

**Title:**

## **Healthy seed for organic production of cereals and legumes.**

**Acronym:**     **ORGSEED**

**Date:**         **21.05.2001**

### **1. 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. 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 are 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.

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### 3. Introduction

Seed borne diseases are a potential problem in production of cereals and legumes. Pathogens can spread and multiply in the fields from seed borne infections or they can, depending on the disease, spread during wet seasons from infected leaves to the seeds. The consequences are low quality of seeds with reduced number of plants in the field or severe reduction in leaves, heads or pods. For some diseases like common bunt also severe reduction in quality can occur.

In conventional agriculture seed borne diseases possesses only a minor problem due to systematic treatment of the seed with effective chemicals. This option is not possible in organic agriculture and for the moment no effective control measures are available.

The seed borne diseases of potential importance to organic production of cereals and legumes are:

Common bunt (*Tilletia tritici/T. caries*) in wheat  
 Seedling blight (*Fusarium spp.*) in wheat, rye, triticale and barley  
 Glume blotch or seedling blight (*Leptosphaeria nodorum/Septoria nodorum*) in wheat and triticale  
 Leaf stripe (*Pyrenophora graminea/Dreschlera graminea*) in barley  
 Net blotch (*Pyrenophora teres/Dreschlera teres*) in barley  
 Loose smut (*Ustilago nuda*) in barley  
 Loose smut (*Ustilago avenae*) in oat  
 Stem smut (*Urocystis occulta*) in rye

Pea-disease complex causing seed and root rot (*Aschocyta pisi, Mycosphaerella pinodes, Phoma medicaginis var. pinodella Ascochyta spp, Fusarium spp. and Botrytis cinerea*).

Growing organic seed without treatment might cause problems of unintentional propagation and spreading of seed borne diseases. It is therefore important that the seed is tested for the occurrence of pathogenic fungi and that those seed lots with unacceptably high infections are being discarded. For the moment, no other possibilities are applicable. The seed (Certified C1) used for production of the ecological Certified C2 (the mother plant of the ecological seed) may be conventionally grown, provided that the seed is untreated.

If organic grown seeds are not available, conventional untreated seed can be used in organic farming according to the current regulations. From January 1<sup>st</sup> 2004 this is no longer an option and from this day on all the seed used in ecological farming must meet the demands regarding organic seed i.e. the mother plant of the seed must be ecologically grown, the only exception being corps grown for propagation until certified seed C1 (EEC 2000).

The thresholds for discarding must be as low as possible in order to prevent propagation of seed borne diseases later on. An essential condition of ecological production of seed is that a relatively large quantity of conventional seed exists of which suitable seed lots can be selected. If this is not the case, a lack of seed for the ecological plant production might arise as it may be necessary to discard a considerable amount of the organic seed due to unacceptable occurrences of seed borne diseases.

The amount of organic seed discarded is unacceptable, and there is several ways to improve the system. The thresholds can be more consistent with the risk of actual yield loss, the cropping system could be better designed to prevent the infection of the seeds, and seed treatments could be implemented to control the diseases in infected seed lots. It is unlikely that one strategy will solve all problems, and it is therefore necessary to develop an integrated strategy using all measures to regulate the development of seed borne diseases in organic agriculture.

Furthermore, it is of vital importance that there is a sufficient and reliable analytical capacity available. Even though accurate threshold values and diagnostic methods are implemented.

#### 4. State of the art.

Chemical seed treatment is not possible in the last generations of organic seed production and the current strategy in production of healthy organic seed in Denmark is to discard seed lots with infections above defined threshold values (Nielsen et al., 1998).

Producers of organic seed analyse their lots of seed for the occurrence of seed-borne diseases according to a voluntary agreement. If the threshold values are exceeded, the seed lot will not be offered as organic seed. This has in practise turned out to cause huge problems. With the present thresholds and analytical methods a very large part of the organic seed has been rejected owing to the occurrence of seed-borne diseases. An investigation from 1999 and 2000 of organic seed (Danish Agricultural Advisory Centre, *unpublished results*) showed that in total 21 % (2000) to 50% (1999) of the winter wheat and 20 % (2000) to 70% (1999) of the spring barley had to be rejected because of the high number of seed-borne diseases. Some seed lots could have been rejected due to several seed borne diseases and the figures are not adjusted for that. In pea the percent seed lots that was above the threshold value was 35 % (1999) to 50 % (2000). This is a serious situation. The variation between years make it difficult to plan the production and the actual loss of valuable seed gives problems with having enough organic seed. Increasing the area for production of organic seeds is at present the only way to ensure enough seeds in quantities and qualities of different varieties.

It is necessary to focus on the following problems:

##### 4.1. Threshold values

The origin of the threshold values was formerly based on conventional grown seed where seed treatment was used more or less on a routine basis. These threshold values was transferred to the organic grown seed in lack of better possibilities. Due to the earlier mentioned high rejection of organic seed lots it was decided in 2000 to change some of the threshold levels temporarily. These changes are probably sufficient, but there is no evidence or practical experiments which proves it.

Common bunt is a disease so serious that the seed is rejected if only one spore occurs in the analysis. In practise it has turned out that this limit can result in the rejection of large quantities of organic seed. Thus analysis of data from 1999 showed that 25-30% of the organically grown winter wheat had to be rejected on the basis of this limit. In 2000 the limit value has, however, been changed to the effect that one spore is acceptable (in practice a threshold of 10 spores per g of kernels). It might be possible to use a higher threshold. In cases of a variety with good resistance for common bunt a certain content of spores in the seeds could probably be acceptable. In Swedish experiments the lowest amount of spores causing infection was established at 5 spores per kernel (=100 spores/g, Johnsson, 1979). It is very important that this threshold value is fixed as realistically as possible, but also without the risk of subsequent multiplication and spreading of the fungus.

Seedling blight can be caused by *Microdochium nivale* and a number of *Fusarium* species and it can cause big problems in wheat and triticale. The disease complex is not fully elucidated, but it is supposed that at least 3-4 species are of importance. It is necessary to examine the seedling blight complex further and evaluate the threshold value on basis of actual loss in the individual year.

Formerly the limit value of glume blotch was 5%, which in practice meant that much wheat had to be rejected as seed. Presumably the limit was too low, and it was recommended that the limit could temporarily be raised to 15%. The limit value can probably be fixed at an even higher percentage in the case of late sowing, but in the case of very early sowing it is probably too high. The need for rejection owing to glume blotch in organically grown wheat was in 1999 estimated at 30%.

The limit value of leaf stripe is 5%, but it should perhaps be lower in the C1 generation to avoid multiplication in C2. The need for rejection in barley owing to leaf stripe/net blotch was estimated at 50% in 1999. For leaf stripe it is to be noted that it is hard to distinguish the fungus from net blotch in the traditional microscopic analysis of spores used presently. Either a slower greenhouse test should be used or complete new methods found. These are, however, not immediately available. Net blotch occurs much more often than leaf stripe, but the actual threshold value for leaf spot is considered to range higher than for leaf stripe.

In peas, the most serious seed borne pathogen is considered to be *Ascochyta spp.* The present threshold value for the pea-disease complex is the mere presence of *Ascochyta spp.* in seed propagation material and 5% for in peas for fodder or a total of 25% for the whole complex of fungi colonising the seed. Also in this case, the thresholds are estimated, and experiments need to be carried to validate threshold values so that unnecessary rejection can be avoided. Seed quality due to infection of fungi greatly influences the likelihood of seed infection from soil borne pathogens. Poor quality seeds exude more exudates than healthy seeds and are more stimulatory to e.g. *Pythium*. Attack by *Pythium spp.* may cause severe seedling rots "damping-off" which imply that rotted seedlings do not emerge from the soil. .

#### 4.2. New Diagnostics

When new threshold values for the above mentioned pathogens are estimated it is essential to have reliable and exact diagnostic methods to measure the level of seed borne pathogens on the seed used for experiments. The existing methods used for seed health testing are not accurate enough especially in cereals, and therefore it is necessary to develop new more specific diagnostic methods

Testing for most seed borne fungi is based on traditional incubation methods, which are time consuming and moreover are the facilities for incubation space-demanding and a limit for the number of samples, which can be tested at the same time. Bottlenecks arise when testing winter cereals, where time is very limited. The analysis of winter wheat is the most prolonged test where kernels has to be incubated in 14 days to examine for seedling blight, and the analysis for glume blotch takes 10 days. Both methods are impossible to carry out in routine tests of samples in large scale.

In addition the analyses for seedling blight and glume blotch of wheat are not specific enough. The outcome of the two analyses is a percentage of brown roots and of fluorescent kernels respectively. The pathogens are not identified and this is not acceptable especially for the *Fusarium*-species which are of very high importance for the establishment of winter wheat in field.

Therefore there is a high demand for new rapid and more precise test methods for winter wheat.

In recent years DNA-based methods have been developed (PCR methods), and their advantage is their sensibility and that they are time-saving. With these methods it would be possible to detect fungi directly on the seeds and avoid the long incubation periods. Besides it is possible to develop the methods to detect specific fungal species or several more closely related species. Also the methods are more simple to standardise. In 2001 The Danish Plant Directorate has implemented the first PCR method for detection of barley leaf stripe, but the method is only qualitative and it is not possible to calculate the percentage of infected kernels.

At present the diagnosis for barley leaf stripe and net blotch could be carried out in a three step procedure: First the seed samples has to be tested with the traditional incubation method where leaf stripe and net blotch are detected in total. Those seed lots which exceed the threshold level for net blotch are discarded and those with a lower percentage are tested with the qualitative PCR for leaf stripe. Then samples with no leaf stripe are released. The remaining samples has to be tested by grow-on tests in greenhouse where the percentage of plants with leaf stripe symptoms can be estimated. This procedure is just possible for spring barley with several months between harvest and sowing. An other possibility is to start directly with grow-on test in greenhouse, but the number of samples which can be tested are limited due to lack of greenhouse capacity and a very long test period (4-5 weeks). It is supposed that if a quantitative PCR method could be implemented it would be more economic than the above described possibilities.

PCR methods have been developed for qualitative analysis of various seed borne diseases, but for detection directly on seed and for quantitative determination most of them has not yet been finished. Therefore there exist a number of methods which in a developed form could be implemented in the routine analyses and in this way shorten the test period considerably as well as an essential improvement of exactness and reproducibility.

Several of the traditional incubation methods require great expertise for the examination, which reduces the possibilities for quick training of personnel. Only few laboratories in Denmark offers tests for seed borne diseases and a ring test has showed large unacceptable differences between the results from the laboratories, - in particular the tests for seedling blight and glume blotch of wheat, which supports the need of a new reproduceable method.

