

# Midterm Status Report 2004 and Application for Continuation in 2005

For research projects financed by grants from  
The Directorate for Food, Fisheries and Agro Business  
under the Danish Ministry of Food, Agriculture and Fisheries

---

## 1. Research program

Research in organic farming 2000-2005 (DARCOF II)

---

## 2. Project title and number

VI.1 ORGSEED

Healthy seed for organic production of cereals and legumes.

---

## 3. Head of project

Bent J. Nielsen, *senior scientist*  
Danish Institute of Agricultural Sciences (DIAS)  
Research Centre Flakkebjerg  
Department of Crop Protection  
4200 Slagelse

---

## 4. Participating institutes

**Danish Institute of Agricultural Sciences (DIAS)**  
Research Centre Flakkebjerg  
Department of Crop Protection  
4200 Slagelse

**Danish Institute of Agricultural Sciences (DIAS)**  
Research Centre Bygholm  
Department of Agricultural Engineering  
8700 Horsens

**Danish Plant Directorate (PD)**  
Skovbrynet 20  
2800 Lyngby

**Scanagri Denmark A/S (SD)** (until March 31st 2004)  
Vester Farimagsgade 6, 5th  
DK-1606 Copenhagen

**Agrologica** (from April 1st 2004)  
Houvej 51  
9550 Mariager

**Danish Agricultural Advisory Service (LR)**  
Udkærsvvej 15, Skejby  
8200 Århus N

**Danish Agricultural University (KVL)**  
Department of Agricultural Sciences  
Agrovej 10  
2630 Taastrup

---

## 5. Project staff

**Danish Institute of Agricultural Sciences , Research Centre Flakkebjerg**  
Bent J. Nielsen, *senior scientist* (BJN)  
Annemarie Fejer Justesen, *senior scientist* (AFJ)  
Lars Bødker, *senior scientist* (LB) On leave 2003-2004.  
Hanne Wolffhechel, *senior advisor* (HW)  
Hans Pinnschmidt, *senior scientist* (HP)

**Danish Institute of Agricultural Sciences (DIAS), Research Centre Bygholm**  
Erik Fløjgaard Kristensen, *scientist* (EFK)

**Danish Plant Directorate**  
Henrik Jørskov Hansen, *senior plant pathologist* (HJH)

**Scanagri Denmark A/S / Agrologica**  
Anders Borgen, *plant pathologist* (AB)

**Danish Agricultural Advisory Service**  
Ghita Cordsen Nielsen, *senior advisor* (GCN)

---

## 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 today 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:**

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.

Scanagri has until March 31<sup>st</sup> 2004, as a subcontractor for DIAS hired Anders Borgen, Agrologica to participate in the project. From April 1<sup>st</sup> 2004, the responsibility in the project of Scanagri has gone to Agrologica and Agrologica (Anders Borgen) is now subcontractor for DIAS. This change has not affected the budget or any staff mentioned in the application.

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

WP No	WP title	Participants <sup>1)</sup>	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. <sup>2)</sup>	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
<b>Total</b>						

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 seed 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 2004:**

Threshold values have 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. A reasonable data base is also available now for net blotch although additional data will be valuable because of the complexity of the pathosystem due to 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 show 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. The 2004 trials yielded a data base for establishing infection thresholds at a given sowing time but at varying seeding rates and for varieties differing in resistance. An additional years' data would be valuable here, too. The trials in peas in 2002-2003 with sowing seed with varying degrees of leaf and pod spot infection showed no significant yield decrease sowing seed with up to 19 percent infected seed. The attack on the harvested seeds was at the same level irrespective of the attack on the seeds sown. The results from 2004 are not yet available. Trials with different seed amounts in peas in 2003 showed no effect of seed amount on leaf and pod spot infection on the harvested seeds. The results from 2004 are not yet available.

Real-time PCR methods for detection and quantification of DNA from *Microdochium nivale* was developed in 2003 and an assay for *Fusarium culmorum* have been developed in 2004. In 2004 the methods have been validated on several dilution series made by diluting pathogen DNA with wheat DNA as well as mixing healthy grain tissue with infected tissue and this showed a significant correlation. The project is now in the process of testing the methods on natural seed samples. A similar assay for *F. graminearum* has been developed in 2004 and is currently being validated.

PCR primers and a TaqMan probe for a real-time PCR assay for *Pyrenophora graminea* have been designed. Seed samples from a barley leaf stripe field experiment in WP1 have been tested by traditional green house test where the percentage of plants with barley leaf stripe was counted. The tested seed samples showed infection levels from 0-11%. In 2004 DNA has been extracted from these seed samples and tested with real-time PCR. Although there is a highly significant correlation between the data from the two methods the measured amount of DNA only explains 59% ( $R^2=0.59$ ) of the variation in the infection level assessed by the green house test. However, it will be possible to use the method in a semi-quantitative test. This approach will also be used for *M. nivale* and *Fusarium*.

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 by 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. The efficacy is dependent on the capacity of the machine and by improving the air stream in the technique, it is expected that the same efficacy can be obtained by higher capacity. The efficacy has, however not been improved yet. The equipment has with help from the project been used in commercial wheat production in 2004 at the seed company Mørdrupgård Korn. It will be investigated if the reduction is dependent on the spore concentration in the seed lot. It has been shown that the infection by 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*. In 2002-3, it has been shown that the infection by 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. No new experiments are made or planned in 2004-5.

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. Treatment of a seed lot infested by common bunt in 2003 was treated in the drum drier and sown in the field. The result indicate that the infection was reduced by about 90%, but only in treatments, where the germination was negatively affected. However, at pre-

sent the results are not finally analysed. 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 have been tested for resistance to leaf stripe (barley) and common bunt (wheat). The results so far show that many of the spring barley varieties grown in Denmark exhibit some resistance to leaf stripe and that a few varieties are quite resistant. Varieties with reasonable resistance against loose smut are currently not available - only one years' data are available for those having displayed some level of resistance. Most of the Danish winter wheat varieties are highly susceptible to common bunt, but some resistance has been identified. Varieties like Stava (late maturing Swedish varieties) was highly resistant and to some degree also Penta and Beder, whereas a few varieties (in 2004 e.g. Malling and Samourai) were moderate resistant. Also in spring wheat, the screening showed great variation in resistance but no varieties have high resistance. In triticale, most of the tested varieties seem to be highly resistant to common bunt. Only the variety Triamant showed susceptibility to common bunt. A number of pea varieties have been screened for susceptibility to seed and foot rot (*Ascochyta* complex). Two years' results of seed health testing indicate considerable differences between varieties in their susceptibility to seed infection by each of the three pathogens.

