

Midterm Status Report 2003 and Application for Continuation in 2004

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The Directorate for Food, Fisheries and Agro Business
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Research program

Research in organic farming 2000-2005 (DARCOF II)

Project title and number

VI.1 ORGSEED

Healthy seed for organic production of cereals and legumes.

Head of project

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6. Project period (month, year)

Start of project: **1st September 2001**

End of project: **31st December 2005**

7. Midterm description of the project, its results and progress, and application for continuation in 2003

A. Project summary

Seed borne diseases can cause serious problems in production of cereals and legumes. In conventional agriculture these diseases are intensively controlled by seed treatment, but this is not an option in organic agriculture.

The threshold levels used are developed under the presumption that pesticides can be used in case of later disease development in the crop, but no experiments have been made to confirm if the same threshold levels apply under organic farming practice. The project will investigate these thresholds in field trials for all relevant diseases in peas and small grain cereals, and evaluate them for use under organic farming conditions.

Seed health analysis is made by methods normally used for survey of seed health status in propagation of seeds. The methods are in general slow and depend in some cases on subjective evaluation of the expression of the diseases. To improve the threshold levels, it also is necessary to have new and more precise methods for seed analysis. The project will develop and implement PCR techniques for Fusarium seedling blight and leaf stripe.

To minimise the development of seed borne diseases and the number of seed lots discarded, control methods will be developed and evaluated. Focus will be put on preventive methods like design of the cropping system, which minimises the risk of seed infection, and on seed treatments, which immediately can be used in organic agriculture and with already existing multipurpose equipment. Focus in seed treatment will be on seed cleaning and seed drying equipment.

The initiatives taken in this project will within the project period of 5 years contribute to development of a sustainable seed production system for organic agriculture. Most knowledge generated in the research can also be used by organic farmers in other countries and in conventional agriculture to reduce the use of seed treatments and other pesticides.

Change in project staff 2003:

Lars Bødker (LB) is on leave for other projects in 2003 and 2004. Hanne Wolffhechel (HW) will replace LB in the project. Hans Pinnschmidt (HP) will from 2003 be a part of the project (WP1 and WP3). Henrik Jørskov Hansen has fully replaced Christiane Scheel in the project.

Table A.1: Work package list (from application)

WP No	WP title	Participants ¹⁾	Budget	Start	End	Deliverable, No
WP1	Threshold values	<u>BJN</u> ,HP, AB,GCN, HJH HW	3.447 mill. Kr.	2001	2005	1-12
WP2	Diagnostic methods	<u>HJH</u> ,AFJ, BJN,GCN	3,201 mill Kr. ²⁾	2001	2005	13-20
WP3	Regulation and control measures	<u>AB</u> , <u>HP</u> ,BJN,GCN, EFK, HW	2,926 mill. Kr.	2001	2005	21-28
WP4	Integrated strategies and dissemination of information	<u>BJN</u> ,GCN,AB, HJH,AFJ, HP, HW, EFK	incl. in WP 1-3	2001	2005	29-33
WP5	Project management	<u>BJN</u>	0,427 mill. Kr.	2001	2005	34-37
Total			10,00 mill. Kr.			

1) Responsible participants are underlined.

2) Danish Plant Directorate is financing WP2 with extra 1,172 mill Kr.

B. Objectives and expected achievements

The overall objective of the programme is to contribute to production of healthy, disease-free organic seeds of cereals and legumes. The principal aim of the project is to reduce the amount of organically produced seed that need to be discarded as organic seed due to unacceptable infection by seed borne diseases.

To achieve this overall aim the following individual project objectives will be fulfilled:

1. Investigation and re-evaluation of existing threshold values (current discard values) in organic seed production.

The expected achievement will be a new set of threshold values suitable for organic production that

- i) Secure no multiplication of harmful seed borne diseases but not so strict that healthy seeds are unnecessarily discarded.
- ii) Substantial reduction in unnecessarily discarded seed especially in wheat due to seedling blight and common bunt, in barley due to leaf stripe/net blotch and in peas due to seed and foot rot.

2. Implementation of new, more precise and fast methods for seed health analysis.

The expected achievement will be implementation of modern PCR based analysis methods that

- i) Enables a more fast and precise detection (less variation in results)
- ii) Reduces the amount of unnecessarily discarded seed

- iii) Enables to discriminate between pathogens that previously could not be distinguished and identified, e.g. different *Fusarium* species and glume blotch in wheat together with leaf stripe and net blotch in barley.

3. Regulation and control measures in the organic production.

3.1. Regulation and control.

The expected achievement will be evaluation of different control measures and factors influencing the development of the diseases in practice and support of the implementation of the methods in organic farming. Based on the evaluation a set of recommendations will be formulated for the prevention of diseases in cereal and legume propagation and for control measures to implement in cases where thresholds are exceeded. The evaluated methods include:

- i) Cropping methods reducing humidity in the canopy to reduce the risk of seed infection
- ii) The effect of harvest time on diseases infection in the seed
- iii) The effect of sowing time and depth on disease development
- iv) Seed cleaning tested under practical conditions.
- v) Drum heat-treatment compared with other heat- and radiation treatment in large-scale equipment.

3.2. Use of host resistance

The expected achievement will be a description of the susceptibility of the current varieties for seed borne diseases. The information about susceptibility/resistance levels in the varieties will be used in the integrated strategies.

The diseases include

- i) Leaf stripe in barley
- ii) Seed and foot rot in peas
- iii) Common bunt of wheat

C Midterm results and progress

C.1 Description (summary) of main results and conclusions

C.1.1. Short project summary 2003:

Threshold values have in 2003 been investigated in cereals for leaf stripe (*Pyrenophora graminea*), net blotch (*P. teres*), *Fusarium* seedling blight (*Fusarium spp.*), common bunt (*Tilletia tritici*) and in pea for seed and foot rot (*Ascochyta complex: Phoma medicaginis var. pinodella, Ascochyta pisi, Mycosphaerella pinodes*). Results from the project together with other information now makes it possible to evaluate the threshold value (5 % infection) for leaf stripe. More trials are needed with net blotch because of the complexity of later spread of the disease from primary infections to other leaves with subsequent yield loss. For common bunt in wheat, the current threshold is 10 spores/g wheat and the preliminary results shows that even at this low infection level later disease attack can be found in the field starting new epidemics. For *Fusarium spp.* in wheat, a great effect of sowing time on the number of seedlings emerging from infected seed could be demonstrated. More trials are needed to establish seed infection thresholds at a given sowing but at varying seeding rates and for varieties differing in resistance.

Growing of peas in combination with barley reduced the attack of leaf and pod spot on the harvested pea seeds, but the effect of cultivation with spring barley was only very little, and the attacks were all far above the recommended threshold values. This may be due to serious attacks of leaf and pod spot disease on the seeds in the trials. No significant differences were found on yields in the specific plots in the trials with different infection levels of leaf and pod spot on the seeds. As to leaf and pod spot on the harvested seeds the attack was at the same level irrespective of the attack on the seeds sown. It could be observed that mixing peas and barley had a delaying effect of the development of net blotch in barley.

A real-time PCR method for detection and quantification of DNA from *Microdochium nivale* has been developed. The PCR primers and the TaqMan probe have been designed from the internal transcribed spacer (ITS) region and the specificity of PCR primers and probe has been tested on a range of different *Microdochium nivale* isolates, Fusarium species as well as other fungi which may be present in the seed. The PCR method quantifies both *M. nivale* var. *majus* and *M. nivale* var. *nivale* DNA.

PCR primers and a TaqMan probe for a real-time PCR assay for *Pyrenophora graminea* have been designed. Ninety-six seed samples from a barley leaf stripe field experiment in WP1 has been tested by traditional green house test where the percentage of plants with barley leaf stripe were counted. The tested seed samples showed infection levels from 0-11%. DNA has been extracted from these seed samples and is now being tested with real-time PCR in order to see if it is possible to find a correlation between the amount of *P. graminea* DNA and percentage infected plants.

In the work packages with regulation and control it has been tested if seed infected by seed borne diseases can be cleaned in a way that reduce the infection level of the seed lot. Under laboratory conditions, the contamination of spores of common bunt can be reduced by 99% in a brush machine. When scaling up to practical volume only 90% of the spores were removed. It will be investigated if the reduction can be improved under practical conditions. Seed infected by seed pathogens may be reduced in size or density compared with healthy seed. It has been shown that the infection of loose smut and leaf stripe in barley in a seed lot can be reduced by seed separation. No effects have yet been observed in seed lots infected by glume blotch, net blotch and Fusarium. It has been shown that the infection of net blotch in barley and seed rot in peas increase during the harvest period. However, the effect was small and in the experiment insufficient to avoid discarding of the seed lot.

Treatments with heated air in a drum dryer showed that it is difficult to control seed-borne diseases without reducing the germination capacity of the seeds. However, promising results from tests with treatment of net blotch infected barley have been obtained. Test results regarding treatment of common bunt in wheat are not yet available, as the trials are not finished. On treatment of pea-disease (*Ascochyta pisi*), no effects could be achieved without damaging the germination capacity of the seeds.

Use of resistance is an important preventive control measure and during the project period most of the Danish barley, wheat and triticale varieties will be tested for resistance to leaf stripe (barley) and common bunt. The results so far shows that most of the barley varieties grown in Denmark are susceptible to leaf stripe, but also that a few varieties are resistant. Most of the Danish winter wheat varieties were highly susceptible to common bunt, but some resistance was identified. Varieties like Stava (late maturing Swed-

ish varieties) was highly resistant and to some degree also Penta whereas a few varieties were moderate resistant and moderate susceptible. Also in spring wheat, the screening showed great variation in resistance but no varieties have high resistance. In triticale, all the tested varieties seem to be highly resistant to common bunt. A number of pea varieties have been screened for susceptibility to seed and foot rot (*Ascochyta* complex). First results of seed health testing indicate considerable differences between varieties in their susceptibility to each of the three pathogens.

C.1.2. Summary for the individual work packages:

Work package 1: Threshold values.

The objectives of the work package is to develop more precise threshold values for organic produced seeds to avoid unnecessary discharge of valuable seeds.

Task 1 Seedling blight in wheat

Seedling blight caused by seed infection with *Fusarium spp.* can significantly affect crop performance by reducing the viability of the germinating seed, plant emergence and over-wintering ability. Two seed lots infected with *Fusarium culmorum* were sown at 3 different times and 2 sowing depths (trial A). Seeds from different origin and with different infection levels of *Fusarium spp.* were also sown and evaluated for seedling blight in the field. Seed treatments were included here to compare to low level of infection (trial B). The trials have been performed under organic conditions.

In trial A, approx. 80% of the variation in the number of emerged plants could be explained based on sowing time. Sowing 20 days later (Oct. 30 instead of Oct. 10) reduced number of emerged plants by 1/3. All other effects (except the replication effect) were non-significant. In trial B, approx. 50% of the variation in number of emerged plants could be explained by the seed lots (which represents a combined effect of the seed infection level and other factors) while only around 30% were explainable by the seed treatment.

New plans: Based on the trials 2002/2003 it was difficult to evaluate the threshold level for *Fusarium*. It is therefore suggested to focus on the effect of the variety and seed infection level and on management options to compensate for *Fusarium*-induced crop damage, such as increasing the seeding rate. Therefore, a trial will be conducted in 2003/2004 where seed lots of different varieties with various degrees of infection by *F. culmorum* and *Microdochium nivale* are sown at 3 seeding rates. The trial will be performed under organic conditions.

Task 2 Net blotch in barley

The net blotch experiments are aimed at determining whether the present threshold value of 15% infected seed is actually applicable for net blotch in organic farming. The important question regarding net blotch is the risk of later spread of the disease from primary infections to other leaves with subsequent yield loss, reduced yield quality and infection of the new seeds. A field trial was established at the research station Flakkebjerg where infections from plant debris in the soil were minimised (no barley in previous crops). Seeds of two spring barley varieties heavily infected with net blotch (Madras: 80% infection, Lux: 57% infection) were used in a randomised trial with nine seed infection levels for the two varieties in three replications. Healthy and infected seeds were mixed to establish seeds with different infection levels. Additional treatments with net

blotch infected straw (four straw levels) were established within the seed infection level representing the present seed infection threshold (15%).

Seeds of five spring barley varieties harvested in 2002 in two other DARCOF (FØJO) trials (BAR-OF) under two cropping conditions (organic versus conventional) at three sites were analysed with respect to seed infection (Plant Directorate), 1000 grain weight and germination ability. The data representing the net blotch severity levels in the respective plots during the generative phase were obtained from BAR-OF.

The relationship between seed infection level and primary infection was highly significantly positive. The slope of this relationship was steeper in the more susceptible variety Madras pointing at higher infection transmission efficiency in Madras as compared to Lux. The relationship between seed infection level and disease severity was positive but rather loose in variety Madras. It became more variable as the season progressed but could still be noticed after heading in the variety Lux. Increasing straw applications highly increased primary infection and disease severity at tillering, especially in variety Lux. However, this effect was highly variable across the replications, especially in the plots received high amounts of straw. Also, the effect faded as the season progressed.

Net blotch severity at the generative phase (after heading), was highly positively correlated with seed infection level and highly negatively with 1000 grain weight. Net blotch severity, adjusted for the growth stage at the time of assessment, explained 73% of the variation in seed infection levels and 71% of the variation in 1000 grain weight, irrespective of the variety. The trial was performed under conventional conditions

New plans: Because of the interactions between variety and seed infection level, that can be assumed based on the results, field trials will be conducted in 2004 in which a range of seed infection levels is tested in combination with a number of varieties of various degrees of net blotch-susceptibility. Because 2003 was a net blotch-prone year, there exists a unique chance of obtaining appropriate seed material from selected locations in Denmark (BAR-OF trials). The trials will be performed under organic conditions.

Task 3 Leaf stripe in barley

Experiments were established 2002 to demonstrate the importance of variety, soil temperature (sowing date) and various levels of infection. The trial gave information on relationship between infection level on the seed and corresponding later attack in the field. No new trials have been planned.

Task 4 Seed and foot rot in peas

The aim in task 4 is to evaluate the relationship between seed infection and severity of disease expression as seed and foot rot.

Trials in peas have been laid out according to three experimental plans. In addition seed samples from variety trials with nine varieties and one variety mixture in the national field trials have been sent in for seed health analysis. All trial series have focus on diseases in peas. Two of the trial plans are a continuation of the trials in 2002, where the effect of sowing seeds with varying degrees of infection by leaf and pod spot pathogens is examined. Also trials were performed where the effect of cultivation of peas in combination with varying amounts of spring barley on the level of leaf and pod spot is tested. These trials refers to WP 3.1 task 1 but is mentioned here to give an overview of the pea trials. In both trial series the effect is examined in a short (Athos) and a long variety (Attika). Both yield and attack of seed borne diseases are

measured at harvest. Furthermore trials have been laid out according to a new experimental plan, which examines the importance of seed amounts in peas to yield and attack of pea disease.

In 2003, 5 trials were performed with cultivation of peas in combination with spring barley (1 trial discarded because of no peas). 6 trials were performed with different infection levels of leaf and pod spot on pea seeds (here 2 trials were cancelled, 1 trial cancelled due to wood pigeon attacks). 5 trials were performed with different amount of seed and leaf and pod spot in peas (1 trial cancelled due to wood pigeon attacks). 11 trials were performed with different pea varieties (1 trial cancelled, 2 cancelled because of too much weeds, 1 because of deers, weed, and pea weevils). Some of the trials have been discarded for various reasons as mentioned after each trial series. Some of the trials were carried out in conventional fields because of problems with placing the trials in fields for organic cultivation.

In September seed samples have been sent to the Plant Directorate for seed health testing, but no results are available as yet.

The trials from 2002 showed, that growing of peas in combination with barley reduced the attack of leaf and pod spot on the harvested seeds, but the effect of cultivation with spring barley was only very low, and the attacks were all far above the recommended threshold values. This may be due to high infection levels of leaf and pod spot disease on the seeds in the trials. No significant differences were found on yields in the specific plots in the trials with different infection levels of leaf and pod spot on the seeds. As to leaf and pod spot on the harvested seeds the infection was at the same level irrespective of the infection on the seeds sown.

New plans: When the analyses for leaf and pod spot on pea seed are available in 2003 it will be decided what kind of trials that will be performed in 2004. See also WP 3.1. task 1.

Task 5 Common bunt in wheat

Pathogen populations have been collected as infection material to be used in field trials with different infection levels 2003. Seed lots of 2 winter wheat varieties (susceptible Herzog and moderate resistant Penta) were infected artificially with various amounts of bunt (5 levels) in order to establish a connection between spores on the kernels and subsequent attacks in the field. Very small amounts of infection are tested to approach the present damage threshold. The lowest infection level where disease could be observed in the field was 15 spores/g (0,5 % attack in field) – 61 spores/g (0,3 % attack in field). In order to get more information on the importance of low infection levels, 43 different seed lots were received from two different laboratories (OK Laboratories and Plant Directorate) and sown in the field. The results showed that even at the lowest infection level (10 spores/g) there was a low attack in the field (0,3-1 % field attack). The dataset will be evaluated and the current threshold discussed.

Due to high risk of infection of organic experimental field with common bunt all trials will be performed in a separate field under conventional conditions.

