Midterm Status Report 2002 and Application for Continuation in 2003

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

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1. Research program

Research in organic farming 2000-2005 (DARCOF II)

Project title and number

VI.1 Healthy seed for organic production of cereals and legumes (ORGSEED)

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3. Head of project

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

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4. Participating institutes

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

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

Danish Plant Directorate (PD)
Skovbrynet 20
2800 Lyngby

Scanagri Denmark A/S (SD)
Vester Farimagsgade 6, 5th
DK-1606 Copenhagen

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

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

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5. Other project staff

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

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

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

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

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

6. Project period (month, year)

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

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

A. Project summary

Seed borne diseases can cause serious problems in production of cereals and legumes. In conventional agriculture these diseases are intensively controlled by seed treatment, but this is not an option in organic agriculture. 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 the EU-regulation concerning the use of conventional seed will be changed, and the access to use conventional seed likely more restricted.

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

Seed health analysis on seed is made by methods normally used for survey of seed health status in propagation of seeds. The methods are in general slow and depend in some cases on subjective evaluation of the expression of the diseases. Resent 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 to have faster techniques, if the analysis shall be used as a basis for rejection of 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 de-

veloped 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.

Change in project staff 2003 for DIAS (Flakkebjerg):
Lars Bødker (LB) is on leave for other projects in 2003 and 2004. Hanne Wolffhechel (HW) will replace LB in the project.
Hans Pinnschmidt (HP) will from 2003 be a part of the project (WP1 and WP3).

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

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

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

B. Objectives and expected achievements

The overall objective of the programme is to contribute to production of healthy, disease-free organic seeds of cereals and legumes.

The principal aim of the project is to reduce the amount of organic produced seed that need to be discarded as organic seed due to unacceptable infection by seed borne diseases.

The target is therefore to improve methods for seed analysis and to adjust the current values for discarding of seed to organic conditions. This will help to optimise the pro-

duction, 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 to leaf stripe/net blotch and in peas due to seed and foot rot.

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

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

i) Enables a more fast and precise detection (less variation in results)
ii) Reduces the amount of unnecessarily discarded seed
iii) Enables to discriminate between pathogens that previously could not be distinguished and identified, e.g. different Fusarium species and glume blotch in wheat together with leaf stripe and net blotch in barley.

3. Regulation and control measures in the organic production.

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

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

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

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

C. Midterm results and progress

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

Work package 1: Threshold values
Objectives: More precise threshold values for organic produced seeds to avoid unnecessary discharge of valuable seeds.

Task 1 Seedling blight in wheat
Seed lots have been collected with different infection levels of *Fusarium culmorum*, *Michrodochium nivale* and *Septoria nodorum* and 3 field trials have been established at Flakkebjerg autumn 2001 at two sowing depths. The trials have been harvested august 2002 and analyse for expression of the diseases under a set of different conditions (soil temperature, sowing depth, soil type).

New plans under task 1: Autumn 2002 trials are under preparation with seed lots infected with Fusarium culmorum. Different infection levels will be established by mixing healthy and diseased seed lots and the trials will be sown at 3 different times. Seeds from different origin and with different infection levels of Fusarium spp. will also be sown and evaluated for seedling blight in the field.

Task 2 Net blotch in barley
The aim of the experiments with net blotch is 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 were therefore established where infections from plant debris in the soil were minimised (no barley in previous crops). Seeds of spring barley heavily infected with net blotch (73 % infection) were used in 2 split plot trials in 2002 at the research stations Flakkebjerg and Borris. Healthy and infected seeds were mixed to establish seeds with different infection levels. The seeds were sown at 3 different times in fields were barley had not been grown for many years. The results showed that net blotch were introduced to the field plots by the seed borne phase and that the disease quickly spread from the small primary infections on the first leaves to the other leaves in the barley crop. The field plots were heavily infected by net blotch during the summer.

New plans under task 2: The trial with different infection levels and sowing conditions will be repeated in 2003.

Task 3 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 %. In the spring 2002 split plot field trial was established at Research Station Flakkebjerg in spring barley heavily infected with leaf stripe. Healthy and infected seeds were mixed to establish seeds with different infection levels and the seeds were sown at 2 sowing depths and at 3 different times. The attack of leaf stripe in the different field plots were moderate to severe depending on the germination conditions and shows the expression of the diseases under a set of different conditions (soil temperature, sowing depth, soil type).

New plans under task 3: The importance of variety resistance against leaf stripe for the actual damage threshold will be examined (with WP 3.2). The trial with different infection levels and sowing conditions will be repeated in 2003. Practices like sowing time etc. will be integrated with corresponding trials in WP3.

Task 4 Seed and foot rot in peas
The aim in task 4 is to establish seed lots of pea with different infection levels and to perform field trials at different soil types to evaluate the relationship between seed infection and severity of disease expression as seed and foot rot.

In 2002 trials have been conducted in cooperation with local agricultural centres (LR) according to two experimental plans. The purpose of the first experimental plan is to examine if growing peas in combination with spring barley and will be explained later under WP 3.1

The purpose of the second plan is to measure the influence of seed borne infection of leaf and pod spot. The significance to the harvest year yield as well as to the leaf and pod spot on the harvested peas is examined. The effect is examined again in two types of varieties (one short and one longer) Five trials have been carried out, but for different reasons data on leaf and pod spot on the harvested seeds are only available from two trials. Data processing is not yet finished. Seed samples have recently been sent to the Plant Directorate for analyses. The results will be published in ”Annual Report of the National Field Trials 2002” in late December.