In 2005, the last year of the project special focus will be on

- Finalizing field trials and data analysis
- Publishing data
- Development of models for propagation and spread of seed borne diseases with emphasis on the importance of threshold values.
- Implementation of official threshold values for organic seed
- Implementation of new diagnostic methods for leaf stripe and Fusarium
- Arrange a final workshop with contribution from Nordic and other European countries with discussion of results and implementation/harmonisation of new threshold values.

### 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 seed to avoid unnecessary discharge of valuable seed.

#### **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.

Based on the 2002/2003 trials, it was difficult to evaluate the threshold level for *Fusarium* and to judge management options aiming at compensating *Fusarium*-related seed infection, such as increased seeding rates. A 4-factorial trial was therefore conducted in 2003/2004 where seed lots of cvs. Ritmo, Bill and Boston with 4 degrees of infection by *F. culmorum* and *Microdochium nivale* were sown at 3 seeding rates (400, 425, 450 plants/m<sup>2</sup>). The trial was run under organic conditions. A general linear model based on additive main effects of the cultivar and an interaction term for [cultivar x germinating seeds/m<sup>2</sup> x fraction of healthy seeds] explained 67% (adjusted R<sup>2</sup> = 0,67) of the variation of the plants/m<sup>2</sup> counted at growth stage 13 (3-leaf stage) with all parameters in the model being highly significant ( $p \leq 0,005$ ) and the R<sup>2</sup> values of the individual cultivar x pathogen combinations ranging from 0,47 (Boston x *M. nivale*) to 0,85 (Ritmo x *F. culmorum*). The parameters of the model suggest that Boston was most responsive to an increasing seeding rate and/or healthy seed fraction, followed by Ritmo and Bill.

*New plans:* The last years' work should focus on data analysis, modelling and publication. Also, yield data from 2004 were not yet analysed because harvesting in 2004 was late. To obtain more data for fine-tuning and/or independent testing of the models that are to be build based on the obtained data, the following trials will be conducted: 1) A replicated single-row field trial using seed lots of several varieties infected by several *Fusarium spp.* 2) A trial will be sown using 5 seed lots of Ritmo at 9 seeding rates. Both trials will be operated 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 key 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.

Seeds of eight spring barley varieties harvested in 2002 and 2003 in two other DARCOF trials (BAR-OF) under two cropping conditions (organic versus conventional) at three sites (Flakkebjerg, Jyndevad, Foulum) where 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.

A field trial was established at the research station Flakkebjerg in 2002 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%). Another trial was conducted under organic conditions in 2004 to study interactions between variety and seed infection level. In this trial, a range of seed infection levels was tested on a number of varieties varying in net blotch susceptibility. The respective seed lots were obtained from individual BAR-OF plots harvested in 2003 (see paragraph above).

Net blotch severity at the generative phase (growth stage 70 – beginning of grain filling)

was highly positively correlated with the infection level of the harvested seeds in both years in the BAROF trials. A general linear model explained 72% of the variation of seed infection based on effects of [variety x year] and [variety x year x net blotch severity at gs 70]. The results indicate that the transmission efficiency of net blotch from the epidemic on the foliage to the grain differs among varieties. Net blotch disease severity was highly negatively correlated with 1000 grain weight and explained, together with [site x year] effects, 58% of its variation.

As a "bycatch" of the BAROF 2003 seed analyses, high seed infestation levels of *Bipolaris* were found in many samples, especially in those from organically managed plots at Flakkebjerg and Foulum. High *Bipolaris* infestation levels were associated with a drastic reduction in the capability of seeds to germinate.

As the 2002 field trial at Flakkebjerg had shown, 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 receiving high amounts of straw. Also, the effect faded as the season progressed. Because of the interactions between variety and seed infection level that were assumed based on the 2003 results, a field trial was conducted in 2004 in which seeds lots of 4 varieties harvested in individual plots of the BAROF trials were sown. The seed lots covered a range of seed infestation levels for each variety. A general linear model based on an interaction term for [variety x seed infestation level] explained 71% of the variation of the net blotch severity at growth stage 70. The results hint at variety-specific effects of seed infestation with respect to net blotch development later during the season.

*New plans:* The main focus in 2005 should be on data analysis (including the yield data from 2004 that are not ready yet due to late harvesting), modelling and publication. A new set of disease data and seed samples from the 2004 BAROF trials shall be obtained and analysed in analogy to those from 2002 and 2003. If more experimental work is deemed to be necessary, it could consist in conducting a similar field trial as in 2004 (seed infestation levels x variety) under organic conditions.

### **Task 3 Leaf stripe in barley**

Previous results in the project (2002) have demonstrated 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.

*New plans:* No new trials were conducted

### **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 seven varieties and one variety mixture in the national field trials will be sent to PD for seed health analysis. In the trials in 2003 the variety Pinocchio was least infected with leaf and pod spot on the harvested seeds. All trial series have focus on diseases in peas. One of the trial plans (5 trials) is a continuation of the trials in 2002-2003, where the effect of sowing seeds with varying degrees of infection of leaf and pod spot pathogens is examined. The trials in 2002-2003 showed no significant yield decrease by sowing up to 19 percent infected seeds. On the harvested seed the attack of leaf and pod spot was at the same level irrespective of the attack on the seeds sown. A new plan (4 trials) has been started in 2004 examining the importance of varying degrees of seed infection of leaf and pod spot pathogens in peas for whole crop (*helsæd*). Another of the trial plans (5 trials) is a continuation of the trials in 2003 which examines the importance of seeding rates in peas to yield and attack of pea disease. The trials in 2003 showed no effect of seeding rates on leaf and pod spot. Some trials refer to WP 3.1 task 1 but are mentioned here to give an overview of the pea trials.

A new experimental plan with "strategic harvest" was started in 2004, but unfortunately no trials were conducted. The purpose is to try harvesting the wettest areas in the field separately and measure the effect on seed infection with leaf and pod spot in the remaining parts of the fields. An other attempt will be made to conduct some of these trials in 2005.

The trials (8 trials) with cultivation of peas in combination with spring barley conducted in 2002-2003 were not continued because of a too low effect on leaf and pod spot by cultivating peas with barley.

Some of the trials in 2004 were carried out again in conventional fields because of problems with placing the trials in fields for organic cultivation.

In September seed samples will be sent to the Plant Directorate for seed health testing, so no results are available as yet. It is also not yet possible to say how many trials will be discarded for various reasons in 2004. The wet weather has resulted in a very late harvest in some trials. In the plan with varying degrees of infection with leaf and pod spot on the seeds there will only be trials with low infections in one of the two varieties because of delivering of the wrong seed for the trials.

*New plans:* When the analyses for leaf and pod spot on pea seed are available in 2004 it will be decided what kind of trials that will be performed in 2005. See also WP 3.1. task 1. Continued experiments in whole crop will be considered. There are no experiments planned in Lupin (according to the application) but because of the importance of this crop, evaluation of the threshold values (based on literature) for antrachnose will be considered.