ISTA (The International Seed Testing Association) do 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 have just only started. It will take some years before the system is fully developed. By initiating development and implementation of moderne analysis methods, Denmark will be able to achieve influence on which of the methods to be international

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.

Furthermore, better test methods will be an essential basis for a reliable revision of the tolerances. They will give a much better basis for decision regarding any rejection of organic seed and they are expected to lead to a reduction in the number of rejected seed lots.

In the table mentioned below the analyses for the individual diseases are listed including the disadvantages, which are limiting the production of organic seed. Besides are listed the wishes and possibilities for use of the new technology for developing new methods of diagnoses. As stated before the need for new methods for seedling blight and glume blotch in wheat are the most necessary, and it could be convenient to combine with a method to detect common bunt in wheat. Then it would be possible to make a test for all seed borne diseases in winter wheat in one step and reduce the analysing time considerably.

Diseases	Seed crop	Disadvantages of existing diagnostic methods	Possibilities of new diagnostic methods
Seedling blight <i>Fusarium spp.</i> complex and <i>Bipolaris sorokiniana</i>	Wheat Triticale	Fungal genera or species are not identified by examining kernels with brown roots. Very slow (14 days) with high unreliability and too big variation.	PCR method, to specify and quantify species of <i>Fusarium</i> .
	Barley Rye Oat	Fungal genera or species are not identified by examining kernels with brown roots. Slow (7 days) with high unreliability and too big variation.	PCR method, to specify and quantify species of <i>Fusarium</i> , (applied from the PCR method on wheat)
Glume blotch, <i>Leptosphaeria nodorum</i>	Wheat	Fungal species is not identified by examining kernels with fluorescence. Very slow (10 days) with high unreliability and too big variation.	PCR method, to specify and quantify glume blotch.
Barley net blotch , <i>Pyrenophora teres</i> and Barley leaf stripe, <i>Pyrenophora graminea</i>	Barley	<u>Incubation method:</u> Fungal species are not identified, - only the genera <i>Pyrenophora spp.</i> Slow (7 days) and difficult to examine.	PCR method, to specify and quantify barley net blotch and barley leaf stripe.
		<u>PCR method:</u> Identification of the genera <i>Pyrenophora spp.</i> and the species <i>P. graminea</i> . Only qualitative	
		<u>Grow-on test in greenhouse:</u> Registration of symptoms of leaf stripe and net blotch. Extremely slow (4-5 weeks) Restricted greenhouse capacity	
Barley loose smut <i>Ustilago nuda</i>	Barley	According to ISTA toxic chemicals should be used in the analysis.	Less toxic chemicals. Test can be replaced by field inspection.
Wheat bunt <i>Tilletia tritici</i> and Rye stem smut <i>Urocystis occulta</i>	Wheat Triticale Rye	Experience needed to identify specific spores.	PCR method can be implemented in combination with other PCR tests for winter wheat
Foot and seed rot <i>Ascochyta spp.</i> , <i>Fusarium</i> and <i>Botrytis cinerea</i>	Pea	Problems with a range of secondary fungi in the seed coat. Difficulties in identification. Slow (7 days) and difficult to examine.	- PCR method can be implemented but not necessarily needed

#### 4.3. Development and implementation of control measures.

In conventional agriculture, seed borne diseases are almost exclusively controlled by seed treatments with synthetic pesticides, ergot in rye being the only exception. In Denmark about 90% of all cereals seeds are treated with fungicides on a routine basis (Nielsen *et al.*, 1998). In peas, no pesticides are at present approved neither in organic, nor in conventional agriculture.

#### Cultural practices and integrated control strategies

The infection of seed by pathogens depend in some cases on the climate during growth and harvest (*Fusarium spp.*, net blotch, glume blotch, pea-diseases) and in others mainly depend on the conditions in the germination period (leaf stripe, common bunt). The dependence on certain conditions during germination can be used in a control strategy, by late or early sowing of the fields for seed propagation. By this strategy, the infection by systemic diseases will be minimised

(except for loose smut).

In the period from seed maturity to harvest, the seeds have a high moisture content, and a low resistance to pathogens. Under Danish climatic conditions, this period has a high rainfall, and the seeds are therefore very susceptible to diseases infecting the seeds. It is therefore possible that the frequency of seed infections could be minimised by precipitating the harvest from the normal time of harvest at about 14-16% m.c. to the time of seed maturity where m.c. are about 25-30% (Olvang 2000). The microclimate in the canopy is affected by the plant density. Growing cereals in rows has gained increased interest in organic agriculture in order to control weed and improve protein content in the seeds. This cropping method also increases the air exchange in the canopy and reduce air humidity with a positive side effect on disease regulation. However, the potential of these strategies in practical seed propagation is uncertain, and a cost-benefit analysis has not been made.

#### Seed cleaning.

Ergot in rye is a pathogen present as sclerotia among the seeds. This pathogen is also in conventional agriculture controlled partly by seed cleaning based on density and size. The same principle can be used to control other pathogens.

Some pathogens like common bunt in wheat, stem smut in rye and to some extent also loose smut in oat and the *Fusarium*-species, the spores are loosely attached to the seed surface. These diseases can therefore be controlled (or reduced) by physical removal of the spores from the seed lot. Preliminary test (results not published) indicate that a brushing machine used for separation of grass seeds can remove 90-99% of the spores on the seed surface combined by an increased germination speed caused by a stratification of the seeds. However, this machine needs further development to improve the effect and stability in spore-removal in cereals.

The *Fusarium*-species, barley loose smut, and glume blotch in wheat will reduce the size and density of the infected seeds, and also the small seeds in the top of the head are more susceptible to some of the diseases. The infection level in the small seed fraction is therefore higher than the larger seed fractions. By removal of the small seeds and/or seeds with low density, the infection level of the seed lot can be reduced. However, the potential of this control strategy in practical seed production is at present unknown.

#### Thermal treatment

Resting plant tissues like seeds and pathogens on seed are sensitive to humid heat. It is possible to develop a seed treatment based on the possibility to hit the pathogens on the seeds without affecting the seed vigour. This has been utilised in the traditional hot water treatment (Jensen 1888), by soaking the seeds into hot water. By the correct combination of time and temperature, this treatment has effect on all known pathogens in cereals (Piorr 1991, Winter *et al.* 1994, Nielsen *et al.* 2000). However, this treatment is impractical in modern seed handling, and the expenses for seed drying are very high. New methods are therefore under development in which only the seed surface are imbibed and heated or radiated, and the expenses for seed drying therefore minimal. Seeds can be beamed with electrons (Burth *et al.* 1991, Lindner *et al.* 1991, Lindner 1992, Schröder *et al.* 1998) or with humid air (Winkelmann 1955, Kristensen and Forsberg 2000, Forsberg 2001) or with a combination of humid air and microwaves (Cwiklinski *et al.* 2001). When drying rye for bread, previous tests have shown that the prevalence of *Fusarium* on grain seeds without affecting the germination of the seeds could be reduced in a drum dryer, especially at high temperatures. (Kristensen E. F. & Sogaard H.T. 2001).

#### Organic products and microbiological products.

The pesticides used in conventional agriculture can not be used in organic production (EEC 1991), but other treatments like organic products or products containing microbiological antagonists, may be used instead. In contrast to thermal methods, these may only be used in cases of acute risk of yield loss, where preventive and thermal methods fail to control the diseases (EEC 2000). However, this compound is not approved for seed treatment in Denmark by the Danish Environmental Agency. A number of micro-organisms are developed as seed amendments to control seed borne diseases, and one product, Cedomon (*Pseudomonas chloraphis*), is in evaluation for approval in conventional agriculture in Denmark. The legal aspects concerning the eventual use of these products in organic agriculture in Denmark are at present unclear, since our knowledge about the thresholds for risk of yield loss and potential preventive methods are limited. The focus in this project will therefore be on preventive and thermal methods before efforts are made to further improve products for seed amendment.

#### Host resistance.

Growing plants that are resistant to seed borne is a strategy with sustainable perspectives. Resistance against serious seed borne diseases in cereals, such as leaf stripe, loose smut and common bunt have been described in the literature, but only little is known about the resistance in modern varieties.

New projects have, however, been started in co-operation with Danish breeders with the objective to ensure availability of healthy seed for pesticide-free and organic growing of cereals. The results so far show great variation in susceptibility to leaf stripe (Nielsen and Christensen, 2000). Most of the tested varieties or lines were susceptible, but some varie-

ties had a moderate level of resistance and a few were highly resistant. The test have been made with different populations collected from Denmark, but only little is known about the variation in leaf stripe.

A number of Danish winter wheat varieties were tested on different populations of common bunt with great variation in resistance (Nielsen and Christensen, 2000). Most of the varieties tested very highly susceptible to common bunt, but some resistance was identified. Varieties like Stava and Tjelvar (late maturing Swedish varieties) were highly resistant whereas a few varieties like e.g. Trintella and Aspect were moderate resistant (attack of 1-2 %). A group of varieties were identified as moderate susceptible. Also in spring wheat, the screening showed great variation in resistance like in winter wheat but no varieties have high resistance. In triticale, 20 varieties have been tested and they were all were highly resistant to common bunt.

The projects screening for resistance to leaf stripe and common bunt ("Cerealieprojekt" and "Pesticidhandlingsplan II") stops in 2002. There is a need to continue the screening and to develop the methods so that new varieties can be tested for resistance. There seems also to be a good basis for introducing resistance against leaf stripe and common bunt in breeding programmes, but it has to be a long lasting and continuous process.

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

The target is therefor 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

## 6. Description of work packages including methods

In order to accomplish these principal objectives in the coming 5 years period and to ensure the primary results of the projects and synergistic effects of joint research effort, the project is divided into 5 work packages.

**Table 1: Work package list**

<b>WP No</b>	<b>WP title</b>	<b>Responsible participant</b>	<b>Budget</b>	<b>Start</b>	<b>End</b>	<b>Deliverable, No</b>
WP1	Threshold values	BJN	3,447 mill. Kr.	2000	2005	1-12
WP2	Diagnostic methods	CS	3,201 mill Kr.	2000	2005	13-20
WP3	Regulation and control measures	AB	2,926 mill. Kr.	2000	2005	21-28
WP4	Integrated strategies and dissemination of information	BJN	incl. in WP 1-3	2000	2005	29-33
WP5	Project management	BJN	0,427 mill. Kr.	2000	2005	34-37
<b>Total</b>			<b>10,00 mill. Kr.</b>			

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

**Table 2: Description of work packages**

<b>WP1: Threshold values</b>	
Workpackage number:	<b>1</b>
Start date or starting event:	<b>August 2001</b>
Responsible person:	<b>BJN</b>
Contributing persons:	<b>AB, GCN, CS</b>
Person-months:	<b>30 month scientists and 60 months technicians.</b>
<b>Objectives:</b>	
<b>More precise threshold values for organic produced seeds to avoid unnecessary discharge of valuable seeds.</b>	
<b>Description of work:</b>	
The work package is divided in 7 specific tasks:	
<ol style="list-style-type: none"> <li>1. Seedling blight in wheat</li> <li>2. Leaf stripe in barley</li> <li>3. Net blotch in barley</li> <li>4. Seed and foot rot in peas</li> <li>5. Common bunt in wheat</li> <li>6. Glume blotch/seedling blight in wheat</li> <li>7. Models</li> </ol>	
General description of working method:	
The present damage/rejection thresholds will be checked and experiments will be established with a view to ensure a better basis for decision. The working method includes:	
<ol style="list-style-type: none"> <li>a) Establishment of seed lots with different infection levels either by natural or artificial infection</li> <li>b) Evaluation of the expression of the diseases under a set of different conditions (soil temperature, sowing depth, soil type and susceptibility of variety)</li> <li>c) Determination of loss in yield and quality corresponding to the actual infection level and susceptibility of variety</li> <li>d) Determination of spread of disease to new seeds (transmission rate to the next generation) under different conditions and varieties. Experiments here are integrated part of WP 3.1.</li> <li>e) Development of models describing multiplication over several seasons</li> <li>f) On the basis of the results the present threshold values will be adjusted to organic conditions. Differentiated thresholds for the various stages of the propagation process of seeds, especially C1 and C2 will be elucidated, including acceptable thresholds for various control measures (WP3). When determining the control threshold it must be taken into consideration that even a slight attack may over some generations turn into serious attacks.</li> </ol>	
The trials will be established at Research Centre Flakkebjerg (DIAS) under semi-field and field conditions. During the project period when important factors etc. are selected, a number of trials will be placed in different localities in Denmark in co-ordination with the Danish Advisory Service (GCN).	
Seed lots with different infection levels will be delivered to WP2 (diagnostics) and WP3 (integrated control).	
New diagnostic methods will be developed in WP2 and during the project period it is expected that higher capacity (more tests) and higher precision will be possible in determine species and infection levels.	

**Task 1. Seedling blight in wheat.**

Several *Fusarium* species can cause seedling blight in wheat but only *M. nivale* and *F. culmorum* are supposed to be of importance under Danish conditions. In the first part of the project experiments will be performed to identify the most important pathogen. Subsequent trials will then be concentrated on selected pathogens. It is very important to co-ordinate this work with the corresponding work on identification of *Fusarium* species in WP2.

Artificial infection will be carried out in field trials with different sowing dates and sowing depths. Through this it will be possible to show any difference between species (particularly temperature requirement) and the importance of infection level to the subsequent crop establishment, development and harvest.

As an increased quantity of seed may to some extent compensate for the effect of the seedling blight diseases, experiments with different seed quantities will also be included.

Characteristics of the wheat are of importance for infection level (e.g. plant height) and severity of symptoms (e.g. emergence rate). The variety factor will therefore be included from 2003 with selected variety types.

The infection level in the small seed fraction is probably higher than the larger seed fractions. This is of great importance for determining the threshold value and experiments with fractionating infected seed lots will be included from 2003 co-ordinated with the activity under WP3.

The ability for an organic soil to compensate for reduction in plant emergence, or to suppress seedling blight pathogens, is different from a conventional soil. This question is of special importance with seedling blight diseases and during the project period a greater part of the trials will be placed under organic conditions.

Seedling blight is also a problem in triticale. It is expected that results obtained in wheat are applicable in triticale and no special trials will therefore be performed in triticale

**Task 2. Leaf stripe in barley**

The germination speed is of great importance to the proportion of kernel infection actually being expressed as disease in the field. Experiments will therefore be established to demonstrate the importance of variety, soil temperature (sowing date) and various levels of infection.

Of special interest is the current threshold in C1 certified seed where no infection is acceptable and C2 where the present threshold is 5 %.

The importance of variety resistance against leaf stripe for the actual damage threshold will be examined in co-ordination with WP 3.2.

Experiments with effect of cultural practices like sowing time etc. will be integrated with corresponding trials in WP3.

**Task 3. Net blotch in barley.**

The aim of the experiments with net blotch is particularly to examine whether the present threshold value of 15% is actually applicable for net blotch in organic farming. The important question regarding net blotch is the risk of later spread of the disease from primary infections to other leaves. Trials will therefore be established where infections from plant debris in the soil are minimised (no barley in previous crops) and where conditions are optimal for infection of net blotch (artificial irrigation)

**Task 4. Seed and foot rot in pea**

Seed lots of pea with different infection levels will be established and used in trials in different soil types. The relationship between seed infection and severity of disease expression as seed and foot rot will be studied. The influence of seed quality and the infection level of other soil-borne fungi e.g. *Pythium* will be included.

The importance of variety resistance for the level of seed infection will be examined in co-ordination with WPx

**Task 5. Common bunt in wheat**

Wheat lots are infected artificially with various amounts of bunt in order to establish a connection between spores on the kernels and subsequent attacks in the field. Very small amounts of infection are tested to approach the present damage threshold. To demonstrate the importance of the germination speed experiments will be carried out with various soil types and sowing times with special focus on early sowing.

The importance of variety resistance for the actual damage threshold will be examined in co-ordination with WPx

**Task 7. Glume blotch in wheat.**

Wheat lots with various infection levels of *Lephtospharia nodorum* will be established/collected. The lots are sown at different time and in the trials registrations are made of germination percentage, multiplication of glume blotch under field conditions, and yield. Trials with different seed quantities will also be included. Glume blotch has a lower priority in the project and experiments will first start in later part of the project period (2003).

Glume blotch can also attack triticale. It is expected that results obtained in wheat are applicable in triticale and no special trials will therefore be performed in triticale.

**Task 8. Models for multiplication and spreading of seed-borne diseases**

Models describing the connection between kernel infection, field infection, and subsequent infection in the harvested yield under different conditions are developed. The models include projecting over several seed propagation generations (pre basic-C2) and years and to evaluate the long-term risk of multiplication and consequences of reduction factors. For some pathogens like loose smut in barley relationship between kernel infection and field infection is quite clear so for this disease field trials have low priority and description of spread and multiplication of disease will only be at model levels using existing data and information. If relevant information will become available from other sources on some of the pathogens during the project period, data will be used in the models as supplement to field trials.

**Deliverables:**

**D1:** Most important seedling blight pathogens identified within the *Microdochium/Fusarium* complex.

**D2:** Seed lots established with different infection levels for use in WP1, WP2 and WP3.

**D3:** Information on disease multiplication/reduction in individual seed lots in the propagation process.

**D4:** Threshold for seedling blight adjusted to organic condition

**D5:** Threshold for seed borne Glume blotch adjusted to organic condition

**D6:** Threshold for leaf stripe adjusted to organic condition

**D7:** Threshold for net blotch adjusted to organic condition

**D8:** Threshold for seed and foot rot disease in pea adjusted to organic condition

**D9:** Models developed describing multiplication over years

**D10:** New recommendations for threshold values to seed producers and farmers

**D10:** Results published in farmer's journals, leaflets, magazines, web-site etc.

**D12:** Results published in international publications.

**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

Workpackage number:	<b>2</b>
Start date or starting event:	<b>August 2001</b>
Responsible person:	<b>CS</b>
Contributing persons:	<b>AFJ, NN, BJN, GCN</b>
Person-months:	<b>50 month scientists and 34 months technicians.</b>

**Objectives:****Implementation of 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*)

**Description of work:**

The work package is divided in 3 task with methods on:

1. Seedling blight
2. Glume blotch
3. Leaf stripe and Net blotch

General description of working method:

- a) Identification of relevant diagnostic methods
- b) Development and test of methods in connection with WP1
- c) Implementation of methods in practise

Precise, reproducible and rapid diagnostic methods are prerequisites for investigation of thresholds and control measures. Such methods will be identified, further developed and implemented for use in WP1, WP3 and also for routine testing purposes. The methods will be tested on seed lots produced in WP1 and compared to results achieved by traditional mycological test methods and green house tests. For the above listed diseases an improvement of the diagnostic methods will most likely be achieved by implementing PCR-based or serological methods. A real-time PCR-machine will be at the disposal and therefore makes it possible to develop quantitative PCR based test methods. For all the above listed diseases, PCR-primers have already been developed and can be used in a qualitative test. However, development of quantitative PCR-methods using these primers have not been completed and may demand additional sequence characterisation of fungal isolates to ensure the most optimal design of PCR-primers for a quantitative assay. Furthermore it is desirable to be able to perform the quantitative PCR test directly on DNA extraction of the seeds to avoid time consuming incubation steps. Therefore suitable DNA-extraction methods have to be identified and tested. Development and implementation of the tests will be performed at DIAS in close collaboration with the Danish Plant Directorate who will supply seed lots and assist in performing traditional mycological tests and green house tests.

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

*Fusarium culmorum*, *F. avenaceum*, *F. graminearum*, *F. poae* and *Microdochium nivale*.

Also for these pathogens the aim will be to develop PCR-based tests. DNA-sequences of DNA-primers are published for *F. graminearum* (Nicholson et al. 1998; Schilling et al. 1996) for *F. poae* (Parry and Nicholson, 1996) and for *F. culmorum* and *F. avenaceum* (Schilling et al. 1996; Nicholson et al. 1998). PCR-primers for *Microdochium nivale* have also been developed (Nicholson et al. 1996). Initially agar plate counts techniques and examination of kernels for brown roots will determine the infection of seed lots from field trials in WP1. Qualitative PCR-tests for the different species and sub-species will be tested in relation to the agar plate test. In parallel development of a quantitative PCR-test, which can be performed directly on kernels, will be initiated for *M. nivale* and *F. culmorum*. Based on the experiments in WP1 it may be possible to put the species in order of priority for development of a quantitative test. PCR-assays will be performed at DIAS. Agar plate tests will be performed at both DIAS and PD where the examination of kernels with brown roots will be performed too.

**Task 2: Glume blotch (Leptosphaeria nodorum)**

PCR-primers designed from the ITS (Internal Transcribed Spacer) region in the ribosomal DNA have been published for the detection of *L. nodorum* in leaf tissue (Beck and Ligon, 1995). Commercial kits are now available for PCR- and ELISA tests and will also be investigated. All methods will be evaluated for their qualification for use in a quantitative test performed on kernels. Seed lots from WP1 will be used, and infection levels determined on these by the fluores-

cence-method and examination for deformities on seedlings will be correlated with results achieved by the new methods.

Initiatives against combining new PCR techniques have been taken in the UK (Cockerell, 2001 personal comm.) where multiplexing primers for both *Microdochium nivale* and *Tilletia caries* has shown promising results. Possibilities for developing a combined test including *Leptosphaeria nodorum* in a one step PCR test will be investigated.

**Task 3: Barley net blotch and leaf stripe**

Recently a real time PCR-assay has been developed for the quantification of *Pyrenophora* species in barley seed (Bates et al., 2001). This method will be implemented and used in combination with a greenhouse test where the percentage of plants with leaf stripe symptoms can be determined. At DIAS, previous work has resulted in the identification of DNA-sequences which are specific for *P. graminea* (Husted, 1993). From these DNA-sequences PCR-primers which specifically amplifies *P. graminea* have been identified. These primers can be used in the development of a quantitative PCR-test for *P. graminea*, which later on can replace the time- and space-consuming greenhouse test.

**D13:** Qualitative PCR-method for *Fusarium spp.* will be identified and developed

**D14:** Priority to *Fusarium spp* for which a quantitative PCR-test should be developed will be given based on results from WP1

**D15:** Quantitative method for *Fusarium sp.* will be tested on seed lots, produced in WP1, with known infection

**D16:** Test for glume blotch will be identified

**D17:** Quantitative method for glume blotch will be tested on seed lots, produced in WP1, with known infection.

**D18:** Combination of methods for detection of seed borne diseases in wheat will be tested

**D19:** Quantitative method for *Pyrenophora teres* will be tested on seed lots, produced in WP1, with known infection.

**D20:** Quantitative method for *Pyrenophora graminea* will be tested on seed lots, produced in WP1, with known infection

**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**

Work package number: **3**  
 Start date or starting event: **August 2001**  
 Responsible person: **AB/BJN**  
 Contributing persons: **GCN, LB, EFK**  
 Person-months: **30,5 month scientists and 34 months technicians.**

**Objectives:**

**Develop and implement different control measures applicable for organic farming**

**Description of work:**

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

- 2.1. Direct and indirect control measures
- 2.2. Host resistance

The work packages will be described in the following:

**WP 3.1: Control measures acceptable in the organic seed production**

Work package number: **3**  
 Start date or starting event: **August 2001**  
 Responsible person: **AB**  
 Contributing persons: **BJN, GCN, EFK**  
 Person-months: **25,5 month scientists and 25 months technicians**

**Objectives: Develop and implement different control measures applicable for organic farming**

The objective of the study is to evaluate the potentials of different measures to prevent and control seed borne diseases in organic farming. The objective is also to assist in the implementation of the methods in practice.

**Description of work:**

The work package has the following tasks:

1. Preventing cropping methods
2. Multiplication of diseases in the propagation process from C1 to ware seed.
3. Seed cleaning
4. Heat treatment

**Task 1. Preventing cropping methods.**

The question with prevention and effect of different cultural practices is very closely related to the activities in WP1 and the experiments will be conducted integrated between the two WP's. For practical reasons the detailed descriptions of methods are under WP1. The aim in WP3 taks 1 is to investigate problems with spread of disease to new seeds under different conditions. There will be specific focus upon:

Experiments with pea/barley mixtures to investigate possible reduction in spread of disease in the pea canopy. Experiments with different variety-types and peas-barley mixtures

In fields infected by net blotch, *Fusarium*, glume blotch and seed and foot rot in pea, harvest will be conducted at different intervals from physiological seed maturity onto the normal time of harvest in order to relate the infection of the diseases in the seeds and the seed vigour with seed moisture and maturity at the time of harvest. The study will include an evaluation of the side effects of the method in terms of cost for seed drying, risk of post-harvest pathogen and mycotoxin development and other factors relevant for the implementation in practice.

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

The many different fields at organic farmers used for propagating seeds can be used for collecting data on disease multiplication/reduction during the different process steps. Individual seed lots will be followed from selected individual fields of wheat, barley and peas (3-4 of each). The C1 field will be characterised for important parameters like e.g. previous crop, plant densities, lodging, N-level, straw-containing manure, neighbouring (infected ?) fields etc. Infection level will be determined in field and later in harvested seeds (raw seed) and then in the clean seed. The seed lot will be followed in the C2-field and then in farmers field using the same procedure. The last test will be in the ware seed leaving the farmers field. In this way it is possible to follow the same seed string from start to end and to evaluate important steps where multiplication and especially reduction takes place and to evaluate different threshold scenarios coordinated with the activities in WP1.

Of special interest is to investigate sampling methods but this subject is technical very complicated and is not within the scope of this project. Different sampling methods will therefore only be investigated in the above mentioned selected propagation fields/seed producers and tested for variation in seed infection.

**Task 3. Seed cleaning.**

The potential of seed cleaning in the regulation of seed pathogens will be tested in large scale installations on seed lots selected from commercial farming practice. Focus will be put on:

3.1 Removal of small infected kernels.

Removal of seeds infected by *Fusarium*, and barley loose smut, but also eventual effect on leaf stripe, net blotch and glume blotch will be tested in a size separator and gravity separator. The effect of the treatments will be tested by seed analysis in lab. Tests will be conducted both in small scale/laboratory seed cleaners and in full-scale equipment.

3.2. Removal of bunt spores

Seed lots natural or artificial contaminated by bunt spores will be treated in a full-scale seed brushing cleaner (available at DJF-Flakkebjerg and Mørdrupgård) and a full-scale Sigma cleaner (available at DAMAS a/s and Carl Rasmussen a/s). The reduction in number of spores and the effect on seed vigour will be tested in lab at KVL. The tests will be coordinated with on going research (by Susanne Elmholt, DJF-Foulum a.o.) investigating the effect of seed handling on the presence of mycotoxins in the seeds (produced by *Fusarium* sp. and *Penicillium* sp).

**Task 4. Heat treatment.**

The effect of heat treatment on seed pathogens will be tested at DJF, Bygholm in a drum dryer. By this technique, the temperature and duration of the treatment can be precisely controlled. The influence of the initial seed moisture on the effect on seed diseases will be tested, and seeds will be imbibed at different intervals before treatment since this is likely to have effect on the treatments effect on the pathogens. Test will be done with seed lots naturally infected by net blotch and the pea disease complex, and the effect on disease infection will be tested in lab tests described in WP1. All treated samples will also be thoroughly assessed for effect on seed vigour. Preliminary tests will be conducted with other methods.

**Deliverables:**

**D21:** Effect of pea/barley mixture on spread of seed and foot rot diseases to pods and new seeds.

**D22:** 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.

**D23:** 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.

**D24:** 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.

**D25:** 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.

**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**

Work package number:	<b>3</b>
Start date or starting event:	<b>August 2001</b>
Responsible person:	<b>BJN</b>
Contributing persons:	<b>LB, GCN</b>
Person-months:	<b>5 month scientists and 9 months technicians.</b>

**Objectives:**

**Develop and implement different control measures applicable for organic farming**

**Description of work:**

The work package has 4 task:

1. Screening for resistance to leaf stripe in Danish barley varieties
2. Screening for resistance to seed and foot rot diseases in pea.
3. Screening for resistance to common bunt in Danish wheat varieties

The screening part utilises the screening facilities and expertise developed in ongoing research projects. In this way high capacity can be obtained already from the start in 2003.

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**

Screening of Danish barley varieties is running until 2002 supported by the projects "Cerealieprojekt" and "Pesticid-handlingsplan II". From 2003 and the rest of the project period, the screening will be a part of the new project. Barley varieties of interest to organic production will be characterised as fully resistant, moderate resistant, moderate susceptible and fully susceptible.

Advanced breeding material from Danish breeders will also be tested in the screening system, which will support ongoing breeding processes.

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

Danish pea varieties will be tested for their level of resistance to *Phoma medicaginis*. A test system will be developed with artificial inoculated small plots of pea under artificial irrigation and varieties of interest to organic production will be characterised

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

Danish wheat varieties will be tested on a set of different *Tilletia* populations for their level of resistance. The test sys-

tem which has been developed in two projects (“Cerealieprojekt” and “Pesticidhandlingsplan II”) will be used, and varieties of interest to organic production will be characterised as fully resistant, moderate resistant, moderate susceptible and fully susceptible. The test is running in 2001 and 2002 financed by the project “Pesticidhandlingsplan II”. In 2003-2005 the test will be a part of the new project which will ensure a continuous test of Danish wheat varieties.

Advanced breeding material from Danish breeders will also be tested in the screening system, which will support ongoing breeding processes.

**Deliverables:**

**D26:** Danish barley varieties with interest for organic production screened for resistance against leaf stripe.

**D27:** Danish pea varieties with interest for organic production screened for resistance against *Phoma medicaginis*

**D28:** Danish wheat varieties with interest for organic production screened for resistance against common bunt.

**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.**

Work package number:	<b>4</b>
Start date or starting event:	<b>November 2002</b>
Responsible person:	<b>BJN</b>
Contributing persons:	<b>GCN, AB, CS, NN, AFJ, LB,</b>
Person-months:	<b>Part of all work packages and part of institutional work of participants.</b>

**Objectives:** **To develop and implement in practice different integrated control strategies, new thresholds and seed analysis methods.**

**Description of work:**

This WP is the synergy between the different WP's and a description of how information is disseminated and new methods implemented. Being an integrated part of all WP's and also a part of several of the participant's institutional work, no specific budget is allocated to this WP.

**Integrated strategies**

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

**Dissemination of information**

Information will be delivered via Danish Advisory Service (GCN) 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 made by LR (GCN)

Information, results and recommendations will be available on a special new web site for organic seed under the information platform "Planteinfo" ([www.planteinfo.dk](http://www.planteinfo.dk)) where information on plant growing are delivered to farmers and extension service by LR and DIAS.

**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 (voluntarily) standard used by seed producers.

**The methods developed in the project.**

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

ISTA (The International Seed Testing Association) do 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 have 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 international 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

**Deliverables:**

**D29:** Integrated strategies with different control measures

**D30:** Leaflets, journals etc. to organic farmers and seed producer's etc. with information on problems with seed borne diseases.

**D31:** Web-site with information on seed borne diseases, thresholds, recommendations etc.

**D32:** Thresholds adopted as a standard in organic seed production of cereals and peas.

**D33:** Test methods standardised in Denmark.

**WP5: Project Management**

Workpackage number: **5**  
Start date or starting event: **August 2001**  
Responsible person: **BJN**  
Contributing persons:  
Person-months: **9 month scientists**

**Objectives: Project management****Description of work:**

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

Project meetings and project reports.

Project workshops.

**Deliverables:**

**D34:** Project reports each year

**D35:** Final project report

**D36:** Project workshop with discussion of preliminary results

**D37:** Project workshop presenting final results and conclusions.

**Milestones:**

**M18:** Project report October each project year.

**M19:** Final project report December 2005.

**M20:** Workshop October 2003.

**M21:** Final Workshop October 2005.

## 7. Implementation and time schedule

**Table 3: Deliverables list (yyyy/yyyy=first results/final results)**

Deliverable, No	Deliverable title	Delivery date	Meeting	Nature
1	Most important seedling blight pathogens identified within the <i>Microdochium/Fusarium</i> complex.	2003		R
2	Seed lots established with different infection levels for use in WP1, WP2 and WP3.	2002/2003		M
3	Information on disease multiplication/reduction in individual seed lots in the propagation process.	2004/2005		R
4	Threshold for seedling blight adjusted to organic condition	2003/2005		T
5	Threshold for seed borne Glume blotch adjusted to organic condition	2003/2005		T
6	Threshold for leaf stripe adjusted to organic condition	2003/2005		T
7	Threshold for net blotch adjusted to organic condition	2003/2005		T
8	Threshold for seed and foot rot disease in pea adjusted to organic condition	2003/2005		T
9	Models developed describing multiplication over years	2005		M
10	New recommendations for threshold values to seed producers and farmers	2003/2005		RE
11	Results published in farmer's journals, leaflets, magazines, web-site etc.	2002-2005		POP
12	Results published in international publications.	2004/2005		PU
13	Qualitative PCR-method for <i>Fusarium spp.</i> will be identified and developed.	2003/2005		ME
14	Priority to <i>Fusarium spp</i> for which a quantitative PCR-test should be developed will be given based on results from WP1	2003		ME
15	Quantitative method for <i>Fusarium sp.</i> will be tested on seed lots, produced in WP1, with known infection	2005		ME
16	Test for glume blotch will be identified	2004/2005		ME
17	Quantitative method for glume blotch will be tested on seed lots, produced in WP1, with known infection	2005		ME
18	Combination of methods for detection of seed borne diseases in wheat will be tested	2004/2005		ME
19	Quantitative method for <i>Pyrenophora teres</i> will be tested on seed lots, produced in WP1, with known infection.	2002		ME
20	Quantitative method for <i>Pyrenophora graminea</i> will be tested on seed lots, produced in WP1, with known infection	2002		ME
21	Effect of pea/barley mixture on spread of seed and foot rot diseases to pods and new seeds.	2003/2005		R
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		R
23	Description of the influence of cropping parameters on the development of epidemics of net blotch, glume blotch and <i>Fusarium</i> . The description will lead to recommendation for propagation and for field inspections.	2004/2005		R
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		R
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 ad-	2003		R

	justed 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.			
26	Danish barley varieties with interest for organic production screened for resistance against leaf stripe.	2003/2005		R
27	Danish pea varieties with interest for organic production screened for resistance against <i>Phoma medicaginis</i>	2003/2005		R
28	Danish wheat varieties with interest for organic production screened for resistance against common bunt.	2003/2005		R
29	Integrated strategies with different control measures.	2004/2005		RE, M
30	Leaflets, journals etc. to organic farmers and seed producer's etc. with information on problems with seed borne diseases.	2002-2005		POP
31	Web-site with information on seed borne diseases, thresholds, recommendations etc.	2002		POP
32	Thresholds adopted as a standard in organic seed production of cereals and peas.	2003/2005		RE
33	Test methods standardised in Denmark.	2004/2005		RE
34	Project reports each year	2002-2005		REP
35	Final project report	2005		REP
36	Project workshop with discussion of preliminary results	2003		W, PRO-na
37	Project workshop presenting final results and conclusions	2005		W

R: Experimental results

T: Experimental results/Threshold value

M: Models

RE: Recommendations

POP: popular papers/information etc.

PU: International publications

ME: Method

W: Workshop

REP: Report

PRO-na: National proceeding

**Table 4: Timetable**

Task	WP1: Thresholds	Priority	2001				2002				2003				2004				2005					
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4		
1	Seedling blight	H			M1				M1	M2			M1	M2	M4		M1	M2			M5	M6		
2	Net blotch	H						M1		M2		M1		M2	M4	M1		M2			M5		M6	
3	leaf stripe	M						M1			M4	M1			M4	M1					M5		M6	
4	seed/foot rot in pea	H						M1		M2		M1		M2	M4	M1		M2			M5		M6	
5	Common bunt	M			M1				M1		M4		M1		M4		M1					M5	M6	
6	Glume blotch in wheat	M							M1				M1									M5		M6
7	Models	H													M3							M3	M6	

**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



Task	WP 3.1: control	2001				2002				2003				2004				2005				
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
1	Cropping strategy							M11	M12			M11	M12			M11	M12			M14		
2	Seed cleaning			M13					M13				M13		M14							
3	Heat treatment				M13				M13				M13			M13	M14					

**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

Task	WP 3.2. Resistance	2001				2002				2003				2004				2005				
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
1	Leaf stripe											M15				M15				M15		
2	Seed and foot rot in peas							M16				M16				M16				M16		
3	Common bunt															M17				M17		

**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.

Task	WP1: Project management	2001				2002				2003				2004				2005				
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
1	Project meeting. Planning																					
2	Project meeting. Status																					
3	Project report				M18				M18				M18				M18					M19
4	Project workshop												M20									M21
5	Publications (internat.)																					

**Milestones:**

**M18:** Project report October each project year.

**M19:** Final project report December 2005.

**M20:** Workshop October 2003.

**M21:** Final Workshop October 2005.

**8. Collaborative partners**

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.

**9. Budget.**

Total budget for the project and budget for each institution:

<b>TOTAL FOR THE PROJECT</b>						
	2001	2002	2003	2004	2005	TOTAL
Months (scientific)	10	33	33	26	20	120
Months (technical)	5	36	39	33	15	128
Salary (scientific)	316.500	1.136.255	1.175.383	957.675	760.502	4.346.315
Salary (technical)	105.000	778.680	868.877	757.260	354.535	2.864.352
Operation – equipment	0	0	0	0	0	0
Operation - other	45.000	230.000	325.000	298.000	100.000	998.000
Overhead	91.300	467.483	513.683	445.793	273.422	1.791.681
<b>Total</b>	<b>557.800</b>	<b>2.612.418</b>	<b>2.882.943</b>	<b>2.458.728</b>	<b>1.488.460</b>	<b>10.000.348</b>

<b>DJF TOTAL</b>						
<b>SUM</b>	2001	2002	2003	2004	2005	TOTAL
Months (scientific)	5	19	19	13	10	66
Months (technical)	5	29	30	25	12	101
Salary (scientific)	171.000	671.210	696.386	497.091	400.902	2.436.590
Salary (technical)	105.000	627.270	668.367	573.682	283.628	2.257.947
Operation – equipment	0	0	0	0	0	0
Operation - other	25.000	120.000	115.000	98.000	45.000	403.000
Overhead	60.200	283.696	295.951	233.755	145.906	1.019.507
<b>Total</b>	<b>361.200</b>	<b>1.702.176</b>	<b>1.775.704</b>	<b>1.402.527</b>	<b>875.437</b>	<b>6.117.044</b>

<b>DANAGRO TOTAL</b>						
<b>Danagro (AB)</b>	2001	2002	2003	2004	2005	TOTAL
Months (scientific)	0	6	6	6	4	22
Months (technical)	0	0	0	0	0	0
Salary (scientific)	0	222.480	229.154	236.029	162.073	849.737
Salary (technical)	0	0	0	0	0	0
Operation – equipment	0	0	0	0	0	0
Operation - other	10.000	30.000	30.000	20.000	10.000	100.000
Overhead	0	88.992	91.662	94.412	64.829	339.895
<b>Total</b>	<b>10.000</b>	<b>341.472</b>	<b>350.816</b>	<b>350.441</b>	<b>236.903</b>	<b>1.289.631</b>

KVL TOTAL						
SUM	2001	2002	2003	2004	2005	TOTAL
Months (scientific)	0	0	0	0	0	0
Months (technical)	0	5	5	4	1	15
Salary (scientific)	0	0	0	0	0	0
Salary (technical)	0	108.150	111.395	91.789	23.636	334.969
Operation – equipment	0	0	0	0	0	0
Operation - other	0	5.000	5.000	5.000	5.000	20.000
Overhead	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>

LR TOTAL						
SUM	2001	2002	2003	2004	2005	TOTAL
Months (scientific)	2	2	2	2	2	8
Months (technical)	0	0	0	0	0	0
Salary (scientific)	55.500	57.165	58.880	60.646	62.466	294.657
Salary (technical)	0	0	0	0	0	0
Operation – equipment	0	0	0	0	0	0
Operation - other	0	50.000	150.000	160.000	30.000	390.000
Overhead	11.100	21.433	41.776	44.129	18.493	136.931
<b>Total</b>	<b>66.600</b>	<b>128.598</b>	<b>250.656</b>	<b>264.776</b>	<b>110.959</b>	<b>821.588</b>

PD TOTAL						
SUM	2001	2002	2003	2004	2005	TOTAL
Months (scientific)	3	6	6	5	4	24
Months (technical)	0	2	4	4	2	12
Salary (scientific)	90.000	185.400	190.962	163.909	135.061	765.332
Salary (technical)	0	43.260	89.116	91.789	47.271	271.436
Operation – equipment	0	0	0	0	0	0
Operation - other	10.000	25.000	25.000	15.000	10.000	85.000
Overhead	20.000	50.732	61.016	54.140	38.466	224.354
<b>Total</b>	<b>120.000</b>	<b>304.392</b>	<b>366.093</b>	<b>324.838</b>	<b>230.799</b>	<b>1.346.122</b>

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

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

Detailed budget for each work package is in appendix 2.

## 10. References

- Bates J.A., Taylor E. J. A., Kenyon D.M., Thomas J.E. (2001). The application of real-time PCR to the identification, detection and quantification of *Pyrenophora* species in barley seed. *Molecular Plant Pathology* 2:49-57
- Beck J. J. , Ligon J.M. (1995). Polymerase chain reaction assays for the detection of *Stagnospora nodorum* and *Septoria tritici* in wheat. *Phytopathology*, 85:319-324.
- Borgen A; L. Kristensen (2001). Use of mustard flour and milk powder to control common bunt (*Tilletia tritici*) in wheat and stem smut (*Urocystis occulta*) in rye in organic agriculture. In: Seed treatment - challenges and opportunities (ed. A.J.Biddle) p. 141-150
- Borgen A; Nielsen B J (2001). Effects of seed treatments with acetic acid for control of seed borne diseases. In: Seed treatment - challenges and opportunities (ed. A.J.Biddle) p. 135-140
- Burth,U, K.Gaber, J.Marga, K.Lindner, G.Motte, S.Panzer, J.Pflaumbaum and F.Scholze 1991: Behandlung von Saatgut mittels Elektronen - Ein neues Verfahren zur Bekämpfung samenüburtiger Schaderreger an Winterweizen
- M. Cwiklinski, D. von Hörsten, W. Lücke, G. Wolf(2001): Alternativen zur chemischen Beizung. Saatgutbehandlung mit Mikrowellen- und Hochfrequenzenergie *Landtechnik* 56(1) 28-29
- (EEC 2000) Council Regulation No. 2091/92, May 2000 on organic production of agricultural and indications referring thereto on agricultural products and foodstuf
- Forsberg, G. 2001: Heat sanitation of cereal seeds with a new, efficient cheap and environmental friendly method. In: Seed treatment - challenges and opportunities (ed. A.J.Biddle) p. 69-72
- Jensen, J.L. 1888a: Nye Undersøgelser og Forsøg over Kornsorternes Brand (Første Meddelelse). Wilhelm Priors Boghandel, Kjøbenhavn. 18 sider
- Husted K. Detektion af plantepatogene svampe exemplificeret ved *Pyrenophora graminea* og *Pyrenophora teres*. Ph.D-thesis. Statens Planteavlsvforsøg, 1993
- Johnsson, L. 1979: Dvärgstinksot (*Tilletia contraversa*) och vanligt stinksot (*Tilletia caries*) i svenskt vete. Växtskyddsrapporter. 6:1-19
- Kristensen, E.F., Søgaard, H.T., (2001), Tørring og varmebehandling af maltbyg og brødkorn i tromletørreri. Driftsparametres betydning for kvalitet. DJF rapport – Markbrug, 43, 36pp.
- Kristensen, L and G.Forsberg 2000: Control of common bunt (*Tilletia tritici*) in wheat by thermal seed treatment. Proceedings 13<sup>th</sup> International IFOAM Scientific Conference 28-31/8-2000 in Basel. p. 134
- Landbrugets Rådgivningstjeneste 2001: (den med antallet af kasserede partier)
- Lindner, K, J.Marga, Uburth, K.Gaber and J.Pflaumbaum 1991: Saatgutbehandlung mit niederenergitischen Elektronen - zur Entwicklung eines neuen physikalischen Beizverfahrens für Winterweizen. *Gesunde Pflanzen* 43:249-252.
- Lindner, K 1992: Untersuchungen zur phytosanitären Wirkung einer Behandlung von Winterweizen mit niederenergetischen Elektronen. Diss. Humbolt.Universtität Berlin.
- Nicholson P., Lees A.K., Maurin N, Parry D.W., Rezanoor H.N. (1996). Development of a PCR assay to identify and quantify *Microdochium nivale* var. *nivale* and *Microdochium nivale* var. *majus* in wheat. *Physiological and Molecular Plant Pathology* 48:257-271.
- Nicholson P, Simpson D.R. Weston G., Rezanoor H.N. Lees A.K. Parry D.W. Joyce D. (1998). Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. *Physiological and Molecular Plant Pathology* 53: 17-38.
- Nielsen B J; Borgen A; Kristensen L (2000). Control of seed borne diseases in production of organic cereals. The Brighton conference - Pest and Diseases pp.171-176. BCPC: Farnham.

- Nielsen, B. J., Borgen, A., Nielsen, G. C. & Scheel, C. 1998. Strategies for controlling seed borne diseases in cereals and possibilities for reducing fungicide seed treatments. The 1998 Brighton Conference - Pest and Diseases, vol. 3: 893-900.
- Nielsen, B. J., Nielsen, G. C., Pedersen, J. B. og Tersbøl, M. 1999 Muligheder for produktion af sygdomsfri, økologisk såsæd. 16. Danske Planteværnskonference 1999, Sygdomme og Skadedyr. DJF Rapport nr. 10 (1999) 29-40.
- Nielsen, B. J., Christiansen, S. og Bagge, J. O. 1999 Ny resistens mod frøbårne sygdomme i korn. 16. Danske Planteværnskonference 1999, Sygdomme og Skadedyr. DJF Rapport nr. 10 (1999) 149-160.
- Olvang, H. 2000: Utsædesburna sjukdommar på jordbruksväxter. Jordbruksinformation 8. p. 98
- Parry D.W., Nicholson P. (1996). Development of a PCR assay to detect *Fusarium poae* in wheat. *Plant Pathology* 45: 383-391.
- Piörr, H.P. 1991: Bedeutung und Kontrolle saatgutübertragbarer Schaderreger an Winterweizen im Organischen Landbau. Bonn 1991. pp 166.
- Schilling A.G., Möller E.M., Geiger H.H. (1996). Polymerase chain reaction-based assay for species specific detection of *Fusarium colmorum*, *Fusarium graminearum* and *F. avenaceum*. *Phytopathology* 86: 515-522.
- Schröder, T, O.Röder og K.Lindner 1998: e-dressing - a unique technology for seed. *ISTA News Bulletin*. 118:13-15.
- Spie□ H (2000). Aktuelle Versuchergebnisse zur Weizensteinbrandbekämpfung. *Lebendige Erde* 5, 41.
- Stephenson, M.M.P., A.C.Kuschalappa og G.S.V.Raghavan 1996: Effect of selected combinations of microwave treatment factors on inactivation of *Ustilago nuda* from barley seed. *Seed Sci.&Technol.*24:557-570.
- Winter, W, I. Bänziger, H.Krebs and A.Rüegger 1994: Warmwasserbehandlung von Weizensaatgut. *Agrarforschung* 1:492-495.
- Winter W; Rogger C; Bänziger I; Krebs H; Rüegger A; Frei P; Gindrat D; Tamm L (1997). Weizenstinkbrand: Bekämpfung mit Magermilchpulver. *Agrarforschung* 4, 153-156.
- Winkelmann, A. 1955: Untersuchungen zur Bekämpfung des Gersten- und Weizenflugbrandes. *Angewante Botanik* 29:3-13

**Appendix 1:**

CV's of central persons, and description of role, qualifications, capacity and experience of each participant including relevant papers:

**DIAS:**

The Danish Institute of Agricultural Sciences has great experiences in biology and control of seed borne diseases, molecular biological analytical methods and development of diagnostic methods. Research Centre Flakkebjerg was built in 1998 and has modern facilities for semi-field and field trials and well-functioning classified laboratories in which mycological as well as molecular biological work can be done. At the department of agricultural engineering, Research Centre Bygholm, there is great expertise into the optimisation of machinery systems and the development of technique for grain drying.

**Bent J. Nielsen** has for almost 20 years worked with seed-borne diseases and various aspects of control, including alternative methods for production of organic seed. Bent J. Nielsen is at present the co-ordinator of a FØJO knowledge synthesis on organic seed.

Role in the Project:

WP1: Investigations of threshold values. Field and green house trials at Research Centre Flakkebjerg. Development of models and integrated systems.

WP2: Deliver infected seed lots, seed material etc. to group working with new methods.

WP3.1: Field trials at Research Centre Flakkebjerg with different methods in co-operation with **AB**.

WP 3.2: Screening system for testing resistance against common bunt and leaf stripe at Research Centre Flakkebjerg. New test system for leaf stripe.

**Annemarie Fejer Justesen** is working with development and implementation of methods for DNA-fingerprinting of fungal isolates both for population studies and for diagnostic purposes. Is currently working with population studies of wheat yellow rust (*Puccinia striiformis* f.sp. *tritici*) and detection methods for *Pyrenophora* spp. Is also experienced in handling fungal isolates, cloning, sequencing and characterisation of fungal genes.

Role in the project:

WP2: Identification and development of new PCR based methods..

WP1: Implementation of methods for use in WP1

**Lars Bødker** has 10 years of experience working with root diseases of vegetables with special emphasise and on vegetables and potatoes in Denmark. The research has mainly focused on mycological studies, resistance and growing systems in both organic and conventional growing systems. In the last three years the work has primarily been focused on integrated disease control of root rot of peas and cavity spot of carrots using organic amendments and improvement of control strategies for late blight in potato.

Role in the project:

WP1: Threshold values with seed and foot rot in peas

WP3.1: Experiments with seed and foot rot of peas in connection to cultural practices and control methods in co-operation with **AB**.

WP 3.2: Screening system for testing resistance against *Phoma medicaginis* at Research Centre Flakkebjerg.

**Erik Fløjgaard Kristensen's** primary research areas are grain drying techniques and techniques for harvesting. His work concerning drying techniques includes research into and development of existing and new techniques for grain and seed crop.

WP4. Task3

EFK will be responsible for the optimisation of the drum dryer for drying and heat treatment of grain for seed.

**PD**

The Danish Plant Directorate perform seed health analyses and other diagnostic work in plant pathology. PD has great experience with traditional diagnostic methods and has implemented some PCR-methods in the diagnostic work. PD is accredited by ISTA for performing seed health tests in linseed and by DANAK for bacterial test in seed potatoes.

**Christiane Scheel** has worked as a plant pathologist in several years including seed borne pathogens. CS is a member of the Plant Disease Committee in ISTA and The Nordic Seed Pathology Group, who exchange test methods and perform ring tests among the Nordic laboratories.

Role in the Project:

WP1: Discussion and implementation of new threshold. Performing 'old' tests on material from WP1 which will also be used in WP2

WP2: Implementation of new diagnostic systems

NN new employed scientist at PD

Role in the Project:

WP1: Use of new diagnostic methods for implementation of new thresholds

WP2: Implementation of new diagnostic systems

## LR

Danish Agricultural and Advisory Centre provides technical knowhow and services to the local advisory centres which are distributed all over Denmark. Each year about 2000 experiments are carried out in close co-operation between local centres and the Advisory Centre. They provide information on factors of importance also in relation to variation in local environment.

**Ghita Cordsen Nielsen** has for many years worked with agricultural guidance and counselling, also including questions concerning seed-borne diseases and the contact to producers of seed concerning damage thresholds and analyses.

Role in the Project:

WP1: Field trials under different conditions in different part of Denmark. (Danish Advisory Service). Discussion and implementation of new threshold

WP2: Results implemented in practice.

WP3: Field trials under different conditions in different part of Denmark. (Danish Advisory Service). Discussion and implementation of results.

## KVL

KVL has facilities for and laboratory experience with seed handling and fields trials with seed health in organic farming.

Role in the project:

WP3: Field and laboratory trials at Research farm Højbakkegård, Tåstrup with different methods under supervision of **AB**.

## DANAGRO

Danagro has expertise within organic and sustainable agriculture and ongoing international projects within organic seed production and regulation.

**Anders Borgen** has 10 years experience in research with organic seed health, and has through this work a network within the organic movement, organic seed producers and within seed research world wide.

Role in the Project:

WP1: Investigations of threshold values in co-operation with **BJN**. Contribution with expertise on special conditions for organic farming and to the development of models and integrated systems.

WP3: Field and laboratory trials at KVL (Research farm Højbakkegård) with different methods. Coordination with organic seed producers and companies producing machinery. Test of seed handling equipment, and survey in commercial cropping.

**Curriculum Vitae**                      **Bent Jørgen Nielsen (BJN)**

**Year of Birth:**                      16-05-1952, Denmark

**Education:**

Name of Institution:                      University of Copenhagen, 1979

Degree or Diploma:                      M. Sc. (Cand. Scient.; Biology/plant pathology)

**Key Qualifications:**

The experience of Bent J. Nielsen is disease control in cereals, potatoes, peas and oil seed rape. The work has been concentrated on development of control strategies and implementation of integrated control strategies in forecasting models. The research projects have included investigations on efficacy of biological/microbiological products (Microbiological and Biological Control of Seed Borne Diseases) and alternative methods (hot water/air, alternative chemicals) on seed borne diseases in cereals (Seeds for Organic Farming, leader of project with alternative methods for controlling seed borne diseases in organic cereal production). In cereals also investigations on host resistance against seed borne diseases and use of host resistance in integrated control strategies under Danish Cereal Network (Leader of project with resistance to seed borne diseases in cereals).

**.Present Employer and Position:**

Senior Scientist

Danish Institute of Agricultural Sciences  
Department of Crop Protection, Research Centre Flakkebjerg  
DK-4200 Slagelse

**List of Major Publications**

Nielsen, Bent J. and Jørgensen, L.N. (1994). Control of Common bunt (*Tilletia tritici* (DC) Tull) in Denmark. In Seed Treatment: Progress and Prospects, BCPC Monograph No. 57, ed. Trevor Martin: 47-52.

Skou, J.P., Nielsen, B.J. and Haahr, V. (1994). Evaluation and Importance of Genetic Resistance to Leaf Stripe in Western European Barleys. Acta Agric. Scand., Sect. B, Soil and Plant Sci 44, 98-106.

Nielsen, B. J. and Scheel, C. S. 1997 Production of quality cereal seed in Denmark. Proceedings of the ISTA Pre-Congress Seminar on Seed Pathology, ISTA, Zürich, 11-17

Nielsen, B. J., Borgen, A., Nielsen, G. C. & Scheel, C. 1998. Strategies for controlling seed borne diseases in cereals and possibilities for fungicide seed treatments. The 1998 Brighton Conference - Pest and Diseases, vol. 3: 893-900.

Nielsen, B. J., Nielsen, G. C., Pedersen, J. B. og Tersbøl, M. 1999 Possibilities for production of healthy organic seeds. 16th Danish Plant Protection Conference, 1999 (in Danish). DJF Rapport nr. 10 (1999) 29-40.

Nielsen, B. J., Christiansen, S. og Bagge, J. O. 1999 New resistance against seed borne diseases in cereals. 16<sup>th</sup> Danish Plant Protection Conference, 1999 (in Danish). DJF Rapport nr. 10 (1999) 149-160.

Nielsen, B. J., 2000. Bekæmpelse af spiringsfusariose i korn. 17. Danske Planteværns-konference, DJF rapport nr. 24, 185-195.

Nielsen, B. J., Christiansen, S. 2000. Resistance against seed borne diseases in Danish wheat and barley varieties. Proceedings from the Third Annual Meeting in the Danish Cereal Network, Danish Institute of Agricultural Sciences: 8-10.

Nielsen, B. J., Borgen, A., Kristensen, L. 2000. Control of seed borne diseases in production of organic cereals. The BCPC Brighton Conference - Pest & Diseases 2000 (1), 171-176.

Nielsen, B. J. 2001. Control of soil-borne common bunt (*Tilletia tritici*) by seed treatment. Proceedings from BCPC Symposium No. 76: "Seed Treatment: Challenges & Opportunities", eds. A. J. Biddle. BCPC, Farnham, 263-266.

Borgen, A. & Nielsen B. J. 2001 Effect of seed treatment with acetic acid for control of seed borne diseases. Proceedings from BCPC Symposium No. 76: "Seed Treatment: Challenges & Opportunities", eds. A. J. Biddle. BCPC, Farnham, 135-140.

**Curriculum Vitae** Anders Borgen

**Year of Birth:** 5-10-1960, Denmark

**Education:**

Ph.D. in Agronomy, KVL 2000. Thesis: Common bunt in wheat – a challenge for the principles of ecological plant protection

M.Sc. in agronomy. KVL, 1992

Basic course for farmers, 1983

Bachelor of philosophy, University of Odense, 1983.

**Key Qualifications:**

The experience of Anders Borgen is organic farming with special attention to problems related to seed production. Research has included studies of epidemiology and plant protection of seed borne pathogens. Besides Anders Borgen has studied the basic principles of ecological agriculture and been involved in the development of the organic sector during 20 years.

**Present Employment and Position:**

Expert in organic agriculture and seed production, Danagro Advisors a/s,

**List of Major Publications**

**Kristensen, L. and A. Borgen 2001:** Reduction of spore spread of common bunt (*Tilletia tritici*) via combining equipment. Accepted by: Biological Agriculture and Horticulture **19**:9-18,

**Borgen, A. & Nielsen B. J. 2001:** Effect of seed treatment with acetic acid for control of seed borne diseases. Proceedings from BCPC Symposium No. 76: "Seed Treatment: Challenges & Opportunities", eds. A. J. Biddle. BCPC, Farnham, 135-140.

**Borgen, A and L. Kristensen 2001.** Use of mustard flour and milk powder to control common bunt (*Tilletia tritici*) in wheat and stem smut (*Urocystis occulta*) in rye in organic agriculture. In: Seed treatment - challenges and opportunities (ed. A.J.Biddle) p. 141-150

**Borgen, A.:** Hvedens stinkbrand - en udfordring for principperne for økologisk jordbrug. Ph.D.-Thesis. ISBN 87-988060-9. 136 pages.

**Borgen, A. and M. Davanlou 2000:** Biological control of common bunt (*Tilletia tritici*) in organic farming. Journal of Crop Production **3**(5):159-174. Also published in: "Nature Farming and Microbial Applications", Ed: H.L. Xu , J. F. Parr and H. Umemura. Haworth Press Inc., New York. ISBN: 1-56022-082-1 / ISBN:1-56022-083-X , side 159-174.

**Borgen, A. 2000:** Perennial survival of common bunt (*Tilletia tritici*) in soil under modern farming practice. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz. **107**(2):182-188

**Curriculum Vitae**                      **Annemarie Fejer Justesen (AFJ)**

**Year of Birth:**                      15-06-1967, Denmark

**Education:**

Name of Institution:                      The Royal Veterinary and Agricultural University and Risø National Laboratory  
Degree or Diploma:                      M. Sc. (Cand. agro; genetics, 1991 ) Ph.D. (molecular genetics, 1995)

**Key Qualifications:**

Research area:

Molecular Plant Pathology, especially the molecular aspects of interactions between plant pathogenic fungi and their host plants, both on the organism and population level: Development of DNA-markers for analysis of genetic variation and population dynamics of pathogen populations, phylogenetic studies, and development of DNA-based methods for detection and diagnosis. Techniques such as AFLP, RFLP, RAPD, UP-PCR and DNA-sequencing are used. Are currently working with PCR-based detection of *Puccinia striiformis* f.sp. *tritici* clones (Funded by Danish Directorate for Development in Food, Agricultural and Fisheries, 1997-2001) and development of DNA-based method for detection of barley leaf stripe (*Pyrenophora graminea*) in the project 'Seeds for Organic Farming' (Funded by Danish Directorate for Development in Food, Agricultural and Fisheries (1999-2000)

**Present Employer and Position:**

Scientist

Danish Institute of Agricultural Sciences  
Department of Crop Protection  
Research Centre Flakkebjerg  
Forsøgsvej 1  
DK 4200 Slagelse

**List of Major Publications**

Justesen A, Somerville S, Christiansen SK and Giese H. (1996) Isolation and Characterization of two novel genes expressed in germinating conidia of the obligate biotroph *Erysiphe graminis* f.sp. *hordei*. GENE 170: 131-135

Justesen AF, Jespersen HM and Welinder KG. (1998) Analysis of two incompletely spliced Arabidopsis cDNAs encoding novel types of peroxidase. Biochimica et Biophysica Acta, 1443, 149-154

Christiansen SK, Justesen AF and Giese H. (1997) Cloning and characterisation of the *Erysiphe graminis* f.sp. *hordei* glyceraldehyde-3-phosphate dehydrogenase gene. Curr Genet 31:525-529

Nielsen K, Justesen AF, Jensen DF, Yohalem DS. (2001) Molecular variability of Botrytis spp. causing onion neck rot. Phytopathology, accepted.

Justesen AF and Hovmøller MS. (1999). Virulens- and DNA-variation in wheat yellow rust. 16<sup>th</sup> Danish Plant Protection Conference, Crop protection in organic farming, Pests and Diseases, DJF rapport Markbrug nr. 10.

Hovmøller MS and Justesen AF. (2000) Evolution in Populations of *Puccinia striiformis* f.sp. *tritici* in Northwest Europe. Acta Phytopathologica et Entomologica Hungarica 35 (1-4), pp299-306.

Hovmøller MS and Justesen AF. (2001) Molecular markers in wheat yellow rust: (I) Development and discussion of their potential use in survey studies. First Regional Yellow Rust Conference for Central & West Asia and North America, 8-14 May 2001, Karaj, Iran.

Hovmøller MS and Justesen AF. (2001) Molecular markers in wheat yellow rust: (II) Long-distance migration in Northwest Europe. First Regional Yellow Rust Conference for Central & West Asia and North America, 8-14 May 2001, Karaj, Iran.

**Curriculum Vitae**                      **Christiane Scheel (CS)****Name:**                                      Christiane Scheel**Year of Birth:**                            28-07-1953, Denmark**Education:**

Name of Institution:                      The Royal Danish Agricultural University, 1978

Degree or Diploma:                      M.Sc. (Cand. hort.; plant pathology)

**Key Qualifications:**

The experience of Christiane Scheel is diagnostics in seed health testing and in pests and diseases in ornamentals, fruit- and vegetable plants with special reference to plant quarantine.

CS is a member of The Plant Disease Committee in The International Seed Testing Association (ISTA).

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**List of Major Publications**

Nielsen, B. J. and Scheel, C. (1997) Production of quality cereal seed in Denmark. Proceedings of the ISTA Pre-Congress Seminar on Seed Pathology, ISTA, Zürich, 11-17.

Brodal, G., Kortema, H., Scheel C. and Sperlingsson K. (1997) Recent Problems with Loose Smut in Oats and Common Bunt in Wheat in the Nordic Countries. Seed Health Testing - Progress Towards the 21st Century, CAB International, pp. 11 - 20.

Scheel C. (1997) Review on Policy Developments with Regard to Seed Health testing and Seed Treatment in the Nordic Countries with Special Reference to Denmark. Seed Health Testing - Progress Towards the 21st Century, CAB International, pp. 107 - 114.

Nielsen, B. J., Borgen, A., Nielsen, G.C. & Scheel, C. (1998) Strategies for controlling seed borne diseases in cereals and possibilities for fungicide seed treatments. The 1998 Brighton Conference - Pest and Diseases, vol. 3: 893-900.

Scheel, C. og Nielsen G.C. (2000) Seed borne fungi of importance in pea. 17<sup>th</sup> Danish Plant Protection Conference. (in Danish) DJF Rapport Markbrug nr. 24. pp 157 – 163.

**Appendix 2: Budget details for the individual work packages****WPI THRESHOLDS:**

<b>SUM</b>	2001	2002	2003	2004	2005	SUM
Months (scientific)	2	8	8	6	6	30
Months (technical)	3	15	17	17	8	60
Salary (scientific)	74.000	303.850	312.966	241.493	248.737	1.181.046
Salary (technical)	63.000	324.450	378.741	390.104	189.085	1.345.380
Operation – equipment	0	0	0	0	0	0
Operation - other	10.000	60.000	105.000	110.000	35.000	320.000
Overhead	29.400	145.076	166.980	156.187	102.668	600.311
<b>Total</b>	<b>176.400</b>	<b>833.376</b>	<b>963.687</b>	<b>897.783</b>	<b>575.491</b>	<b>3.446.737</b>
	176.400	833.376	963.687	897.783	575.491	3.446.737

<b>DJF</b>	2001	2002	2003	2004	2005	
Months (scientific)	1	6	6	4	4	21
Months (technical)	3	15	15	15	6	54
Salary (scientific)	37.000	228.660	235.520	161.724	166.575	829.479
Salary (technical)	63.000	324.450	334.184	344.209	141.814	1.207.657
Operation – equipment	0	0	0	0	0	0
Operation - other	10.000	30.000	30.000	30.000	15.000	115.000
Overhead	22.000	116.622	119.941	107.187	64.678	430.427
<b>Total</b>	<b>132.000</b>	<b>699.732</b>	<b>719.644</b>	<b>643.119</b>	<b>388.067</b>	<b>2.582.562</b>

<b>LR (GCN)</b>	2001	2002	2003	2004	2005	
Months (scientific)	1	1	1	1	1	5
Months (technical)	0	0	0	0	0	0
Salary (scientific)	37.000	38.110	39.253	40.431	41.644	196.438
Salary (technical)	0	0	0	0	0	0
Operation – equipment	0	0	0	0	0	0
Operation - other	0	30.000	75.000	80.000	20.000	205.000
Overhead	7.400	13.622	22.851	24.086	12.329	80.288
<b>Total</b>	<b>44.400</b>	<b>81.732</b>	<b>137.104</b>	<b>144.517</b>	<b>73.973</b>	<b>481.726</b>

<b>PD</b>	2001	2002	2003	2004	2005	
Months (scientific)	0	0	0	0	0	0
Months (technical)	0	0	2	2	2	6
Salary (scientific)	0	0	0	0	0	0
Salary (technical)	0	0	44.558	45.895	47.271	137.724
Operation – equipment	0	0	0	0	0	0
Operation - other	0	0	0	0	0	0
Overhead	0	0	8.912	9.179	9.454	27.545
<b>Total</b>	<b>0</b>	<b>0</b>	<b>53.469</b>	<b>55.073</b>	<b>56.726</b>	<b>165.268</b>

<b>Danagro (AB)</b>	2001	2002	2003	2004	2005	
Months (scientific)	0	1	1	1	1	4
Months (technical)	0	0	0	0	0	0
Salary (scientific)	0	37.080	38.192	39.338	40.518	155.129
Salary (technical)	0	0	0	0	0	0
Operation – equipment	0	0	0	0	0	0
Operation - other	0	0	0	0	0	0
Overhead	0	14.832	15.277	15.735	16.207	62.052
<b>Total</b>	<b>0</b>	<b>51.912</b>	<b>53.469</b>	<b>55.073</b>	<b>56.726</b>	<b>217.180</b>

**WP2: NEW DIAGNOSTIC METHODS**

<b>SUM</b>	2001	2002	2003	2004	2005	
Months (scientific)	5	14	14	10	7	50
Months (technical)	1	12	11	7	3	34
Salary (scientific)	150.000	437.400	455.562	337.553	244.457	1.624.971
Salary (technical)	21.000	259.560	245.068	160.631	70.907	757.166
Operation – equipme	0	0	0	0	0	0
Operation - other	20.000	85.000	85.000	65.000	30.000	285.000
Overhead	38.200	156.392	157.126	112.637	69.073	533.427
<b>Total</b>	<b>229.200</b>	<b>938.352</b>	<b>942.756</b>	<b>675.820</b>	<b>414.436</b>	<b>3.200.565</b>
	229.200	938.352	942.756	675.820	414.436	3.200.565

<b>DJF</b>	2001	2002	2003	2004	2005	
<b>AFJ</b>	1	6	7	4	3	
<b>NN</b>	1	2	1	1	0	
Months (scientific)	2	8	8	5	3	26
Months (technical)	1	10	9	5	3	28
Salary (scientific)	60.000	252.000	264.600	173.644	109.396	859.639
Salary (technical)	21.000	216.300	200.510	114.736	70.907	623.453
Operation – equipme	0	0	0	0	0	0
Operation - other	10.000	60.000	60.000	50.000	20.000	200.000
Overhead	18.200	105.660	105.022	67.676	40.061	336.619
<b>Total</b>	<b>109.200</b>	<b>633.960</b>	<b>630.132</b>	<b>406.056</b>	<b>240.363</b>	<b>2.019.711</b>

<b>PD</b>	2001	2002	2003	2004	2005	
<b>CS</b>	1	1	1	1	1	
<b>NN</b>	2	5	5	4	3	
Months (scientific)	3	6	6	5	4	24
Months (technical)		2	2	2	0	6
Salary (scientific)	90.000	185.400	190.962	163.909	135.061	765.332
Salary (technical)	0	43.260	44.558	45.895	0	133.712
Operation – equipme	0	0	0	0	0	0
Operation - other	10.000	25.000	25.000	15.000	10.000	85.000
Overhead	20.000	50.732	52.104	44.961	29.012	196.809
<b>Total</b>	<b>120.000</b>	<b>304.392</b>	<b>312.624</b>	<b>269.764</b>	<b>174.073</b>	<b>1.180.853</b>

**WP3: CULTURAL PRACTICE AND REGULATION/CONTROL.**

<b>SUM</b>	2001	2002	2003	2004	2005	
Months (sci)	1,5	8,5	8,5	7,5	4,5	30,5
Months (tec)	1	9	11	9	4	34
Salary (sci)	55.500	318.785	328.349	297.768	184.021	1.184.422
Salary (tec)	21.000	194.670	245.068	206.525	94.543	761.806
Operation -	0	0	0	0	0	0
Operation -	15.000	85.000	135.000	123.000	35.000	393.000
Overhead	16.300	150.771	173.876	160.797	85.024	586.767
<b>Total</b>	<b>107.800</b>	<b>749.226</b>	<b>882.292</b>	<b>788.090</b>	<b>398.587</b>	<b>2.925.996</b>
	107.800	749.226	882.292	788.090	398.587	2.925.996

<b>DJF</b>	2001	2002	2003	2004	2005	
<b>BJN</b>	1	2	2	2	1	
<b>LB</b>						
<b>EFJ</b>		1	1			
Months (sci)	1	3	3	2	1	10
Flakkebjerg	1	3	5	5	3	
Bygholm		1	1			
Months (tec)	1	4	6	5	3	19
Salary (sci)	37.000	114.330	117.760	80.862	41.644	391.596
Salary (tec)	21.000	86.520	133.673	114.736	70.907	426.837
Operation -	0	0	0	0	0	0
Flakkebjerg	5.000	20.000	20.000	20.000	20.000	
Bygholm		10.000	10.000			
Operation -	5.000	30.000	25.000	18.000	10.000	88.000
Overhead	12.600	46.170	55.287	42.720	24.510	181.286
<b>Total</b>	<b>75.600</b>	<b>277.020</b>	<b>331.720</b>	<b>256.318</b>	<b>147.061</b>	<b>1.087.719</b>

<b>KVL</b>	2001	2002	2003	2004	2005	
Months (sci)	0	0	0	0	0	0
Months (tec)	0	5	5	4	1	15
Salary (sci)	0	0	0	0	0	0
Salary (tec)	0	108.150	111.395	91.789	23.636	334.969
Operation -	0	0	0	0	0	0
Operation -	0	5.000	5.000	5.000	5.000	20.000
Overhead	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>

<b>LR (GCN)</b>	2001	2002	2003	2004	2005	
Months (sci)	0,5	0,5	0,5	0,5	0,5	2,5
Months (tec)	0,0	0,0	0,0	0,0	0,0	0,0
Salary (sci)	18.500	19.055	19.627	20.215	20.822	98.219
Salary (tec)	0	0	0	0	0	0
Operation -	0	0	0	0	0	0
Operation -	0	20.000	75.000	80.000	10.000	185.000
Overhead	3.700	7.811	18.925	20.043	6.164	56.644
<b>Total</b>	<b>22.200</b>	<b>46.866</b>	<b>113.552</b>	<b>120.259</b>	<b>36.986</b>	<b>339.863</b>

<b>PD</b>	2001	2002	2003	2004	2005	
Months (sci)	0	0	0	0	0	0
Months (tec)	0	0	0	0	0	0
Salary (sci)	0	0	0	0	0	0
Salary (tec)	0	0	0	0	0	0
Operation -	0	0	0	0	0	0
Operation -	0	0	0	0	0	0
Overhead	0	0	0	0	0	0
<b>Total</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>

<b>Danagro (</b>	2001	2002	2003	2004	2005	
Months (sci)	0	5	5	5	3	18
Months (technical)						0
Salary (sci)	0	185.400	190.962	196.691	121.555	694.608
Salary (tec)	0	0	0	0	0	0
Operation -	0	0	0	0	0	0
Operation -	10.000	30.000	30.000	20.000	10.000	100.000
Overhead	0	74.160	76.385	78.676	48.622	277.843
<b>Total</b>	<b>10.000</b>	<b>289.560</b>	<b>297.347</b>	<b>295.367</b>	<b>180.177</b>	<b>1.072.451</b>

