



Annual Status Report 2001 and Application for Continuation in 2002

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 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)
Vester Farimagsgade 6, 5th
DK-1606 Copenhagen

Danish Agricultural Advisory Centre (LR)
Udkærvej 15, Skejby
8200 Århus N

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

5. Contact persons

Bent J. Nielsen, *senior scientist* (BJN)
Danish Institute of Agricultural Sciences

Christiane Scheel, *senior plant pathologist* (CS)
Danish Plant Directorate

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

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

6. Other project staff

Annemarie Fejer Justesen, *scientist* (AFJ), Danish Institute of Agricultural Sciences, Research Centre Flakkebjerg, Department of Crop protection, 4200 Slagelse

Lars Bødker, *senior scientist* (LB), Danish Institute of Agricultural Sciences, Research Centre Flakkebjerg, Department of Crop protection, 4200 Slagelse

Erik Fløjgaard Kristensen, *scientist* (EFK), Danish Institute of Agricultural Sciences (DIAS), Research Centre Bygholm, Department of Agricultural Engineering, 8700 Horsens

Henrik Jørskov Hansen, *senior plant pathologist* (HJH), Danish Plant Directorate, Skovbrynet 20, 2800 Lyngby

7. **Start of project: 1st September 2001**
End of project: 31st December 2005
-

8. Annual report/Application for continuation in 2002

A. Objectives and expected achievements (from application)

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 organic produced seed that need to be discharged as organic seed due to unacceptable infection by seed borne diseases.

The target is therefore to improve methods for seed analysis and to adjust the current values for discharge of seed to organic conditions. This will help to optimise the production, but seed lots still can be infected and effective control measures are necessary in these cases to obtain further reduction in actual loss of organic seed.

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

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

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

- i) Secure no multiplication of harmful seed borne diseases but not so strict that healthy seeds are unnecessarily discarded.
- ii) Substantial reduction in unnecessarily discarded seed especially in wheat due to seedling blight and common bunt, in barley due 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 no 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) Seed cleaning tested under practical conditions.
- iii) 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

B. Project summary (from application)

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. Current practice in organic agriculture is to analyse the seed by seed health testing and to discard the seed lot if the infection by diseases exceed the threshold levels, where seed treatment are recommended in conventional agriculture. A huge number of propagated organic seed lots are discarded using this practice; in some crops and years almost all seed lots are discarded. Most years, the quantities of organic seed are insufficient to supply the market because seed lots are discarded for infections by seed borne diseases. In these cases it is allowed for the organic farmers to use conventional propagated seeds. However, after December 2003 this will no longer be accepted by certifying bodies, and only organic seeds can be used in the EU.

The threshold levels used are developed under the presumption that pesticides can be used in case of later disease development in the crop, and no experiments has 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 on seed 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. Recent studies have shown that huge differences in results exist between the results from different laboratories. To improve the threshold levels, it is necessary with new and more precise methods for seed analysis. Especially in winter cereals in Northern Europe, where the time from harvest to sowing is very short, it is necessary with faster techniques, if the analysis shall be used as a basis for rejection seed lots. The project will develop and implement PCR techniques for seedling blight, glume blotch and leaf stripe, since the PCR technique is quick and unambiguous and the biggest problems are related to the investigation of these diseases.

The development of more correct threshold values based on improved analytical methods will minimise the development of seed borne diseases in organic farming which is relevant especially in the propagation phase, and it will minimise the number of seed lots unnecessarily discarded. To further 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 for design of the cropping system, which minimise the risk of seed infection, and on seed treatments, which immediately apply, 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 significantly 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.

Table 1: Work package list (from application)

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

1) Responsible participants are underlined.

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

C. Progress

C.1 Annual description (resume) of main results and conclusions

Work package 1: Threshold values.

Task 1 Seedling blight in wheat

Seed lots have been collected with different infection levels of *Microdochium nivale* and *Septoria nodorum* and 3 field trials have been established at Flakkebjerg at two sowing depths. The aim is to evaluate the expression of the diseases under a set of different conditions (soil temperature, sowing depth, soil type) [Milestone 1]

Task 2 Net blotch in barley

Will start 2002 (see section D)

Task 3 Leaf stripe in barley

Will start 2002 (see section D)

Task 4 Seed and foot rot in peas

Two field trials in peas with different varieties have been established in co-operation with other projects. Seeds have been harvested and will be tested for infection of seed borne diseases and data analysed (variety, precropping interaction). Different seed lots have been collected with different infection levels to be used in field trials that will start 2002 (see section D)

Task 5 Common bunt in wheat

Pathogen populations have been collected as infection material to be used in field trials with different infection levels 2002 (see section D)

Task 6 Glume blotch/seedling blight in wheat

Will start 2002 (see section D)

Task 7 Models

Will start 2002 (see section D)

Work package 2: Diagnostic methods

Task 1: *Fusarium spp.* and *Microdochium nivale*

Real-time PCR-machine (ABI Sequence detection system 7000 HT) for quantitative PCR has been installed in the lab. Identification and implementation of PCR-test for *Fusarium spp./Microdochium, nivale* and will start 2002. Production of 'reference seed lots' with known infection levels will start 2002.

Samples of commercial wheat seed from the 2000-2001 seasons are examined by traditional methods. Seed samples of value for the project will be selected and used as reference seed samples for future PCR testing.

Task 2: Glume blotch (*Leptosphaeria nodorum*)

Identification of PCR- or ELISA test will start 2002. Production of 'reference seed lots' with known infection levels will start 2002.

Samples of commercial wheat seed from the 2000-2001 seasons are examined by traditional methods. Seed samples of value for the project will be selected and used as reference seed samples for future PCR and/or ELISA testing.

Task 3: Barley net blotch and leaf stripe

Optimisation of quantitative PCR procedure on barley seeds using the ABI 700 HT will start 2001 by using the quantitative PCR-method for *Pyrenophora spp.* described by Bates et al. *Molecular Plant Pathology*, 2001, 2, 49-57).

Samples of commercial barley seed samples from the 2000-2001 season are examined by traditional methods. Seed samples of value for the project will be selected and used as reference seed samples for future PCR testing.

Work package 3: Regulation and control measures

WP 3.1. Control measures acceptable in the organic seed production

This work package is a scientific continuation of activities in an ongoing grass-root project also funded by DFFE ("Landbrugsplanter – udvikling af strategi til forebyggelse og behandling af frøbårne sygdomme i fremavlskorn"). Activities are planned to start when the grass-root project is ended in beginning of 2002. No activities are therefore made in this project at present, but the grass root project is adjusted in order to optimise the continuation of the activities.

Task 1. Preventing cropping methods.

Will start 2002 (see section D)

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

Seed samples and data are available from the grass root project.

Task 3. Seed cleaning.

Seed samples, data and new development of equipment are available from the grass root project. The level of some diseases have been low in the year 2001, and some of the current collected seed samples may be insufficient for large scale experiment.

Task 4. Heat treatment.

No experiments in the current period. Experiments will start 2002 (see section D).

Contacts, not planned in the original application, are made to Force Institutet, a private company in Denmark. At this company, equipment are recently developed able to treat legumes and other seeds with a combination of steam and ultra-waves very promising in control of seed pathogens. This equipment has not yet been tested for fungal seed borne pathogens relevant for organic seed production. Discussions are made to coordinate activities relevant for the development of the organic seed production. The contact does not change the priority in the activities planed in the current project.

WP 3.2. Control measures acceptable in the organic seed production

Task 1. Screening for resistance to leaf stripe in Danish barley varieties
Will start 2003 (see section D)

Task 2. Screening for resistance in Danish pea varieties to seed and foot rot.
Will start 2002 (see section D)

Task 3. Screening for resistance to common bunt in Danish wheat varieties
Will start 2003 (see section D)

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

Task 2: Dissemination of information

Task 3: Official regulations

Task 4: The methods developed in the project.

Work package 5: Project management

Project planning, co-ordination and reports.

C.2 Fulfilment of tasks and deadlines in individual work packages

In the following are listed specific tasks for the different work packages with deliverables and milestones. Over view is given in section 8 D.

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	
2	Seed lots established with different infection levels for use in WP1, WP2 and WP3.	2002/2003	
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	
5	Threshold for seed borne Glume blotch adjusted to organic condition	2003/2005	
6	Threshold for leaf stripe adjusted to organic condition	2003/2005	
7	Threshold for net blotch adjusted to organic condition	2003/2005	
8	Threshold for seed and foot rot disease in pea adjusted to organic condition	2003/2005	
9	Models developed describing multiplication over years	2005	
10	New recommendations for threshold values to seed producers and farmers	2003/2005	
11	Results published in farmer's journals, leaflets, magazines, web-site etc.	2002-2005	
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: <i>Fusarium</i> species and <i>Microdochium nivale</i> 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	
14	Priority to Fusarium spp for which a quantitative PCR-test should be developed will be given based on results from WP1	2003	
15	Quantitative method for Fusarium sp. will be tested on seed lots, produced in WP1, with known infection	2005	
16	Test for glume blotch will be identified	2004/2005	
17	Quantitative method for glume blotch will be tested on seed lots, produced in WP1, with known infection	2005	
18	Combination of methods for detection of seed borne diseases in wheat will be tested	2004/2005	
19	Quantitative method for Pyrenophora teres will be tested on seed lots, produced in WP1, with known infection.	2002	
20	Quantitative method for Pyrenophora graminea will be tested on seed lots, produced in WP1, with known infection	2002	

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

Milestones:

M7: Identification and test of new diagnostic method for selected Fusarium species and glume blotch

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:

2.1. Direct and indirect control measures

2.2. 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.

Deliverable, No	Deliverable title	Time schedule according to application	Deviations
21	Effect of pea/barley mixture on spread of seed and foot rot diseases to pods and new seeds.	2003/2005	
22	The potential of early, pre-optimal harvest time will be evaluated as a control strategy in fields for propagation and integrated in strategies in developed in WP1.	2004/2005	
23	Description of the influence of cropping parameters on the development of epidemics of net blotch, glume blotch and Fusarium. The description will lead to recommendation for propagation and for field inspections.	2004/2005	
24	The potential of seed cleaning as a strategy for reduction in disease frequency in infected seed lots will be estimated. The results will be integrated in strategies and recommendations in developed in WP1	2003/2005	
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	

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	
27	Danish pea varieties with interest for organic production screened for resistance against <i>Phoma medicaginis</i>	2003/2005	
28	Danish wheat varieties with interest for organic production screened for resistance against common bunt.	2003/2005	

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

Milestones:

M15: Relevant barley varieties screened for resistance against leaf stripe

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

M17: Relevant wheat varieties screened for resistance against bunt.

WP4: Integrated strategies and dissemination of information.

Task 1: Integrated strategies

Task 2: Dissemination of information

Task 3: Official regulations

Task 4: The methods developed in the project.

Deliverable, No	Deliverable title	Time schedule according to application	Deviations
29	Integrated strategies with different control measures.	2004/2005	
30	Leaflets, journals etc. to organic farmers and seed producer's etc. with information on problems with seed borne diseases.	2002-2005	
31	Web-site with information on seed borne diseases, thresholds, recommendations etc.	2002	
32	Thresholds adopted as a standard in organic seed production of cereals and peas.	2003/2005	

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

WP5: Project Management

Task 1 Co-ordinating the different element ensuring a coherent and integrated project.

Task 2 Project meetings and project reports.

Task 3 Project workshops.

Deliverable, No	Deliverable title	Time schedule according to application	Deviations
34	Project reports each year	2002-2005	
35	Final project report	2005	
36	Project workshop with discussion of preliminary results	2003	
37	Project workshop presenting final results and conclusions	2005	

Milestones:

M18: Project report October each project year.

M19: Final project report December 2005.

M20: Workshop October 2003.

M21: Final Workshop October 2005.

C.3 Discussion on the progress, incl. deviations and achievements in the project as a whole and in the individual work packages.

The project started 1st September 2001 and progress is according to the plan. The first activities are to collect seed samples with different levels of infection and to establish the first field trials (seedling blight). A PCR machine for running quantitative PCR has been purchased and installed and according to the plan a quantitative PCR-test, which is already developed and described in the literature, will be used to run in the machine.

The Danish Plant Directorate has employed Ph.D. Henrik Jørskov Hansen for development of diagnostic methods. Since his employment from 1st October seed samples of wheat, barley and pea have been tested by traditional methods. The purpose of testing seed samples by traditional methods is to become familiar with the present methods and to identify seed samples of relevance to the project.

E. Project publications

No publications yet.

F. Scientific education (ph.d. and post doc.), including visiting scientists and visits abroad

G. National and international co-operation

Danish research Institutions (Risø, KVL)

Danish Plant breeders

Producers of machinery for seed cleaning and heat treatment

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

H. Possible elaboration of project and achieved results

The project has just started and no results have been achieved so far.