### **Task 5 Common bunt in wheat**

In order to get more information on the importance of low infection levels, 27 different seed lots were received autumn 2003 from two different laboratories (OK Laboratories and Plant Directorate) and sown in the field (43 samples in autumn 2002). The trial in 2003 showed that even at the lowest infection level (10 spores/g) there was a low attack in the field (0,3-1 % field attack). In 2004 the results were similar but the lowest infection level that gave measurable infections in the field were 30 spores pr g wheat. 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 relatively 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 were 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**

*Leptosphaeria 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 more trials with *Leptosphaeria nodorum*.

#### **Task 8 Models**

The data collected under task 1 can be used to construct a model estimating the no. plants/m<sup>2</sup> (and, later when the yield data have been included in the analysis, yield parameters) to the no. planted germinating seeds/m<sup>2</sup> as well as to the seed health status (fraction of healthy seeds = 1 – % infected seeds/100). This model and the decision support tools to be developed based on it (e. g. iso-curves, look-up tables) will enable the user to quantitatively determine how stand- and/or yield reducing effects of *Fusarium* attack in winter wheat seeds can be compensated by increasing the seeding rate.

The data collected under task 2 serve as the basis for developing two types of models: 1) a model describing the relationships between seed infection, field infection, and subsequent infection of the harvested seed of different cultivars and 2) a model relating seed infection and varietal resistance to yield related variables. While the type 1 model can be used on a strategic level to project the level of seed infestation and to evaluate the long-term risk of multiplication of net blotch in seed production over several seed propagation generations, the type 2 model can be used on a more tactical level to judge the value of particular seed batches and the associated yield risks of using these batches in a particular cropping season.

Task 8 shall receive highest priority in 2005.

#### *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. A real time PCR method for quantification of *M. nivale* has been developed. The specificity of the assay 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. In 2004 the quantification of *M. nivale* DNA has been validated on several dilution series made by diluting pathogen DNA with wheat DNA as well as mixing healthy grain tissue with infected tissue followed by DNA extraction and PCR. An infection gradient obtained by mixing *M. nivale* infected seeds with healthy seeds was produced for a field experiment in WP1 and was also used in this experiment. The samples were tested on brown roots with a traditional seed health test and with PCR. A correlation between the amount of *M. nivale* DNA and the percentage of seeds having brown roots in a seed health test was found. Approximately 70% ( $R^2=0.70$ ) of the 'percentage brown roots' can be explained by the variation in the amount of *M. nivale* DNA. More samples need to be tested. In 2004 a quantitative PCR assay for *F. culmorum* has been developed and similar experiments have been performed. Here no correlation was found between the amount of *F. culmorum* DNA and the level of brown roots probably because the seed lots have been infected with other species causing brown roots. This has to be further investigated. A quantitative PCR assay for *F. graminearum* has also been developed and is being tested at the moment. A real time PCR method for *F. avenaceum* has just recently been published and we will try to implement this for seed testing.

The mycoflora developed on brown roots has 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 minimise the number of primers simultaneously used in each test it is necessary to investigate the relative contribution of the 4 species to seedling blight development.

*New plans:* The relative importance of the 4 species causing seedling blight has to be further investigated. Seeds harvested from a field trial artificially inoculated with the four species will be analysed for plant emergence and over-wintering ability in the field as well as being tested in the laboratory by classical seed health test (number of kernels with brown roots) and by PCR (DNA amount of each of the four species).

### **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*. A quantitative PCR method for *P. graminea* has been developed. Seed samples from a barley leaf stripe field experiment in WP1 have 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%. In 2004 DNA has been extracted from these samples and has been tested by PCR. A significant correlation between the amount of *P. graminea* DNA and the number of plants with barley leaf stripe symptoms was seen. However, only 60% of the infection level could be explained by the amount of *P. graminea* DNA determined by PCR. The method will allow quantification in relation to a certain threshold, but more seed samples need to be tested.

*New plans:* So far all the tested seed samples have been of the same barley variety. More samples of different varieties will be tested in order to investigate whether this has an influence on the correlation.

#### *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 were made in 2002-3 in pea and barley. 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 was made in both mono-cropping and mixed cropping systems. The disease incidence was significantly lower in the mixed cropping system, but the effect of the harvest time was independent of cropping system. Based on these results, it was decided not to continue the experiments in 2004-5.

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 after 3 experimental plans (see WP 1, task 4).

### **Task 2. Multiplication of diseases in commercial seed lots**

After the harvest 2003 a questionnaire by Danish Agricultural Advisory Service was 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 47 percent of the C2 pea seed lots had more than 5 percent seed with leaf and pod spot and 26 percent of the C2 pea seed lots had more than 20 percent seed with leaf and pod spot. 31 percent of the spring barley C2 lots was rejected because of seed borne diseases. Lupin and broad bean were included in the questionnaire in 2003. The questionnaire will be sent to the companies again after harvest 2004.

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.**

Spores of common bunt contaminate the surface of wheat seed, and can be removed by different seed cleaning equipment. A traditional basic cleaning with air stream and size separation is shown to remove 80% of the spores. A brush cleaner has been tested for the effect of further spores removal. The experiments are mainly made in seed lots with high spore contamination (60,000-300,000 spores/gram). It is demonstrated that a laboratory brush cleaner can reduce the contamination of spores by 99.9%, but in commercial seed plants, the efficacy was only about 90%. By changing the air-stream in the equipment, the efficacy was improved to about 97% without reduction in seed vigour, and at the same time the capacity of the machine ( $\varnothing=400\text{mm}$ ) was improved to about 700kg/hour. A Sigma cleaner was tested in a commercial seed plant. The equipment gave no improved effect compared with traditional seed cleaning.

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 was highly significant in trials in 2003, and the infection level being reduced by 50-60% by discarding the 15% of the smallest seeds. The effect can be further improved by seed separation based on density by a gravity separator or a table separator. New field trials are made in 2004, also including leaf stripe. The results seem to confirm previous results, but data are at present not finally analysed. In seed lots infected by *Fusarium*, net blotch or glume blotch no significant effects have been seen. This difference in effects indicates that the infection can only be reduced by seed separation in seed lots, where the seed size and density is very uniform. In seed lots, where the seed are very diverse 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 barley 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 2003, organically 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 were sowed in experimental plots at Højbakkegård this autumn and assessed in July 2004. Data are not finally analysed, but indicates that the infection was reduced from 31% in the control plots to 1.3% in the most effective treatment. However, this treatment reduced also the vigour of the seeds.

ORGSEED has followed the development of heat treatments in other projects, particular the Thermoseed project in Sweden (warm humid air) and FRØTU in Denmark (steam and ultrasound), but we are also in contact with researchers involved with electron beam treatment in Germany and steam-vacuum treatment in Germany. The Thermoseed project is now close to commercial implementation, whereas FRØTU has been tested on some seed, but has not been subject to systematic research. These systems may have a higher efficacy and specificity than the drum dryer, but the drum dryer has the advantage of being very cheap in cases, where equipment is available and drying is needed for other reasons.

*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 of this potentially devastating disease. In spring 2003 and 2004, the spring barley varieties of the official variety testing program were sown in small-plot nurseries at Flakkebjerg in three replications. The seed lots had been inoculated by means of highly infected spreader rows of susceptible varieties in the preceding seasons. The trials were performed under conventional conditions. The leaf stripe level on the most susceptible varieties was relatively low in 2003 and 2004 in comparison to 2002 and reached a maximum of ca. 5 and 8% disease incidence (= infected tillers/100 tillers) in the years 2003 and 2004, respectively. 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 val-

ues below 20% of the most susceptible genotypes. However, relatively few varieties were tested in 3 or more years and some genotypes expressed relatively high susceptibility with disease incidence levels reaching 40% or more of the most susceptible genotypes.

Data on loose smut incidence were obtained in the above mentioned trials whenever the disease occurred in the trials. Loose smut incidence was high in 2004 (7% on the susceptible cultivar Ceylon). Generally, there appear to be far less spring barley varieties resistant to loose smut than to leaf stripe .

*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 and 2004 in 2005. However, an effort shall be made to create higher disease pressure. This can perhaps be accomplished by applying overhead-irrigation during heading/flowering. Loose smut will be monitored in this trial as well. This trial will be the only available source of information about the leaf stripe- and loose smut 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 partly responsible for 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. Tests 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.**

The objective is to test Danish pea varieties for their level of resistance to the three pathogens of the *Ascochyta* complex (*Phoma medicaginis* var. *pinodella*, *Ascochyta pisi*, *Mycosphaerella pinodes*). For this, a test system was to be developed with artificially inoculated small plots of pea under irrigation in order to characterize varieties of interest to organic production.

In 2002, 2003 and 2004 ten different varieties were tested in field trials at Research Centre Flakkebjerg, in separate trials for each pathogen. Initially, inoculation with infected barley grain, as well as a suspension of conidiospores of *P. medicaginis* var *pinodella*, *A. pisi* and *M. pinodes*, respectively, was carried out. The final inoculation system used in 2004 was: Infected barley grain was spread out in the plots shortly after emergence of the peas. The plots were covered with fiber cloth to prevent birds from eating the inoculum, and sprinkler irrigation was installed to produce a microclimate conducive to infections. Significant symptoms of leaf or pod spot were generally not seen before the podding stage, and they developed most clearly in the *M. pinodes* trial at maturity.

However, the seed health tests carried out on the harvested seed showed considerable infection percentages, especially with *A. pisi* and *M. pinodes*. Seed infection with *Phoma medicaginis* was generally low. During the first year of seed health testing, the testing capacity was low which meant that seed lots from some of the replications were stored for more than half a year. When this seed was tested, infection percentages of *M. pinodes* had decreased considerably whereas infection with *A. pisi* was still at the same level. This may explain why *A. pisi* is the pathogen detected most commonly in naturally infected seed.

The 2004 field trial has only just been harvested so the seeds have not been tested, yet. Seed health testing results from the first two years of experiments indicate differences in resistance between varieties: *Jackpot*, *Brutus* and *Faust* had low seed infection in both years, whereas *Sponsor* and *Athos* had high infections. For these varieties, the reactions towards both *A. pisi* and *M. pinodes* were similar, whereas for other varieties there was a differential reaction towards the pathogens. For still other varieties the ranking in the two years was not clear. Seed health testing of the seeds from 2004 should give more conclusive results.

There is a hypothesis that low varieties should be more susceptible to seed infection than high varieties, because the pods in the maturing stage are closer to the soil. *Athos* which was found to be susceptible is a low variety, however, this is not the case for *Sponsor*.

Trials in 2002 and 2003 were carried out under conventional conditions, since spreading of inoculum in the plots made weed control difficult. Anyhow, it was decided to carry out the 2004 trials under organic conditions. Because of risk of cross contamination the trials had to be carried out in separate fields. The *Phoma* and *Ascochyta* trials were located in a field which unfortunately had a high and uneven weed infestation with especially thistles which could not be controlled by harrowing. Therefore, the yield of several plots was very low.

*New plans:* Seeds of the 2004 trials will be tested during autumn and winter. Activities in 2005 will be decided based on the results.

### **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. In 2004 31 different new varieties from breeders (and varieties of special interest for organic wheat production) were inoculated with two different bunt isolates. 3 new varieties had very low attack (*Penta*, *Beder* and 798-389A), 2 varieties had moderate attack (*Malling*, *Samourai*) and the rest had different level of high attack (susceptible). 17 varieties that in previous test had shown some resistance were tested again on a new set of 3 isolates. Only the variety *Stava* was highly resistant while 5 varieties were resistant (*Penta*, *Hereward*, *Veronica*, *Globus*, and 3540). A group of 6 varieties expressed moderate resistance (*Miller*, *Bill*, *Tommi*, *Statur*, *Watson* and *Lancelot*). 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 2004, 11 different triticale varieties were tested on two isolates of *T. caries* and 6 varieties (showing some attack in 2003 test) were tested again on 3 new isolates. It seems that triticale is resistant to common bunt and only low attack was observed in the trials. Only one variety, *Triamant* was attacked in the test and seems to be susceptible to common bunt. Of the 6 varieties that were tested again on new isolates only the line HE115-98 was susceptible to common bunt.

In spring wheat 13 varieties were tested in 2004 sown at 3 different times 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 than in the 2003 test. This will be further analysed.

*New plans:* New varieties of winter wheat and triticale will be tested and the varieties that in 2004 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.**

In 2005 more focus will be on integrating the different control strategies. 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.

### **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](http://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. ISTA (The International 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.

A workshop on organic legumes was arranged 4th December 2003 in cooperation

with other DARCOF projects on legumes (GRAINLEG and GENESIS). At the workshop results from ORGSEED were discussed and new initiatives in peas for 2004 were 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 Nordic/European workshop on organic seed in 2005 where the results from the project will be discussed and new initiatives coordinated. Especially initiatives regarding harmonization of threshold in Europe values will be discussed.

## C.2 Fulfilment of deliverables and milestones

*Deviations will be further discussed in D*

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

### WP1: Threshold values

The work package is divided in 7 specific tasks:

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	

Deliverable, No	Deliverable title	Time schedule according to application	Deviations
13	Qualitative PCR-method for Fusarium spp. will be identified and developed.	2003/2005	OK (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 OK but will continue 2005
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	Has been taken out of the project
17	Quantitative method for glume blotch will be tested on seed lots, produced in WP1, with known infection	2005	Has been taken out of the project
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	OK
20	Quantitative method for Pyrenophora graminea will be tested on seed lots, produced in WP1, with known infection	2002	2002/2003 OK but will continue 2004/2005

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	exper-ments termi-nated
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 <i>Phoma medicaginis</i>	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.

<b>WP4: Integrated strategies and dissemination of information.</b>			
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)

<b>WP5: Project Management</b>			
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, another 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. 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 2005 when data from the 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 will be performed 2005 on all the trial data.

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 final evaluation of trial data. It was only possible in 2003 and 2004 to establish good data on common bunt and first conclusions can first be made early 2005

## 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.

D18: Multiplexing has not been investigated in 2004 as we have been using all resources on design of quantitative methods for single species and to test these on as many seed lots as possible. It will only be relevant to investigate multiplexing when it has been decided which species are most important and relevant in relation to field emergence.

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*, *Microdochium nivale* and *F. culmorum* have been developed. A Q-PCR method for *F. graminearum* is under development. A Q-PCR method for *F. avenaceum* has recently been published and will be implemented in the project. A Q-PCR method for glume blotch will not be developed in this project as it has been decided to put more focus on the other disease

M8 and M9: The Q-PCR methods are being tested on a range of different seed samples which is a step in the implementation process but implementation will have to continue beyond this project.

M10: A publication on detection and quantification of *P. graminea* will be produced in 2005. Furthermore results from WP1 and WP2 concerning the relative importance of the different *Fusarium* species and the detection methods will be combined in one or two papers 2005/2006.

**WP 3.1 Regulation and control.**

Deliverables:

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. No further experiments are planned.

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.

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.

**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 2004 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: A workshop was arranged 4<sup>th</sup> December 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. Products from Organic Eprints archive

### English

Borgen, Anders and Kristensen, Lars (2003) [Macroscopic leaf symptoms in wheat infected by \*Tilletia tritici\*](#). *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 110(5):432-436.\*

Dromph, Karsten and Borgen, Anders (2001) [Reduction of viability of soil borne inoculum of common bunt \(\*Tilletia tritici\*\) by collembolans](#). *Soil Biology and Biochemistry* 33(12-13):1791-1795.\*

Kristensen, Lars and Borgen, Anders (2001) [Reduction of spore spread of common bunt \(\*Tilletia tritici\*\) via combining equipment](#). *Biological Agriculture and Horticulture* 19:9-18.\*

### Not peer-reviewed

#### English

Borgen, A. and Gustavsson, A.-M. D. and Kieksi, J. and Johnsen, T. and Andersson, R. and Eriksen, R. (2002) [Factors affecting the development of the organic seed sector](#). Paper presented at European Symposium: 'Organic Seed Production and Plant Breeding - strategies, problems and perspectives, Humbolt University, Berlin, 21-22/11-2002; Published in Wilbois, K.P., Eds. *Organic Seed Production and Plant Breeding - strategies, problems and perspectives* 1(1), page 6. European Consortium for Organic Plant Breeding.\*

Borgen, Anders (2004) [Control of seed borne diseases in organic seed propagation](#). Paper presented at The First World Conference on Organic Seed. Challenges and Opportunities for Organic Agriculture and the Seed Industry., FAO Headquarters, Rome, Italy, 5th-7th, 2004; Published in Osborn, Thomas and van Bueren, Edith Lammerts and Ranganathan, Radha, Eds. *Proceedings of the First World Conference on Organic Seed. Challenges and Opportunities for Organic Agriculture and the Seed Industry* 1, page 170-171. IFOAM.

Borgen, Anders (2004) [Strategies for regulation of seed borne diseases in organic farming](#). Paper presented at 27th ISTA Congress, Seed Symposium, 2004, Budapest, May 17th-19th, 2004; Published in Alison, Powell, Eds. *Abstracts, 27th ISTA Congress, Seed Symposium, 2004(27)*, page 10.

Borgen, Anders (2004) [Strategies for regulation of seed borne diseases in organic farming](#). *Seed Testing International - ISTA News Bulletin* 127:19-21.

Borgen, Anders (2003) [Seed separation to control barley loose smut \(\*Ustilago nuda\*\)](#). Paper presented at Healthy seed for healthy crop. 2nd International Seed Health Conference, Poznan, Poland, September 16th-18th 2003; Published in *Healthy seed for healthy crop. proceedings of the 2nd International Seed Health Conference 2*, page 27-28.

Borgen, Anders (2002) [Control of seed borne diseases in organic cereals and legumes](#). Paper presented at The 4th ISTA - PDC seed health symposium: Healthy seeds, the basis for sustainable farming, Wageningen, The Netherlands, 29th April-1st May 2002; Published in *Proceedings of The 4th ISTA - PDC seed health symposium: Healthy seeds, the basis for sustainable farming*, page 18. ISTA.

Borgen, Anders (2002) [Control of seed borne diseases in organic cereals and pulses](#). Paper presented at The 4th ISTA - PDC seed health symposium: Healthy seeds, the basis for sustainable farming, Wageningen, Netherlands, 29th April-1st May 2002, page 18. ISTA, International Seed Testing Association.

Borgen, Anders (2002) [Organic seed production and seed regulation](#). Paper presented at International Scientific and Practical Conference: Scientific aspects of organic farming, Jelgeva, Latvia, 21-22th March 2002.

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](#). Paper presented at 8th International Congress of plant Pathology, Christchurch, New Zealand; Published in *Abstracts of Offered Papers*, page 321.\*

Nielsen, B.J. (2003) [Strategies for avoiding seed borne diseases](#). Paper presented at 11th Australian Barley Technical Symposium and 53rd Australian Cereal Chemistry Conference, University of Adelaide; Published in *Conference Proceedings*, page 75-79.

Nielsen, B.J. (2003) [The science of increasing production of organic grains](#). Paper presented at 11th Australian Barley Technical Symposium and 53rd Australian Cereal Chemistry Conference, University of Adelaide; Published in *Conference Proceedings*, page 19-22.

Nielsen, B.J. (2002) [Threshold values for seed borne diseases of cereals and legumes](#). Paper presented at ECO-PB 1st International symposium on organic seed production and plant breeding, Berlin, Germany, November 2002; Published in *Proceedings of ECO-PB 1st International symposium on organic seed production and plant breeding*, page 28-31.

Nielsen, Bent J. (October 2004) [Threshold levels for seed borne diseases in organic cereals](#). Online at <<http://www.darcof.dk/enews/sep04/threshold.html>>. Accessed October 2004

#### Dansk - Danish

Borgen, Anders (2001) [Strategier til bekæmpelse af udsædsbårne sygdomme](#) [Strategies to control seed borne diseases]. Paper presented at Ekologiskt Lantbruk . Centrum för Uthålligt Lantbruk. Ultuna, SLU, Ultuna, Sweden, 13-15 november 2001; Published in *Ekologiskt Lantbruk - Sammenfattninger av föredrag og postrar*, page 134-139.

Pinnschmidt, Hans O. and Nielsen, Bent J. and Hansen, Henrik J. (2004) [Resistente bygsorter begrænser problemer med bladplet i økologisk såsæd](#) [Resistant barley varieties limit the problems related to net blotch in organic seed production]. *FØJOe-nyt*(3). Online at <<http://www.foejo.dk/enyt2/enyt/juni04/bladplet.html>>

Kristensen, Erik Fløjgaard (2003) [Tromletørring skal sikre kvalitetskorn](#) [Drum drying, a method to ensure high-quality grain]. In *Økologisk Jordbrug*, August, No 293, page 6-6. Økologisk Landsforening.

Pinnschmidt, Dr. Hans O. and Nielsen, Bent J. and Hansen, Dr. Henrik J. (2004) [Sygdom truer økologisk udsæd](#) [Disease threatens organic seeds]. In *Økologisk Jordbrug*, Volume 313, No 24, page 6.

Nielsen, G.C. (2003) [Kassationsprocenter som følge af udsædsbårne svampe i økologisk udsæd af korn og bælgssæd](#). Landbrugets Rådgivningscenter.

Nielsen, Bent J. and Kristensen, Lars, Eds. (2001) [Forædling af korn og bælgssæd samt produktion af såsæd i økologisk jordbrug](#). FØJO-rapport no. 15, Forskningscenter for Økologisk Jordbrug.

Jørgensen, L.N. and Pinnschmidt, H. and Nielsen, B.J. and Nielsen, G.C. (2004) [Bygbladplet, biologi og bekæmpelse](#) [Biology and control of Barley Net Blotch (Pyrenophora teres)]. Grøn Viden Markbrug no. 289, Danish Institute of Agricultural Sciences.\*\*

Nielsen, B.J. (2002) [Udsædsbårne sygdomme i korn – bejdsemidler og andre behandlingsmetoder. 19. Danske Planteværnskonference 2002, Sygdomme og Skadedyr](#), in *DJF rapport Markbrug*. Report no. 64, page 43-58.\*\*

## **2. Other products (oral presentations, public meetings, field days, etc.)**

### Papers presented at congresses, symposiums etc.

Borgen, A. 2004 Organic seed treatment to control common bunt (*Tilletia tritici*) in wheat. ISTA Seed Symposium, Abstracts. Budapest, Hungary, 17-19th May, 2004. ISBN 3-906549-44-6. page 10

\*\*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

\*Nielsen, B. J. 2004. Screening for Resistance to Leaf Blight (*Pyrenophora graminea*) in Barley. Proceedings of the second International Workshop on barley Leaf Blights,

7-11 April 2002, ICARDA, Aleppo, Syria. Eds. Yahyaoui, A. H., Brader, L., Tekauz, A., Wallwork, H., Steffenson, B. ICARDA, Aleppo, 277-280.

Pinnschmidt, H. O., Nielsen, B. J. & Hansen, H. J., 2004: Thresholds for net blotch infestation in organic barley seed production. In: Lammerts van Bueren, E., Ranganathan, R. & Sorensen, N. (eds.). Proc. of the The First World Conference on Organic Seed: Challenges and Opportunities for Organic Agriculture and the Seed Industry, p. 184.

Reports, articles in agricultural journals, etc.

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

\*\*Anon.2004. Vejledning i Planteværn 2004. Danmarks JordbrugsForskning og Landscentret, Planteavl. Udgivet af Dansk Landbrugsrådgivning, Landscentret, Landbrugsforlaget.

Kristensen, E.F., 2004. Tørring og rensning af korn. Månedsmagasinet MARK 1, p. 66.

Nielsen, B. J. 2002. Resistens mod stinkbrand i vinterhvede & resistens mod stribesyge i vårbyg. I "Sortsforsøg 2002. Korn, bælgæd og olieplanter", Deneken G. & Pedersen J. B. (eds), Danmarks Jordbrugsforskning & Landbrugets Rådgivningsscenter, 8, 22-23, 36-37.

Nielsen, B. J. 2003. Resistens mod stinkbrand i vinterhvede. I "Sortsforsøg 2003. Korn, bælgæd og olieplanter", Deneken G. & Pedersen J. B. (eds), Danmarks Jordbrugsforskning & Landbrugets Rådgivningsscenter, 7, 19

Nielsen, B. J. 2004. Resistens mod stinkbrand i vinterhvede. I "Sortsforsøg 2004. Korn, bælgæd og olieplanter", Deneken G. & Pedersen J. B. (eds), Danmarks Jordbrugsforskning & Landbrugets Rådgivningsscenter, (in press)

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

Nielsen, G. C. 2003. Oversigt over Landsforsøgene 2003, p.230-233.

Nielsen, G. C. 2003. Oversigt over Landsforsøgene 2004. Under preparation.

Nielsen, G. C. 2004. Results from field trials at Danish Advisory Service available at: <http://www.lr.dk/applikationer/kate/viskategori.asp?ID=ka00400012000080000200>

Nielsen, G. C. 2004. Undersøgelse af ærtesyge ved økologisk dyrkning. FØJOenyt nr. 2 April 2004.

Nielsen, G. C. 2004. Planteavlsorientering no.09-664. Kassationsprocenter som følge af udsædsbårne svampe i økologisk udsæd af korn og bælgæd.

Nielsen, G. C. 2004, afgrødenyt nr. 4. Undersøg økologisk vårsæd og bælgæd for

udsædsbårne svampe.

Pinnschmidt, H. O., 2004: Sortsforsøg 2004. Landbrugets Rådgivningscenter, Landskontoret for Planteavl (in press).

\*\*Pinnschmidt, H. O & Hovmøller, M. S. 2004: Resistens afhænger af sort (Resistance depends on the variety). Mark, Januar 2004:31.

\*\*Pinnschmidt, H. O & Hovmøller, M. S. 2004: Resistance against net blotch, scald and Ramularia of barley (Resistens mod bladplet, skoldplet og Ramularia i byg). DJF rapport nr. 98 (2004), pp. 61-71.

\*Pinnschmidt, H. O., 2003: Sortsforsøg 2003. Landbrugets Rådgivningscenter, Landskontoret for Planteavl, pp. 28.

\*\*Pinnschmidt, H. O., 2002: Sortsforsøg 2002. Landbrugets Rådgivningscenter, Landskontoret for Planteavl, pp. 35-37.

Oral presentations, public meetings, field days, etc.

Borgen, A. 2004: "Science and the organic movement". Presentation at the SOAR-education programme for organic ph.d. students. Middelfart, 22.9.2004

Borgen, A. 2004: Organic seed treatment to control common bunt (*Tilletia tritici*) in wheat. Oral presentation at the ISTA Seed Symposium. Budapest, Hungary, 17-19th May, 2004. ISBN 3-906549-44-6. page 10.

\*Borgen, A. 2004: Økologi og miljø - perspektiver for fremtidens generationer. Den Økologiske efterskole. 11/8-2004

Borgen, Anders (2004) Control of seed borne diseases in organic seed propagation. Poster presentation at The First World Conference on Organic Seed. Challenges and Opportunities for Organic Agriculture and the Seed Industry., FAO Headquarters, Rome, Italy, 5th-7th, 2004.

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

\*Borgen, Anders (2004): Gamle og nye kornsorter til Økologisk dyrkning. Oral presentation at a field day at Mørdrupgård 14/7-2004

\*Borgen, Anders (2004): Gamle og nye kornsorter til Økologisk dyrkning. Oral presentation at a field day at Kåre Birk, Lægdsgrdvej 2, 7430 Ikast 22/6-2004.

\*Borgen, Anders (2004): Gamle og nye kornsorter til Økologisk dyrkning. Field day Kåre Birk, Lægdsgrdvej 2, 7430 Ikast 10/8-2004.

Borgen, Anders (2004): Participation in public meeting on quality in organic cereals. General assembly, Organic farmers association.

**\*\*Borgen, Anders (2004):** Frøbårne sygdomme i korn. Oral presentation at a public meeting for organic farmers at Hovborg Kro, 26th. Nov. 2003

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

Justesen A. F. Detektion af svampe med molekylære teknikker. DJF, Forskningscenter Flakkebjerg 7. oktober 2003 ved kursus i kornsvampenes biologi

Justesen A. F. Detektion af svampe med ny teknik. DJF, Forskningscenter Flakkebjerg 4. maj 2004 ved besøgsdag arrangeret for Plantedirektoratet

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.

Kristensen, E.F., 2004. Tørring og rensning af korn. "Planteproduktion 2004" Kongrescenteret Herning. 14 januar 2004.

Nielsen, B.J (årligt) Forelæsning på KVL kursus "Disease Resistance in Plants" med titel "Resistance to seed borne diseases in cereals"

Nielsen, B.J. 2003 Udsædsbårne sygdomme i korn. Skælskør vandrehjem, 7. oktober 2003, kursus i "Kornsvampenes biologi"

Nielsen, B. J. 2004 Orientering om ORGSEED. DJF, Forskningscenter Flakkebjerg 4. maj 2004 ved besøgsdag arrangeret for Plantedirektoratet

Nielsen, G. C. 2003 Oral presentation: Produktion af sund økologisk såsæd – skadetærskler og samdyrkning. Workshop DIAS, Research Centre Flakkebjerg 4. december 2003.

Nielsen, G. C. 2003 Oral presentation : Skadevoldere i økologisk produktion af bælgplanter. Workshop DIAS, Research Centre Flakkebjerg 4. December 2003.

Wolffhechel, H. 2003. Resistens over for ærtesyge. Legume workshop at Research Centre Flakkebjerg. 4 December 2003.

### **Plans for publications:**

In the following section are listed the publications that are planned in the last year of the project, 2005 (first author in bracket):

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

- "Novel development for heat treatment in organic cereals" (ISTA symposium Angers, France 2005) (AB)
- "Control of seed borne diseases in organic cereals by seed treatment" (IFOAM/ISO FAR conference, Adelaine 2005). (AB)
- "Brush cleaning to remove fungal spores in cereals" (journal not decided) (AB)
- "Seed separation to reduce diseases incidence of barley leaf stripe" (journal not decided) (AB)
- Kvantitative sammenhæng i smittecyklus af frøbårne bladplet (Plantevluskongress 2005) (HP)
- Quantitative relationships in the infection cycle of seed-borne net blotch (DARCOF news sent 2004/early 2005) (HP)
- Quantitative relationships in the infection cycle of seed-borne net blotch (A publication 2005) (HP)
- Models to compensate damage effects of seed-borne Fusarium in winter wheat by increased seeding rates (DARCOF news early 2005) (HP)
- Quantitative relationships in the infection cycle of seed-borne net blotch (fordrag ISTA symposium Angers, France 2005) (HP)
- Models to compensate damage effects of seed-borne Fusarium in winter wheat by increased seeding rates (poster ISTA symposium Angers, France 2005) (HP)
- Resistance to common bunt (*Tilletia tritici*) in Danish wheat and triticale varieties (BJN)
- Threshold values in organic cereal seed (BJN and all others)
- Results from the pea trials in 2004 will be published in December in the "Annual Report of the National Field Trials 2004". (GCN)
- A quantitative PCR assay for *Pyrenophora graminea* 2005 (HJH, AFJ)
- Quantitative detection of Fusarium species associated with seedling blight 2005/2006 (AFJ, HJH, HP)
- Resistance to seed borne pea diseases in Danish pea varieties (HW)

---

## F. Scientific education

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

Bent J. Nielsen is giving a lecture on resistance to seed borne diseases in cereals at the Royal Agricultural University, Copenhagen each year in the course "Disease Resistance in Plants"

Anders Borgen has in 2004 participated in SOAR-education programme for organic ph.d. students, Middelfart, 22.9.2004 with presentation: "Science and the organic movement".

## **G. National and international cooperation.**

Danish research Institutions (Risø, DTU)  
Danish Plant breeders

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

ThermoSeed project, SLU, Sweden, on the test for the effect of moist air on pea disease. Anders Borgen participates in a steering comity of an EU-project of the equipment

Force Technologies is involved in the test for the effect of steam/ultrasound on seed borne diseases. Anders Borgen act as an external consultant for Force Technologies.

Westrup A/S and Mørdrupgård Korn on development of brush cleaner to control common bunt and stem smut. The companies provide the equipment for the experiments in ORGSEED.

DAMAS a/s and DLG(Herregård) has provided equipment for SIGMA cleaning.  
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.

ORGSEED takes part in the new EU COST action 860 on "Sustainable low-input cereal production: required varietal characteristics and crop diversity" which has just started.

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 has 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, may be different from a conventional soil. This question is of special importance with seedling blight diseases and these trials (2002/2003, 2003/2004, 2004/2005) 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 were 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. Also, not all weed management options (mainly mechanical) available in organic production can be implemented in inoculated field trials because of the risk of disease contamination across plots.

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 placed 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 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 dataset with correlations between seed infection and later damage in the field. New approaches with appropriate statistical designs covering the varietal effect were undertaken in 2004 and will continue in 2005 if deemed necessary to obtain data sets for at least two seasons. There will also be continued cooperation with the DACROF project BAR-OF to get a better understanding of the whole disease cycle and to make better use of resistance data and seed lots obtained from this project.

### **WP2 Diagnostic methods\_**

There have been no major changes of the plans. 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 *Cochliobo-*

*lus 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 can be difficult to obtain very good correlations between the total amount of fungal DNA assessed by real-time PCR and the number of infected seeds/plants. This will apply to all diagnostic tests which measure the total amount of fungal mass e.g. as total DNA (PCR) or as antigen (ELISA). We have investigated the possibility for using a sequential test where several samples will have to be tested for the presence of DNA (a qualitative test).. 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. In stead we envisage that the quantitative PCR-method developed in this project can be used in a semi-quantitative way in which a fast pre-selection of seed lots with infection levels far from the threshold level can be identified. If necessary additional tests can then be performed on those seed lots which have infection levels within the range of the threshold. This will save time in the test procedure.

### **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 ultra sound 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 these methods, especially combination of steam and ultra sound.

### **WP 3.2 Resistance**

After relatively low barley leaf stripe infection levels in two consecutive years (2003 and 2004) of resistance testing, it should be considered to develop procedures that yield higher infection levels and are therefore more reliable. These could include the use of sprinkler-irrigation during the critical time of infection (heading/flowering) to provide better infection conditions. After DJF Flakkebjerg has greatly expanded its irrigation capabilities, this option will be available in most fields from 2005 on.

After having discovered heavy seed infections by *Bipolaris sorokiniana* and, associated with this, a sharp decline in germination ability of heavily affected seeds in the spring barley seed lots of the 2003 BAROF trials, especially in seed lots from organically managed plots, one should consider to test seeds for *Bipolaris*-infestation in addition to net blotch testing.

Although loose smut resistance testing had low priority, the recurrence of this disease in the leaf stripe resistance tests in 2004 demands continued awareness of its destructive potential. Its occurrence will therefore be monitored along with the regular

leaf stripe assessments.

The pea variety trials were this year conducted under organic conditions. Since artificial inoculation was carried out with three different pathogens, the trials had to be located in separate sites to avoid cross contamination. Especially one of the sites had heavy weed infestation with thistles and couchgrass. Furthermore, as inoculum is spread on the soil after crop emergence, the time for carrying out mechanical weeding is limited, and since pea is a poor competitor, this resulted in a high weed infestation of the plots. The uneven distribution of weeds will give a high variation between plots.

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

Year:	2001	2002	Expected 2003	2004	2005	Total
Salaries	267.000	2.045.653	1.958.572	1.770.293	1.131.917	7.173.435
Scientific personnel	216.000	1.218.313	1.167.637	949.034	777.382	4.328.366
Technical personnel	51.000	827.340	790.935	821.259	354.535	2.845.069
Other operational costs	25.000	305.000	375.000	348.000	108.000	1.161.000
Equipment	0	0	0	0	0	0
Others (please specify)	0	0	0	0	0	0
Direct costs	292.000	2.350.653	2.333.572	2.118.293	1.239.917	8.334.435
Indirect costs (20% of direct costs)	58.200	469.531	466.915	423.459	247.583	1.665.688
<b>Total</b>	<b>350.200</b>	<b>2.820.184</b>	<b>2.800.487</b>	<b>2.541.752</b>	<b>1.487.501</b>	<b>10.000.123</b>

## 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	2003	Expected 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	2003	Expected 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
<b>Total</b>	<b>193.200</b>	<b>2.356.792</b>	<b>2.183.393</b>	<b>1.951.914</b>	<b>1.146.702</b>	<b>7.832.001</b>

#### Comments:

Scanagri has until March 31<sup>st</sup> 2004, as a subcontractor for DIAS hired Anders Borgen, Agrologica to participate in the project. From April 1<sup>st</sup> 2004, the responsibility in the project of Scanagri has gone to Agrologica and Agrologica (Anders Borgen) is now subcontractor for DIAS. This change has not affected the budget or any staff mentioned in the application.

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

**Danish Plant Directorate (PD):**

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

Year:	2001	2002	2003	Expected 2004	2005	Total
Salaries	90.000	228.660	280.078	255.698	182.332	1.036.768
Scientific person- nel	90.000	185.400	190.962	163.909	135.061	765.332
Technical person- nel	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
<b>Total</b>	<b>120.000</b>	<b>304.392</b>	<b>366.094</b>	<b>324.838</b>	<b>230.798</b>	<b>1.346.122</b>

**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	2003	Expected 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	31.000	58.000	59.000	61.000	62.000	271.000
Scientific personnel	31.000	58.000	59.000	61.000	62.000	271.000
Technical personnel	0	0	0	0	0	0
Other operational costs	0	75.000	150.000	160.000	30.000	415.000
Equipment						0
Others (please specify)						0
Direct costs	31.000	133.000	209.000	221.000	92.000	686.000
Indirect costs (20% of direct costs)	6.000	26.000	42.000	44.000	18.000	136.000
<b>Total</b>	<b>37.000</b>	<b>159.000</b>	<b>251.000</b>	<b>265.000</b>	<b>110.000</b>	<b>822.000</b>

**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 / Agrológica

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	2003	Expected 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	2003	Expected 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	<b>651.075</b>	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
<b>Total</b>	<b>193.200</b>	<b>1.785.852</b>	<b>1.607.065</b>	<b>1.485.326</b>	<b>875.436</b>	<b>5.946.880</b>

Comments:

**II) Department of Agricultural Engineering, Research Centre Bygholm**

Year:	2001	2002	2003	Expected 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	2003	Expected 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
<b>Total</b>	<b>0</b>	<b>83.688</b>	<b>85.839</b>	<b>0</b>	<b>0</b>	<b>169.527</b>

**III) Scanagri Denmark A/S (SD) and Agrologica:**

Year:	2001	2002	2003	Expected 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	2003	Expected 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
<b>Total</b>	<b>0</b>	<b>351.472</b>	<b>350.816</b>	<b>350.441</b>	<b>236.903</b>	<b>1.289.632</b>

**Comments:**

The budget compiles cost at Scanagri until March 31st, 2004 and costs at Agrologica from April 1st, 2004.

**IV) Danish Agricultural University (KVL), Tåstrup:**

Year:	2001	2002	2003	Expected 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	2003	Expected 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
<b>Total</b>	<b>0</b>	<b>135.780</b>	<b>139.673</b>	<b>116.147</b>	<b>34.363</b>	<b>425.963</b>

**Comments:**

The work done at Højbakkegaard, KVL are field trials and laboratory work under supervision by Anders Borgen, Scanagri/Agrologica.

**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):

**21.05.01**

WP1 (skadetÅ tskler)

<b>SUM</b>	2001	2002	2003	2004	2005
Months (scientific)	2	8	8	6	6
Months (technical)	3	15	17	17	8
Salary (scientific)	74.000	303.850	312.966	241.493	248.737
Salary (technical)	63.000	324.450	378.741	390.104	189.085
Operation â€ equipment	0	0	0	0	0
Operation - other	10.000	60.000	105.000	110.000	35.000

**Comments:**