

Annex 1

Scientific description of the project

Title: High Quality Seed – Maintaining Integrity in Organic Farming

Acronym: SEED

Duration: From: 1 January 2006 to: 31 December, 2009

Participating scientists:

(Titles, names and brief institutional affiliations of scientists in the project. Head of project is written in bold, participants responsible for work packages are underlined).

Birte Boelt (BB), Head of Research Unit, Senior Scientist, Ph.D.
DIAS-GBI

Hanne Østergård (HØ), Senior Research Specialist, Ph.D.
RISØ

Bjarne Jørnsgård (BJO), Associate Professor, Ph.D.
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Bent J. Nielsen (BJN), Senior Scientist
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Johannes R. Jørgensen (JRJ), Scientist
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English summary (1 page, suitable for publication):

The axiom of organic farming that only organic seed is to be used often contradicts with insufficient accessibility of high quality seed. Despite recent achievements, organic seed supply for spring wheat, spring triticale, field pea, beets, maize, white clover and a number of vegetable species is insufficient. This may be due to inadequate production and/or processing, poor quality or lack of seed in varieties with recognised agronomic traits for production in Denmark and needs further investigation. Genes of cultivated plants spread in time and space through pollen and seed dispersal, as well as through handling during transport and processing. To maintain the integrity of organic farming, the availability of organically produced GM-free seed of varieties adapted to organic production systems is of the utmost importance. Therefore, in order to elucidate the potential of recently developed knowledge-based technology for organic seed production in Denmark, it is proposed to undertake a multidisciplinary research approach for the production of high quality organic seed production.

One of the organic farming principles is to enhance diversity at all levels including bio-diversity at the field level and genetic diversity at the crop level. Growing species mixtures has the potential of increasing yield as well as increasing the yield stability. However, it is not yet quantified how production in species mixtures affects seed quality.

Near infrared spectroscopy technologies (NIRS/NITS) are fast and reliable calibrated methods used in determining seed quality. This technique has further been used for discrimination between kernels of different species and varieties. Kernel size and shape parameters contain information relevant for end-use quality. Automated Image Analysis has therefore become a promising analytical tool for the cereal industry.

Grain legumes have an important role in supplying high quality protein feed for monogastric animals and ruminants. The organic grain legume protein production in Denmark is challenged due to severe problems in seed production; soil borne root rot diseases in peas, seed borne anthracnose and grey mould in lupines and *Ascochyta* blight in faba beans.

Common bunt (*Tilletia tritici*) is a potential serious seed borne disease in organic wheat and triticale. Seed health analysis have shown that *T. tritici* occurs relative commonly on organic seed and there is a need for identifying resistant varieties that can be used in integrated production systems.

Our hypothesis is that elucidating the potential of recently developed knowledge-based technologies on the exploitation of genetic diversity, the production in crop mixtures, the improvement of seed quality by utilising new integrated methods in seed technology will improve the supply of high quality organic seed for organic farming to remain independent and viable.

Due to the logistic nature of seed production, seed movements across borders and the future introduction of GM-crops in European farming systems, the project aims at establishing a platform for international scientific collaboration in order to maintain the integrity in organic farming – by the production of high quality, GMO-free seed.

A.0 Introduction, state of the art and objectives of the project (max. 3 pages):

Introduction

The axiom of organic farming that only organic seed is to be used often contradicts with insufficient accessibility of high quality seed. To maintain the integrity of organic farming, the availability of organically produced GM-free seed of varieties adapted to organic production systems is of the utmost importance. Due to climatic conditions, grower's expertise and location of the seed processing industry, seed is often multiplied in specific geographic regions. This logistic structure of seed multiplication and the regulation and certification of organic farming implies that this aspect needs to be addressed at an European level.

With more than 80% of the total European white clover seed production, Denmark is world leading in the production and export of forage seed and is the largest exporter of grasses (The Danish Seed Council, 2003), and among the only countries exporting organic grass seed. In addition, Denmark has a recognised vegetable seed production with 70-80% of the world production of spinach seed, and also for a number of vegetable species organic seed production is either established or under development.

In 2005, new regulations imply that no conventionally grown feedstuff can be used in organic farming systems. Before the implementation of these regulations, the allowed quota of conventional feed was used to import protein feed. The importance of grain legumes is accentuated by the increasing problem in the supply of guaranteed GMO free organic soybean. Thus integrity of organic farming will require a stable Danish production of quality grain legumes, and hence organic seed of grain legumes is required.

Finally, one of the organic farming principles is to enhance diversity at all levels including biodiversity at the field level and genetic diversity at the crop level (Kristensen et al. 2002, Lammerts van Bueren 2003).

State of the art

Despite recent achievements, organic seed *supply* for spring wheat, spring triticale, field pea, beets, maize (early varieties), white clover and a number of vegetable species is insufficient. This may be due

to inadequate *production*, poor *quality* or lack of *varieties* with recognised agronomic traits for production in Denmark.

Insufficient supplies

The inadequate provision of seed may occur because seed is used in limited quantities and therefore multiplication is not profitable for seed companies. This will often be the case for vegetable crops. Some species may be under introduction due to specific interest in organic farming, but an organic seed multiplication may not be established yet – such as grain legumes, spring wheat and spring triticale. In forage seed crops an organic seed production has been under establishment since 1992, however, organic white clover seed is still in request (The Danish Seed Council, 2003), one of the main obstacles being pest infection resulting in dramatic yield reductions (Hansen, 2002).

Quality of seed

The major difference between the organic and conventional proportion of seed lots discarded is the infection by seed borne diseases, as the access to control measures in organic farming is limited. In 2004, 72% of all organic field peas in Denmark were discarded due to seed borne diseases, mainly *Ascochyta pisi*. In winter wheat and barley, 0-38% of the organic seed lots were discarded in the period 2000-2004 primarily due to common bunt (wheat), net blotch and/or leaf stripe (barley), loose smut (barley) and *Fusarium spp.* respectively *Microdochium nivale* (Nielsen 2005).

Maintaining integrity in organic farming

Co-existence is defined as the farmers being able to choose between conventional, organic and genetically modified (GM) crop production, in compliance with the relevant legislation on labelling rules and purity standards. Genes of cultivated plants spread in time and space through pollen and seed dispersal, and in addition handling during transport and processing may also disperse seeds.

Consequently, suitable measures during cultivation, harvest, transport, storage, and processing are necessary to ensure co-existence. With the increasing area of GM-crop production as well as the number of crops in which GM-varieties are available the need for guidelines to ensure coexistence is essential. At present no control measures or guidelines are identified to ensure coexistence in organic seed production of clover seed and grass for grasslands mixtures (Tolstrup, *et al.*, 2003).

Developing varieties / identifying species of specific value for organic farming

The organic grain legume protein production in Denmark is challenged due to severe problems in seed production; lack of stable early ripening varieties of faba bean and lupins suited for mixture with cereals, soil borne root rot diseases in peas, seed borne anthracnose and grey mould in lupins and *Ascochyta* blight in faba beans. Crop mixtures with cereals are effective in reducing the severe weed problems seen in pure stands, and are also stabilising yield and significantly reducing epidemic leaf diseases (Kinane and Lyngkjær 2003). The *Ascochyta* blight disease on faba bean (*Ascochyta fabae*) and anthracnose (*Colletotrichum lupini*) in lupin (Helgard *et al.* 2002) have worldwide distributions with a major impact on yield and quality of the seeds. So far, there has been no focus on *A. fabae* in Denmark, but observations among breeding lines show clear and devastating epidemics of this pathogen (Knudsen pers. medd.). However, large differences in plant resistance have been demonstrated in faba bean accessions under field conditions (Maurin & Tivoli 1992, Sillero 2001). There is a need for a determination of the threshold values for the important diseases *A. fabae* in faba bean and *C. lupini* because the existing values are only based on an estimate and not validated under field conditions.

Improving seed quality and yield - by the production in crop mixtures

Resistance to seed borne diseases have been described, especially resistance to common bunt (*Tilletia tritici*) in wheat. Common bunt is the major specific problem in winter wheat seed production and at least 15 specific resistance genes (Bt) against common bunt are known to exist. There is a need for a more focused breeding for resistance against common bunt and to provide triticale and wheat, spring and winter types, which are safe to multiply and grow for organic farmers (<http://www.foejo.dk/enyt2/enyt/aug05/resistens.html>). It is important to quantify the effects of genetic diversity and of production in crop mixtures.

This synthesis results in models from which decision support tools such seed infection thresholds can be derived and risks associated with the propagation of seed-borne diseases can be assessed (Nielsen, 2003a, Nielsen 2003c, Cockerell et al., 2004).

Previous DARCOF supported studies have shown that the vitality in organic seed lots is significantly lower than comparable conventional seed (Borgen and Kristensen 2001, Kristensen 2001). The differences could partly, but not completely be explained by a lower seed size or density caused by lower nutrient availability of the mother plants. A way to compensate for the lower vitality in organic seed lots can be a mechanical size separation of the seed, as it is known that the vitality of larger seeds is higher (Guberac et al., 1999).

Additional information about seed quality in the form of image and spectral measurements may lead to better use of seed sorting equipment in improving seed quality and reduce the number of discarded seed lots. Near infrared spectroscopy technologies (NIR/NIT) are fast and reliable calibrated methods that will be used in detecting seed quality. NIT has become a widely used method in the analysis of cereals and cereals-based products, as extensively reviewed by Osborne et al., (1993), Williams and Norris, (1987), Williams, (2002) and Meurens and Yan (2002). Today, NIT instrumentation for whole kernel analysis for protein, starch and moisture in grains on bulk samples is used worldwide in combination with advanced multivariate regressions methods (Büchmann et al., 2001). Kernel size and shape parameters contain information relevant for end-use quality. Automated Image Analysis has therefore become a promising analysis for the cereal industry. This technique has been used for discrimination between kernels of different species (Chtioi et al., 1996), discrimination between wheat classes and varieties (Zayas et al., 1986), and, used in combination with physical measurements, for variety identification (Zayas et al., 1996). Mechanical seed scarification is reported to be as effective as cold treatment and more effective than seed treatment with gibberelic acid on breaking post-harvest dormancy by improving the germination rate (Taillefer et al., 1996).

Developing strategies for the production for high quality, GMO-free organic seed

At present organic farmers are not fully self-sufficient in organic products. For organic farming to remain viable in an agricultural landscape where GM-crop production is included in future (Boelt, 2004; O'Doherty Jensen K., 2001; Lassen et al., 2002) it will be necessary to stimulate the production of high quality organic seed. Also to maintain Denmark's position as the leading clover and grass seed producer in the EU in organic seed production, the development of cropping systems to maintain varietal purity in seed fields will be of great importance (Boelt & Gislum, 2004). White clover and grasses are cross-pollinators and almost completely self-incompatible. Grasses are wind-pollinators, and clover is insect-pollinated by honeybees and naturally occurring bumblebees. Due to the wide distribution of white clover and grasses in the landscape, more knowledge on factors determining cross pollination and the extent of gene dispersal at the field level will be necessary to propose effective separation distances (Tolstrup, *et al.* 2003). This requires that the degree of invasion, establishment and introgression of genes and plants into perennial or permanent clover/grass swards is accounted for (Hauser et al., 2003, Jørgensen and Wilkinson, 2005), and related to the nature of the sward and its management.