Pure cultures of the 3 pathogens involved in the disease complex have been established under WP 2. They have been supplied to WP 1 for field experiment purpose.

New plans under task 3: The importance of variety resistance for the level of seed infection will be examined.

Task 5 Common bunt in wheat
Pathogen populations have been collected as infection material to be used in field trials with different infection levels 2002.

New plans under task 5: Wheat lots are infected artificially with various amounts of bunt in order to establish a connection between spores on the kernels and subsequent at-

tacks 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 sowing times. The importance of variety resistance for the actual damage threshold will also be examined. The trial will start autumn 2002 at Research Centre Flakkebjerg with 2 varieties (susceptible Herzog and moderate resistant Penta), 5 infection levels of bunt and 3 sowing times.

Task 6 Tan spot (*Drechslera tritici-repentis*) in winter wheat
Tan spot ("hvedebladplet", "DTR") is a relative new disease in wheat in Denmark and have occurred quite frequently the last years. Especially in 2002 many fields were attacked by the disease with extending spotting of the leaves. Normally the disease is spread from plant debris on the soil and if wheat is grown quite regularly, this is the most important transmission. But tan spot can also be seed borne and can –like *Leptospharia nodorum*- be introduced in to a new area by the seed borne phase. To investigate the importance of the seed borne phase it was decided to make a field trial where wheat with different infection levels were used. The field plots will be monitored for primary infections and multiplication of the disease.

Task 7 Glume blotch/seedling blight in wheat
Glume blotch has a lower priority in the project and experiments will first start in later part of the project period (2003).

New plans under task 6: Lephtospharia nodorum will be multiplied under field conditions in the summer 2003 to establish seed lots with natural infections. In the autumn 2002 only a small field trial in winter wheat will be performed with seeds artificially inoculated with *Lephtospharia nodorum*. The trial will be assessed for later multiplication and spread of the disease in the field plots. Autumn 2003 trials will be established with different infection levels.

Task 8 Models
Models describing the connection between seed infection, field infection, and subsequent infection in the harvested yield under different conditions will be developed later in the project when more data are available. The models will include projecting over several seed propagation generations (pre basic-C2) and years and to evaluate the long-term risk of multiplication and consequences of reduction factors.

Work package 2: Diagnostic methods
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*). The General working method is 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 developing or implementing PCR-based or serological methods. A real-time PCR-machine is 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 has 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 spp. and Microdochium nivale
Qualitative PCR methods for *Fusarium culmorum*, *Fusarium avenaceum*, *Fusarium poae* and *Fusarium graminearum* have been identified and implemented. These methods will be used during the whole project period to assist in the diagnostics of *Fusarium* species when necessary.

Samples of commercial winter wheat seed lots from the 2000-2001 seasons have been examined by traditional seed health testing methods. Testing continues in 2002 to be followed by isolation and identification of fungal species associated with the seedling blight symptom in wheat. Seed samples of value for the project will be selected and used as reference seed samples for future PCR testing. Isolates of *Microdochium nivale* as well as *Fusarium* spp. are being used in the implementation of PCR methods. Based on experiences from previous years as well as literature studies it has been decided to give the highest priority to the development of a quantitative PCR method for *Microdochium nivale*. A real-time PCR-machine (ABI Sequence detection system 7000 HT) for quantitative PCR has been installed in the laboratory and development of a quantitative PCR assay for *Microdochium nivale* has been started. At the moment the specificity of the assay towards *M. nivale* is being tested on a range of *M. nivale* isolates, *Fusarium* isolates as well as other fungal species which may be present in wheat seed lots.

New plans under task 1: Development of the quantitative PCR method specific for *M. nivale* will continue in 2003. Implementation and optimisation of a suitable method for DNA extraction from wheat seed will be started. Test of seed samples from the season 2001-2002 with traditional methods will continue and different fungal species will be isolated to be used in the development of the PCR method.

Task 2: Glume blotch (*Leptosphaeria nodorum*)
Identification of PCR- or ELISA test will start 2004.Samples of commercial wheat seed from 2000, 2001 and 2002 are examined by traditional methods. Seed samples of value for the project will be selected and used as reference seed samples for future PCR and/or ELISA testing.

Task 3: Barley net blotch and leaf stripe
The quantitative method for *Pyrenophora* spp (both *P. teres* and *P. graminea*), described by Bates et al. (Molecular Plant Pathology, 2001, 2, 49-57) has been tested in the ABI Sequence detection system 7000 HT on a range of different isolates of *P. graminea*, *P. teres*, *Cochliobolus sativus*, *Fusarium* spp, *Aspergillus* spp, *Alternaria* spp. The method was found not to be specific for *Pyrenophora* spp. under the conditions used in the ABI Sequence detection system as also *C. sativus* was detected by this method. The implementation of this specific test in the ABI Sequence detection system will not be possible without extensive development. Therefore a higher priority has been given to the development of a PCR method which is specific for *P. graminea* as it is more important to be able to distinguish *P. graminea* from *P. teres* . To-day the only alternative to this test is a time-consuming green house test. In this work we are taking advantage of the results from a a previous project 'Seeds for Organic Farming' (Funded by DFFE, 1999-2001), where a unique DNA sequence from *P. graminea* was identified and sequenced. PCR primers for a quantitative PCR assay for *P. graminea* have been designed and a quantitative PCR method is being developed. The method has been tested on a few selected seed lots from WP1 as well as one selected commercial seed lot. It has been possible to detect *P. graminea* in a seed lot with 5% *P. graminea*, and it seems likely that it should be possible to detect less infection.

Samples from the 2001-2002 season are examined by traditional methods as well as by a qualitative PCR procedure. Different methods for DNA extraction from seed lots is being tested. At the moment the most efficient method is grinding of seeds in a 'coffee mill' followed by grinding with mortar and pestle in liquid nitrogen and then DNA extraction using a DNA kit from QIAGEN.

New plans under task 2: The quantitative PCR method for *P. graminea* is now awaiting to be tested on a range of different seed lots when these have been examined and the percentage of *P. graminea* infection has been determined by traditional methods. The detection limit of the method has to be determined either by construction of seed lots with less than 5% infection or by using commercial seed lots if some will be available. The optimisation of the DNA extraction method from barley seed will continue.

Work package 3: Regulation and control measures.

Objectives: Develop and implement different control measures applicable for organic farming. The work packages is divided in to two sub work packages:

3.1. Direct and indirect control measures
3.2. Host resistance

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

This work package started in the beginning of year 2002.

Task 1. Preventing cropping methods.
The aim in task 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 n 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.

In 2002 trials have been conducted in cooperation with local agricultural centres (LR) . The purpose of the experimental plan is to examine if growing peas in combination with spring barley can reduce the attack of leaf and pod spot. The trial plan includes two types of pea varieties (one short stemmed and one long stemmed variety). Different mixing ratios between spring barley and pea are used in the trial plots. Five trials have been conducted, that seem to be well carried out. The data processing is not yet finished. Seed samples will be analysed at the Danish Plant Directorate. The results will be published in the ”Annual Report of the National Field Trials 2002” in late December.

Experiments on the effect of harvest time on the development of seed borne diseases has been established at Højbakkegård. Field trials have been conducted in pea and barley. The fields are harvested, but seed is not yet analysed for infection frequency.

New plans under task 1: Experiments with the effect of harvest time will be repeated in 2003. Detailed planning of this will be made when the results from this year are examined.

Task 2. Multiplication of diseases in the propagation process from C1 to ware seed.
Seed lots for sale have been assed for seed borne diseases. Seed lots below the current threshold level are sown commercially for propagation purpose. Seed lots above the threshold level for pea disease and net blotch are sown at Højbakkegård, Bygholm and Flakkebjerg, and seed samples are collected for seed analysis.

New plans under task 2: No new plans

Task 3. Seed cleaning.
A brush cleaner has been redesigned at Westrup a/s. This equipment has been tested, but the improvement in reduction of bunt spores was insignificant. New ajustment of the equipment will be made later in the winter 2002-2003 Seed lots infected with loose smut and net blotch have been sorted according to seed size. The result showed that seed above 2.8 mm had significant lower infection of Ustilago nuda than seed below 2.8 mm. The result of the effect of seed separation on net blotch is still not available.

Seven wheat seed lots from the harvest 2001 were sorted in three different kernel sizes. Then the non-sorted and the sorted samples were analysed for *Stagonospora nodorum*

and *Fusarium spp*. On two of the samples the attack was so severe that estimation of the Fusarium attacks was not possible. In the other samples the attacks were relatively mild, and the effect of sorting was small. Statistics of the results has not yet been made.

New plans under task 3: Further tests on seed separation will be made later this year.

Task 4. Heat treatment.
The drum dryer was tested in the spring 2002 for effect on pea disease and netblotch with different temperatures (seed temperature 41-99 °C) and different durations (3-8 minutes). The effect on pea disease was limited, and no combinations of parameters were able to reduce the infection level below the threshold level without negative effect on germination ability. One combination of time and temperature reduced the level of net-blotch without damaging the germination ability. The first series of treatments was conducted on dry seed whereas the equipment is developed for seed drying. It is therefore the hope that the effect will be significantly improved when moist seed are used, as will be the case in practice. This is recently done, but the results not yet available.

The effect of traditional hot water treatment on the infection of seed borne diseases in cereals are well known, but the method has not previously been tested for effect on seed on foot rot in pea. This has been tested, and the result showed that both pathogen and pea seed are very heat resistant compared to cereal seed. No treatments were able to reduce the disease frequency below threshold level without negative effect on germination ability.

As for hot water treatment, the effect of moist air on the infection of seed borne diseases in cereals are well known, but the method has not previously been tested for effect on seed on foot rot in pea. This has been tested, and the result showed promising effect compared with other methods. One treatment was able to reduce the disease frequency below the threshold level without negative effect on germination ability.

To combine steam and ultra sound as heat treatment has not previously been tested for the effect on seed infections. This is now tested for the effect on Fusarium, glume blotch, net blotch, leaf stripe, common bunt and seed rot in pea. The equipment was tested at only one temperature (100 °C) with a range of different durations (0-120 seconds). The effect on glume blotch and seed rot in pea was insignificant, whereas the other diseases were reduced by 40-90%. On this background it is concluded that this method has promising potential for development compared with other heat treatments, but that is not the purpose of this project to do so. It has therefore been decided to search for funds for a separate project on this particular method.

New plans under task 4: Further trials with drum drying will be conducted. On the basis of the results obtained this year new tests using the most promising drying regimes will be made during harvest 2003.

WP 3.2. Resistance against seed borne diseases.
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 "Pesticidhandlingsplan 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. The work will start 2003.

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

In spring 2002, isolates of *Phoma medicaginis var pinodella, Ascochyta pisi* and *Mycosphaerella pinodes* received from Plant Directorate were tested for pathogenicity in greenhouse study on a susceptible variety. These isolates showed only to be slightly pathogenic under these artificial conditions. The most pathogenic isolates were used in the production of large quantities of artificial inoculum (infected barley grain) for field inoculation. Ten different varieties with a large variation in tolerance against leaf and pod spot caused by *Ascochyta* spp. were obtained from the Danish breeders. These ten varieties were sown in three replicate field trials at Research Centre Flakkebjerg and dual inoculated with both infected barley grain and a slurry of conidiospores of *P. medicaginis var pinodella, A. pisi* and *M. pinodes,* respectively. Despite sprinkler irrigation were installed in all three field trials, no leaf or pod spot caused by *Ascochyta* spp. could be seen in any of the trials. All seeds are harvest and seed from the controls will be tested for occurrence of *Ascochyta* spp

New plans under task 2:
In 2003, the field trials will be repeated. More isolates will be screened in greenhouse in winter and infected barley seeds will be distributed on soil just after pea emergence. Natural infected pea straw will be applied in one of the three trials at flowering stage.

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 system 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 2002 financed by the project "Pesticidhandlingsplan II". In 2003-2005 the test will be a part of the new project which will ensure a continuos 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.

Work package 4: Integrated strategies and dissemination of information

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

Task 1: Integrated strategies.
During the project period the different control strategies and models will be integrated and combined with different cropping practices. The aim is to combine methods with moderate to good efficacy to get a combined system with over all good and in practice acceptable effect. This could for example be combining heat treatment or seed cleaning methods with varieties with moderate resistance. The effect of the soil temperature, harvest times and plant density on the development of the seed borne diseases will also be used.

Task 2: Dissemination of information
Information will be delivered via Danish Advisory Service to organic farmers through leaflets, farmer's journals and other publications. Information on seed borne diseases, thresholds, control and regulation methods will also be published in a grower manual to organic farmers

It is discussed to make a special new web site for organic seed under the information platform "Planteinfo" (www.planteinfo.dk) where informations on plant growing is delivered to farmers and extension service by LR and DIAS. It is, however decided in another project at LR to make a special new web site for organic seed in 2003 and this will be operating from 2004. Information, recommendations etc. from ORGSEED could be placed here. Until 2004 the information under ORGSEED could then be published on "Landbrugsinfo" (www.lr.dk) where information on plant growing are delivered to farmers and extension service by LR.

Task 3: Official regulations
The project will be the basis for making new threshold values that can be implemented in the official system as recommended thresholds. These thresholds will be used for selecting available organic seed and will be a (voluntarily) standard used by seed producers.

After the harvest 2001 a questionnaire (LR) was sent to companies that grow organic seed. Information has been collected on pct rejected lots of the grain varieties and peas as a result of the seed borne diseases. The results are published in the information series published by the Danish Agricultural Advisory Centre "Planteavlsorientering" no. 09-532 of March 25, 2002.

Task 4: The methods developed in the project.
The methods that are developed and implemented in the project will be part of the official test for organic seed made by PD. Other laboratories in Denmark are also offering seed test and is the intention that seed testing procedures will be standardised and harmonised on the basis of the results obtained in this project. ISTA (The 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 im-

plementation 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

Work package 5: Project management

Project planning, co-ordination and reports.

Workshop on organic seed will be arranged in 2003/2004 (co-ordinated with other FØJO activities).

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

C.2 Fulfilment of deliverables and milestones

Deviations are to be further discussed in D

In the following are listed specific tasks for the different work packages with deliverables and milestones.

WP1: Threshold values
The work package is divided in 7 specific tasks: 1. Seedling blight in wheat 2. Leaf stripe in barley 3. Net blotch in barley 4. Seed and foot rot in peas 5. Common bunt in wheat 6. Glume blotch/seedling blight in wheat **7. Models**

De-liver-able, No	Deliverable title	Time schedule according to applica-tion	Devia-tions
1	Most important seedling blight pathogens identified within the Microdochium/Fusarium complex.	2003	
2	Seed lots established with different infection levels for use in WP1, WP2 and WP3.	2002/2003	
3	Information on disease multiplication/reduction in individual seed lots in the propagation process.	2004/2005	
4	Threshold for seedling blight adjusted to organic condition	2003/2005	

5	Threshold for seed borne Glume blotch adjusted to organic condition	2003/2005	
6	Threshold for leaf stripe adjusted to organic condition	2003/2005	
7	Threshold for net blotch adjusted to organic condition	2003/2005	
8	Threshold for seed and foot rot disease in pea adjusted to organic condition	2003/2005	
9	Models developed describing multiplication over years	2005	
10	New recommendations for threshold values to seed producers and farmers	2003/2005	
11	Results published in farmer's journals, leaflets, magazines, web-site etc. The results from the pea trials will be published in "Annual Report of the National Field Trials 2002" in late December.	2002-2005	
12	Results published in international publications.	2004/2005	

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

Milestones:
M1: Seed lots established with different infection levels
M2: Preliminary report quantifying relationship between disease intensity and yield loss
M3: Models describing multiplication and spread of diseases
M4: First adjustment of threshold
M5: Final threshold operational
M6: International publications

WP 2	**Diagnostic methods**

Task 1: Fusarium species and Microdochium nivale

Task 2: Glume blotch (*Leptosphaeria nodorum*)

Task 3: Barley net blotch and leaf stripe

De-liver-able, No	Deliverable title	Time schedule according to applica-tion	Devia-tions
13	Qualitative PCR-method for Fusarium spp. will be identi-fied and developed.	2003/2005	2002[1]
14	Priority to Fusarium spp for which a quantitative PCR-test should be developed will be given based on results from WP1	2003	2002/ 2003[2]
15	Quantitative method for Fusarium sp. will be tested on seed lots, produced in WP1, with known infection	2005	
16	Test for glume blotch will be identified	2004/2005	
17	Quantitative method for glume blotch will be tested on seed lots, produced in WP1, with known infection	2005	
18	Combination of methods for detection of seed borne dis-eases in wheat will be tested	2004/2005	
19	Quantitative method for Pyrenophora teres will be tested on seed lots, produced in WP1, with known infection.	2002	2002[3]
20	Quantitative method for Pyrenophora graminea will be tested on seed lots, produced in WP1, with known infec-tion	2002	2002/ 2003[4]

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

Milestones:
M7: Identification and test of new diagnostic method for selected *for Pyrenophora graminea*, *Fusarium* species and glume blotch (*Leptosphaeria nodorum*)
M8: Implementation of the new methods for routine practise
M9: First step against validation of new methods in ISTA
M10: Publications

WP 3. Regulation and control measures
The work packages is divided in to two sub work packages: 3.3. Direct and indirect control measures 3.4. Host resistance

WP 3.1: Control measures acceptable in the organic seed production

Task 1. Preventing cropping methods.
Task 2. Multiplication of diseases in the propagation process from C1 to ware seed.
Task 3. Seed cleaning.
3.1 Removal of small infected kernels.
3.2. Removal of bunt spores
Task 4. Heat treatment.

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

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

Milestones:
M11: Results with pea/barley mixtures.
M12: Results with cropping parameters on the development of epidemics of net blotch, Fusarium spp., glume blotch and seed and foot rot in pea.
M13: Seed lots tested by heat treatments and seed cleaning equipment and diagnosis of diseases status and seed vigour. Results will lead to recommendations for seed handling
M14: Publication phase

WP 3.2. : Screening for resistance

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

Deliv-erable, No	Deliverable title	Time schedule according to applica-tion	Devia-tions
26	Danish barley varieties with interest for organic produc-tion screened for resistance against leaf stripe.	2003/2005	
27	Danish pea varieties with interest for organic production screened for resistance against Phoma medicaginis	2003/2005	
28	Danish wheat varieties with interest for organic produc-tion screened for resistance against common bunt.	2003/2005	

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

Milestones:
M15: Relevant barley varieties screened for resistance against leaf stripe
M16: Relevant pea varieties screened for resistance against Phoma medicaginis.
M17: Relevant wheat varieties screened for resistance against bunt.

WP4: Integrated strategies and dissemination of information.			
Task 1: Integrated strategies **Task 2: Dissemination of information** **Task 3: Official regulations** Task 4: The methods developed in the project.			
Deliv-erable, No	Deliverable title	Time schedule according to applica-tion	Devia-tions
29	Integrated strategies with different control measures.	2004/2005	
30	Leaflets, journals etc. to organic farmers and seed pro-ducer's etc. with information on problems with seed borne diseases.	2002-2005	
31	Web-site with information on seed borne diseases, thresholds, recommendations etc.	2002	2003/04
32	Thresholds adopted as a standard in organic seed pro-duction of cereals and peas.	2003/2005	
	The results from the questionare to seed producers will be published in the information series published by the Danish Agricultural Advisory Centre "Planteavlsorientering" no. 09-532 of March 25, 2002.	2002	

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

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

Milestones:
M18: Project report October each project year.
M19: Final project report December 2005.
M20: Workshop October 2003.
M21: Final Workshop October 2005.

D. Description of deviations and subsequent adjustments of plans

WP 1 Thresholds

Tan spot of wheat have been included in the trials 2002/2003 as described under section C1, task 6.

WP2 Diagnostic methods

(the numbers refers to the table about deliverables)
1) It has been possible to implement qualitative PCR methods for *F. culmorum*, *F. avenaceum*, *F. poae* and *F. graminearum*. These methods cannot directly be converted into quantitative PCR methods. However, they can be used in the present project to assist in diagnostics of *Fusarium* species.

2) Based on the present project it has not been possible to give priority to *Fusarium* species for which a quantitative PCR method is most needed. However based on previous experience from other projects as well as literature studies it has been decided first to develop a quantitative PCR method for *Microdochium nivale*.

3) A PCR method for the quantification of *Pyrenophora* spp. have previously been described by Bates *et al*., (Molecular Plant Pathology, 2001, 2, 49-57). However, this method cannot distinguish between the species *P. teres* causing net blotch and *P. graminea* causing leaf stripe. The method has been tested in the ABI Sequence detection system 7000 HT on a range of different isolates of *P. graminea*, *P. teres*, Cochliobolus sativus, *Fusarium* spp, *Aspergillus* spp, *Alternaria* spp. The method was not specific for *Pyrenophora* spp when performed in the ABI Sequence detection system. The implementation of this specific test will not be possible without extensive development. Therefore a higher priority has been given to the development of a PCR method which is specific for *P. graminea*. It is not possible to distinguish *P. graminea* from *P. teres* with the traditional microscopic methods and the only way to distinguish these two species is at present by looking at symptoms in a green house test. However, such a test is very slow and time-consuming. Furthermore, it is assumed that it is extremely important to be able to distinguish these species as they do not have the same threshold values.

4) Very few seed lots with known infections of *P. graminea* have been available so far. After this growing season more seed lots from WP1 as well as commercial seed lots should be available and the development and test of the quantitative PCR method will be continued in 2003.

WP 3.1 Regulation and control.

It has been much more expensive to get hold on infected seed lots than expected, especially for experiments with the drum dryer, where huge quantities are required (4 tons/test series). Even so, it has been decided to conduct the experiments with the drum dryer on the expense of scientific and technical time consumption. Less priority has been given to experiments with the effect of seed separation, canopy humidity and harvest time.

Expenses for the assessment of seed rot in pea exceeded the presumptions made in the budget.

E. Project publications and other products

1. Articles in international, scientific journals with review procedures

2. Papers presented at congresses, symposiums, etc.

Borgen, A. 2002: Control of seed borne diseases in organic cereals and legumes. Abstracts of the 4th ISTA - PDC seed health symposium: Healthy seeds, the basis for sustainable farming. Wageningen, Netherlands, 29th April-1th May 2002. Page 18.

Borgen, A. 2001: Strategier til bekæmpelse af udsædsbårne sygdomme. Ekologiskt Lantbruk - Sammenfattninger av föredrag og postrar. Centrum för Uthålligt Lantbruk. Ultuna. 134-139.

**Borgen, A. 2002: Organic seed production and seed regulation. Book of abstracts. International Scientific and Practical Conference: Scientific aspects of organic farming. 21-22th March 2002, Jelgeva, Latvia.. Page 17.

**Nielsen, B. J. 2002. Udsædsbårne sygdomme i korn – bejdsemidler og andre behandlingsmetoder. 19. Danske Planteværnskonference 2002, Sygdomme og Skadedyr. Danmarks JordbrugsForskning Rapport, Markbrug nr. 64, 43-58.

3. Reports, articles in agricultural journals, etc.

**Anon. 2002. Vejledning i Planteværn 2002. Danmarks JordbrugsForskning og Landskontoret for Planteavl. Udgivet af Landbrugets Rådgivningscenter, Landskontoret for Uddannelse.

*Nielsen, G. C. 2002 Planteavlsorientering no.09-532. Landbrugets Rådgivningscenter.

**Nielsen, G. C. 2002. Forekomst og betydning af udsædsbårne sygdomme i økologisk planteavl Forædling af korn og bælgsæd samt produktion af såsæd i økologisk jordbrug. Vidensyntese om økologisk såsæd og forædling. Forskningscenter for Økologisk Jordbrug, Foulum, side 39-49.

**Nielsen, B. J. 2002. Vidensyntese om økologisk såsæd. Økologisk Jordbrug, 238 (6/4), 6

**Nielsen, B. J. og Kristensen, L. 2002. Forædling af korn og bælgsæd samt produktion af såsæd i økologisk jordbrug. Vidensyntese om økologisk såsæd og forædling. Forskningscenter for Økologisk Jordbrug, Foulum, 168pp.

**Nielsen, B. J. 2002. Tolerancer for forekomst af udsædsbårne sygdomme i økologisk såsæd. I Nielsen og Kristensen (eds.) ”Forædling af korn og bælgsæd samt produktion af såsæd i økologisk jordbrug”. Vidensyntese om økologisk såsæd og forædling. Forskningscenter for Økologisk Jordbrug, Foulum, 51-63.

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

Nielsen, G. C. 2002 Kursus for planteavlskonsulenter 2.- 4. oktober : Kornsvampenes biologi herunder Udsædsbårne svampe i korn - biologi og forebyggelse

Borgen, A. 2002: Control of seed borne diseases in organic cereals and legumes. The 4th ISTA - PDC seed health symposium: Healthy seeds, the basis for sustainable farming. Wageningen, Netherlands, 29th April-1th May 2002.

Borgen, A. 2002: 14th IFOAM Organic World Congress: Cultivation Communities. Victoria, Canada 20-28 August 2002: Poster on organic seed regulation an several seminars and meetings on organic seed

*Borgen, A. 2002: Ekologisk Lantbruk Konferense Ultuna, Sweden 13-15 november 2001. Oral presentation of a paper on control strategies for seed borne diseases in organic agriculture.

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

F. Scientific education

G. National and international cooperation

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
Gustaf Forsberg, SLU, on the test for the effect of moist air on pea disease.
Force Technologies is involved in the test for the effect of steam/ultrasound on seed borne diseases.
Westrup A/S and Mørdrupgård Korn on development of brush cleaner to control common bunt and stem smut.
NIAB (National Institute of Agricultural Botany), Cambridge, UK
Regarding technical construction and development of the drum drying technique, contact to the firm Cimbria A/S has been established.

Critical reflection on the project

WP2 diagnostic methods.

There have been no major changes of the plans. The developed PCR methods are now to be tested on a range of seed samples. Due to lack of suitable seed samples the test has to be continued in 2003 where more samples from the harvest in 2002 are available. It will be necessary to invest a substantial amount of time in the test of

seed batches with traditional mycological methods perhaps combined with qualitative PCR on single seeds to make sure that the percentage infection is determined as precise as possible.
In collaboration with the Danish Plant Directorate it has been decided to focus on *Pyrenophora graminea* and *Microdochium nivale* in the first phase of the project and a lower priority has been given to the implementation of an already published method for the quantification of *Pyrenophora* spp. We have tested this method in our real-time PCR system and it showed that it was not specific to *Pyrenophora* species but did also detect *Cochliobolus sativus*. The implementation of this specific test will not be possible without an extensive development. Therefore it has been decided to concentrate on the development of new assays for *P. graminea* and *M. nivale* as there is an urgent need for faster and more specific and sensitive test methods for these two species. *Pyrenophora teres* and *Pyrenophora graminea* are very closely related and at present it is not possible to distinguish these with traditional mycological methods. We have developed a PCR assay which can distinguish these two species. The main challenge now is to test whether it is possible to find a good correlation between the results achieved with the quantitative PCR method and the percentage of infected seeds determined by green house test.
For *Microdochium nivale* we have developed several primer pairs which are now being checked for specificity towards *M. nivale*.

WP 3.1
The experiments on control and regulation has followed the overall plans, even then the expenditure for infected seed lots exceeded the budget. Only few experiments are conducted with seed separation (4) and effect of harvest time (2) on the infection on seed borne diseases.
It was expected that a reconstruction of a brush cleaner would improved the effect of removal of fungal spores from the seed surface. However, the effect was insignificant from the current reduction potential of about 90%. It is still the ambition to improve the effect to 95-98%. This will be tried in 2003.
The effect of heat treatment on the pea diseases has not been assessed previously. The results from this project indicate, that peas differ considerable from cereals, most likely because of the seed size. Of the tested methods, only humid air gave a promising reduction in disease infection. The effect of the drum dryer was disappointing, but may be due the initial humidity in the seed tested. New experiments are made to test this.

WP 3.2
The infected grain with *Ascochyta* spp. was maybe applied too late to the crop be infectious. Therefore, a slurry of conidiospores was applied at the beginning of pod setting under very moist conditions due to heavy sprinkler irrigation. However, no infection was registered. In 2003, the infected barley grain will be added to the soil just after pea emergence, which gives the inoculum a longer period of time to infect the plant canopy. Natural infected pea haulm will be added in one of the three field trials with different varieties.

8. Budget

A. Account for any change in budgets

There has been no change in the over all budget but small changes have been made within budget for DIAS, Scanagri and KVL. The changes are explained under the comments for each department.

B. Budget for the whole project (1.000 DKK)

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

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months	9,6	73,8	71,5	58,8	35,0	248,7
Scientific personnel	7,1	34,6	32,7	26,0	20,0	120,4
Technical personnel	2,5	39,2	38,8	32,8	15,0	128,3

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries	266.600	2.025.651	2.027.452	1.695.940	1.117.383	7.133.026
Scientific personnel	215.600	1.198.311	1.163.575	943.681	762.848	4.284.015
Technical personnel	51.000	827.340	863.877	752.259	354.535	2.849.011
Other operational costs	26.540	322.868	375.000	353.000	123.000	1.200.408
Equipment	0	0	0	0	0	0
Others (please specify)	0	0	0	0	0	0
Direct costs	293.140	2.348.519	2.402.452	2.048.940	1.240.383	8.333.434
Indirect costs (20% of direct costs)	58.628	469.704	480.548	409.787	247.617	1.666.566
Total	**351.768**	**2.818.223**	**2.883.000**	**2.459.000**	**1.488.000**	**10.000.000**

Comments:

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project Bent J. Nielsen	Danish Institute of Agricultural Sciences (DIAS), Research Centre Flakkebjerg Department of Crop protection, 4200 Slagelse	15.11.2002	

Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)

Danish Institute of Agricultural Sciences, DIAS

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months	5,5	63,8	59,5	47,8	27,0	203,6
Scientific personnel	3,0	26,6	24,7	19,0	14,0	87,3
Technical personnel	2,5	37,2	34,8	28,8	13,0	116,3

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries	146.000	1.738.991	1.688.494	1.379.596	872.585	5.825.666
Scientific personnel	95.000	954.911	913.733	719.126	565.321	3.248.091
Technical personnel	51.000	784.080	774.761	660.470	307.264	2.577.575
Other operational costs	15.000	225.000	200.000	178.000	83.000	701.000
Equipment	0	0	0	0	0	0
Others (please specify)	0	0	0	0	0	0
Direct costs	161.000	1.963.991	1.888.494	1.557.596	955.585	6.526.666
Indirect costs (20% of direct costs)	32.200	392.798	377.699	311.520	191.117	1.305.334
Total	**193.200**	**2.356.790**	**2.266.193**	**1.869.115**	**1.146.702**	**7.832.000**

Comments:

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

Danish Plant Directorate (PD):

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months	3	8	10	9	6	36
Scientific personnel	3	6	6	5	4	24
Technical personnel	0	2	4	4	2	12

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

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

Danish Agricultural Advisory Centre (LR):

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

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries	30.600	58.000	58.880	60.646	62.466	270.592
Scientific personnel	30.600	58.000	58.880	60.646	62.466	270.592
Technical personnel	0	0	0	0	0	0
Other operational costs	1.540	72.868	150.000	160.000	30.000	414.408
Equipment						0
Others (please specify)						0
Direct costs	32.140	130.868	208.880	220.646	92.466	685.000
Indirect costs (20% of direct costs)	6.428	26.174	41.776	44.129	18.493	137.000
Total	**38.568**	**157.042**	**250.656**	**264.775**	**110.959**	**822.000**

Comments:

A total of KR 28.032 has been transferred from budget 2001 to budget 2002 and placed under cost. The over all budget is the same.

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

The budget for DIAS includes:
I)Department of Crop Protection, Flakkebjerg
II)Department of Agricultural Engineering, Research Centre Bygholm
III)Scanagri A/S
IV)Danish Agricultural University (KVL), Tåstrup.

In the following are listed the budget for each group:

I) Department of Crop Protection, Flakkebjerg:

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

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries	146.000	1.343.208	1.278.221	1.050.773	669.530	4.487.732
Scientific personnel	95.000	683.908	632.133	477.092	385.902	2.274.035
Technical personnel	51.000	659.300	646.088	573.681	283.628	2.213.697
Other operational costs	15.000	145.000	130.000	118.000	60.000	468.000
Equipment						0
Others (please specify)						0
Direct costs	161.000	1.488.208	1.408.221	1.168.773	729.530	4.955.732
Indirect costs (20% of direct costs)	32.200	297.642	281.644	233.755	145.906	991.147
Total	**193.200**	**1.785.850**	**1.689.865**	**1.402.528**	**875.436**	**5.946.879**

Comments:

The project started 1th September 2001 and only few field trials could be arranged. A total of KR 178.000 have therefore been transferred from budget 2001 to budget 2002 (note of 7.06.2002)

Due to unexpected high cost there has been a change within the budget 2002 and some expenditures to salaries have been transferred to cost. Expenditures to salaries have also been transferred to cost in 2003-2004. The over all budget is the same.

II) Department of Agricultural Engineering, Research Centre Bygholm

Year:	Consumption before 2002	Expected consump-tion 2002	2003	2004	2005	Total
Man-months	0	2	2	0	0	4
Scientific personnel	0	1	1	0	0	2
Technical personnel	0	1	1	0	0	2

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

III) Scanagri Denmark A/S (SD):

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months	0	6,3	6,4	6,5	4,4	23,6
Scientific personnel	0	6,3	6,4	6,5	4,4	23,6
Technical personnel	0		0	0	0	0

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries	0	232.893	242.347	242.034	179.419	896.693
Scientific personnel	0	232.893	242.347	242.034	179.419	896.693
Technical personnel						0
Other operational costs	0	60.000	50.000	50.000	18.000	178.000
Equipment						0
Others (please specify)						0
Direct costs	0	292.893	292.347	292.034	197.419	1.074.693
Indirect costs (20% of direct costs)	0	58.579	58.469	58.407	39.484	214.939
Total	**0**	**351.472**	**350.816**	**350.441**	**236.903**	**1.289.632**

Comments:
The project started 1th September 2001 and no field trials could be arranged. A total of KR 10.000 (cost) has therefore been transferred from budget 2001 to budget 2002.

Due to unexpected high cost there has been a change within the budget 2002 and some expenditures to salaries have been transferred to cost. Expenditures to salaries have also been transferred to cost in 2003-2004. The over all budget is the same (see note under section D).

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

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

IV) Danish Agricultural University (KVL), Tåstrup:

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

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries	0	103.150	106.394	86.789	23.636	319.969
Scientific personnel	0	0	0	0	0	0
Technical personnel	0	103.150	106.394	86.789	23.636	319.969
Other operational costs	0	10.000	10.000	10.000	5.000	35.000
Equipment						0
Others (please specify)						0
Direct costs	0	113.150	116.394	96.789	28.636	354.969
Indirect costs (20% of direct costs)	0	22.630	23.279	19.358	5.727	70.994
Total	**0**	**135.780**	**139.673**	**116.147**	**34.363**	**425.963**

Comments:

Comments: Due to unexpected high cost especially for analysis of Ascochyta spp. there has been a change within the budget and expenditure to salaries have been transferred to cost. The over all budget is the same (see note under section D)

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

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

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

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

Comments: