



Progress Report 2007 and Application for Continuation in 2008

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Research in Organic Food and Farming
International Research Co-operation and Organic Integrity
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High Quality Seed – Maintaining Integrity in Organic Farming (SEED)

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Lars Bødker is no longer working in this area of research, and the activities he was involved with and in charge of, are now taken care of by Bent J. Nielsen.

7. Midterm description of the project, its results and progress, and application for continuation in 2008

A. Project summary

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.

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

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			

B. Objectives and expected achievements

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.

C. Midterm results and progress

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

WP No.: 1. Management and network administration

Task 1. Organising workshops where preliminary results are presented

The project staff met twice during 2006, and during the growing season of 2007 meetings have been organised specific to the crops included in the project. Next common meeting and workshop are planned for late 2007.

It was originally planned to have yearly workshops where also representatives for organic farmers

were invited. Until now project staff has had two common meetings in 2006, and it has been decided, that until we have more experimental data i.e. from two growing seasons, we want to meet in relation to the individual crops (growing season 2007) and then have the next common meeting late 2007. It was concluded not to organise meetings for breeders, advisers, farmers etc. until we have evaluated the results from at least two growing seasons.

During the project meeting in December 2006 it was decided not to have a workshop on the basis of one year trial-results. In addition several scientists from SEED are involved in DanSeed (www.DanSeed.dk) which organised a seed symposium on March 2007 with 88 participants day one and 45 participants day 2, where focus groups were formed. During the symposium activities on seedborne diseases was reported.

Task 2. Planning communication and publication

During the common meetings plans for communication and publication have been and will continue to be discussed. Project staff has been involved national and international seminars and conferences during the first two years of the project.

Task 3. Organising network meetings

During the project meeting in December 2006 it was decided not to have a workshop on the basis of one year trial-results. In addition several scientists from SEED are involved in DanSeed (www.DanSeed.dk) which organised a seed symposium on March 2007. During the symposium activities on seedborne diseases was reported.

Task 4. International activities

In March 2006 a NJF-seminar on 'Aspects of growing transgenic crops' was held in Denmark (GBI-Flakkebjerg). The seminar had 23 participants from Finland, Norway, Sweden and Denmark. <http://www.njf.nu/site/seminarRedirect.asp?intSeminarID=379&p=1293>

During 2006 a NJF-seminar on Herbage Seed was organised in Denmark including a session on organic seed production. The seminar was attended by scientists, advisers and seed growers from the Scandinavian countries and the seminar had in total 93 participants.

<http://www.njf.nu/site/seminarRedirect.asp?intSeminarID=395&p=1293>

In 2007 International Herbage Seed Conference was organised in Norway including a post-conference tour in Denmark. The conference had close to 100 participants and included a session on gene flow. Following the post-conference tour in Denmark a meeting was held to discuss areas of collaboration with AgResearch, New Zealand. However, activities in New Zealand are scarce due to lack of funding in this area.

<http://cropandsoil.oregonstate.edu/ihsg/conference2007/welcome.php>

In addition project staff has provided information on national activities to Organic Revision.

Task 5. Project web administration

Project website is open including information on each workpackage and activities. It has been discussed to establish/utilise the intranet facilities, however, this is not yet decided.

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

Task 1. Prevalence and severity of seed borne infections of *Ascochyta fabae* in faba bean

Transmission of *A. fabae* from infected seed to new seeds has been estimated in field trials 2007 at AU, Flakkebjerg. In trial A three different mixtures of healthy and diseased seeds with *A. fabae* (0, 5 and 10% infected seeds) have been sown in field trials to estimate the yield and seed infections of *A. fabae* in the harvested seed. In trial B, twelve seed lots of the same variety (Columbo) with different levels of seed vitality and seed infections of primarily *A. fabae* have been sown in field plots to estimate the variation in yield due to difference in seed vitality and seed infections. Despite the use of infected seeds only little infection could be observed in the field. The trials have been harvested August 2007 and the seeds are currently being analysed for infections of *A. fabae*.

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 was available and further multiplication of susceptible and resistant lines were performed in 2006 (the lines Boruta, Teo Prima, Tanjil Polonez Rose, Illyarie, Eranti, Sonet Kalya, Boral, Lae8 and Danja). Due to the dry conditions in 2006, Anthracnose development in the field was insignificant. Three lines were multiplied in Chile for

production of infected seeds during winter 2006-2007. In 2007 the first field trial with variable infection level was carried out. Seeds with 0%, 2,5%, 5% and 7,5% anthracnose infection of the resistant Lav8-5, the susceptible Prima and the highly susceptible Eranti were evaluated in a 4 replicate block experiment. Due to the dry and warm spring the initial transmittance from seed to plant did not occur and anthracnose symptoms were not observed later in the season. The plots were harvested in September 2007 and seeds are being prepared for analysis for infection of *Coletotrichum lupini*.



Fig 1. Attack of anthracnose in lupin. Left is shown pods with symptoms and necrosis on young tissue.
To the right is shown destruction of upper branches.

First year testing indicates that a zero threshold value for anthracnose infection in seeds is too low at least for commercial seeds. In earlier generations of seed multiplications this low tolerance is presumably an important precaution to avoid infection build up. However, threshold values recommendations can not be based on one year and one location and further testing is needed to verify if threshold values can be modified.