Objectives

The objective is to maintain the integrity in organic farming – by the production of high quality, GMO-free seed of varieties and species of specific value in organic farming.

This goal is achieved by the

- identification and development of improved species/varieties with specific value in organic farming
- improvement of seed quality and seed yield by production in crop mixtures
- development of strategies to restrict gene flow in out-breeding species of important seed crops.

A.1 Relevance for DARCOF III and argumentation for the subject (max. ½ page):**3.4.2 Forædling og production af økologisk udsæd** (breeding and production of organic seeds)

A.2 Technical content of the research activity

The project consists of three thematic workpackages (WP2-WP4) and one workpackage describing the organisation of the project and a platform for networking and cooperation (WP1). Prior to the description of this project, cereal and forage crop breeders, seed multipliers, advisers, regulatory institutes as well as representatives from university and research institutes active in the area of breeding and production of organic seed met at Research Centre Flakkebjerg. It was agreed to collaborate in this broad initiative – either as collaborate partners or as members of the project networking group.

Work Package list

WP No.	WP title	Responsible scientist	Budget DKK	Start	End	Deliverable No.
1	Project management and network administration	BB	0,668	01/2006	12/2009	D1.1; D1.2; D1.3; D1.4; D1.5
2	Exploitation of genetic resources for enhanced seed quality and yield	LB	1,709	01/2006	12/2009	D2.1; D2.2; D2.3
3	Enhancing yield and seed quality in variety and species mixtures of cereals and grain legumes	BJ	2,437	01/2006	12/2009	D3.1; D3.2; D3.3; D3.4; D3.5; D3.6
4	Developing strategies for the production of high quality, GMO free organic seed	RBJ	2,186	01/2006	12/2009	D4.1; D4.2; D4.3; D4.4; D4.5
Total			7,000			

Deliverables list

Deliverable No	Deliverable title	Lead scientist	Delivery date	Allocated scientific person months	Type of deliverable
D1.1	Meetings in the project group – at a minimum twice a year	BB	2006-09	1	O/C
D1.2	Yearly communication plans	BB	01/2006 - 01/2009	0,5	O
D1.3	Yearly workshops	BB	2006-09	1	R/C
D1.4	International workshop	BB	2007	0,5	R/C
D1.5	Project web-site established	BB	06/2006	1	O
D2.1	Manuscript on influence of seed vitality and seed infections with <i>A. fabae</i> in faba bean has been published	BJO/LAB	05/2009	5	P/O
D2.2	Manuscript on threshold values of <i>C. lupini</i> in lupin in Denmark has been submitted	BJO	05/2009	5	P/O
D2.3	Manuscript on resistance to common bunt (<i>Tilletia tritici</i>) in Danish wheat and triticale varieties and <i>T. tritici</i> virulence survey in Denmark has been published	BJN	05/2009	4	P/O
D3.1	FØJOenyt. Disease development, and seed quality in pure stands and mixtures of cereals and grain legumes	BJO	12/2007	2	R/P
D3.2	Paper on disease development and seed infection in pure stands and variety and species mixtures of cereals and grain legumes	BJO/LAB /BJN	12/2009	4	R/P
D3.3	Paper on grain quality for human and animal nutrition in pure stands and mixtures of cereals and grain legumes	JRJ	06/2009	3	R/P
D3.4	Paper on crop development and general and specific combining ability species mixtures.	BJO/JRJ	08/2009	2	R/P
D3.5	FØJOenyt. Yield and quality of pure stands and mixtures of cereals and grain legumes	JRJ	12/2007	2	R
D3.6	Manuscript on meta analysis of mixture effects of selected characteristics of species mixtures as part of PhD thesis	HØ	12/2008	4	P
D4.1	Yearly reports on white clover seed and clover-grass forage yield in mixed crop production	BB	12/2007 12/2008 12/2009	3	R/P
D4.2	Decision support system to minimise yield reduction due to pests produced, report and publication written and data available on the internet	LMH	12/2009	4	R/S/O
D4.3	Report on the frequency and distribution of white clover and clover-grass on organic farms	RBJ	12/2006	1	S/C
D4.4	Guidelines to reduce gene flow in white clover and clover-grass	BB + RBJ	11/2009	1,3	P/S/C

Deliverabl No	Deliverable title	Lead scientist	Delivery date	Allocated scientific person months	Type of deliverab le
D4.5	Report on the use of morphological markers to analyse gene flow in white clover	RBJ + BB	01/2009	4	R

Description of work packages

WP No.: 1. Management and network administration

	Start date or starting event: 01/2006									
Partner id.	DIAS- GBI									
Person-months per participant	VIP: 4,0 TAP: 1,0									
Total PM VIP:	4,0									
Total PM:	5,0									

Objectives:

- To facilitate collaboration within the project to obtain results of relevance to organic farming
- To stimulate communication and publication
- To create a platform for dialog and networking between breeders, seed companies, advisers, organic farmers and regulatory bodies
- To stimulate the dissemination of the Danish experiences internationally
- To administrate the project in accordance with guidelines

Description of work:

Task 1. Organising workshops where preliminary results are presented

To obtain reliable results from scientific investigations often takes a long time and collaboration between different fields of experience is needed to obtain good results. In order to stimulate collaboration a workshop is held every year, and all project staff is invited as well as representatives for organic farmers.

Task 2. Planning communication and publication

Each year a plan for publication of project results (final as well as preliminary) in FØJOenyt, DARCOFenews and ISO FAR are produced.

Task 3. Organising network meetings

Even before the project was described it was agreed to establish a network group consisting of breeders, seed companies, advisers, organic farmers, regulatory bodies and scientists in order to facilitate the production of high quality seed of varieties/species in request in organic farming. In some crops seed companies hesitate to invest in organic seed because production costs and risks are high and the potential consumption of the commodity may not be clear. It is necessary to create commitment in the chain – farmers should concentrate their request on a few, well adapted, high product quality varieties and organic seed production of these should be stimulated. It is important for seed companies to have a clear indication of the potential market before an organic seed production in a ‘new’ crop is established. In these new crops information on the specific value in organic farming may be missing. The network group may propose to have specific quality traits analysed, i.e. in the set-up for an organic variety testing.

Task 4. International activities

In Denmark there has been a very successful implementation of the mandatory use of certified organic seed due to a very successful dialog between the regulatory bodies, the seed industry and the organic farmers. In order to stimulate the movement of seed across borders – to meet the requirements for organic seed in all European countries – certification schemes and regulations should be interpreted in a harmonized way. International activities are stimulated to disseminate the Danish experiences and to create a science-based discussion on the need for derogations. In this aspect project data on threshold values in seed may prove very important. In addition discussions on guidelines on maintaining GMO-free seed are initiated.

Task 5. Project web administration

A project-website will be created to facilitate the production of status reports and to ensure that rapid and updated project information is available.

Deliverables:

- D1.1 Meetings in the project group – at a minimum twice a year
- D1.2 Yearly communication plans are available 01/2006; 01/2007; 01/2008; 01/2009
- D1.3 Yearly workshops are held in January or February
- D1.4 The project will organise at least one international workshop where the Danish experiences are presented
- D1.5 Project home page

Milestones:

- M1.1 Project website open 05/2006
- M1.2 International workshop is held 07/2007

WP No.: 2. Exploitation of genetic resources for enhanced seed quality and yield

	Start date or starting event: 01/2006								
Partner id.	RVAU	DIAS IPM							
Person-months per participant	VIP: 6,0 TAP: 3,0	VIP: 8,0 TAP: 12,0							
Total PM VIP:	14,0								
Total PM:	29,0								

- Objectives:**
- To identify the importance of seed vitality and seed borne infections of *Ascochyta fabae* in Faba bean
 - To identify the resistance and threshold values in Lupine against *Colletotrichum lupini*
 - To identify and characterise disease resistance in wheat and triticale against *Tilletia tritici*
 - To obtain information on virulence structure in Danish populations of *T. tritici*

Description of work:

Task 1. Prevalence and severity of seed borne infections of *Ascochyta fabae* in faba bean
 Infected haulm and seed with *A. fabae* will be collected and produced from field plots in 2005 and 2006. Thresholds for seed borne *A. fabae* will be estimated based on two types of field trials in 2007 and 2008. Trial A: Four different mixtures of healthy and diseased seeds with *A. fabae* (e.g. 5, 10, 15% infected seeds) will be sown in field trials to estimate the yield and seed infections of *A. fabae* in the harvested seed. Trial B: Parallel to trial A, twelve seed lots of the same variety (e.g. Columbo) with different levels of seed vitality and seed infections of primarily *A. fabae* will be sown in field plots at DJF Flakkebjerg to estimate the variation in yield due to difference in seed vitality and seed infections.
 Responsible: DIAS-IPM

Task 2. Threshold values for seed borne infections of *Coletotrichum lupini* in lupine
 At the project start, infected seed lots of varieties varying in resistance will be available, and other relevant varieties with infection will be multiplied in the first year. Threshold value for *C. lupini* is estimated in field trials in 2007-08 where infected seeds in susceptible and resistant varieties are mixed with healthy seed of the respective varieties in different proportions (e.g. 1, 5, 10% infected seed). Disease development, yield losses and infection of harvested seed will be assessed. In cooperation with activities in WP3 the influence of mixture with cereals on the disease development will be investigated. Threshold values will be assessed and recommendations prepared.
 Responsible: RVAU

Task 3. Screening for resistance to common bunt (*Tilletia tritici*)
 Major resistance genes (*Bt*) give complete resistance to common bunt caused by *T. tritici*. Varieties of wheat and triticale suitable for organic production will be characterised for the resistance level against common bunt in 2006-2008 using artificial inoculation in field experiments. In 2006, the test will be performed in varieties already inoculated and drilled in autumn 2005. The varieties will not only be Danish varieties but also suitable varieties from other countries. Information of resistance to common bunt in wheat varieties for organic growers.
 Responsible: DIAS-IPM

Task 4. Virulence survey
 To determine which major resistance genes that are effective against common bunt, a set of wheat lines with different *Bt* genes will be inoculated with different bunt isolates. In 2006, wheat lines with specific *Bt* genes will be propagated and infected seeds with *T. tritici* will be collected. In 2007-2008 these populations will be tested on the differential lines with the different resistance genes. Included in the test

will also be different populations of *T. tritici* sampled from Danish seed producers during the last 5 years. Information will be provided for breeders for selection of resistance genes for breeding programmes (task 5).

Responsible: DIAS-IPM

Task 5. Contribute to the development of new varieties

Breeders at Pajbjergfonden will carry out back crossings of effective genes for bunt resistance into bread wheat varieties with a good general resistance to other major diseases. The expected output is bunt resistant bread wheat safe to multiply and grow for organic farmers in DK and Europe. Responsible: Pajbjerg (financed by Pajbjergfonden).

Deliverables:

- D2.1 Manuscript on influence of seed vitality and seed infections with *A. fabae* in faba bean has been published (05/2009) P/O.
- D2.2 Manuscript on threshold values of *C. lupini* in lupin in Denmark has been submitted (05/2009) P/O.
- D2.3 Manuscript on resistance to common bunt (*Tilletia tritici*) in Danish wheat and triticale varieties and *T. tritici* virulence survey in Denmark has been published (05/2009) P/O.

Milestones:

- M2.1 Infected seed and haulm with *A. fabae* have been produced and collected (09/2006)
- M2.2 First year field trials on host resistance against *A. fabae* in faba bean are completed (12/2007)
- M2.3 Second year field trials on thresholds and host resistance against *A. fabae* in faba bean are completed (12/2008)
- M2.4 Infected seeds have been multiplied and first year field trial on variety resistance and thresholds against *Coletotrichum lupini* in lupin is completed (09/2006).
- M2.5 Second year field trials on thresholds and host resistance against *Coletotrichum lupini* in lupin are completed (12/2007)
- M2.6 Third year field trials on thresholds and host resistance against *Coletotrichum lupini* in lupin are completed (12/2008)
- M2.7 Wheat differential lines with specific *Bt* genes propagated and seeds with *T. tritici* have been collected (10/2006)
- M2.8 First year field trials on host resistance against *T. tritici* are completed (12/2006)
- M2.9 Second year field trials on host resistance against *T. tritici* and first virulence survey are completed (12/2007)
- M2.10 Third year field trials on host resistance against *T. tritici* and second virulence survey are completed (12/2008)

WP No.: 3. Enhancing yield and seed quality in variety and species mixtures of cereals and grain legumes

	Start date or starting event: 01/2006								
Partner id.	RVAU	DIAS- IPM	DIAS- GBI	RISØ					
Person-months per participant	VIP: 2,0 TAP: 4,0	VIP: 5,0 TAP: 5,0	VIP: 6,0 TAP: 7,0	VIP: 4,0 (+ 6 PhD Riso financed)					
Total PM VIP	17,0								
Total PM:	33,0								

Objectives:

- To improve quality and yield of organically produced seed by increased crop diversity using variety and species mixture
- To quantify disease epidemiology in species mixtures
- To assess crop development and general and specific combining ability in species mixtures
- To evaluate grain quality for human and animal nutrition

Description of work:

At two locations, Flakkebjerg and Højbakke, a diversification trial will be performed over three years. The objective is to elucidate the potentials for improved seed quality by increased genetic diversity of cereals and grain legumes and to get information about general and specific combining abilities at species and variety level. Disease development, canopy development and weed infestation will be monitored in pure stands of wheat, triticale, oat, barley, pea, fababean, lupine and in combinations of wheat, triticale, oat and barley with pea, faba bean and lupin. In the trials, healthy seeds of all varieties are used in the study of the influence of crop mixtures on disease development in the canopy and disease transfer to seeds. However, for pea, faba bean and lupin both healthy and infected seeds with *Ascochyta pisi*, *Ascochyta fabae* and *Colletotrichum lupini* respectively are included.

Task 1. Disease development during the growing season and in seeds

In the first year, different triticale varieties suitable for organic growth conditions will be evaluated to find the best variety for the species mixtures. Infected seed with *A. fabae* in faba bean, *Ascochyta* spp. on pea and *C. lupini* in lupin will be collected and produced in field plots in 2006 and 2007 in WP2 and included in the field trials in 2007 and 2008, respectively. The disease development in the cereals and grain legumes in pure stand and in mixtures will be scored continuously during the growing season. The prevalent diseases to observe are in triticale: Mildew (*Erysiphe graminis*), yellow rust (*Puccinia striiformis*), leaf blotch (*Septoria nodorum*) and Fusarium head blight (*Fusarium spp.*), in wheat: Mildew (*E. graminis*), yellow rust (*P. striiformis*), leaf blotch (*S. nodorum*, *S. tritici*) and Fusarium head blight (*Fusarium spp.*), and in the legumes: *Botrytis* sp. and *Ascochyta* sp. in faba bean, *Ascochyta* spp., *Botrytis* spp. and *Peronospora* sp. in pea and Anthracnose (*Colletotrichum lupini*), Grey mould (*Botrytis cinerea*), Brown spot (*Pleiochaeta setosa*) and Fusarium (*Fusarium spp.*) in lupin. After harvest, the seeds will be cleaned and species mixtures separated in fractions representing individual crop species. Separated seeds from all relevant plots will be analysed for seed borne diseases.

Task 2. Crop development yields and general and specific combining abilities

Crop development will be recorded during the growing season including emergence and plant stands, phenological development stages, plant height during vegetative growth and canopy development. Measurement of canopy development will be performed with non-destructive methods e.g. leaf area

index (LAI) by Licor 2000 and normalized difference vegetation index (NDVI) by remote sensing. Weed infestations will be registered during the growing season and at harvest. At full ripening the plots will be combine harvested, mixtures separated and component yield will be measured.

Task 3. Grain quality for human and animal nutrition

Samples of cereal and grain legumes from the field trials carried out in 2006-2008 will be processed by appropriate cleaning and grading techniques and analysed for:

- Seed size distributions and shape parameters using standard methods and Image Analysis.
- Gross chemical composition using NITS (Near-Infrared Transmission Spectroscopy) and NIRS (Near-Infrared Reflectance Spectroscopy) technologies.
- Gross composition of seed C and N (protein) for those grain legumes where no NITS or NIRS calibrations have been developed yet.
- Vitality testing

The potential of using Image Analysis of physical seed characteristics in order to get a rapid description of seed lots will be attempted.

The NITS analyses include seed protein, starch and gluten for wheat; protein and starch for barley and triticale; protein for oat.

In vitro enzyme digestibility of organic matter (EDOM) will be analysed by NIRS for wheat and triticale. Germination Index is determined according to EBC method (European Brewery Convention: Analytica-EBC, Fachverlag Hans Carl: Nürnberg, 1998.) on cereal seed samples, in order to evaluate seed vitality.

Task 4. Meta analysis of mixture effects of selected characteristics of species mixtures

With the aim of generalizing the knowledge obtained from other Tasks in WP3, results from a number of different studies on variety and species mixtures each considering questions addressed in this WP will be synthesized by the use of meta analysis. Mixture effect variables (= difference between measurement in mixture and average of components) will be determined from each study along with an estimate of its variance. Combining results will make it possible to compare effect sizes as a function of explanatory variables. The studies analyzed will be based on WP3 data, published data as well as unpublished data made available within the SUSVAR network where collaboration on analysis of crop diversity is a main aim. A PhD student studying meta analyses with special emphasis on variety mixtures will be connected to the work.

Deliverables:

- D 3.1. FØJOenyt. Disease development, and seed quality in pure stands and mixtures of cereals and grain legumes (12/2007).
- D 3.2 Paper on disease development and seed infection in pure stands and variety and species mixtures of cereals and grain legumes (2009).
- D3.3 Paper on grain quality for human and animal nutrition in pure stands of species and variety mixtures of cereals and grain legumes (2009).
- D 3.4 Paper on crop development and general and specific combining ability of variety and species mixture (2009).
- D 3.5 FØJOenyt. Yield and quality of pure stands and mixtures of cereals and grain legumes (12/2007).
- D 3.6 Manuscript on meta analysis of mixture effects of selected characteristics of species mixtures as part of PhD thesis(12/2008).

Milestones:

- M 3.1 Production of seed lots infected with *A. fabae* in faba bean, *C. lupini* in lupin and *A. pisi* in pea (12/2006).
- M3.2 Field experiment with species and crop mixtures (diversification trial) conducted first year at two locations (08/2006).

- M 3.3 Harvested seeds from 1st year diversification trial are cleaned and separated. The seed of cereals and grain legumes are tested for seed borne diseases. Physical seed characteristics and quality parameters are analysed (12/2006).
- M3.4 Field experiment with species and crop mixtures conducted second year at two locations (08/2007).
- M 3.5 Harvested seeds from second year diversification trial are cleaned and separated. The seed of cereals and grain legumes are tested for seed borne diseases. Physical seed characteristics and quality parameters are analysed (12/2007).
- M3.6 Harvested seeds from third year diversification trial are cleaned and separated (08/2008).
- M 3.7 Harvested seeds from third year of field trials with sole crops and species mixtures cleaned and separated. The seeds of cereals and grain legumes are tested for seed borne diseases. Physical seed characteristics and quality parameters are analysed (12/2008).
- M 3.8 Principles for including data in the meta analysis (9/2006).

WP No.: 4 Developing strategies for the production of high quality, GMO free organic seed

	Start date or starting event: 01/2006								
Partner id.	DIAS GBI	RISØ	DIAS IPM						
Person-months per participant	VIP: 4,0 TAP: 9,0	VIP: 5,3 TAP: 7,0	VIP: 4,0 TAP: 5,0						
Total PM VIP	13,3								
Total PM:	34,3								

- Objectives:**
- To investigate production systems of crop mixtures of white clover and grass species with regards to seed and forage production
 - To develop a decision support system to minimise yield reduction in white clover due to pests
 - To develop strategies to restrict gene flow in white clover between seed production fields, grasslands and natural habitats
 - To establish national and international collaboration on the development of strategies to improve the availability of organic, GMO-free seed in varieties of specific value to organic farmers

Description of work:

Task 1. Producing white clover seed in crop mixtures with grass species
 In field trials at Research Centre Flakkebjerg white clover for seed production will be established both in a pure stand and in a crop mixture with grass species. The latter will allow for combining seed and forage production, and effects on seed yield will be registered. Different defoliation strategies will be performed in order to optimise the production system and in addition this will provide data to be used in task 2. Seed yield components in white clover will be analysed with regard to the influence of mixed cropping and defoliation.
 Responsible: DIAS-GBI

Task 2. Developing a decision support system to minimise yield reduction in white clover due to weevils
 In lab tests, glasshouse tests and field experiments the biology of pests in white clover seed fields are investigated. Host-plant preferences of the weevils as well as a simple method for monitoring weevils in the field will be produced. Changes in seed yield due to management strategies asynchronising flowering by defoliation are registered. The changes may be seen as a reduction of potential seed yield due to removal of flowerbuds and/or as a reduction of pest infestation due to the asynchronisation of flowering with the biology of the pests. The prevalence and activity of pollinators are registered.
 In a lab test the efficacy of natural pyrethrum is tested on the pests, the clover seed weevil (*Apion fulviceps* Geoff.) and the lesser clover leaf weevil (*Hypera nigrostris* Fabr.). If results prove promising the product are tested under field conditions. If other control agents internationally accepted in organic farming are identified these will be evaluated similarly.
 Moreover the influence of the clover cyst nematode (*Heterodera trifolii*) on germinating and small clover plants will be investigated.
 Responsible: DIAS-IPM

Task 3. Collection of base-line information on the prevalence of white clover in organic farming systems and the surrounding natural habitats
 To quantify the presence of white clover and clover-grass on organic farms, two types of organic farms representing different gene dispersal scenarios are selected for analysis: 1) farms with both seed production and clover-grass fields and 2) farms without clover-grass. In surveys on the farms the frequency (plants/m²), flower number, flowering time, pollen and seed production are analysed for white

clover. The distribution of the reservoir populations will be related to the position of the fields for seed production, and studies will be performed to analyse the behaviour of pollinators visiting the reservoir populations of clover. A combined fitness estimate for the natural or semi-natural populations can be calculated. The farm survey will be carried out during the first year of the project.

Responsible: RISØ + DIAS-GBI

Task 4. To develop management methods and recommendations for reduction of gene flow in white clover and perennial ryegrass for seed production

Field experiments are set up to evaluate the effect from species proportions on gene flow between clover-grass areas and seed multiplication fields. Clover-grass plots are established with different densities and proportions of white clover and grass. Pure plots of white clover and rye grass will mimic the GM donor field or the organic seed multiplication field. The species proportion in clover-grass mixtures, the plot-size, the distance between pure plots and clover-grass plots and the frequency and nature of pollinators should account for (without being identical to) the natural scenarios. The varieties used as donor and recipient will be chosen to have differences in DNA markers (diagnostic markers or different marker frequencies) that will allow analysis of gene flow to the offspring. The most suitable marker system (i.e. SSR, ISSR, AFLP) and bulking method will be selected during a screening of varieties. The diagnostic markers in combination with assignment tests (STRUCTURE (Prichard et al. 2000), NEW HYBRID (Anderson and Thompson 2002) will reveal the gene flow.

In the plots described above the degree of self-incompatibility/the capacity for self-pollination is analysed in clover-grass. Seeds from individual plants will be analysed and the paternity determined should it be 1) individuals of the same variety, 2) selfing or 3) other varieties in the experimental area. Highly variable AFLP markers developed previously by Risø determine outcrossing/selfing in the varieties. This knowledge will bring about recommendation of mitigating measures for co-existence of clover-grass and GM-grass.

From the distribution and fitness of white clover and clover-grass “on farm”, and the correlations found between gene flow, proportion, insect behaviour and field size of clover-grass, strategies are proposed for reduction of gene flow at the farm level.

Responsible: RISØ

Task 5. Establishing a common procedure and set-up for the studying of gene flow in seed crops between international partners

An international collaboration is agreed upon between IGER-Aberystwyth, UK and AgResearch-Christchurch, New Zealand to test different non-GMO marker systems in white clover as a common test set-up for internationally linked investigations.

In this project DIAS-GBI will be quantifying seed loss and the establishment of volunteer white clover. Using a red-leaf morphotype (dominant, homozygous) transplanted into a green-leaf seed production field or a grazing field will provide information on the number of volunteers establishing in consecutive years and crops. This non-GMO marker is provided by IGER-Aberystwyth. Partners in UK and NZ will also test the fitness of the marker system, and seek funding for a similar trial.

Responsible: DIAS-GBI, RISØ

Deliverables:

- D4.1 Yearly reports on white clover seed and clover-grass forage yield in mixed crop production 12/2007, 12/2008 and 12/2009.
- D4.2 Decision support system to minimise yield reduction due to pests produced, report and publication written and data available on the internet 12/2009
- D4.3 Report on the frequency and distribution of white clover and clover-grass on organic farms 12/2006
- D4.4 Guidelines to reduce gene flow in white clover and clover-grass fields 11/2009

D4.5 Guidelines on the use of morphological markers to analyse gene flow in white clover 01/2009

Milestones:

- M4.1 Seed yields in white clover and forage yields are registered in three consecutive years of field experimentation 08/2007, 08/2008 and 08/2009.
- M4.2 Validation of data for decision support system 12/2008
- M4.3 Quantification of white clover and clover-grass on organic farms completed 12/2006
- M4.4 Field plots established for pest analysis and gene flow investigations 12/2006
- M4.5 Molecular analysis of gene flow between clover-grass fields and white clover in pure stand plots finalised 03/2008
- M4.6 Data for the recommendation of guidelines to reduce gene flow analysed 09/2009

A.3 Project resources and budget overview

RVAU, The Department of Agricultural Sciences, has a long-standing research experience in crop development, plant breeding, crop physiology and plant population dynamics with focus on variety and species mixture. Emphasis is on sustainable use of natural resources and environmental protection. The department integrates a number of disciplines, spanning from the molecular biology level to the more applied and systems oriented levels. In the last decade extensive expertise has been build up in organic farming and plant adaptation using lupin as a model crop. Within the plant breeding effort of the Department molecular markers have successfully been applied to a broad spectrum of agricultural crops.

RISØ National Laboratory, Biosystems Department, has a long and estimated experience in the field of modelling and experiments on gene flow, co-existence, environmental risk assessment of GM crops, biodiversity aspects, organic plant breeding with special emphasis on cereals and resistance biology. At present we are involved in the EU projects ANGEL and SIGMEA focused on GMP risk assessment and co-existence, two Nordic studies on biodiversity and leading the management of the COST network SUSVAR (COST860). The participants from Risø were responsible for and contributed to the oilseed rape chapter in the Danish co-existence report (Tolstrup et al. 2003), as well as we have participated as experts at coexistence meetings with the EU commission and the EU parliament. Further, we have had the leadership of the FØJO II project BAROF on Characteristics of spring barley varieties for organic farming.

DIAS-GBI, Department of Genetics and Biotechnology holds an outstanding tradition in experimentation in seed production research – in grasses, clover and vegetable seed crops. At present the research group *Crop Ecology and Product Quality* focuses on the optimisation of crop production systems, which enhance crop yield and/or product quality in environmentally sustainable and economically feasible farming systems. The national coordinators in Cereals and Seed Production research in Denmark are in this research group. DIAS coordinated the Danish work on co-existence including the Danish co-existence report (Tolstrup et al. 2003) and organising the 1st European Co-existence Conference (GMCC-03), and we have participated as experts at coexistence meetings with the EU parliament and the Danish parliament. We are involved in the EU project SIGMEA (mentioned by RISØ), and will be involved in the organisation of GMCC-05.

DIAS-IPM, Department for Integrated Pest Management has substantial expertise within identification, modelling and control of pests and seed-borne diseases using a combination of classic methods and new molecular tools for identification and characterisation. Research Centre Flakkebjerg was built in 1997 and has excellent facilities for field and semi-field experiments, climate chambers and green houses for controlled experiments, as well as well functioning classified laboratories for both studies in classic mycology and molecular biology.

Table for person month allocated on WP's and partners

Partner	1 RVAU	2 RISØ	3 DIAS- GBI	4 DIAS- IPM	Total
WP1			4,0		4,0
WP2	6,0			8,0	14,0
WP3	2,0	4,0	6,0	5,0	17,0
WP4		5,3	4,0	4,0	13,3
Total	8,0	9,3	14,0	17,0	48,3

(Please give Institution or Department as partner id.)

Table for breakdown of total budget on partners and different cost categories (DKK)

Participating institution	Responsible scientist	Salaries		Equip-ment	Operational expenses	Total budget DKK
		Acade-mic	Techn. adm.			
RVAU	BJO	407	211		323	1.130
RISØ	RBJ	482	219		142	1.012
DIAS-GBI	BB	754	472	224	612	2.474
DIAS-IPM	LAB	754	620		613	2.384
						7.000

A.4. Dissemination of scientific results

Please enclose information on:

- Planned education of scientists including Ph.D. and post-docs. Risø cofinances a PhD student (6 months in total).
- Stays abroad, guest researchers etc. Project staff will visit relevant collaborate partners, and specifically in WP4 stays abroad / guest researchers are planned – however, only short term.
- Communication of results. Publication strategy, contributions to national or international workshops etc.

Results from the work packages will be presented at field days and in popular farmers magazines as they are obtained during the project period and presented in DARCOF-enews, at national and international meetings and conferences for advisers and researchers. At the end of the project the results will be published in peer-reviewed journals.

A.5. Scientific collaborations

Please enclose information on:

- Collaboration with other research institutes (national and international collaborative partners etc. please include a brief description of the collaborative scheme)

HØ is Head of the COST Action 860: Sustainable low-input cereal production: required varietal characteristics and crop diversity' (SUSVAR www.cost860.dk). The main aims of the network are to ensure stable and acceptable yields of good quality for low-input, especially organic, cereal production in Europe. This will be achieved by developing ways to increase and make use of crop diversity (e.g. variety mixtures, crop populations or intercropping) and by establishing methods for selecting varieties, lines and populations with special emphasis on the influence of genotype-environment interactions. As a member of the board for the Consortium for Organic Plant Breeding (ECO-PB), HØ interacts with scientists and breeders all over Europe within the organic area. This interaction is extended globally through being vice head for Plant Breeding and Seed Production Section in the International Society for Organic Agriculture Research (ISOFAR).

In 2004 collaboration was decided with Athole Marshall, IGER, UK and Phil Rolston, AgResearch, New Zealand to establish a common platform for the studying of gene flow in white clover. Until

now none of us has been able to attract funding, but hopefully experimentation can start in 2006 (WP4-DIAS-GBI). IGER perform white clover breeding – both with traditional and biotechnologically methods, in addition Athole Marshall is responsible for the multiplication of breeders material and new varieties. AgResearch is also breeding white clover. New Zealand holds the largest white clover seed production in the world.

DIAS-IPM (BJN) and DIAS-GBI (BB) will participate in the EU-project ORGANIC-REVISION with the overall objective to provide recommendation for the revision and further development of the EU Regulation 2092/91 and other standards for organic agriculture. BB has established contact to the manager of WP4 Cristina Micheloni.

Nikolai Kuptsov, from Minsk in Belarus, is expected to participate as guest researcher on the proposed project. Nikolai is author breeder of a number of lupin varieties registered in Eastern and Western Europe and he has extensive knowledge of lupin genetic resources and breeding. Contacts are established to Chile. E.g. Semilas Baer, where selected material can be multiplied during winter in nurseries.

EUGRAINLEGUMES. From contacts with the only Danish partner RISØ and by studying the project home page, it can be concluded that there is no overlap between the proposed project in SEED and the EUGRAINLEGUMES. However, activities in the two programmes will be discussed and coordinated with partners in EUGRAINLEGUMES and synergistic effects are foreseen.

- Co-ordination with other projects (relations with other projects within the same field, which have been granted public funding)

There will be a synergism between SEED and another DARCOFIII project proposal QUALCER. For the grain legumes, QUALCER will focus on diversity, improved organic growing systems for quality bread and feed and the soil-borne diseases in relation to crop rotation and intercropping. In SEED, the focus will be on breeding of new ideo-types better adapted for the organic production systems and impact studies of seed health of grain legumes.

- Collaboration with private business partners or networks
Signed letters from collaborate partners are provided

A.6 Other issues

(E.g. special considerations concerning conducting of experiments in relation to principles of organic food and farming).

The project group will discuss principles of locating field trials with regards to seed borne diseased and the prevalence of pests. If the project group finds it optimal to do part of the experimentation outside an organic cropping area DARCOF will be consulted.

A.7 References

WP1

WP2

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WP 3

- Borgen, A and L. Kristensen 2001: Spiringsvitalitet af økologisk såsæd. Kapitel 7 i Videnssynthesen "Forædling af korn og bælgssæd samt produktion af såsæd i økologisk jordbrug" Ed: B.Nielsen og L.Kristensen. FØJO-rapport.
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A.8 Curriculum vitae

(CV's of central persons describing their key qualifications for the project. The CV for each person should be 1 page, including a list of the most significant publications during the last five years).