New plans: In the autumn a new set of different seed lots will be sown out, but no experiments with artificial infection levels will be performed.

Task 6 Tan spot (*Drechslera tritici-repentis*) in winter wheat

Tan spot ("hvedebladplet", "DTR") is a relative new disease in wheat in Denmark and has occurred quite frequently during the last years. To investigate the importance of the seed borne phase it was decided to make a preliminary field trial where seed lots with different infection levels were used. The field plots was monitored for primary infections and multiplication of the disease but no attack from seed borne infection could be observed.

Task 6 was not part of the original application and because of need to make priorities, no new trials will be performed.

Task 7 Glume blotch/seedling blight in wheat

Lephtosphaeria nodorum (*Septoria nodorum*) was multiplied under field conditions in the summer 2003 to establish seed lots with natural infections but no seedling infection by *L. nodorum* could be found. Moreover, due to down-prioritising this task and to be able to focus more on tasks 1 and 2, there will be no trial with *Lephtosphaeria nodorum* in autumn 2003.

Task 8 Models

Models describing the connection between seed infection, field infection, and subsequent infection in the harvested seed under different conditions will be developed later in the project when more data are available. The models will include projecting over several seed propagation generations (pre basic-C2) and years and to evaluate the long-term risk of multiplication and consequences of reduction factors.

Work package 2: Diagnostic methods.

The objectives are to implement new, more precise and fast methods for seed health analysis for Seedling blight (*Fusarium* spp. complex), Glume blotch (*Leptosphaeria nodorum*), Leaf stripe (*Pyrenophora graminea*) and Net blotch (*Pyrenophora teres*).

Task 1: *Microdochium nivale* and *Fusarium* species

The aim is to develop PCR methods which can detect and quantify fungal species causing seedling blight. *Microdochium nivale* is the most common species causing seedling blight. An automated ABI Prism 7000 sequence detection system has been used to develop a real-time PCR method for the quantification of *Microdochium nivale* DNA. The PCR primers and the TaqMan probe have been designed from the internal transcribed spacer (ITS) region and the specificity of PCR primers and probe has been tested on a range of different *Microdochium nivale* isolates, *Fusarium* species as well as other fungi which may be present in the seed. The PCR method quantifies both *M. nivale* var. *majus* and *M. nivale* var. *nivale* DNA.

A DNA extraction method has been developed and it has been possible to detect *M. nivale* in winter wheat seed lots infected with *M. nivale* and *Fusarium* species. The same samples were tested for percentage of brown roots. Samples with high percentage of brown roots contained high amounts of *M. nivale* DNA as compared to those samples with low percentage of brown roots. More samples where percentage infection is determined for each fungal species separately need to be tested to see whether it is possible to find a correlation between percentage infected seeds and the amount of fungal DNA. An infection gradient obtained by mixing *M. nivale* infected seeds with un-

infected seeds is produced for field experiments in WP1 and will also be used in this WP to test the method.

The mycoflora developed on brown roots have been studied over two seasons. During the 2001 and 2002 season the most common species associated with seedling blight were, besides *M. nivale*, *Fusarium culmorum*, *F. avenaceum*, and *F. graminearum* (based on commercial seed lots analysed by the Microbiological Laboratory, Danish Plant Directorate). The relative importance of the mentioned species as well as other fungi found associated with brown roots may vary from season to season as well as from location to location. The established seed health testing method is revealing the presence of the root damaging fungi irrespective of the species involved. A PCR based test method needs to be conducted with several primers to avoid false negative results. To identify the needed primers the mycoflora of a larger number of samples must be studied over more seasons and locations. Quantification of the infection level of a seed sample with this complex nature of infection is a second problem to solve before the established seed health test may be replaced with a PCR test.

PCR primers for the detection of different *Fusarium species* are described in the literature and these have been used in the project to assist in the diagnostics of *Fusarium species*. However, it is not possible directly to develop a quantitative PCR (Q-PCR) method from the above PCR methods due to the specific PCR conditions, PCR-product size etc. Therefore new PCR primers and probes for Q-PCR have to be developed. It will not be possible to design primers and probes based on the ITS-regions due to the low variation in this region between the *F. culmorum* and *F. graminearum*. The aim is to use other sequences already available in GenBank. It is the plan to develop a method for *F. avenaceum* as well.

Task 2: Glume blotch (*Leptosphaeria nodorum*)

Glume blotch has from the beginning of the project been given lower priority and it has now in collaboration with the other WPs been decided to exclude glume blotch from WP2 and to focus on the development of Q-PCR methods for *Fusarium* seedling blight, as it is more urgent to have improved methods for this complex.

Task 3: *Pyrenophora* spp.

The aim is to develop a PCR method which can distinguish *Pyrenophora graminea* from *P. teres* and quantify *P. graminea*. In a previous project a DNA sequence specific for *P. graminea* was identified and sequenced. PCR primers and a TaqMan probe for a Q-PCR assay for *P. graminea* have been designed. The specificity of the primers and probe has been tested on a range of different *P. graminea* isolates as well as *P. teres*, *Cochliobolus sativus*, *Fusarium spp* and a range of other species which can be expected to be found on barley seed. Different DNA extraction methods have been tested. The most efficient method seems to be grinding of whole seeds followed by DNA extraction using a commercial available kit. It has been possible to detect *P. graminea* in constructed seed lots with 1% infection. Ninety-six seed samples from a barley leaf stripe field experiment in WP1 has been tested by traditional green house test where the percentage of plants with barley leaf stripe were counted. The tested seed samples showed infection levels from 0-11%. DNA has been extracted from 50 of these samples and is now waiting to be tested with Q-PCR in order to see if it is possible to find a correlation between the amount of *P. graminea* DNA and percentage infected plants.

Work package 3: Regulation and control measures.

The work packages is divided in to two sub-work packages:

- 3.1. Direct and indirect control measures
- 3.2. Host resistance

WP 3.1. Control measures acceptable in the organic seed production.

The objectives are to develop and evaluate different control measures applicable for organic farming. The objective is also to assist in the implementation of the methods in practice.

Task 1. Preventive cropping methods.

Experiments on the effect of harvest time on the development of seed borne diseases have been established at Højbakkegård. Field trials have been conducted in pea and barley. In order to insure infection in the field, infected seed was used. As these were only available in conventional quality, the experiment was conducted under unsprayed but conventional conditions. In peas, there was a tendency of increased infection of the seed by *Ascochyta pisi* from the first early harvest until normal harvest time. However, the infection incidence was already at the first harvest time >70% which is far above the threshold level. In barley, the infection level was at all harvest times below the threshold level, and the tendency to increased infection insignificant. The experiment is repeated in 2002-3, but the seed is not analysed yet.

It has been assessed if the plant density has an influence on disease incidence of *Fusarium*. The experiment gave no significant result.

The trials in task 1 (Højbakkegård, KVL) were performed under conditions equivalent to first year conversion except for the use of partly conventional seed

Field trials under the Danish Agricultural Advisory Service have been performed where the effect of cultivation of peas (short variety Athos and a long variety Attika) in combination with varying amounts of spring barley on the level of leaf and pod spot is tested (see WP 1, task 4). The results showed, that the mixture pea and barley reduced the attack of leaf and pod spot on the harvested seeds, but the effect of cultivation with spring barley was only very low, and the attacks were all far above the recommended threshold values. A number of experiments have also been conducted in co-operation with DACROF (FØJO) project GENESIS with mixtures of barley and pea where the seed analyses were done at Højbakkegård (KVL). The results indicate that the infection of leaf and pod spots on pea and barley net blotch decreases in both the canopy and on the seed compared to cultivation of each crop separately.

New plans: The disease complex in pea (*Ascochyta sp*) is still an unsolved problem. and still a large amount of seed lots are expected to be discarded because of infection. More priority will therefore be given to these diseases. As topics for 2004 are discussed soil pH and influence on the seedlings and later disease development in the crop. Other suggestions are effect of plant density and strategic harvest in pea. Also effects of seed treatments on seedling infection is considered. However, new plans will first be decided after evaluation of the results from 2003 experiments (see WP 1, task 4) and the planned workshop on legumes.

In the DARCOF project BAR-OF three-component barley variety mixtures is constructed and these mixtures provides a good way to test the effect of mixtures on spread of net blotch from seed borne infections (via primary infections). In cooperation with BAR-OF analysis of seed borne infections will be made in the resown mixtures. Resources will be allocated from BAR-OF for assessment of the seed borne diseases.

Task 2. Multiplication of diseases in the propagation process from C1 to ware seed. After the harvest 2002 a questionnaire by Danish Agricultural Advisory Service were again sent to companies that grow organic seed. Information has been collected on percent rejected lots of the different cereals and peas as a result of the seed borne diseases. It showed that 85 percent of the C2 pea seed lots had more than 5 percent seed with leaf and pod spot. 13 percent of the spring barley C2 lots was rejected which was relatively low compared to the previous years.

In order to get more information on the importance of low infection levels of common bunt in wheat, 43 different seed lots were received from two different laboratories (OK Laboratories and Plant Directorate) and sown in the field (se WP 1 task 5).

Task 3. Seed cleaning.

A brush cleaner and a Sigma cleaner is planned to be tested for the effect of spores removal from seed lots contaminated with spores of common bunt. Due to lack of contaminated seed lots in large quantities, new experiments have not been conducted. A seed lot harvested in 2003 is now available, and will be tested in autumn 2003. The effect will be tested in field trials at Højbakkegård. The trial will not be placed in organic area in order to avoid soil contamination.

Seed lots infected by loose smut (*Ustilago nuda*), leaf stripe and *Fusarium spp.* have been cleaned according to seed size and seed density by different cleaning equipment. In seed lots infected by loose smut, the effects of seed separation is highly significant, and the infection level can be reduced by 50-60% by discarding the 15% of the smallest seeds. The effect can be further be improved by seed separation based on density by a gravity separator or a table separator. There seem to be an effect on infection of leaf stripe, but results are not finally analysed. In seed lots infected by *Fusarium* or glume blotch no significant effects have been seen. This difference in effects indicates that the infection can only be reduced be seed separation in seed lots, where the seed size and density is very uniform. In seed lots, where the seed are very divers due to differences in water or nutrient supply, other factors will influence the seed size, and the effect on the disease will be insignificant.

Task 4. Heat treatment.

In March 2003, the drum dryer was applied for drying and heat treatment of spring barely infected with *Fusarium spp.* Prior to the test, the grain was re-wetted to moisture content of about 17.5%. The grain (variety Odin, 37% infection with germination inhibiting fungi) was grown conventionally. The drying regime included 11 different combinations of treatment temperature and time. The maximum seed temperature was 48-97°C and, the treatment duration was from 4.5 to 10.5 minutes. The effect was evaluated by means of seed vigour analyses and by determination of number of kernels with fungi inhibiting seed germination.

No combinations of temperature and time were able to reduce the level of germination inhibiting fungi (mainly *Fusarium*) essentially without affecting the germination ability negatively. One combination of time and temperature (10.5 minutes and 71°C seed temperature) reduced the number of kernels with germination inhibiting fungi by about 50%, but at the same time the germination percentage was halved.

In August, organic grown wheat infected with common bunt was treated. The drying regime included 24 different combinations of treatment temperature and time. The maximum seed temperature was 48-75°C, and the duration of the treatments was from 4.5 to 13 minutes. Samples from all treatments will be analysed and sowed in experimental plots at Højbakkegård this autumn, and therefore, the results are not yet available.

New plans: According to the application the experiments with drum drying will stop in 2003 and no new trials will be performed.

WP 3.2. Resistance against seed borne diseases.

Information from the screening of varieties relevant to organic cereal and pea production will be integrated in the work in WP1 with threshold values and development of strategies.

Task 1. Screening for resistance to leaf stripe in Danish barley varieties

Monitoring the stability of leaf stripe resistance of relevant commercial (contemporary) barley varieties over the years as well as assessing the leaf stripe resistance in new germplasm continues to be an important task that ensures a sound, resistance-based management. In spring 2003, 100 spring barley varieties inoculated with leaf stripe in 2002 were sown in a small-plot nursery at Flakkebjerg in three replications. The leaf stripe level on the most susceptible variety was low in comparison to 2002 and reached a maximum of ca. 8% disease incidence (infected tillers/100 tillers). More than half of the genotypes expressed extremely high resistance with disease incidence values of zero or around zero. Most of the other genotypes expressed fairly high resistance levels with disease incidence values below 20% of the most susceptible genotypes. However, ca. 10% of the genotypes expressed relatively high susceptibility with disease incidence levels reaching 40% or more of the most susceptible genotypes. The trials were performed under conventional conditions

New plans: Because of the continuing threat of the leaf stripe disease for organically produced seeds in Denmark, it is proposed to continue the same trial as in 2003 in 2004. It will be the only available source of information about the leaf stripe resistance performance of varieties currently used in organic farming in Denmark. The trial will be conducted under organic conditions.

In the DARCOF project BAR-OF there is a focus on the influence of varietal variation on yield and yield stability. Seed borne diseases are part of this variation and data on leaf stripe (and to some extent loose smut) on the same varieties as tested in BAR-OF are available from ORGSEED. Data on seed borne diseases will be included in later analyses in BAR-OF. Test of resistance to other seed borne diseases than leaf stripe will be discussed with BAR-OF and new funding for this work will be investigated.

Task 2. Screening for resistance in Danish pea varieties to seed and foot rot.

Danish pea varieties will be tested for their level of resistance to the three pathogens of the *Ascochyta* complex (*Phoma medicaginis* var. *pinodella*, *Ascochyta pisi*, *Mycosphaerella pinodes*). A test system will be developed with artificially inoculated small plots of pea under artificial irrigation and varieties of interest to organic production will be characterised.

In 2002 ten different varieties with a variation in tolerance against leaf and pod spot were obtained from the Danish breeders. These ten varieties were sown in three replicate field trials at Research Centre Flakkebjerg and dually inoculated with both infected barley grain and a suspension of conidiospores of *P. medicaginis* var. *pinodella*, *A. pisi* and *M. pinodes*, respectively. Even though sprinkler irrigation was installed in all three field trials, hardly any leaf or pod spot caused by the *Ascochyta* complex could be seen in any of the trials. However, the seed health testing carried out during winter showed high seed infection percentages, especially with *A. pisi* (up to 44%). There was also an indication of some varieties being more resistant to infection than others. Because of capacity problems not all seeds have been tested. The remaining seeds will be tested this winter together with seeds from the 2003 field trials.

Field trials in 2003 were similar but the infected barley grain was spread out earlier in order to obtain a high disease attack. Unfortunately, a large number of grains was eaten by birds, and disease symptoms on plants were again quite low. Seed health testing will show the degree of seed infection by the *Ascochyta* complex.

Because of the very specific requirements, the trials were conducted under conventional conditions.

New plans: Trials in 2002 and 2003 have focused on varietal differences regarding susceptibility in the reproductive stage and regarding infection of the new seed generation. In 2004 there will also be focus on varietal differences regarding foot rot developing from infected seeds. More isolates will be screened in the greenhouse in winter and the method of inoculation will be optimised. The trials will be carried out under conventional conditions, because application of inoculum to the plots and installation of irrigation will make mechanical weeding impossible.

Task 3. Screening for resistance to common bunt in Danish wheat varieties

Danish wheat varieties are tested on a set of different *Tilletia* populations for their level of resistance. 58 different new varieties from breeders (and varieties of special interest for organic wheat production) were inoculated with two different bunt isolates. 4 varieties had very low attack (highly resistant), 4 varieties had moderate attack and the rest had different level of high attack (susceptible). 8 varieties that in previous test had shown some resistance were tested again on a new set of 4 isolates. Only two varieties (Stava and Penta) were highly resistant while 3 varieties were moderate resistant. It seems from test with the same varieties performed over several years that some varieties are getting a higher attack during the years (becoming more susceptible). This will be further analysed.

The test for resistance to common bunt is also performed in triticale in the same way. In 2003, 12 different triticale varieties were tested on two isolates of *T. caries* and 5

varieties (showing some attack in 2002 test) were tested again on 4 new isolates. It seems that triticale is resistant to common bunt and only low attack was observed in the trials.

In spring wheat 5 varieties were tested and sown at 3 different times and 2 sowing depths to investigate correlations with emergence rate in the field. In general the varieties were more attacked in early and deep sowing (slow emergence) but some varieties had a different pattern. This will be further analysed.

New plans: New varieties of winter wheat and triticale will be tested and the varieties that in 2003 test showed some resistance will be tested again on new isolates. In spring wheat the same test will be performed again

Due to high risk of infection of organic experimental field with common bunt all trials will be performed in a separate field under conventional conditions.

Work package 4: Integrated strategies and dissemination of information.

This WP is the synergy between the different WP's and the different tasks will be developed according to the progress of the other WP.

Task 1: Integrated strategies.

During the project period the different control strategies and models will be integrated and combined with different cropping practices. The aim is to combine methods with moderate to good efficacy to get a combined system with over all good and in practice acceptable effect. This could for example be combining heat treatment or seed cleaning methods with varieties with moderate resistance. The effect of the soil temperature, harvest times and plant density on the development of the seed borne diseases will also be used.

Task 2: Dissemination of information

Information will be delivered via Danish Advisory Service to organic farmers through leaflets, farmer's journals and other publications. Information on seed borne diseases, thresholds, control and regulation methods will also be published in a grower manual to organic farmers. The Danish Agricultural Advisory Service has made a special new web site for organic seed (www.lr.dk). Information, recommendations etc. from ORGSEED can be placed here

Task 3: Official regulations

The project will be the basis for making new threshold values that can be implemented in the official system as recommended thresholds. These thresholds will be used for selecting available organic seed and will be a (voluntary) standard used by seed producers.

Task 4: The methods developed in the project.

The methods that are developed and implemented in the project will be part of the official test for organic seed made by PD. Other laboratories in Denmark are also offering seed test and is the intention that seed testing procedures will be standardised and harmonised on the basis of the results obtained in this project. ISTA (The Inter-

national Seed Testing Association) does accredit laboratories for validated test methods. At present only a small number of the methods for seed borne diseases are validated and the work with the rest of the methods has just only started. It will take several years before the system is fully developed. By initiating development and implementation of modern analysis methods, Denmark will be able to achieve influence on which of the methods to be internationally validated. Subsequently it will be possible to be accredited fast by ISTA and in this way secure the standardisation of analyses in all of the laboratories. In the future this can be a basis for approval of analysing laboratories

Work package 5: Project management

Project planning, co-ordination and reports.

Workshop on organic legumes will be arranged in 2003/2004 in cooperation with other DARCOF projects on legumes (GRAINLEG and GENESIS). On the workshop results from ORGSEED will be discussed and new initiatives in peas for 2004 will be coordinated with the other DARCOF projects.

ORGSEED will also take part in workshops together with the DARCOF project BAR OF (organic barley) and coordinate work especially on seed borne barley diseases.

Initiative will be taken to arrange a European workshop on organic seed in 2004/05.

C.2 Fulfilment of deliverables and milestones

Deviations will be further discussed in D

In the following are listed specific tasks for the different work packages with deliverables and milestones. Overview of the different task, deliverables and milestones can be found in the original application

WP1: Threshold values
<p>The work package is divided in 7 specific tasks:</p> <ol style="list-style-type: none"> 1. Seedling blight in wheat 2. Leaf stripe in barley 3. Net blotch in barley 4. Seed and foot rot in peas 5. Common bunt in wheat 6. Glume blotch/seedling blight in wheat 7. Models

Deliverable, No	Deliverable title	Time schedule according to application	Deviations
1	Most important seedling blight pathogens identified within the Microdochium/Fusarium complex.	2003	2004
2	Seed lots established with different infection levels for use in WP1, WP2 and WP3.	2002/2003	OK
3	Information on disease multiplication/reduction in individual seed lots in the propagation process.	2004/2005	
4	Threshold for seedling blight adjusted to organic condition	2003/2005	2004/2005
5	Threshold for seed borne Glume blotch adjusted to organic condition	2003/2005	2004/2005
6	Threshold for leaf stripe adjusted to organic condition	2003/2005	2004/2005
7	Threshold for net blotch adjusted to organic condition	2003/2005	2004/2005
8	Threshold for seed and foot rot disease in pea adjusted to organic condition	2003/2005	2004/2005
9	Models developed describing multiplication over years	2005	
10	New recommendations for threshold values to seed producers and farmers	2003/2005	2004/2005
11	Results published in farmer's journals, leaflets, magazines, web-site etc. The results from the pea trials will be published in "Annual Report of the National Field Trials 2002" in late December.	2002-2005	OK Re. Web see WP4, task 2
12	Results published in international publications.	2004/2005	

Deliverables list (yyyy/yyyy=first results/final results)

Milestones:

M1: Seed lots established with different infection levels

M2: Preliminary report quantifying relationship between disease intensity and yield loss

M3: Models describing multiplication and spread of diseases

M4: First adjustment of threshold

M5: Final threshold operational

M6: International publications

WP 2	Diagnostic methods
Task 1: Fusarium species and Microdochium nivale Task 2: Glume blotch (<i>Leptosphaeria nodorum</i>) Task 3: Barley net blotch and leaf stripe	

De-liver-able, No	Deliverable title	Time schedule according to applica-tion	Devia-tions
13	Qualitative PCR-method for Fusarium spp. will be identified and developed.	2003/2005	2002
14	Priority to Fusarium spp for which a quantitative PCR-test should be developed will be given based on results from WP1	2003	2003/2004
15	Quantitative method for Fusarium sp. will be tested on seed lots, produced in WP1, with known infection	2005	
16	Test for glume blotch will be identified	2004/2005	
17	Quantitative method for glume blotch will be tested on seed lots, produced in WP1, with known infection	2005	
18	Combination of methods for detection of seed borne diseases in wheat will be tested	2004/2005	
19	Quantitative method for Pyrenophora teres will be tested on seed lots, produced in WP1, with known infection.	2002	2002
20	Quantitative method for Pyrenophora graminea will be tested on seed lots, produced in WP1, with known infection	2002	2002/2003

Deliverables list (yyyy/yyyy=first results/final results)

Milestones:

M7: Identification and test of new diagnostic methods for *Pyrenophora graminea*, *Fusarium* species and glume blotch (*Leptosphaeria nodorum*)

M8: Implementation of the new methods for routine practise

M9: First step against validation of new methods in ISTA

M10: Publications

WP 3. Regulation and control measures

The work packages is divided in to two sub work packages:

- 3.1. Direct and indirect control measures
- 3.2. Host resistance

WP 3.1: Control measures acceptable in the organic seed production

Task 1. Preventing cropping methods.

Task 2. Multiplication of diseases in the propagation process from C1 to ware seed.

Task 3. Seed cleaning.

3.1 Removal of small infected kernels.

3.2. Removal of bunt spores

Task 4. Heat treatment.

De-liver-able, No	Deliverable title	Time schedule according to applica-tion	Devia-tions
21	Effect of pea/barley mixture on spread of seed and foot rot diseases to pods and new seeds.	2003/2005	
22	The potential of early, pre-optimal harvest time will be evaluated as a control strategy in fields for propagation and integrated in strategies in developed in WP1.	2004/2005	
23	Description of the influence of cropping parameters on the development of epidemics of net blotch, glume blotch and Fusarium. The description will lead to recommendation for propagation and for field inspections.	2004/2005	
24	The potential of seed cleaning as a strategy for reduction in disease frequency in infected seed lots will be estimated. The results will be integrated in strategies and recommendations in developed in WP1	2003/2005	OK
25	The potential of heat treatment in a drum dryer as a strategy for reduction in frequency of a range of diseases in infected seed lots will be evaluated, and the equipment will be adjusted to improve the effect and selectivity. Based on the results, the fundament for decisions of implementation of this equipment in practice will be improved as for other measures included for comparison in the study.	2003	OK

Deliverables list (yyyy/yyyy=first results/final results)

Milestones:

M11: Results with pea/barley mixtures.

M12: Results with cropping parameters on the development of epidemics of net blotch, Fusarium spp., glume blotch and seed and foot rot in pea.

M13: Seed lots tested by heat treatments and seed cleaning equipment and diagnosis of diseases status and seed vigour. Results will lead to recommendations for seed handling

M14: Publication phase

WP 3.2. : Screening for resistance

Task 1. Screening for resistance to leaf stripe in Danish barley varieties

Task 2. Screening for resistance in Danish pea varieties to seed and foot rot.

Task 3. Screening for resistance to common bunt in Danish wheat varieties.

Deliverable, No	Deliverable title	Time schedule according to application	Deviations
26	Danish barley varieties with interest for organic production screened for resistance against leaf stripe.	2003/2005	OK
27	Danish pea varieties with interest for organic production screened for resistance against Phoma medicaginis	2003/2005	OK
28	Danish wheat varieties with interest for organic production screened for resistance against common bunt.	2003/2005	OK

Deliverables list (yyyy/yyyy=first results/final results)

Milestones:

M15: Relevant barley varieties screened for resistance against leaf stripe

M16: Relevant pea varieties screened for resistance against Phoma medicaginis.

M17: Relevant wheat varieties screened for resistance against bunt.

WP4: Integrated strategies and dissemination of information.			
Task 1: Integrated strategies Task 2: Dissemination of information Task 3: Official regulations Task 4: The methods developed in the project.			
Deliverable, No	Deliverable title	Time schedule according to application	Deviations
29	Integrated strategies with different control measures.	2004/2005	
30	Leaflets, journals etc. to organic farmers and seed producer's etc. with information on problems with seed borne diseases.	2002-2005	
31	Web-site with information on seed borne diseases, thresholds, recommendations etc.	2002	2003/04
32	Thresholds adopted as a standard in organic seed production of cereals and peas.	2003/2005	
33	The results from the questionnaire to seed producers will be published in the information series published by the Danish Agricultural Advisory Centre "Planteavlsorientering"	2002	OK

Deliverables list (yyyy/yyyy=first results/final results)

WP5: Project Management			
Task 1 Co-ordinating the different element ensuring a coherent and integrated project. Task 2 Project meetings and project reports. Task 3 Project workshops.			
Deliverable, No	Deliverable title	Time schedule according to application	Deviations
34	Project reports each year	2002-2005	
35	Final project report	2005	
36	Project workshop with discussion of preliminary results	2003	
37	Project workshop presenting final results and conclusions	2005	

Milestones:

M18: Project report October each project year.

M19: Final project report December 2005.

M20: Workshop October 2003.

M21: Final Workshop October 2005.

D. Description of deviations and subsequent adjustments of plans

In the following are comments to deviation from the original plan (the numbers refers to the table about deliverables):

WP 1 Thresholds

Deliverables: Most of the deliverables in the project will according to the plan come late in the project.

D1: Due to the complexity of the brown root mycoflora, a minimum of one more season will be required for continued investigation of the brown root mycoflora of commercial seed lots.

D4: More data are needed before threshold can be adjusted. New trials are planned this year (WP1, task 1)

D5: It has been decided in the project to put more focus upon other diseases and not to continue with *Lepthospharia nodorum*

D6: Data from the leaf stripe trials need to be analysed and first discussion of new threshold will be early 2004. No new trials with leaf stripe are expected.

D7: Data from the net blotch trials need to be analysed and first discussion of new threshold will be late 2004.

D8: More data are needed before threshold can be adjusted. New trials are planned this year (WP1, task 4)

D10: First discussion on revision of thresholds will be early 2004 en when data from the first trials have been analysed.

Tan spot of wheat was included in the trials 2002/2003 but no attack appeared in the trials. Tan spot is not a part of the original proposal and will be taken out again.

Milestones:

M2: Quantification of relationship between disease intensity and yield loss were expected in 2002 and 2003. There will first be enough good data in 2003 to make a preliminary report and it will be expected early 2004

M4: First adjustment of threshold was expected early 2003 for leaf stripe and bunt. Data from the trials with leaf stripe suggest that the threshold should not be changed but final conclusions wait for further trials. It was only possible in 2003 to establish good data on common bunt and first conclusions can first be made early 2004

WP2 Diagnostic methods

Deliverables:

D13: Qualitative PCR-methods for *Fusarium spp.* and *Microdochium nivale* have

been implemented and are being used in the project for diagnostic purposes.

D14: Based on experiences from test of commercial seed lots it has been decided to give higher priority to the development of quantitative PCR tests for *Fusarium* species causing seedling blight. This means that a higher priority will be given to *F. culmorum*, *F. avenaceum* and *F. graminearum* besides *M. nivale*.

D16 and D17: It has been decided to leave out glume blotch from WP2 and then invest the resources on the development of Q-PCR methods for *Fusarium* spp.

D19 and D20: The quantitative method for *Pyrenophora* spp (both *P. teres* and *P. graminea*), described by Bates et al. (Molecular Plant Pathology, 2001, 2, 49-57) has been tested in the ABI Sequence detection system 7000 HT on a range of different isolates of *P. graminea*, *P. teres*, *Cochliobolus sativus*, *Fusarium* spp, *Aspergillus* spp, *Alternaria* spp. The method was found not to be specific for *Pyrenophora* spp. under the conditions used in the ABI Sequence detection system as also *C. sativus* was detected by this method. The implementation of this specific test in the ABI Sequence detection system will not be possible without extensive development. Therefore a higher priority has been given to the development of a PCR method which is specific for *P. graminea* as it is more important to be able to distinguish *P. graminea* from *P. teres*. To-day the only alternative to this test is a time-consuming green house test

Milestones:

M7: Q-PCR methods for *Pyrenophora graminea* and *Microdochium nivale* have been developed. Both methods are waiting to be tested on seed samples. Glume blotch will be left out of the project. More effort will be put into developing methods for *Fusarium* species. No Q-PCR methods are at the moment public available.

WP 3.1 Regulation and control.

Deliverables:

D21 Results from the trial available but many seed lots still need to be analysed for seed borne diseases (winter 2003/2004). There seem to be a contradiction between results obtained in cooperation with GENESIS and the results from the advisory service (landsforsøgene). This will be discussed in a common workshop in autumn 2003.

D22 The effect is likely to be limited, and will therefore only have an influence when the infection is close to the threshold. As these pathogens are all affected by climatic conditions, it has been very difficult to establish fields near the threshold. These experiments have been done in fields rather than plots. It is a possibility that the harvested areas have not been randomly selected in the fields, but rather biased for areas without lodging. This may have affected the results in both 2002 and 2003.

D24: Infection of seed pathogens does affect seed size and density of loose smut and maybe also other diseases. It has been identified that this may give problems in assessment, as selection of seed in the laboratory for testing of effects of seed separation have may not been random, but instead been biased on large seeds that are easier to analyse. Some experiments therefore need to be repeated with new instructions for selection of seed in the testing.

tions for selection of seed in the testing.

D25: The trials with the drum dryer is finishing 2003 and the potential of heat treatment in the drum dryer will be evaluated and possible adjustments to improve the effect and selectivity will be proposed.

Milestones:

M11: Seed analysis from the trials is not finished yet and will first be available winter 2003/2004. See also comments for D21

M12: Results with cropping parameters on the development of epidemics of net blotch, *Fusarium spp.*, glume blotch and seed and foot rot in pea expected 2002.

M13: Seed lots have been tested by heat treatments and seed cleaning equipment. First results available winter 2003/2004

WP 3.2 Screening for resistance

Deliverables:

First part of deliverables achieved.

Milestones:

M15: Relevant barley varieties screened for resistance against leaf stripe were expected in 2003 and the milestone was achieved.

M16: 10 varieties were screened both years against the three leaf and pod spot pathogens. Unfortunately, plant symptoms were very low, but the seed health testing is expected to show clearer differences. Probably testing will have to continue another year.

M20: Workshop will be arranged 2003 on legumes in co-operation with other DARCOF projects (GRAINLEG, GENESIS)

E. Project publications and other products

No marking: 75 – 100 % financed by DARCOF , * 25 – 75 % financed by DARCOF DAR and ** 5 – 25 % financed by DARCOF

1. Articles in international, scientific journals with review procedures

**Borgen, A. and L. Kristensen 2003 *in press*: Macroscopic leaf symptoms in wheat infected by *Tilletia tritici*. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz. 110(5): ??-??

**Borgen, A. and L. Kristensen *in submission*: Spore contamination of *Tilletia tritici* in seed lots as affected by field disease incidence. European Journal of Plant Pathology

2. Papers presented at congresses, symposiums, etc.

Borgen, A. 2003: Seed size separation to control loose smut (*Ustilago nuda*) in barley. 2nd International Seed Health Conference, Healthy Seed For Healthy Crop, Poznan, 16 – 18 September 2003 page 27-28

**Borgen A., A.-M. D. Gustavsson, J. Kieksi, T. Johnsen, R. Andersson and R. Eriksen 2003: Organic seed in the Nordic countries. TemaNord:588. 64 pages

*Borgen A., A.-M. D. Gustavsson, J. Kieksi, T. Johnsen, R. Andersson and R. Eriksen 2002: Factors affecting the development of the organic seed sector. In: K.P.Wilbois (ed.): proceedings of the European Symposium: 'Organic Seed Production and Plant Breeding - strategies, problems and perspectives', Berlin, 21-22/11-2002

Borgen, A. 2002: Organic seed production and seed regulation. The proceedings of the International Scientific and Practical Conference: Scientific aspects of organic farming. 21-22th March 2002, Jelgeva, Latvia.

Nielsen B.J. 2002 Threshold values for seed borne diseases of cereals and legumes. Proceedings of ECO-PB 1st International symposium on organic seed production and plant breeding, Berlin, Germany November 2002. [E.T. Lammerts van Bueren and K-P. Wilbois editors](#), 28-31.

*Nielsen, B. J. 2003. Resistance to leaf stripe (*Pyrenophora graminea*) in barley and common bunt (*Tilletia tritici*) in wheat and use of integrated strategies to control seed-borne diseases. 8th International Congress of plant Pathology, Christchurch, New Zealand, Abstracts of Offered Papers: 321.

Nielsen, B.J. 2003. The science of increasing production of organic grains. 11th Australian Barley Technical Symposium and 53rd Australian Cereal Chemistry Conference. University of Adelaide, Conference Proceedings: 19-22. **Invited paper.**

*Nielsen, B.J. 2003. Strategies for avoiding seed borne diseases. 11th Australian Barley Technical Symposium and 53rd Australian Cereal Chemistry Conference. University of Adelaide, Conference Proceedings: 75-79 **Invited paper.**

3. Reports, articles in agricultural journals, etc.

**Anon. 2003. Vejledning i Planteværn 2003. Danmarks JordbrugsForskning og Landscentret, Planteavl. Udgivet af Landbrugets Rådgivningscenter, Landbrugsforlaget.

Kristensen, E.F., 2003. Tromletørring skal sikre kvalitetskorn. Økologisk Jordbrug 293, p. 6.

Nielsen, G. C. 2003, Planteavlsorientering no. 09-603. Kassationsprocenter som følge af udsædsbårne svampe i økologisk udsæd af korn og bælgæd. Landbrugets

Rådgivningscenter

Nielsen, G.C. 2002. Oversigt over Landsforsøgene 2002", p. 251-253.

Results from field trials at Danish Advisory Service available at:
<http://www.lr.dk/applikationer/kate/viskategori.asp?ID=ka00400012000080000200>

Nielsen, G.C. 2003 "Oversigt over Landsforsøgene 2003". *Under preparation*

4. Oral presentations, public meetings, field days, etc.

Borgen, A., Oral presentation: Seed size separation to control loose smut (*Ustilago nuda*) in barley. 2nd International Seed Health Conference, Healthy Seed For Healthy Crop, Poznan, 16 – 18 September 2003

Kristensen, E.F., 2002. Varmebehandling til kvalitetsforbedring af frø. Workshop: Frø- og kornkvalitet. DIAS, Research Centre Flakkebjerg. 18 December 2002.

Kristensen, E.F., 2003. Heat treatment for disease control. Research Centre Flakkebjerg. 22 January 2003.

F. Scientific education

The project have been very important and valuable for improving the competence of the project staff

G. National and international cooperation.

Danish research Institutions (Risø, KVL, DTU)

Danish Plant breeders

Producers of machinery for seed cleaning and heat treatment

Collaborative partners will also involve researchers within and outside DARCOF, including foreign researchers

Gustaf Forsberg, SLU, on the test for the effect of moist air on pea disease.

Force Technologies is involved in the test for the effect of steam/ultrasound on seed borne diseases.

Westrup A/S and Mørdrupgård Korn on development of brush cleaner to control common bunt and stem smut.

NIAB (National Institute of Agricultural Botany), Cambridge, UK

Regarding technical construction and development of the drum drying technique, contact to the firm Cimbria A/S has been established.

A new EU COST action "Improving quality of plant propagation material in support of organic farming" have been proposed in 2003. It is expected that the COST action will start 2004 and several of the elements in the action will be parallel to the work packages in the Danish ORGSEED project. Several of the project members from ORGSEED will take part in the COST action.

ORGSEED cooperates and coordinates research activities with other DARCOF projects on legumes (GRAINLEG and GENESIS) and with DARCOF project BAR OF (organic barley, especially on seed borne barley diseases).

Critical reflection on the project

General for ORGSEED

In the project there have been a thorough discussion of the basis of the project and to what extent the trials should be performed under strict organic conditions. The ability of an organic soil to compensate for reduction in plant emergence, or to suppress seedling blight pathogens, is different from a conventional soil. This question is of special importance with seedling blight diseases and these trials (2002/2003 and 2003/2004) have and will be performed under full organic conditions. With the other diseases it was not specifically stated in the original application but the plan was to start with focusing on single factors and then gradually perform the trials under more organic conditions. For example, the multifactorial trial 2003 with net blotch in barley was conducted under conventional conditions. In 2004, trials with selected factors will be done under organic conditions to test the reaction in this more complex environment. However, concerns remain about introducing the disease into organically cultivated areas because the pathogen can survive on crop residues in the soil for at least two consecutive seasons.

For some diseases it is more problematic to carry out the trials in an organic field. With common bunt there is a high risk that spores will contaminate the organic soil and stay there for years. That has been the reason for keeping these experiments far away from the organic fields.

In the variety trials in pea artificial inoculum is put on the soil and irrigation is installed to favour disease development. Therefore it is not possible to carry out mechanical weeding.

In the trials with pea it was not possible to get all trials on organic farms because the localities with organic trials was not available.

WP1 Thresholds

Because of the ability of diseases such as net blotch to spread in between fields during the season and because environmental and varietal resistance effects are affecting the disease development to a large extent, it is difficult to establish a good data-set with correlations between seed infection and later damage in the field. New approaches with appropriate statistical designs covering the varietal effect will be undertaken to obtain data sets for at least two seasons. There will also be more cooperation with the DACROF project BAR-OF to get a better understanding of the whole disease cycle and better use of resistance data.

WP2 Diagnostic methods.

There have been no major changes of the plans. The developed PCR methods are now to be tested on a range of seed samples. Due to the lack of suitable seed sam-

ples the test has to be continued in 2003 where more samples from the harvest in 2002 are available. It will be necessary to invest a substantial amount of time in the test of seed batches with traditional mycological methods perhaps combined with qualitative PCR on single seeds to make sure that the percentage infection is determined as precise as possible.

In collaboration with the Danish Plant Directorate it has been decided to focus on *Pyrenophora graminea* in the first phase of the project and a lower priority has been given to the implementation of an already published method for the quantification of *Pyrenophora* spp. We have tested this method in our real-time PCR system and it showed that it was not specific to *Pyrenophora* species but did also detect *Cochliobolus sativus*. The implementation of this specific test will not be possible without an extensive development. Therefore it has been decided to concentrate on the development of a new assay for *P. graminea*.

Due to the variation in the amount of fungal DNA on each seed it may be difficult to find an acceptable correlation between the amount of fungal DNA measured by Q-PCR and the percentage infected seeds. As an alternative to direct quantification an alternative test based on sequential methods have been designed with the assistance of the Biometry Research Unit at DIAS. With a threshold of 5% 9 independent samples each with 21 seeds should be tested first. If 3 or less of these samples contain disease, e.g. *P. graminea* the seed lot can be accepted. If more than 8 of the nine samples are infected the seed lot can be rejected. If 4, 5, 6, or 7 of the samples are positive another 9 samples have to be tested etc. However, the disadvantage of this method is the high number of DNA extractions and PCR reactions which have to be performed for each seed lot.

WP 3.1 regulation and control

The focus in WP3 has been on preventive, "soft" methods of control of seed borne pathogens as these were seen as best in line with the principles of organic farming. The preliminary results indicate that the risk of infection can be reduced by propagation in mixed cropping and by early harvest, but that the effect of these measures are limited, and insufficient for control of the diseases. Seed separation and drum drying has a significant effect on some diseases, but without effect on other diseases. Hence, the preliminary results indicate a need for direct methods in cases, where preventive measures fail to control the seed borne diseases.

The effect of traditional hot water treatment, hot air or combination of steam and ultrasound on the infection of seed borne diseases has been tested. The results are promising but it is not within the objectives of this project to continue the work. It has therefore been decided to search for funds for a separate project on this methods, especially combination of steam and ultrasound.

WP 3.2 Resistance

Again in 2003 it was difficult to establish clear symptoms of leaf and pod spot of peas in the field trials. The inoculum was applied earlier than the previous year, but birds ate a large part of the grain inoculum. In the *M. pinodes* trial a second inoculation was carried out by application of a spore suspension, and higher disease attack occurred. However, despite low symptoms in 2002, seed infection levels were considerable. Seed health testing will show whether this is also the case this year.

8. Budget

A. Account for any change in budgets

There has been no change in the over all budget

B. Budget for the whole project (1.000 DKK)

Total consumption of funds from DARCOF and expected consumption this year and coming years

Year:	2001	2002	Expected 2003	2004	2005	Total
Man-months	6,6	73,8	71,5	57,3	34,6	243,8
Scientific person- nel	4,1	34,6	32,7	24,5	19,6	115,5
Technical person- nel	2,5	39,2	38,8	32,8	15,0	128,3

Year:	2001	2002	Expected 2003	2004	2005	Total
Salaries	261.480	2.038.388	1.964.105	1.769.939	1.132.383	7.166.295
Scientific person- nel	210.480	1.211.048	1.173.170	948.680	777.848	4.321.226
Technical person- nel	51.000	827.340	790.935	821.259	354.535	2.845.069
Other operational costs	26.540	307.593	375.000	348.000	108.000	1.165.133
Equipment	0	0	0	0	0	0
Others (please specify)	0	0	0	0	0	0
Direct costs	288.020	2.345.981	2.339.105	2.117.939	1.240.383	8.331.428
Indirect costs (20% of direct costs)	58.628	470.971	467.822	423.588	248.077	1.669.085
Total	346.648	2.816.952	2.806.927	2.541.527	1.488.460	10.000.513

Comments:

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project Bent J. Nielsen	Danish Institute of Agricultural Sciences (DIAS), Research Centre Flakkebjerg Department of Crop protection, 4200 Slagelse		

Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)
Danish Institute of Agricultural Sciences, DIAS

Year:	2001	2002	Expected 2003	2004	2005	Total
Man-months	5,5	63,8	59,5	46,3	26,6	201,7
Scientific personnel	3,0	26,6	24,7	17,5	13,6	85,4
Technical personnel	2,5	37,2	34,8	28,8	13,0	116,3

Year:	2001	2002	Expected 2003	2004	2005	Total
Salaries	146.000	1.758.993	1.619.494	1.453.595	887.585	5.865.667
Scientific personnel	95.000	974.913	917.675	724.125	580.321	3.292.034
Technical personnel	51.000	784.080	701.819	729.470	307.264	2.573.633
Other operational costs	15.000	205.000	200.000	173.000	68.000	661.000
Equipment	0	0	0	0	0	0
Others (please specify)	0	0	0	0	0	0
Direct costs	161.000	1.963.993	1.819.494	1.626.595	955.585	6.526.667
Indirect costs (20% of direct costs)	32.200	392.799	363.899	325.319	191.117	1.305.334
Total	193.200	2.356.792	2.183.393	1.951.914	1.146.702	7.832.001

Comments:

The budget for DIAS is inclusive Scanagri A/S and KVL. Detailed budget for Scanagri and KVL is under section B.

Danish Plant Directorate (PD):

Year:	2001	2002	Expected 2003	2004	2005	Total
Man-months	0	6	8	7	5	26
Scientific personnel	0	6	6	5	4	21
Technical personnel	0	2	4	4	2	12

Year:	2001	2002	Expected 2003	2004	2005	Total
Salaries	90.000	228.660	280.078	255.698	182.332	1.036.768
Scientific personnel	90.000	185.400	190.962	163.909	135.061	765.332
Technical personnel	0	43.260	89.116	91.789	47.271	271.436
Other operational costs	10.000	25.000	25.000	15.000	10.000	85.000
Equipment						0
Others (please specify)						0
Direct costs	100.000	253.660	305.078	270.698	192.332	1.121.768
Indirect costs (20% of direct costs)	20.000	50.732	61.016	54.140	38.466	224.354
Total	120.000	304.392	366.094	324.838	230.798	1.346.122

Comments:

The above mentioned is the funding from FØJO to Danish Plant Directorate (PD). PD is **extra** co-financing total 15 scientific and 28 technical month to development of diagnostic methods the project (WP2), for details se appendix section C.

Danish Agricultural Advisory Centre (LR):

Year:	2001	2002	Expected 2003	2004	2005	Total
Man-months	1,1	2	2	2	2	9,1
Scientific personnel	1,1	2	2	2	2	9,1
Technical personnel	0	0	0	0	0	0

Year:	2001	2002	Expected 2003	2004	2005	Total
Salaries	25.480	50.735	64.533	60.646	62.466	263.860
Scientific personnel	25.480	50.735	64.533	60.646	62.466	263.860
Technical personnel	0	0	0	0	0	0
Other operational costs	1.540	77.593	150.000	160.000	30.000	419.133
Equipment						0
Others (please specify)						0
Direct costs	27.020	128.328	214.533	220.646	92.466	682.993
Indirect costs (20% of direct costs)	6.428	27.440	42.907	44.129	18.493	139.397
Total	33.448	155.768	257.440	264.775	110.959	822.390

Comments:

There has been a change within the budget 2001-2003. The over all budget is the same

B. Budget for each participating departments under the financing of DIAS (1.000 DKK)

The budget for DIAS includes:

I) Department of Crop Protection, Flakkebjerg

II) Department of Agricultural Engineering, Research Centre Bygholm

III) Scanagri A/S

IV) Danish Agricultural University (KVL), Tåstrup.

In the following are listed the budget for each group:

I) Department of Crop Protection, Flakkebjerg:

Year:	2001	2002	Expected 2003	2004	2005	Total
Man-months	5,5	49,8	46,3	37,5	21,6	160,7
Scientific personnel	3,0	19,3	17,3	12,5	9,6	61,7
Technical personnel	2,5	30,5	29,0	25,0	12,0	99,0

Year:	2001	2002	Expected 2003	2004	2005	Total
Salaries	146.000	1.368.210	1.224.221	1.139.772	684.530	4.562.733
Scientific personnel	95.000	708.910	651.075	497.091	400.902	2.352.978
Technical personnel	51.000	659.300	573.146	642.681	283.628	2.209.755
Other operational costs	15.000	120.000	115.000	98.000	45.000	393.000
Equipment						0
Others (please specify)						0
Direct costs	161.000	1.488.210	1.339.221	1.237.772	729.530	4.955.733
Indirect costs (20% of direct costs)	32.200	297.642	267.844	247.554	145.906	991.147
Total	193.200	1.785.852	1.607.065	1.485.326	875.436	5.946.880

Comments:

II) Department of Agricultural Engineering, Research Centre Bygholm

Year:	2001	2002	Expected 2003	2004	2005	Total
Man-months	0	2	2	0	0	4
Scientific person- nel	0	1	1	0	0	2
Technical person- nel	0	1	1	0	0	2

Year:	2001	2002	Expected 2003	2004	2005	Total
Salaries	0	59.740	61.532	0	0	121.272
Scientific person- nel	0	38.110	39.253	0	0	77.363
Technical person- nel	0	21.630	22.279	0	0	43.909
Other operational costs		10.000	10.000			20.000
Equipment						0
Others (please specify)						0
Direct costs	0	69.740	71.532	0	0	141.272
Indirect costs (20% of direct costs)	0	13.948	14.307	0	0	28.255
Total	0	83.688	85.839	0	0	169.527

III) Scanagri Denmark A/S (SD):

Year:	2001	2002	Expected 2003	2004	2005	Total
Man-months	0	6,3	6,4	5,0	4,0	21,7
Scientific personnel	0	6,3	6,4	5,0	4,0	21,7
Technical personnel	0		0	0	0	0

Year:	2001	2002	Expected 2003	2004	2005	Total
Salaries	0	227.893	227.347	227.034	179.419	861.693
Scientific personnel	0	227.893	227.347	227.034	179.419	861.693
Technical personnel						0
Other operational costs	0	65.000	65.000	65.000	18.000	213.000
Equipment						0
Others (please specify)						0
Direct costs	0	292.893	292.347	292.034	197.419	1.074.693
Indirect costs (20% of direct costs)	0	58.579	58.469	58.407	39.484	214.939
Total	0	351.472	350.816	350.441	236.903	1.289.632

Comments:

Due to unexpected high cost there has been a change within the budget 2002-2005 and some expenditures to salaries have been transferred to cost. The over all budget is the same

In the original application indirect cost was set to 40 %. This has been changed to 20 %. The surplus has been transferred to salaries in the budget for Scanagri.

There is no co-financing from Scanagri A/S.

IV) Danish Agricultural University (KVL), Tåstrup:

Year:	2001	2002	Expected 2003	2004	2005	Total
Man-months	0	5,71	4,78	3,78	1	15,27
Scientific personnel		0	0	0	0	0
Technical personnel	0	5,71	4,78	3,78	1	15,27

Year:	2001	2002	Expected 2003	2004	2005	Total
Salaries	0	103.150	106.394	86.789	23.636	319.969
Scientific personnel	0	0	0	0	0	0
Technical personnel	0	103.150	106.394	86.789	23.636	319.969
Other opera- tional costs	0	10.000	10.000	10.000	5.000	35.000
Equipment						0
Others (please spec- ify)						0
Direct costs	0	113.150	116.394	96.789	28.636	354.969
Indirect costs (20% of di- rect costs)	0	22.630	23.279	19.358	5.727	70.994
Total	0	135.780	139.673	116.147	34.363	425.963

Comments:

The work done at Højbakkegaard, KVL will be field trials and laboratory work under supervision by Anders Borgen, Scanagri.

C. Budget for co-financing from each participating institute (1.000 DKK)

Danish Plant Directorate (PD) is financing extra total 15 scientific and 28 technical month to development of diagnostic methods the project (WP2):

Financing to the project from PD						
	2001	2002	2003	2004	2005	TOTAL
Months (scientific, HJH)	1	2	3	4	5	15
Months (technical)	3	6	6	6	7	28
Salary (scientific)	30.000	63.000	99.225	138.915	182.326	513.466
Salary (technical)	63.000	132.300	138.915	145.861	178.679	658.755
Operation - other	0	0	0	0	0	0
Operation – equipment	0	0	0	0	0	0
Overhead	0	0	0	0	0	0
Financing from PD	93.000	195.300	238.140	284.776	361.005	1.172.221

Comments: