. (ed.), (Adelaide University, Adelaide, 2000) p. 19-21

Kollist, K.; Nielsen, T.; Kirso, U., The distribution of polycyclic aromatic compounds between natural humic substances and water. In: Proceedings. Secotox 99. 5. European conference on ecotoxicology and environmental safety, Munich (DE), 15-17 Mar 1999. Kettrup, A.; Schramm, K.-W. (eds.), (GSF-Forschungszentrum, Neuherberg, 1999) (GSF-Bericht 02/99) 3 p. (P E4)

Laturnus, F.; Grøn, C., Occurrence of chlorinated solvents in a pristine aquifer system. In: Groundwater 2000. International conference on groundwater research, Copenhagen (DK), 6-8 Jun 2000. Bjerg, P.L.; Engesgaard, P.; Krom, T.D. (eds.), (A.A. Balkema, Rotterdam, 2000) p. 173-174

Mortensen, G.K.; Cassani, G.; Verge, C.; Volfing, M.; Bennetzen, S., LAS in soil samples - Results from an interlaboratory study. In: Proceedings. 5. World surfactants congress, Firenze (IT), 29 May - 2 Jun 2000. (CESIO, Firenze, 2000) p. 1558-1565

Nielsen, B.J.; Christiansen, S.K., Resistance against seed borne diseases in Danish wheat and barley varieties. In: Proceedings. 3. Annual meeting in the Danish Cereal Network, Slagelse (DK), 3-4 Nov 1999. (Danish Cereal Network, Slagelse, 2000) p. 8-10

Rasmussen, L., Forests under mixed inputs of pollutants and nutrients. In: Proceedings forum. Forests and atmosphere - water - soil, Soltau (DE), 2-5 Jul 1999. (1999) (NNA Reports, v. 12, spec. issue 4) p. 7-10

Rosendahl, L.; Rudbeck, A.; Scharff, A.M.; Mouritzen, P., Assimilation of fixed nitrogen studies by membrane vesicle transport and in vivo 15N-NMR spectroscopy in pea. In: Proceedings. 12. International congress on nitrogen fixation, Foz do Iguacu (BR), 12-17 Sep 1999. Pedrosa, F.O.; Hungria, M.; Yates, G.; Newton, W.E. (eds.), (Kluwer Academic Publishers, Dordrecht, 2000) (Current Plant Science and Biotechnology in Agriculture, 38)) p. 359-361

Samsøe-Petersen, L.; Mortensen, G.K., Optag af PAH'er i planter samt hvilke analysemetoder der kan benyttes hertil. In: Tjære og PAH-forureninger under et. ATV møde, Gentofte (DK), 21 Nov 2000. (2000) p. 23-36

Schiemann, A.; Backes, G., The use of molecular markers in practical barley breeding. In: Barley genetics 8. Proceedings. Vol. 3 - Contributed papers. 8. International barley genetics symposium, Adelaide (AU), 22-27 Oct 2000. Logue, S. (ed.), (Adelaide University, Adelaide, 2000) p. 42-44

Sip, V.; Ovesná, J.; Vacke, J.; Chrpová, J.; Nováková, I.; Jahoor, A., Detection and molecular identification of BYDV resistance genes in barley. In: Barley genetics 8. Proceedings. Vol. 2 - Contributed papers. 8. International barley genetics symposium, Adelaide (AU), 22-27 Oct 2000. Logue, S. (ed.), (Adelaide University, Adelaide, 2000) p. 175-177

Tandrup Poulsen, T.; Olesen, A.; Bryngelsson, T.; Collinge, D.; Nielsen, K.; Oliver, R.; Thordal-Christensen, H., Towards transgenic barley with enhanced disease resistance. In: Proceedings. 3. Annual meeting in the Danish Cereal Network, Slagelse (DK), 3-4 Nov 1999. (Danish Cereal Network, Slagelse, 2000) p. 30-38

Acronyms

AFLP	Amplified Fragment Length Polymorphism
AP1	APETALA1
CAL	CAULIFLOWER
EUROFLUX	Effects of CO2 exchange over European forests
EXAMINE	Exchange of Atmospheric Ammonia with European ecosystems
F	Floral
FOREXNOX	Effectc of nitrogen oxides on European forests
FØTEK	Danish Food Technology and Development Programme
GUS	Glucornidase
HR-ICP-MS	High Resolution Inductively Coupled Plasma Mass Spectrometry
I	Inflorescence
LFY	LEAFY
Lp	*Lolium perenne*
PEBPs	Phosphatidylethanolamine-binding proteins
PR	Pathogenesis-related
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAM	Shoot apical meristem
TFL1	TERMINAL FLOWER 1
UBI	Maize Ubiquitin Promoter
UidA	Beta-Glucornidase gene
V	Vegetative
35S	Cauliflower virus mosaic promoter

Bibliographic Data Sheet

Risø-R-1229 (EN)

Title and authors

Plant Biology and Biogeochemistry Department
Annual Report 2000

J. Kossmann, G. Gissel Nielsen
K.K. Nielsen, K. Pilegaard, L. Rasmussen,
S. K. Rasmussen, H. Thordal-Christensen, H. Østergård

ISBN 87-550-2798-9 (internet)

ISSN 0106-2840
ISSN 1397-8977

Pages html documents: 13

Date: April 2001