Task 3. Screening for resistance to common bunt (*Tilletia tritici*)

Screening for resistance to common bunt (*Tilletia tritici*) have been performed at AU, Flakkebjerg 2007 with 31 new varieties of wheat and 24 new varieties of triticale using artificial inoculation in field experiments with two different isolates of *T. tritici*. Wheat varieties that in previous trials had shown some resistance were tested again on two different test isolates of *T. tritici*. In the test of new wheat varieties only two varieties (Penta and 702-1102C) were highly resistant against common bunt on the same levels as the standard variety Stava. Four varieties were resistant/moderate resistant (11-25 % attack in field trials) while the rest of the varieties were susceptible to bunt (see figure below). In winter triticale most of the varieties were resistant to common bunt. However a few varieties were attacked at a low level (e.g. Trigold, Cando, NORD 00754/10, SW Talentro, HE Ti 318, Tritikon, HE 115-01 and Kortego). Three varieties were characterised as moderate susceptible (Triamant, Trignatus and HE TI 301). The level of attack in triticale varieties (even the most susceptible) is in general lower than in wheat.

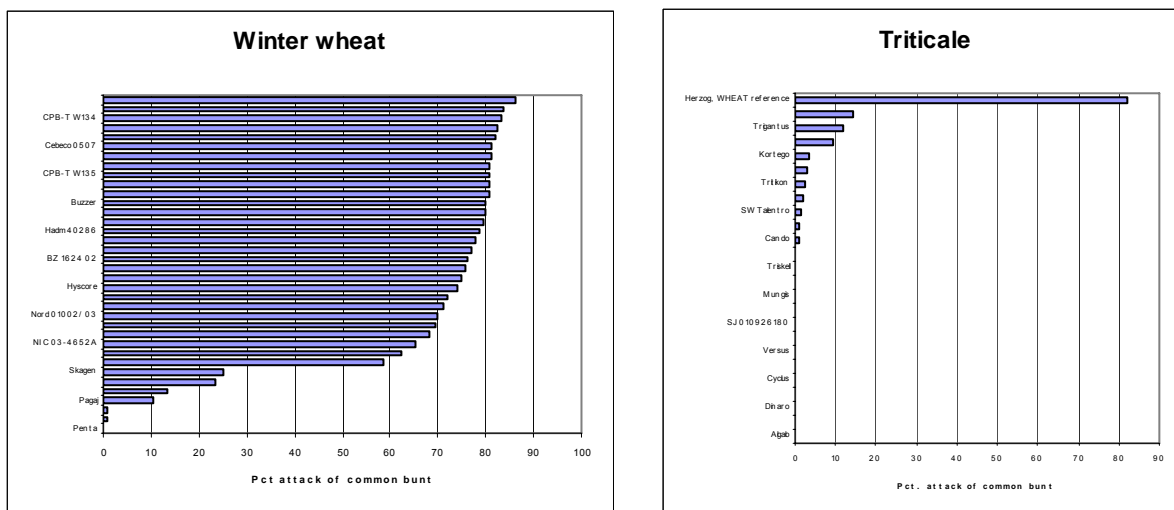


Fig 2. Percent attack of common bunt (*Tilletia tritici*) in 31 new varieties of winter wheat (left) and 24 varieties of winter triticale (right). The susceptible wheat variety Herzog is included in the triticale test as reference. See text for variety names of highly resistant and moderate resistant varieties. Artificial inoculation. AU, Flakkebjerg 2007.

The resistance tests were also performed in spring wheat and spring triticale varieties and the results show that most of the spring wheat varieties are susceptible to common bunt. A few varieties had some resistance in the test 2007 (e.g. Dragon and leguan). The lines 707-4001 and 707-4002 are crossing materials from Pajbjerg (see task 5). In spring triticale the varieties were highly resistant to common bunt. Only one variety were attacked at a moderate level (Dublet).

Table 1. Percent attack of common bunt (*Tilletia tritici*) in spring wheat and spring triticale varieties. Artificial inoculation. AU, Flakkebjerg 2007.

Spring wheat and spring triticale			% attack of common bunt
707-4001 A	Spring wheat	Pajbjerg R	0,0
707-4001 B	Spring wheat	Pajbjerg R	0,0
707-4001 C	Spring wheat	Pajbjerg R	0,2
Dragon	Spring wheat		1,2
Leguan	Spring wheat		4,5
Vinjett	Spring wheat		8,1
707-4002 C	Spring wheat	Pajbjerg S	8,1
707-4002 A	Spring wheat	Pajbjerg S	10,1
Trappe	Spring wheat		10,3
707-4002 B	Spring wheat	Pajbjerg S	10,6
Zircon	Spring wheat		15,2
Sensas	Spring wheat		23,7
Amaretto	Spring wheat		26,9
Safrani	Spring wheat		29,1
Tybalt	Spring wheat		31,9
SW Kungsjet	Spring wheat		32,4
Taifun	Spring wheat		35,0
He 104-03	Spring triticale		0,0
Legalo	Spring triticale		0,0
Logo	Spring triticale		0,0
Granador	Spring triticale		0,0
Trado	Spring triticale		0,3
Nilex	Spring triticale		0,5
Dublet	Spring triticale		11,8

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 was propagated and infected seeds with *T. tritici* 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. It was not possible to perform the test 2007 and the virulence survey have been postponed to 2008. (See deviation and budget change later).

Task 5. Contribute to the development of new varieties

Breeders at Pajbjergfonden have carried out back crossings of effective genes for bunt resistance into spring wheat varieties with a good general resistance to other major diseases. In 2007 six sublines from two lines (7007-4001 A,B,C and 707-4002 A,B,C) have been tested at AU, Flakkebjerg using artificial inoculation in field experiments with two different isolates of *T. tritici*. The results were very promising, two lines were susceptible and two lines were highly resistant to *T. tritici*. (table above). The lines are now F9 and in the first step of multiplication.

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

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

In 2006, different varieties suitable for organic growth conditions have been evaluated to find the best variety for the species mixtures. Infected seeds with *A. fabae* in faba bean, *Ascochyta* spp. on pea and *C. lupini* in lupin have been collected.

Diversification trials have been performed at two locations, AU, Flakkebjerg and KU, Højbakkegård 2006 and 2007. The objective is to elucidate the potentials for improved seed quality by increased genetic diversity of cereals and grain legumes and as well as to get information about general and specific combining abilities at species level. The disease development in the cereals and grain legumes in pure stand and in mixtures have been scored during the growing season.

In 2006 the summer was hot and dry (July) and disease development in the trial was only moderate and late with approximately 10 % attack of net blotch (*D. teres* and *D. avenae*) in barley and oat. In barley and triticale late attack of rust was observed. There were no differences in leaf attack between the treatments. In legumes there was only significant attack in faba bean (chocolate spot). In the trial at KU, Højbakkegård there was less attack of chocolate spot in the cereal/faba bean intercrop while at AU, Flakkebjerg the opposite was observed.

In 2007 spring and early summer was dry and relatively hot. Conditions for disease development were not favourable and the transmission of diseases from seeds to plant and development of diseases in general on the leaves was late. There were no significant differences in development of seed borne diseases in the crop between infected and healthy seed lots.

Separated seeds from all relevant plots are currently being analysed for seed borne diseases.

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

Crop development has been 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 has been performed with leaf area index (LAI) by Licor 2000 and normalized difference vegetation index (NDVI) by remote sensing. Weed infestations have been registered during the growing season and at harvest. The plots have been combine harvested, mixtures separated and component yield measured.

The spring 2006 was cold and wet. As a consequence the establishment of the field trials was postponed to start of May and not as planned in start of April at both Flakkebjerg and Højbakkegård. The wet conditions made it difficult to control the weed mechanically. The late time of sowing and problems with weeds have influenced the yields negatively, and reduced the attack of plant diseases. Intercropped and cereal sole crops were applied 50 kg N/ha of organic manure (Binadan). This is another limiting factor for the obtained cereal yields.

The yield level 2006 in the cereal crops is 2-3,5 t/ha and intercropping with the pulses reduces cereal yield up to 35%. Intercropping with cereals has a much larger effect on the pulses with reduction in yield up to 80%. In faba bean intercropping with wheat, oat, barley or triticale influence the total yield negatively in the 2006 trials. Intercropping with lupin did not show the same effect. The combining ability of lupine with cereals seems to be better in this experiment.

The trials 2007 was established mid April. The spring and early summer was dry and relatively hot. Crop establishment and weed control was good. However, due to the dry conditions disease development was reduced. Mid summer to harvest was wet. After harvest, the seeds have been cleaned and species mixtures separated in fractions representing individual crop species. Quality and seed analysis are currently being performed.

Table 2. Yield (kg/ha) in diversity trials 2006

Crop combination	KU, Højbakkegård			AU, Flakkebjerg		
	Cereal kg/ha	Legume kg/ha	Total kg/ha	Cereal kg/ha	Legume kg/ha	Total kg/ha
Pea		816	816		1.809	1.809
Pea-infected		793	793		1.489	1.489
Faba		453	453		877	877
Fabia-infected		385	385		844	844
Lupine		1.507	1.507		1.701	1.701
Lupine-infected		1.507	1.507		1.837	1.837
Wheat	2.663		2.663	2.801		2.801
½Wheat + ½Pea	2.408	181	2.590	2.319	315	2.634
½Wheat + ½Faba	2.340	91	2.431	1.962	156	2.118
½Wheat + ½Lupin	2.295	385	2.680	2.030	708	2.738
Oat	2.437		2.437	3.211		3.211
½Oat + ½Pea	1.961	136	2.097	2.379	351	2.729
½Oat + ½ Faba	1.796	79	1.876	2.394	158	2.552
½Oat + ½ Lupin	2.227	289	2.516	2.391	627	3.017
Barley	3.117		3.117	3.485		3.485
½Barley + ½Pea	2.374	198	2.573	2.717	328	3.045
½Barley + ½Faba	2.493	85	2.578	2.233	157	2.390
½Barley + ½Lupin	2.403	306	2.709	2.785	449	3.235
Triticale	2.187		2.187	2.061		2.061
½Triticale + ½Pea	1.921	232	2.153	1.789	426	2.215
½Triticale + ½Pea-infected	1.859	266	2.125	1.579	457	2.036
½Triticale + ½Faba	1.655	147	1.802	1.234	241	1.476
½Triticale + ½Faba-infected	1.751	108	1.859	1.444	222	1.666
½Triticale + ½Lupin	1.734	448	2.182	1.462	755	2.217
½Triticale + ½Lupin-infected	1.791	521	2.312	1.487	668	2.155

Yield in 100% dry matter

Task 3. Grain quality for human and animal nutrition

Samples of cereal and grain legumes from the field trials carried out in 2006 have been processed by appropriate cleaning and grading techniques and analyzed for 1) Seed size distributions and shape parameters using standard methods and Image Analysis, 2) Gross chemical composition using NIRS (Near-Infrared Reflectance Spectroscopy, The analyses include seed protein, starch and gluten for wheat; protein and starch for barley and triticale; protein for oat.), 3) Gross composition of seed C and N (protein) for those grain legumes where no NIRS calibrations have been developed yet. 4) In vitro enzyme digestibility of organic matter (EDOM) has been analysed by NIRS for wheat and triticale in order to determine nutritional values for non-ruminant animals.

Analysis of vitality (Germination Index according to EBC method, European Brewery Convention: Analytica-EBC, Fachverlag Hans Carl: Nürnberg, 1998.) on cereal seed samples are currently being performed.

In order to test the potential of image analysis of physical seed characteristics seeds will be analysed using Videometer Lab. in cooperation with other projects.

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

In the first two years we have focused on revealing the problems related to making meta-analyses based on data from field trials. The problems are specifically related to how to establish the degree of certainty in each of the effect estimates from trials with very different experimental error, i.e. how to weight each estimate in the procedure for making the combined meta-estimate of the effect to be studied. This differs from the established knowledge on meta-analyses in medical data. A possibility is

to use a range of weights from equal to observed inverse variances either to form a prior distribution of weights in a Bayesian setting or as a kind of sensitivity analysis. Incorporation of structural information on the individual studies, such as extent in time and space, may also be considered as a criterion for weighing.

In 2008, these ideas will be used to make a meta-analysis on a specific question of importance for the valuation of the WP3 intercropping data.

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

Task 1. Producing white clover seed in crop mixtures with grass species

Field experiment was established in spring 2006 with white clover/grass species undersown in a cover crop of spring barley. After harvest of spring barley and in spring 2007 the establishment of white clover/grass species was evaluated. All crops were established successfully and the experimental site had a very low number of weeds. All experimental treatments have been performed during the growing season and in spite of the very poor weather conditions during harvest the white clover seed plots have been harvested, however, they are still not cleaned and therefore we do not have any results on seed yields yet. The results will be included in our report on preliminary results from herbage seed experiments 2007 and provided to the advisers in December 2007.

Task 2. Developing a decision support system to minimise yield reduction in white clover due to weevils

To make out which meteorological factors are important for colonizing white clover fields from the clover seed weevil (*Apion fulvipes*) 4-5 white clover fields were monitored in 2006 and 2007 1-2 times a week from mid April to colonization time. The preliminary results shows that if more than 4-5 hours in succession with average temperatures more than 18 °C takes place the clover seed weevil will colonize the white clover fields. However, if precipitation or high wind velocity at the same time takes place it seems that no colonization will happen. The interaction of temperature, precipitation and wind velocity are still not unambiguous and the research will continue in 2008.

Furthermore, in 2008 a lab test to test the efficacy of natural pyrethrum on the pests, the clover seed weevil (*Apion fulvipes*.) and the lesser clover leaf weevil (*Hypera nigrostris*) is carried out.

In 2006 a population of the clover cyst nematode (*Heterodera trifolii*) was propagated and we have now a population with about 40000 eggs/larvae per kg soil.

A glasshouse trial to investigate the influence of the clover cyst nematode on germinating and small clover plants is started with 7 treatments and 4 replicates. The treatments are white clover in combination with 3 infestation levels of the clover cyst nematode under influence of ploughed in clover or not. The trial will be repeated in 2008.

Task 3. Collection of base-line information on the prevalence of white clover in organic farming systems and the surrounding natural habitats

In the first year of the project, three organic farms with clover seed production and clover-grass fields were visited to determine the distribution of white clover in and outside fields. White clover was found outside the cultivated areas, but in frequencies much lower than within fields or pastures. It was judged that the seed set of clover in semi-cultivated places would be rather low, as management of field borders is often accomplished, and seed set controlled. Therefore it was decided to focus on the frequency of clover at the cultivated land including clover-grass fields in rotation and permanent pastures.

In 2006, the genetic diversity was analyzed in clover grass fields by collecting 25 plants x 5 m² in each field (125 plants/field). AFLP analysis of plants from three fields showed that the genetic diversity of the white clover was highest in recently established clover-grass and least in old fields. The results support the hypothesis that pronounced vegetative propagation in white clover gave rise to large clones that were most abundant in the older fields suppressing establishment of plants from sexual reproduction. If this is a general trend, then seeds with transgenic paternity (from pollination by GM plants in neighboring fields) are most likely to establish in young fields. However, grazing patterns and trampling are also supposed to influence sexual reproduction and establishment by affecting number of flower heads and creating open patches for seedling establishment. Frequency of flowering head was counted in the visited clover-grass fields.

In 2008, a larger survey of organic clover-grass fields will take place as farms with both seed production and clover-grass fields and farms without clover-grass will be visited. Flower heads and seed set will be accounted for in the clover grass fields (quantification of potential for pollen and seed dispersal) in relation to age of field, and grazing pressure. In selected barley fields with previous clover seeds or clover grass the number of white clover volunteers will be quantified, and compared to farmers' information on rotation schemes and volunteer control.

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

White clover

In 2006 and 2007 we have performed molecular analysis of the three white clover varieties, Klondike, Milo and Rivendale that are sown out in a larger field experiment at AU, DJF, Flakkebjerg. AFLP primer combinations have been selected that will allow us to detect gene flow between different plots (and treatments) in this experiment.

A master study has been carried out in the frame of WP 4. The title of the master thesis "Gene flow in white clover, *Trifolium repens* - modeling inter-field dispersal and experiments on gene flow in small populations". The master consists of:

- Modelling, using a mathematical model, which specifically account for gene flow between fields caused by pollinators. The model - the Portion-dilution-model - is based on three observable parameters, E = the fraction of pollinators that arrive in a recipient field from an extrinsic pollen donor source, ψ = the paternity shadow and b = mean number of flower heads a pollinator visits in one foraging bout. The model (Cresswell et al., Oikos 98, 375-384) has previously been applied to oilseed rape and red clover, and we now use it on white clover, where the honey bee (*Apis mellifera*) is the predominant pollinator. The foraging behavior of *Apis* has required some changes in the original model. The paternity shadow has been determined in an experiment, where we used a pollen donor with red leaf marker.
- Experimental results on gene flow between small populations of white clover. In an 11 experimental plots with different genotypes (propagation plots at DLF-Trifolium, St. Heddinge), DNA markers (AFLP) were determined in maternal plants and their offspring. Using assignment test (AFLPop and STRUCTURE) we determine to what extent outcrossing takes place within plots and from other plots at different distances from the recipient. Pollinator behavior has also been observed and frequency of selfing estimated.
- The master student, Christina Løjtnant, KU, is expected to defend her master thesis in December this year. Results from the master project will be presented in 1-2 peer reviewed international publication.

Ryegrass

Analysis of gene flow distances in fields of ryegrass has been performed in a natural field situation with neighboring rye grass fields cultivated with different varieties. Using AFLP markers and assignment tests, we found a relatively high frequency of impurities, e.g. 300 meter from the adjacent border of the two fields more than 3 % impurities were detected. However, we need to analyze an array of reference varieties before we can say if these impurities derived from outcrossing between the two fields or if they derived from the seed (volunteers or certified seed).

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

During the 6th International Herbage Seed Conference in Norway/Denmark June 2007 areas of common collaboration was discussed between scientists from IGER-Aberystwyth, UK; AgResearch, NZ and AU, DK. Already now the red-leaf morphotype of white clover has been used for pollination trials (collaboration RISØ and AU). Especially the area of pollination seems to be of common interest especially between New Zealand and Denmark. However, in each country resources for activities related to the use of GM-crops are very limited, and activities are quite restricted.

C.2 Fulfilment of deliverables and milestones

Deliverables list (from application)

Workpackage 1						
Deliverable No	Deliverable title	Lead scientist	Delivery date	Allocated scientific person moths	Type of deliverable	Fulfilled (ok) or deviations (d)*
D1.1	Meetings in the project group – at a minimum twice a year	BB	2006-09		O/C	OK (partly, d1)
D1.2	Yearly communication plans	BB	01/2006 - 01/2009		O	OK (partly)
D1.3	Yearly workshops	BB	2006-09		R/C	OK (partly, d2)
D1.4	International workshop	BB	2007		R/C	OK
D1.5	Project web-site established	BB	06/2006		O	OK

* *Deviations are to be further discussed in D*

Milestones list (from application)

Workpackage 1			
Milestone No	Milestone title	Delivery date	Fulfilled (ok) or deviations (d)*
M1.1	Project website open	05/2006	OK
M1.2	International workshop is held	07/2007	OK

* *Deviations are to be further discussed in D*

(The nature of the deliverables must be indicated by S = publication in scientific journal with peer review; P = publication in journals without peer review; R = reports; C = presentation at meetings and congresses or O = other types of deliverables, e.g., prototypes, models, websites, etc.).

Deliverables list (from application)

Workpackage 2						
Deliverable No	Deliverable title	Lead scientist	Delivery date	Allocated scientific person moths	Type of deliverable	Fulfilled (ok) or deviations (d)*
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		P/O	
D2.2	Manuscript on threshold values of <i>C. lupini</i> in lupin in Denmark has been submitted	BJO	05/2009		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		P/O	

* Deviations are to be further discussed in D

Milestones list (from application)

Workpackage 2			
Milestone No	Milestone title	Delivery date	Fulfilled (ok) or deviations (d)*
M2.1	Infected seed and haulm with <i>A. fabae</i> have been produced and collected	09/2006	OK
M2.2	First year field trials on host resistance against <i>A. fabea</i> in faba bean are completed	12/2007	OK
M2.3	Second year field trials on thresholds and host resistance against <i>A. fabea</i> 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 <i>Coletotrichum lupini</i> in lupin is completed	09/2006	OK d3
M2.5	Second year field trials on thresholds and host resistance against <i>Coletotrichum lupini</i> in lupin are completed	12/2007	d3
M2.6	Third year field trials on thresholds and host resistance against <i>Coletotrichum lupini</i> in lupin are completed	12/2008	d3
M2.7	Wheat differential lines with specific Bt genes propagated and seeds with <i>T. tritici</i> have been collected	10/2006	OK d4
M2.8	First year field trials on host resistance against <i>T. tritici</i> are completed	12/2006	OK
M2.9	Second year field trials on host resistance against <i>T. tritici</i> and first virulence survey are completed	12/2007	OK
M2.10	Third year field trials on host resistance against <i>T. tritici</i> and second virulence survey are completed	12/2008	

* Deviations are to be further discussed in D

Deliverables list (from application)

Workpackage 3						
Deliverable No	Deliverable title	Lead scientist	Delivery date	Allocated scientific person moths	Type of deliverable	Fulfilled (ok) or deviations (d)*
D3.1	FØJOenyt. Disease development, and seed quality in pure stands and mixtures of cereals and grain legumes	BJO	12/2007		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		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		R/P	
D3.4	Paper on crop development and general and specific combining ability species mixtures.	BJO/ JRJ	08/2009		R/P	
D3.5	FØJOenyt. Yield and quality of pure stands and mixtures of cereals and grain legumes	JRJ	12/2007		R	
D3.6	Manuscript on meta analysis of mixture effects of selected characteristics of species mixtures as part of PhD thesis	HØ	12/2008		P	

* Deviations are to be further discussed in D

Milestones list (from application)

Workpackage 3			
Milestone No	Milestone title	Delivery date	Fulfilled (ok) or deviations (d)*
M3.1	Production of seed lots infected with <i>A. fabea</i> in faba bean, <i>C. lupini</i> in lupin and <i>A. pisi</i> in pea	12/2006	OK
M3.2	Field experiment with species and crop mixtures (diversification trial) conducted first year at two locations	08/2006	OK
M3.3	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	OK d5
M3.4	Field experiment with species and crop mixtures conducted second year at two locations	08/2007	OK
M3.5	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	
M3.7	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	
M3.8	Principles for including data in the meta analysis	9/2006	OK

* Deviations are to be further discussed in D

Deliverables list (from application)

Workpackage 4						
Deliverable No	Deliverable title	Lead scientist	Delivery date	Allocated scientific person moths	Type of deliverable	Fulfilled (ok) or deviations (d)*
D4.1	Yearly reports on white clover seed and clover-grass forage yield in mixed crop production	BB	12/2007 12/2008 12/2009		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		R/S/O	
D4.3	Report on the frequency and distribution of white clover and clover-grass on organic farms	RBJ	12/2006		S/C	Partly fulfilled, (see d7)
D4.4	Guidelines to reduce gene flow in white clover and clover-grass	BB + RBJ	11/2009		P/S/C	
D4.5	Report on the use of morphological markers to analyse gene flow in white clover	RBJ + BB	01/2009		R	OK and d6

* Deviations are to be further discussed in D

Milestones list (from application)

Workpackage 4			
Milestone No	Milestone title	Delivery date	Fulfilled (ok) or deviations (d)*
M4.1	Seed yields in white clover and forage yields are registered in three consecutive years of field experimentation	08/2007, 08/2008, 08/2009	OK
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	d7 (partly fulfilled)
M4.4	Field plots established for pest analysis and gene flow investigations	12/2006	d8
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	

* Deviations are to be further discussed in D

D. Description of deviations and subsequent adjustments of plans

Workpackage 1

D1: There were two meetings in the project group in 2006. It was decided (Dec. 2006) that meetings during the growing season of 2007 should be held crop-specific and there will be a common meeting in the project group late 2007.

D2: It was originally planned to have yearly workshops where also representatives for organic farmers were invited. It was decided, that until we have more experimental data i.e. from two growing seasons, we will not organise meetings for breeders, advisers, farmers etc. until we have evaluated the results from at least two growing seasons.

Still due to other meetings (DanSeed symposium, Cerealienetværket and others) collaborators are still informed about project and preliminary results.

Workpackage 2

D3: There is a discrepancy between work package description and milestone 2.4, 2.5 and 2.6. According to the work package description, year 2006 is used for production of infected seed lots and 2007 + 2008 for threshold experiments in the field. Milestones in milestone list have been corrected according to work package description (see below)

Milestones corrected according to original work package description

Workpackage 2			
Milestone No	Milestone title	Delivery date	Fulfilled (ok) or deviations (d)*
M2.4	Infected seeds have been multiplied	09/2006	OK
M2.5	First year field trials on thresholds and host resistance against <i>Coletotrichum lupini</i> in lupin are completed	12/2007	OK
M2.6	Second year field trials on thresholds and host resistance against <i>Coletotrichum lupini</i> in lupin are completed	12/2008	

D4: The virulence test should have been performed 2007 but due to lack of differential lines the test have to be postponed to 2008. Budget has been corrected.

Workpackage 3

Description of work: In the original application it is mentioned that diversification trials will be performed over three years to get information about general and specific combining abilities at species and *variety level* (line 4) For wheat, a variety mixture will be included to study the combined effect of species and variety mixtures (line 6). In the revision of the original application (after budget cut down), work on variety level and variety mixtures were taken out. The sentences were by mistake not excluded in the text in the introduction to WP 3.

Corrected text is:

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 as well as to get information about general and specific combining abilities at species 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.

D5: Analysis of seed bore diseases and test of vitality are currently being performed.

D6: This deliverable has a delivery date in 2008, but has actually already been partly fulfilled as we used a red leaf marker to determine the paternity shadow in white clover. The paternity shadow is one of the parameters used in the modelling of gene flow between clover fields.

D7: During the 2006 analysis of distribution of white clover on organic farms we found that the task could not be fulfilled within the time allocated, as white clover was distributed almost in every habitat on the farms. As we evaluated that white clover individuals in semi-cultivated habitats (field margins and borders) would only constitute a minor sink and source of gene flow, we have chosen to concentrate the analysis on clover-grass fields and fields where clover-grass or clover seed production has previously been grown. Monitoring of distribution in these fields will take place in 2008. In 2006 we instead analysed the vegetative propagation in clover-grass fields of different age. The data are publishable in an international journal.

D8: During spring 2006 very concentrated infestation of thistles were observed on the experimental site planned for the field experiment. It was concluded, that hand-weeding would be very, very time consuming – and most likely it would not be enough to avoid influence on the results in 2007. It was therefore decided to postpone establishment of the field trial for gene flow until 2007. Instead soil was kept 'black' and mechanical weed control was performed during the growing season of 2006 – and in 2007 establishment was successful.

Instead a field with small plots of different clover genotypes at DLF-TRIFOLIUM, St. Heddinge was used to study gene flow in 2006. Results will be published as part of a master thesis (ultimo 2007) and as a peer reviewed international publication. The field plots of white clover for pest analysis and gene flow investigations were established in 2007.

E. Project publications and other products

1. Products from Organic Eprints archive

Fernandez-Aparicio, M; Jørnsgård, B and Rubiales, D (2006) [Effects of crop mixtures on pest of faba bean under organic agricultural conditions](#). Paper presented at International workshop on Faba bean breeding and agronomy, Cordoba Spain, 25-27 October 2006; Published in Andalusia, Junta de, Eds. *International Workshop on faba bean breeding and agronomy*, page pp. 140-142. Viceconsejería. Servicio de Publicaciones y Divulgación.**

Jørgensen, RB ; Løjtant, C ; Andersen, NS and Andersen, BA (2007) [Co-existence with GM crops: grasses, clover and fodder beet](#). Paper presented at XXVIIth EUCARPIA Fodder Crop and Amenity Grass Section meeting, Copenhagen, Denmark, August 19-23 2007.

Kiær, Lars; Skovgaard, Ib and Østergård, Hanne (2007) [Yield of spring barley mixtures as a function of varietal and environmental characteristics](#). Paper presented at Varietal characteristics of cereals in different growing systems with special emphasis on below ground traits, Velence, Hungary, 29-31 May, 2007.**

Kiær, Lars; Skovgaard, Ib and Østergård, Hanne (2006) [Meta-analysis is a powerful tool to summarize variety mixture effects - exemplified by grain yield and weed suppression of spring barley](#). Paper presented at COST SUSVAR workshop, La Besse, France, 12-15 June 2006; Published in Østergård, H. and Fontaine, L., Eds. *Cereal crop diversity: Implications for production and products*, page pp. 49-52. ITAB, Paris, France.**

Løjtant, Christina L.; Damgaard, Christian F. and Jørgensen, Rikke B. (2007) [Modelling pollen dispersal - relevant to co-existence in white clover \(*Trifolium repens*\)](#). Poster presented at 6th International Herbage Seed Conference AND GMCC 2007, Norway AND Seville, Spain, 17-20 June 2007 AND 20-21 November 2007.

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

* 25-75% financed by DARCOF

** 5-25% financed by DARCOF

F. Scientific education

Marie Daniels has as a part of her master studies studied the disease development in the diversity trials and published the above-mentioned report.

PhD student Lars Kiær is associated to the project without being funded (task 4.4)

Master student Christina Løjtnant, KU and Risø. Thesis will be submitted ultimo 2007.

SOAR PhD: Naja Steen Andersen, KU-Life and Risø. Thesis on co-existence with GM oilseed rape will be submitted medio 2008.

G. National and international cooperation

Mirela Cordea, University of Cluj, Rumania has been working as guest researcher at KU, Højbakkegård summer 2007 and has participated in the experimental work.

Contribution to COST 860 SUSVAR network: poster for workshop (proceedings under preparation)

Senior Scientist Christian Damgaard, DMU, Aarhus University

Senior Scientist Per Kryger, DJF, Aarhus University

Participating in the EU-project on co-existence, SIGMEA

Participating in the European Academy on Zoopharming and Phytopharming

H. Critical reflection on the project

Although the present application was revised following the budget-cut of approximately 50% (in relation to the initial application) there are quite limited resources to investigate some of the major problems in this area. This has led to a clear focus into the scientific experiments – and during the last meeting in the project group (Dec. 2006) it was decided to limit the number of common meetings during 2007 down to one – and that information activities should be postponed until we have solid data.

To some extent this seems an obvious consequence, however, it should be discussed, how we still can utilise all experience 'common' to the project and utilise the benefit of combining different disciplines. In particular it will be of importance to include and involve the theory of meta analysis in conjunction with data from the present field experiments with species mixtures, where there now are results from two experimental years on yield and analysis of seed infection, seed size distribution, chemical composition etc. are in process.

Transmission of seed borne diseases in grain legumes from seed to plants is very dependent on climatic conditions and the expression of the diseases in the crop needs high humidity and rain splash. The weather conditions 2006 and 2007 has not been favourable for disease establishment and spread in early spring. Consequently is difficult to analyse the effect of seed borne diseases on later establishment of the diseases in the crop. Another problem is to get seed lots with the necessary high infection levels for the field trials. This is often seen in field trials with plant diseases, but field trials is the only way forward to analyse transmissions of seed borne diseases and different control measures in seed production. Field trials over several years and locations are the only way to overcome this constraint.

8. Budget

A. Account for any change in budgets

B. Budget for the whole project (1.000 DKK)

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

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Man-months							
Scientific personnel	48,25	12,85	11,65	14,75	9		
Technical personnel	53	14,90	19,10	16,5	6		

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Salaries							
Scientific personnel	2395	683,8	625,7	783,3	440		
Technical personnel	1522	308,5	349,4	559	166		
Other operational costs	1690	163,7	771,2	627	129		
Equipment	224		154	55	15		
Others (please specify)							
Direct costs	5831	1156	1852,5	2024,3	750		
Indirect costs (20% of direct costs)	1166	231,2	380	404,9	150		
Total	7000	1386,2	2281,5	2429,2	900		

Comments:

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project Birte Boelt		1 October 2007	

Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)

Name of Institute and department: LIFE (RVAU)

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Man-months							
Scientific personnel	8	2	2	3	1		
Technical personnel	9	3	3	3			

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Salaries							
Scientific personnel	407	176	182	136	51		
Technical personnel	211			73			
Other operational costs	323	58	154	107	5		
Equipment							
Others (please specify)							
Direct costs	941	234	288	316	56		
Indirect costs (20% of direct costs)	188	47	67	63	11		
Total	1130	280	403	380	67		

Comments:

A. Budget for each participating institute (1.000 DKr)Name of Institute and department: **RISØ**

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Man-months							
Scientific personnel	9,25	3,25	3,25	2,75			
Technical personnel	7	2	2,5	2,5			

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Salaries							
Scientific personnel	482	163	169,5	149,3			
Technical personnel	219	60	78	81			
Other operational costs	142	35	61	46			
Equipment							
Others (please specify)							
Direct costs	843	258	308,6	276,3			
Indirect costs (20% of direct costs)	169	51,6	61,7	55,3			
Total	1012	309,6	370,3	331,6			

Comments:

A. Budget for each participating institute (1.000 DKr)Name of Institute and department: **DIAS GBI**

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Man-months							
Scientific personnel	15	3	4	4	4		
Technical personnel	17	5	6,5	5	4		

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Salaries							
Scientific personnel	753	151,8	186,2	216	199		
Technical personnel	472	118,5	101,4	131	121		
Other operational costs	612	17,7	316,2	185	93		
Equipment	224		154	55	15		
Others (please specify)							
Direct costs	2061	288	757,9	587	428		
Indirect costs (20% of direct costs)	412	57,6	151,3	117	86		
Total	2474	345,6	909,2	704	514		

Comments:

A. Budget for each participating institute (1.000 DKr)Name of Institute and department: **DIAS IPM**

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Man-months							
Scientific personnel	16	4,6	2,4	5	4		
Technical personnel	20	4,9	7,1	6	2		

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Salaries							
Scientific personnel	753	193	88	282	190		
Technical personnel	620	130	170	274	45		
Other operational costs	613	53	240	289	31		
Equipment							
Others (please specify)							
Direct costs	1986	376	498	745	266		
Indirect costs (20% of direct costs)	397	75	100	169	53		
Total	2384	451	599	1014	319		

Comments:

C. Budget for co-financing from each participating institute (1.000 DKK)Name of Institute and department: **RISØ**

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Man-months							
Scientific personnel	8	3	3	2			
Technical personnel							

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Salaries							
Scientific personnel	260	95	98	67			
Technical personnel							
Other operational costs							
Equipment							
Others (please specify)							
Direct costs	260	95	98	67			
Indirect costs (20% of direct costs)	52	19	19,6	13,4			
Total	312			80,4			

Comments:

Person months from the institution also include education of student(s) within the project.

C. Budget for co-financing from each participating institute (1.000 DKK)Name of Institute and department: **DIAS, GBI**

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Man-months							
Scientific personnel	11	0,25	0,5	4	6		
Technical personnel							

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Salaries							
Scientific personnel	546	11	25	195	317		
Technical personnel							
Other operational costs							
Equipment							
Others (please specify)							
Direct costs	546	11	25	195	317		
Indirect costs (20% of direct costs)	109	2,2	5	39	63		
Total	655	13,2	27	234	380		

Comments:

C. Budget for co-financing from each participating institute (1.000 DKK)Name of Institute and department: **DIAS IPM**

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Man-months							
Scientific personnel	3	1	1	1			
Technical personnel							

Year:	Original budget	Consumption 2005/2006	Expected consumption 2007	2008	2009	2010	Total
Salaries							
Scientific personnel	150	49	49	52			
Technical personnel							
Other operational costs							
Equipment							
Others (please specify)							
Direct costs	150	49	50	52			
Indirect costs (20% of direct costs)	30	10	10	10			
Total	180	59	59	62			

Comments: