Danish Research Centre
for Organic Farming

Midterm Status Report 2002 and Application for Continuation in 2003

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The Directorate for Food, Fisheries and Agro Business
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1. Research program

Research in organic farming 2000-2005 (DARCOF II)

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2. Project title and number

I.12 Preventing Mycotoxin Problems (PREMITOX)

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3. Head of project

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6. Project period (month, year)

Start of project: July 1st 2000
End of project: December 31st 2004

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

A. Project summary

Mycotoxins are naturally occurring compounds and some of them constitute a severe threat to the health of humans and animals. In Danish grown small grain cereals, ochratoxin A (OTA) and trichothecenes are considered to be the most important mycotoxins. Opposed to compounds like pesticides and antibiotics, which are excluded from organic farming, mycotoxin problems cannot be totally eliminated. They can, however, be reduced if conditions stimulating fungal growth and mycotoxin formation are inhibited by suitable management practices.

Several reports and observations indicate that organically produced cereals are sensitive to mycotoxin contamination, stressing the relevance of this subject within the framework of DARCOF. The 'PREMYTOX' project is designed to increase our knowledge on the ecology of mycotoxin producing fungi and provide the farmer with information on the importance of mycotoxin producing fungi as well as practical means to reduce their dissemination, proliferation and toxin formation.

The experimental part of PREMYTOX is focusing on management practices, which are relevant to the general practice in organic farming and which are known or assumed to affect the OTA producing *P. verrucosum* and the trichothecene producing species of *Fusarium*. In short our work will address both pre-harvest and post-harvest aspects with a main emphasis on seed quality, harvest practice, and drying facilities. One objective is to evaluate the effect of a new drum drying technique on the occurrence of fungi on bread grain.

Based on a state of the art of the literature as well as previous results and experience, the following hypotheses were defined at the initiation of PREMYTOX. Focus is placed on rye, which is known to be sensitive to OTA contamination, and on the elucidation and evaluation of the control points, which appear to be the most relevant for organic farmers (taken from the application).

- Hypothesis 1 *Exclusion of seed-treatment fungicides in organic farming favours the dissemination and maintenance of* P. verrucosum *and* Fusarium *in the field environment.* This hypothesis is based on the fact that organic farmers often use their own grain for next year's crop and that the farming system does not allow seed-treatment with fungicides. The OTA-producing *P. verrucosum* as well as trichothecene-producing species of *Fusarium* may survive storage and be present on the seed and they are known to survive well in the field environment. In combination, the exclusion of seed-treatment and the ecology of the mycotoxin producing fungi would seem to favour the dissemination and maintenance of *P. verrucosum* and *Fusarium* in organically cultivated soil.

- Hypothesis 2 *Harvest practice is an important control point in organic farming in the prevention of mycotoxin problems.* This hypothesis is based on the fact that many mycotoxin-producing fungi are saprophytes or facultative pathogens that will be favoured by damaged or broken kernels. As harvest time and harvest practice are known to affect the exposure of the grain to damage, these practices are very likely to constitute important control points in the prevention of mycotoxin problems during drying and storage.

- Hypothesis 3 *Drum drying at high temperatures will reduce the number of surface dwelling fungal spores and prevent mycotoxin problems.* This hypothesis is based on results of a new technique of drum drying grain. Preliminary results indicated that fungal colonisation of the grain surface was reduced very much with this texhnique. This may significantly reduce the risk that surface-dwelling fungal spores of *e.g. Penicillium* and *Fusarium* cause mycotoxin problems given of course that the grain is subjected to the proper environmental conditions during the proceeding storage. The technique was not fully implemented for bread grain at the beginning of PREMYTOX.
- Hypothesis 4 *The drying practice in commercial organic farming needs improvement to prevent mycotoxin problems.* This hypothesis is based on the well-established fact that drying of grain must be fast and well controlled and that the grain has to be sufficiently cooled afterwards. Nevertheless we know that many organic farmers (and many conventional farmers too) have drying facilities, which do not meet these demands. There is, however, a lack of documentation to show how the important mycotoxin producing fungi react to differences in the prevailing drying techniques.
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In the following, we shortly present the contents of the five work packages as outlined in the application. Abbreviations for projects participants: RCF = Research Centre Foulum; RCB = Research Centre Bygholm; DTU = Danish Technical University.

WP1: Project co-ordination, synthesis and dissemination of existing knowledge and *PREMYTOX* results to farmers and extension service (2000-2004)

Task WP1-1: The objective is to co-ordinate the co-operation within the research group, regarding the experimental part of the project (the field trials in WP4 and WP5). This co-ordination will be the subject of annual project meetings and address the subjects of sampling strategy, distribution of samples to contributing persons, parameters to be analysed, methodology, analysis of results and publication. Furthermore, the annual meetings will evaluate the progress of the project with regard to milestones and deliverables.

Task WP1-2: The objective is to disseminate information to farmers and extension service on the importance of mycotoxin producing fungi. This work will be performed continuously during the course of the project. In the initial phase, we'll (RCF) focus on the OTA-producing *P. verrucosum* and make a synthesis of the knowledge on its ecology obtained so far. This knowledge will be related to the general practice in organic farming in Denmark today regarding seed control, harvest, drying and storage as elucidated in WP2. Mid-term conclusions on critical control points will be drawn and related to the focus points of the field trials of WP4 and WP5. In the later phase, the focus will be on both *P. verrusosum* and the trichothecene producing *Fusarium* species. Results of the experimental part of the project will be included and together with earlier results and experiences form the contents of a video, the target audience of which will be farmers and extension service. Its objective is to disseminate knowledge on the importance of mycotoxin producing fungi as well as to focus on those management practices, which have been shown by the project to reduce the risk of dissemination and proliferation of mycotoxin producing fungi.

WP2: General practice in organic farming regarding sowing, harvest, transportation, drying and storage of cereals (2000-2001)

Task WP2-1: The Danish Agricultural Advisory Centre (DAAC) will function as a subcontractor on this work package. In co-operation with the research group of PREMYTOX, a questionnaire will be made. It will address those farming practices, which are supposed at the moment to be critical control points in the dissemination and proliferation of mycotoxin producing *Penicillium* and *Fusarium* species in organic farming. Among these are sowing practice (use of home grown seed, storage of seed, seed treatment if any), harvest practice (time, combine harvesting technique), transportation of grain and the drying and storage practice. DAAC will distribute the questionnaires to representatives among the organic farmers' local advisors and be responsible for the collection of the questionnaires. As evident from the amount of money to be used for WP2, the report will not give an exhaustive answer to the raised questions. It is rather meant to secure that the control points, which we'll agree to focus on in the experimental part of the project, are actually relevant to general practice in organic farming

WP3: Implementation of drum dryer facilities for bread grain and the effect of drum drying on the grain mycobiota with special regard to OA- and trichothecene-producing species (2000-2002)

Task WP3-1: The objective is to modify the existing drum dryer pilot plant at RCB for bread grain. The modifications include changes of construction and incorporation of new equipment for recording of temperatures as well as alterations of the PLC control unit and the automatic data collection unit. The system for supply of grain to the drum dryer will be adjusted, so that accumulation of grain at the inlet is avoided. Furthermore, a valve for regulation of the air supply will be installed in the pipe system to the dry air fan. Temperature sensors (type PT-100 or similar) will be installed at different positions in the drum dryer for temperature registrations throughout the drying drum. An extension of the automatic control system should allow selection of a constant temperature of the drying air, and the data collection unit will be adjusted, so that continuous registration of the fuel consumption and the temperatures inside the drum dryer can be made.

Task WP3-2: By applying different procedures, it is the objective to optimise the drum drying technique for comparison with platform dryers and continuous dryers in WP4. The following independent variables will be included: time of harvesting, temperature of drying air, time for the grain to stay in the drum dryer, air-flow in the dryer and the maximum temperature of the grain. Grain will be obtained from the fields at RCB. Drabæks Mill will function as subcontractor for RCB in the analysis of the effects of the drum drying procedure on the baking quality of the flour.

Task WP3-3: The extent of the mycological analyses to be performed in relation to WP3-2 will be determined at the annual project meeting in the spring of 2001. At the moment they are planned to consist of dilution platings to assess the number of viable propagules on the grain surface (RCF) in order to follow up on the initial results with the pilot plant. Furthermore the ergosterol content in the grain will be assessed (RCF) and screenings will be performed on DYSG for *P. verrucosum* (RCF) and on CZID for *Fusarium* spp (DTU). The work on *Fusarium* will focus on trichothecene-producing species. Thermophilic fungi will be assessed on selected samples. Thermophilic fungi may be able to survive the drum drying, thus being potential mycotoxin producers (though unable to produce OTA or trichothecenes).

WP4: Effect of drying practice on OA- and trichothecene-producing fungi (2002-2003)

Task WP4-1: The objective is to study the effect of platform drying, continuous drying and drum drying on mycotoxin producing fungi. Rye will be obtained from RCB. Following harvest, the grain will be processed in the different pilot plants: platform dryer, continuous dryer and drum dryer.

Task WP4-2: The extent of the mycological analyses to be performed in relation to consist of dilution platings to assess the number of viable propagules on the grain surface (RCF) or measurements of ergosterol content in the grain (RCF) to elucidate the level of fungal contamination. Screenings will be performed on DYSG for *P. verrucosum* (RCF) and on SNA/CZID for *Fusarium.* spp (RCF/DTU). The work on *Fusarium* will focus on trichothecene-producing species. Isolates of *F. culmorum* will be determined with regard to chemotype (DON or NIV producer). The grain will be screened for OTA and DON.

WP5: Effect of seed quality, harvest practice and other critical control points on OA- and trichothecene-producing fungi (2002-2004)

Task WP5-1: It is the objective to obtain seed, which is naturally contaminated with *P. verrucosum* and *F. culmorum*. Grain lots will be screened for their occurrence of *P. verrucosum* and *F. culmorum*. If it is not possible with a reasonable amount of work to obtain this grain, we will consider artificial inoculation with condia of the relevant species.

Task WP5-2: A field experiment will be set up in the DARCOF-initiated 'Danish Crop Rotation Experiment' at Research Centre Foulum. Contaminated seed will be sown in a number of the miniplots. The field experiment will be evaluated with regard to plant growth (RCF). Samplings of soil and plant material during the growth season and harvesting as well as samplings of harvested grain will be collected and distributed to DTU.

Task WP5-3: The extent of the mycological analyses in WP5-2 will be determined at the annual project meetings in the spring of 2002 and 2003. At the moment they are planned to consist of monitoring the level of fungal contamination at different stages of plant development. Screenings on soil and plant material will be performed on DYSG for *P. verrucosum* (RCF) and on SNA for *Fusarium* spp (RCF/DTU). The work on *Fusarium* will focus on trichothecene-producing species. Isolates of *F. culmorum* will be determined with regard to chemotype (DON or NIV producer). Screening for *Alternaria* may be included if resources will allow. If so, they will be performed on DRYES agar (DTU). The grain will be screened for OTA and DON.

Task WP5-4: The effect of threshing damage and moisture pockets on the formation of 'hot spots' of *P. verrucosum* and *Fusarium* spp. will be elucidated in laboratory conditions. Grain from the field experiment will be used and damaged to simulate the detrimental effects of too vigorous threshing by a combine harvester. The extent of the damage of the kernels will be established using indigo carmine staining. Laboratory experiments to elucidate the effect of moisture pockets or other critical control points will be planned in accordance with the conclusions obtained in WP1 and WP2. Mycological procedures as in WP5-3 with regard to *P. verrucosum* and *Fusarium*.

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

No.	**Work package title**	Participants*	Budget (1.000 DKr)	Start	End	Deliverable No.
1	Project co-ordination, synthesis and dissemination of existing knowledge and PREMYTOX results to farmers and extension service	SE, HEH, EFK, UT	1078	2000	2004	WP1-D1-D8
2	General practice in organic farming regarding sowing, harvest, transportation, drying and storage of cereals	SE, DAAC	50	2000	2000	WP2-D1
3	Implementation of drum dryer facilities for bread grain and the effect of drum drying on the grain mycobiota with special regard to OTA- and trichothecene-producing species	EFK , SE, UT, Drabæks Mølle	201	2000	2001	WP3-D1
4	Effect of drying practice on OTA- and trichothecene-producing fungi	EFK, SE, UT, HEH, Drabæks Mølle	964	2001	2003	WP4-D1-D2
5	Effect of seed quality, harvest practice and other critical control points on OTA- and trichothecene-producing fungi	HEH, SE, UT	1558	2001	2004	WP4-D1

* Responsible participants are underlined

B. Objectives and expected achievements (from application)

PREMYTOX aims to prevent mycotoxin problems in cereals. The project has two major objectives:

- to increase our knowledge on the ecology of mycotoxin producing fungi
- to provide the farmer with information on the importance of mycotoxin producing fungi and practical means to reduce the dissemination and proliferation of these fungi

It is the objective of PREMYTOX to identify some of the control points in the primary production, which are critical in the prevention of mycotoxin problems in organic farming in Denmark. This knowledge will be achieved on the basis of mycological analyses of cereal samples from field experiments. The achieved knowledge will regard both pre-harvest and post-harvest aspects. Focus will be put on species producing ochratoxin A and deoxynivalenol, the two mycotoxins currently regarded to be the most important in Danish cereals. Most of the obtained results will also be of use in conventional farming, which is also subjected to mycotoxin problems.

An important achievement of PREMYTOX will be the dissemination of knowledge on the importance of mycotoxin producing fungi and practical means to reduce the spreading and proliferation of these fungi.

C. Midterm results and progress

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

WP1
Based on so-far obtained results (Elmholt, 2003), Critical Control Points regarding OTA prevention to be further studied are the field environment (contaminated seed, soil contamination of standing crop), threshing (insufficiently cleaned harvester, damaged kernels), drying and storage facilities (carry-over of conidia from last year via non-cleaned equipment) and drying process (time, efficiency). The latter is especially relevant for in-bin and platform dryers.

WP2
Most of the reported on-farm drying facilities (Høy, 2001) were in-bin drying and storage or natural air-drying systems. Only one had a continuous flow dryer. A few of the farmers had no drying facilities but left their grain to dry on the floor (approx. 0.75 m depth) with frequent inversions with a shovel. In conclusion it is highly relevant to organic farming to address in-bin drying and platform dryers using natural air-drying (cf. design of WP4).

WP3
In 2000, the existing pilot drum dryer plant was successfully modified to make it suitable for investigations on the influence of temperature and retention time on the quality of bread grain. The PLC based monitoring program was enlarged and changed so the drying process can be performed at either a Fixed constant drying Air Temperature (FAT) or a fixed constant Maximum Grain Temperature (MGT). Preliminary trials with drying of rye were carried out in 2000 with two harvest dates (early and late) and drying for 3 or 5 minutes, respectively, at FATs of 100 to 300°C or MGTs of 49 to 87°C. Preliminary tests in 2000 showed large effects on surface dwelling fungi.

In 2001 drum drying experiments were performed with rye, grown at The Ecological Research Station, Rugballegård. The design aimed to elucidate the influence of harvest time, retention time and drying temperature on the grain quality during the drum drying process. The rye was harvested early (August 15^{th}) or late (August 30^{th}) with moisture contents about 17.5% at both harvest times. 48 drying or heat treatment tests were carried out as listed in Table 1.

Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Time, min.	5	5	5	5	11	11	11	11	15	15	15	15	5	5	5	5	11	11	11	11	15	15	15	15
Drying air,°C	149	181	216	244	152	179	215	247	147	180	216	255	-	-	-	-	-	-	-	-	-	-	-	-
Grain, °C	51	54	58	63	53	57	61	66	45	51	55	59	53	61	71	77	51	56	64	74	53	60	71	79
Sample No.	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	40	42	43	44	45	46	47	48
Time, min.	5	5	5	5	11	11	11	11	15	15	15	15	5	5	5	5	11	11	11	11	15	15	15	15
Drying air,°C	118	237	248	287	126	222	244	286	132	192	248	263	-	-	-	-	-	-	-	-	-	-	-	-
Grain, °C	32	47	51	60	29	50	48	57	28	49	45	59	50	59	67	74	48	59	63	77	43	52	62	76

Table 1. Drying regimes as performed in WP3, 2001 (Kristensen & Elmholt, 2002)

Using three retention periods (5, 11 or 15 minutes), we tested FATs of 118 to 287°C and MGTs of 43 to 79°C. The results were presented at the European Agriculture and Engineering conference in Budapest in June (Kristensen & Elmholt, 2002). Drum drying very efficiently reduced the fungi colonising the grain (Figure 1).

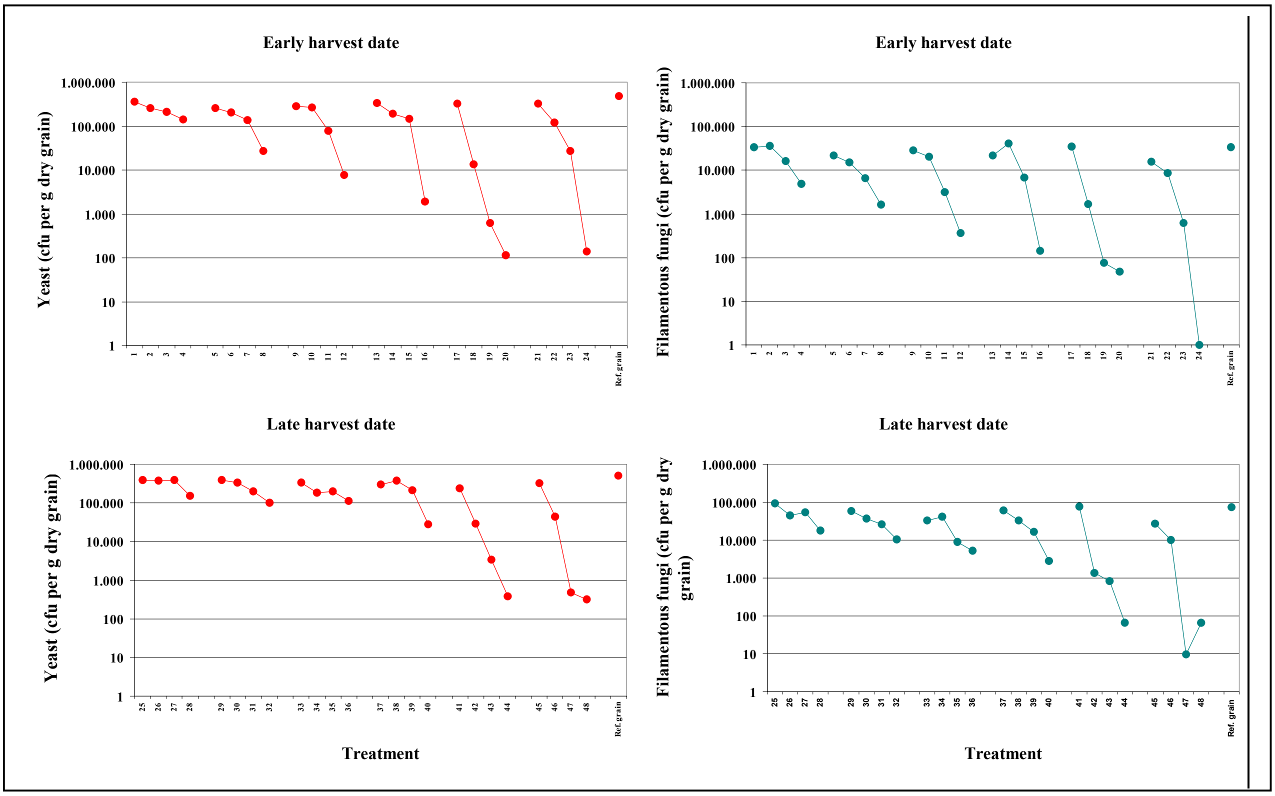

Figure 1. Fungi isolated from drum-dried grain by dilution plating and calculated as colony forming units (cfu) g^{-1} dry grain. Results from different drying regimes show yeast at the early harvest date (upper left), filamentous fungi at the early harvest date (upper right), yeast at the late harvest date (bottom left), and filamentous fungi at the late harvest date (bottom right) (Kristensen & Elmholt, 2002).

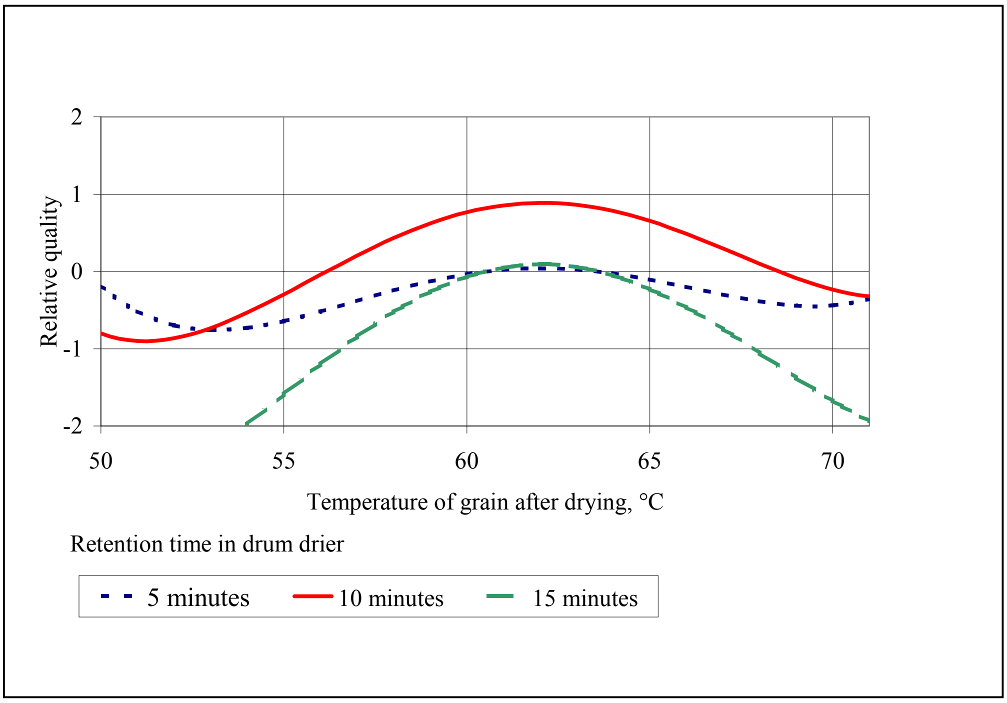

Figure 2. Baking quality of rye treated at different drying temperatures and at short, medium and long retention times in the drum drier. A relative quality number is calculated on the basis of the statistical analyses on falling number and amylograph values for max. viscosity and max. temperature (Kristensen & Elmholt, 2002)

When using an MGT regime, a retention time of *e.g.* 10.5 min and a temperature of 64ºC resulted in less than 1% of the yeast propagules and less than 2% of the filamentous fungi, which were found in the reference grain. At these treatments the moisture content of the grain was reduced from about 17% to about 12%. This reduction of living fungal propagules will significantly reduce the risk of grain deterioration during storage. The highest baking quality in rye was obtained at grain temperatures of about 62°C (Figure 2). Only in samples where the grain temperature had been higher than 70°C, the baking tests showed visual quality changes. In conclusion drum drying offers the possibility to obtain a very high reduction in fungi colonising the grain and at the same time maintain a high baking quality (Kristensen & Elmholt, 2002). It was shown that

- MGT drying regimes reduced the fungi more efficiently than FAT drying regimes
- MGT temperatures of more than 70ºC reduced the baking quality
- Optimum concerning effect on fungi and baking quality was found at a retention time of 10.5 min. and a maximum grain temperature about 62ºC

P. verrucosum was not found in any of the rye samples, and the effect of drum drying on this important species could not be deduced from the above experiment. Therefore we decided to deviate from our original plans and include a drum drying experiment on grain, which we knew was heavily infested with *P. verrucosum*. This grain was obtained in the spring of 2002 from a farmer on Sealand. Eight non-drum dried reference samples from this batch contained an average of 78% +/-9% kernels with growth of *P. verrucosum* (from each sample, 300 kernels are tested on dichloran yeast extract sucrose agar). Figure 3 shows that an MGT regime extremely efficiently reduces *P. verrucosum* in the grain and much more so than a FAT regime.

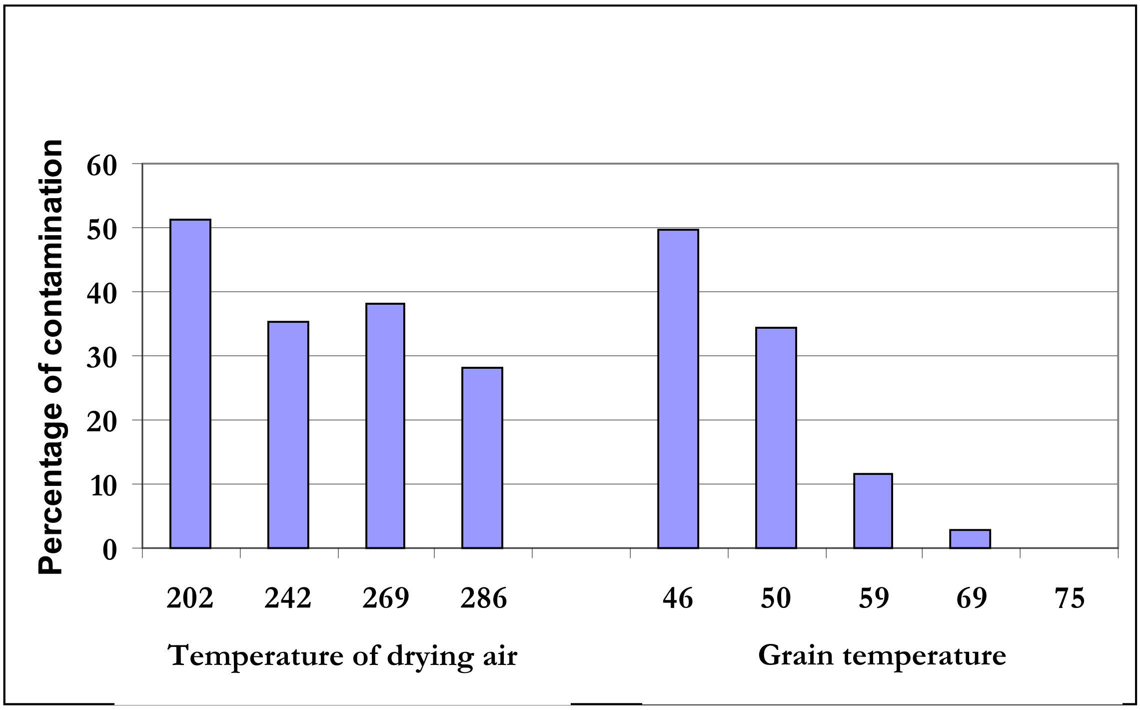

Figure 3. Percentage of *Penicillium verrucosum* in heavily infested, drum dried samples using FATs of 202 to 286ºC and MGTs of 46 to 75ºC.

This experiment with heavily infested grain confirms that drum drying (especially MGT) very efficiently reduces the contamination with *P. verrucosum*.

WP4
Comparative drying tests using different drying techniques have been made. Samples of the treated grain are being analysed at the moment, but no mycological results or results on baking quality have been finished yet.

WP5
No results yet of field experiments with *P. verrucosum* contaminated seed, which have been performed summer 2002 with 4 different batches of naturally contaminated oats and four of spring wheat.

During autumn 2000, a case study was performed at an organic farm at which we had observed problems with *P. verrucosum* in earlier studies. The problems were seemingly related to drying practice (Elmholt, 2003). The case study is part of a M.Sc. project (Maiken Haase (MH), biology, University of Århus), which addresses Hypothesis 4, *i.e.* hot spot contaminations in natural (on-farm) and experimental storage conditions. The farm has a natural air-drying system where the grain is placed in a pile on the floor of the barn. Floor and side-wall are made of concrete, main and side ducts of chipboard and the perforated bottom of the side ducts of plywood. Side ducts are covered with old grain sacks of hessian. Sampling was performed in rye and oats to study if conidia of *P. verrucosum*, which we knew survived in ducts and hessian sacks, could contaminate the new harvest. The results showed increasing numbers of contaminated kernels during the drying period and significantly higher numbers of contaminated kernels near the bottom of the grain heap than in the upper layer, indicating a conidial contamination via the drying facilities (*i.e.* the drying air). However, OTA analyses showed the largest values in the upper layer of the grain, especially in rye, probably because environmental conditions here favour germination, growth and OTA production. Actually the OTA values in the upper layer did not increase until sampling in late October. But when they did, we found values between 52 and 353 μg/kg, *i.e.* up to 70 times the limit of 5 μg/kg grain set by the Danish authorities. This stresses that contamination with *P. verrucosum* is a latent risk and that contamination percentages need not be high to imply severe problems if the grain is subjected to environmental conditions that stimulate mycotoxin production! Figure 4 shows a few results from this study: The bars in Figure 4A show kernel contamination (%) with *P. verrucosum* and marks show OTA concentration in grain samples (ng/g grain). Results are shown for two side ducts (3 and 6) at the first four samplings (sampl. 2 missing for SD 6). Contamination lie below 4 % and OTA below 0.25 ng/g, *i.e.* well below the limit. At the fifth sampling (October 25th), however, concentrations almost exploded to levels 10-70 times the limit of 5 ng/g grain (Figure 4B). These results show very clearly that more attention should be paid to spatial variations in OTA contamination of grain that is subjected to slow and insufficient drying in *e.g.* platform dryers. In this case there are more conidia in the bottom layer, probably transferred to the grain via contaminated drying facilities, but the environmental conditions in the top layer have obviously been more conducive to growth and OTA production of *P. verrucosum*.

Concurrently with PREMYTOX, DTU is engaged in an EU project on OTA headed by Monica Olsen at Livsmedelsverket in Uppsala, Sweden. During the preceding year the two projects have collaborated on genetic diversity in *P. verrucosum* using amplified fragment length polymorphism (AFLP) fingerprinting (primer set EcoRI-P(G) + MseI-P(GG)). 87 pure cultures of *P. verrucosum,* which have been isolated during our case study work at two organic farms, one of which being the above mentioned farm, will be compared with isolates from other European countries, primarily Sweden and the U.K. This is the first time such a study of genetic diversity in *P. verrucosum* is being performed. The aim is to elucidate whether different genetic groups exist within this important species as previously shown at DTU for other *Penicillium* species, *e.g. P. commune*, a contaminant in cheese dairies. In agricultural terms the molecular methods can help reveal isolates with a common origin. This will give very valuable information regarding distribution of this species in the environment, both on the large scale (countries, regions) and on the small scale (field, storage). It may also initiate studies of whether certain strains of *P. verrucosum* are more potent OTA producers and thus more detrimental than others.

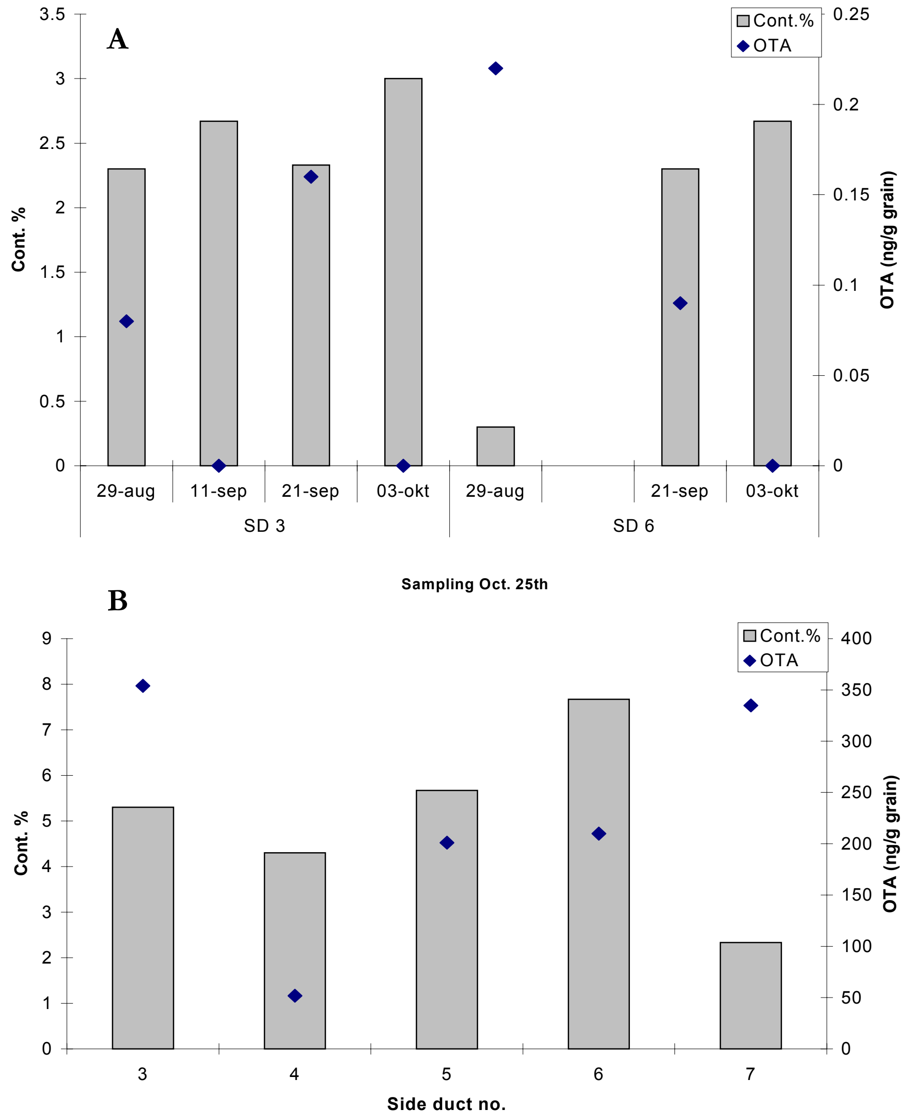

Figure 4. Contamination (Cont. %) by *P. verrucosum* and OTA concentration of rye samples from a case study at an organic farm. A: Results from 4 samplings (Aug. 8th, Sept. 11th, Sept. 21st and Oct. 3rd) in the top layer of the grain above two side ducts (SD 3 and SD 6). B: Results from the fifth sampling (Oct. 25th) in the top layer above five side ducts.

C.2 Fulfilment of deliverables and milestones

WP1: Project co-ordination, synthesis and dissemination of existing knowledge and PREMYTOX results to farmers and extension service

WP1	Time schedule according to application	Deviations, if any*
Deliverables		
WP1-D1-D4: 1st to 4th annual report 2000-2003	2000 - 2003	
WP1-D5: Final report	2005	
WP1-D6: DARCOF report, which summarises the so-far obtained results on the ecology of the OTA-producing *P. verrucosum*	2001	Substituted by scientific paper, BAH
WP1-D7: Video on the prevention of mycotoxin problems	2004	Video may be substituted by other source of communication
WP1-D8 Popular paper summarizing contents of video	2004	
Milestones		
WP1-M1-M5: Annual project meeting 2000-2003	2000 - 2003	
WP1-M6: Mid-term conclusions on critical control points and their implementation into WP4 and WP5	2001	
WP1-M7: Collection of material for use in the video presentation on how to prevent mycotoxin problems	2004	

** Deviations are discussed in D*

Annual meetings (**WP1-M1 and M2**) and annual reports (**WP1-D1, D2 and D3**) were held and delivered according to plan. A synthesis of the so-far obtained results on the ecology of the OTA-producing *P. verrucosum* was intended for publication as a DARCOF report (**WP1-D6**). Instead the results will appear as a scientific paper in *Biological Agriculture and Horticulture* (BAH volume 20(4), Elmholt, 2003). The paper is entitled *"Ecology of Penicillium verrucosum: Natural occurrence in field soil and grain with special attention to farming system and on-farm drying technique"*.

Critical control points (**WP1-M6**) based on so-far obtained results are discussed in Elmholt (2003). These points relate both to pre-harvest, harvest and post-harvest conditions. The conclusions have been disseminated to farmers and extension service at field days, meetings and seminars as well as in short, popular publications (Elmholt & Kristensen, 2001; Elmholt, 2001b; Elmholt, 2002). The role of some of these critical control points is currently being studied in WP4 and WP5, *e.g.* effect of insufficiently drying regimes and the effect of seed quality on the contamination of the standing crop.

Documentation for use in dissemination of results to farmers and extension service (**WP1-M7**) is continuously being collected.

WP2: General practice in organic farming regarding sowing, harvest, transportation, drying and storage of cereals

WP2	Time schedule according to application	Deviations, if any*
Deliverables		
WP2-D1: Report on the results drawn from the questionnaire	2001	
Milestones		
WP2-M1: Questionnaire prepared and distributed to local advisors	2000	
WP2-M2: Questionnaires filled in and returned	2000	

A report - based on a questionnaire - on general harvesting and drying practice in organic farming was made in co-operation with subcontractor Danish Agricultural Advisory Centre (**WP2-D1**, Høy, 2001). The questionnaire (**WP2-M1 and M2**) was distributed by DAAC to fifty-six farmers, chosen by postal code and assumed by DAAC to be representative of organic farmers in Denmark. The farmers were asked questions concerning management (farm size, livestock, crop rotation, seed treatment, harvesting, drying facilities (materials used, fan size, aeration, heating, cooling). Results have been used in the planning of drying regimes in WP4.

WP 3. Implementation of drum dryer facilities and the effect of drum drying on the grain mycobiota with special regard to OTA- and trichothecene-producing species

WP3	Time schedule according to application	Deviations, if any*
Deliverables		
WP3-D1: Report and/or popular paper	2002	
Milestones		
WP3-M1: Technical modifications completed	2000	
WP3-M2: Analyses of baking quality completed	2000/2001	
WP3-M3: Mycological analyses completed	2000/2001	Extended with *P. verrucosum* contaminated samples from commercial farmer
WP3-M4: Drum drying procedure for use in 2002 in WP4 established	2001	

Activities in 2000-2001 (**WP3-M1**) focused on technical modifications and changes of the existing pilot drum dryer plant to make it suitable for investigations on the influence of temperature and retention time on the quality of bread grain. The results confirmed that the drum drying plant was suitable for the intended investigations. Test samples from the trials were analysed during the winter of 2000/2001 with regard to their content of fungi and quality for bread production (**WP3-M2 and M3, 2000**). The findings showed large variation between samples of grain processed at different drying regimes. These preliminary results were used to design the 2001 drying regimes. In 2001, organically grown rye (cv. Dominator) was used for the trials (**WP3-M2 and M3, 2001**).

The experimental work in WP3 is finished. According to plans, the results from WP3 were used at the annual meeting in 2002 to design the drum drying regime in WP4 (**WP3-M4**).

WP 4. Effect of drying practices on OTA- and trichothecene-producing fungi

WP4	Time schedule according to application	Deviations, if any*
Deliverables		
WP4-D1: Popular paper on the results from WP4-1 and WP4-2 (*e.g.* Grøn Viden)	2003	
WP4:D2: Scientific paper on the effect of harvest time and drying practice on the grain mycobiota with special regard to OTA- and trichothecene-producing species of *Penicillium* and *Fusarium*	2004	
Milestones		
WP4-M1: Grain samples from different drying procedures distributed to RCF and DTU	2002	
WP4-M2: Mycological analyses completed	2003	
WP4-M3: Analyses for OTA and DON completed	2003	

Based on results from WP2 and WP3 we designed the following four drying regimes (organically grown rye from The Ecological Research Station, Rugballegård :

1. Drum drying (retention time 10.5 min., MGT 62ºC)
2. Continuous drying with a maximum temperature of the drying air at 65°C and at the same time a maximum grain temperature at 45°C
3. In-bin drying with continuous operation of the drying fan and optimal supply of heat to ensure sufficient low air humidity in the drying air
4. In-bin drying with no supply of heat. The drying fan is only in operation when the humidity of the ambient air is sufficiently low for drying

Treatments 1, 2 and 3 were finished in August and samples have been distributed to project partners (**WP4-M1**). The drying process of Treatment 4 is deliberately being delayed in order to simulate a 'worst case' situation. It will be finished within the coming month.

WP 5. Effect of seed quality, harvest practice and other critical control points on OTA- and trichothecene-producing fungi

WP 5	Time schedule according to application	Deviations, if any*
Deliverables		
WG5-D1: Scientific paper(s) on the effect of seed quality, harvest practice and 'hot-spot' formation on *P. verrucosum* and *Fusarium*	2004	
Milestones		
WG5-M1: Obtaining naturally or artificially contaminated seed for use in 2002 and 2003	2001-2002	
WG5-M2: Performance of field experiment 2002 and 2003	2002-2003	Adjustments according to so-far obtained results and changes in project manning
WG5-M3: Mycological analyses completed	2003-2004	

In 2002 we have performed a field trial at Foulumgård (**WP5-M2,2002**) with naturally contaminated samples of oats and spring wheat. These experiments address Hypothesis 1 and 2 (please cf. project summary). The samples were obtained from commercial farms via related project activities and contamination percentages ranged between 3% and 92% of *P. verrucosum* (**WP5-M1**). Emphasis was placed on this OTA-producing species rather than on *Fusarium* for reasons discussed in section D. Analyses of soil, standing crop and harvested grain are presently being performed.

During autumn 2000, a case study was performed at an organic farm at which we had observed problems with *P. verrucosum* in earlier studies, seemingly related to drying practice (Elmholt, 2003). This study is part of a M.Sc. project (Maiken Haase (MH), biology, University of Århus), which addresses Hypothesis 4, *i.e.* hot spot contaminations in natural (on-farm) and experimental storage conditions.

D. Description of deviations and subsequent adjustments of plans

In WP1 the **WP1-D6** (a DARCOF report) has been substituted by scientific paper, which appears in BAH in 2003. This decision was taken because we realized that our results on the ecology of the OTA-producing *P. verrucosum* in soil and in the field ecosystem are in fact quite unique and relevant to the international scientific community. This assumption was confirmed in the two reviewers' comments on the paper (please cf. Section H).

At the meeting with FØJO in May 2002 it was decided to substitute the video with another way of comunicating the importance of the critical control points to farmers and extension service. Possibilities are the production of a CD-rom or a web-based product. The photo documentation so far collected in PREMYTOX (**WP1-M7**) can be used with these options.

In WP3 we included a drum drying experiment with grain that was heavily infested with *P. verrucosum* (**WP3-M3**). This was considered very important because the rye from Rugballegård had no natural contamination with *P. verrucosum*. The results are described above. It was possible to include this grain because we had access to heavily contaminated batches of grain via other project activities on mycotoxins.

We have adjusted WP5 (**WP5-M2**) according to professional reasons as well as because of changes in project manning. Project participant Helle Hestbjerg, who was to head WP5 and who is a specialist in *Fusarium* ecology, resigned by April 1st 2002. The last month of her employment period, Helle Hestbjerg finished a scientific publication on mycotoxin production by the two important species, *Fusarium culmorum* and *F. equiseti*, which has been accepted for publication in Journal of Agricultural and Food Chemistry. Mycotoxin production is interpreted in relation to the role of the fungi in the field ecosystem and therefore relevant in relation to WP5. Susanne Elmholt has taken over as head of WP5. This change combined with the so-far obtained results has resulted in an increased focus on *P. verrucosum* in WP5.

E. Project publications and other products

1. Articles in international, scientific journals with review procedures

***Elmholt, S. (2003)** Ecology of the ochratoxin A producing *Penicillium verrucosum*: Occurrence in field soil and grain with special reference to farming system and on-farm drying. *Biological Agriculture and Horticulture,* **20(4)** (*In press*).

****Hestbjerg, H., Nielsen, K.F., Thrane, U. & Elmholt, S.** Production of trichothecenes and other secondary metabolites by *Fusarium culmorum* and *F. equiseti* on common laboratory media and a new soil organic matter agar: An ecological interpretation. *Journal Agricultural and Food Chemistry* (accepted).

2. Papers presented at congresses, symposiums, etc.

Kristensen, E.F. & Elmholt, S. (2002) High-temperature drying of organically grown bread rye. Proceedings of EurAgEng2002, Budapest 30 June-4 July. ISBN 963 9058 12 2ö, ISBN 963 9058 13 0, Abstracts Part 1, 189-190. CD Paper: http://www.gte.mtesz.hu

***Elmholt, S. (2001a)** Environmental pertubations as revealed by shifts in fungal populations. Invited speaker at workshop "Fate and Effects of Microbial Inoculants" at LO-skolen, Helsingør, 6th May 2001.

3. Reports, articles in agricultural journals, etc.

Elmholt, S. (2002) Pas godt på kornet (Take care of your grain) (in Danish). Økologisk Jordbrug, 9. august, side 6.

Elmholt, S. & Kristensen, E.F. (2001) Korn uden mykotoksiner (Grain without mycotoxins), pp. 45-55. In: Waagepetersen, J., Petersen, J.B., Knudsen, L., Deneken, G. & Jørgensen, J.R. Produktion af kvalitetshvede i Danmark. En oversigt over problemer og muligheder (Production of high quality wheat in Denmark. A survey of problems and possibilities) (in Danish). DJF rapport 53. Danmarks JordbrugsForskning, Foulum.

Elmholt, S. (2001b) Forebyg svampegift i korn (how to prevent mycotoxins in grain) (in Danish). Den faglige baggrund, Landsbladet, 10. august.

Høy, J.J. (2001) Økologisk kornopbevaring (Organic handling of grain). Rapport fra spørgeskemaundersøgelse (Report from questionaire) (in Danish). Landbrugets Rådgivningstjeneste, Skejby. 2 pp.

Kristensen, E.F. (2001a) Ny tørringsteknik kan gøre økologisk korn bedre (New drying technique may lead to improvements in organically grown grain) (in Danish). Jordbrugs-Forskning, 5, p. 12.

Kristensen, E.F. (2001b) Tromletørring god til øko-korn. Ny tørringsteknik kan fjerne svampe fra økologisk korn (Drum drying suitable for eco-grain. New drying technique for elimination of fungi from organically grown grain) (in Danish). Økologisk Jordbrug, 243, p. 9.

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

Elmholt, S. (2002) Svampe i kornlagre (Storage fungi). Project Farm4U 'Researcher for a day' (please cf. http://www.farm4u.dk/sw91.asp). Five student teams (January 30th, February 5th, February 27th, August 13th, August 14th .

Elmholt, S. (2001e) Svampe og toksiner. Oral presentation at seminar for dlg employees, FAF, Gamle Havnekaj 25, Odense 10th October 2001

Elmholt, S. (2001d) Mykotoksinproducerende svampe på korn. Markvandring, Foulumgård, 14th June 2001.

Elmholt, S. (2001c) Hvad ødelægger kvaliteten af økologisk korn? Oral presentation at seminar on Grain Quality for dlg ØKOLOGIs øko-salgskonsulenter, FAF, Gamle Havnekaj 25, Odense, 9th January 2001.

F. Scientific education

G. National and international cooperation

National cooperation: During the project (**WP2)** we cooperated with the Danish Agricultural Advisory Centre (Jens J. Høy; Michael Tersbøll) regarding general pre- and postharvest management practice in organic farming.

During the project period we have had a very good co-operation with a biodynamic/organic mill/bakery (Aurion, Hjørring). Aurion has supplied us with a large number of samples and this cooperation has strengthened our conclusions regarding general practice for drying of bread grain in organic farming (**WP2**). It has furthermore enabled us to obtain the naturally *P. verrucosum* contaminated seed, which has been used in **WP3** and in the field experiments of **WP5**.

We have cooperated with RISØ National Laboratory (Senior Scientist Gerda Krogh Mortensen, GKM) and the Royal Veterinary and Agricultural University (Prof. Hans Christian Bruun Hansen), who are studying occurrence of naturally produced toxins from plants and fungi in the environment. In connection with the field experiments in **WP5**, GKM has analysed a number of our soil samples for OTA.

Analyses of OTA in grain **(WP3, WP5)** have been performed in co-operation with the Danish Veterinary and Food Administration (Peter Have Rasmussen; Kevin Jørgensen), which is accredited to perform these analyses.

Analysis for *Fusarium* species at DTU (**WP3-WP5**) is conducted in a no-cost collaboration with Anne Svendsen (Biotechnological Institute, Kolding) as part of a formal Letter of Agreement between the two institutions.

The evaluation of the baking quality of rye treated at different drying regimes in the drum dryer (**WP3**) has been made in co-operation with Cerealia Danmark, Drabæks Mølle.

Regarding technical construction and further development of the drum drying technique, contact to the firm Cimbria A/S has been established.

International cooperation: At the international level, the Mycology Group at DTU secures a close contact to the ongoing EU project on OTA (http://www.epsoweb.org/catalog/EU/fp5/OTA.htm), in which Jens Frisvad and Flemming Lund from DTU are participating. The overall aim of the EU-project is to implement a general HACCP for cereal production in the EU. A direct co-operation on some of our pure cultures of *P. verrucosum* is currently taking place as discussed above. Our cultures will be compared with cultures obtained from different European countries.

DTU (Ulf Thrane) has collaborated within the EU-supported COST835 action "Agriculturally important fungi" on characterisation on trichothecene producing Fusarium species on cereals. The aim of the collaboration is species delimitation around *F. poae* and *F. sporotrichioides* and has resulted in discovery of a new trichothecene producing species, provisionally named F. "powdery poae". DJF (Susanne Elmholt) has also participated in the EU-supported COST835 action as a national delegate of WG 3 (Ecology and pathogenicity of toxigenic Fungi).

H. Critical reflection on the project

Mycotoxins are hazardous compounds and their possible occurrence in agricultural commodities is extremely relevant in animal production as well as human nutrition. Ochratoksin A (OTA) is for example regarded carcinogenic and has a high thermostability. It is therefore essential that OTA is not present in flour meant for human bread production. It is detrimental to organic farming that several studies and surveys of cereal commodities show that this compound and its producer are found more frequently and in larger amounts in samples from organic than conventional farms. The aim of PREMYTOX is to elucidate why this is so. Our experiments aim to identify and study 'critical control points' in farming practice, which affects the fungus mostly (analog to 'Hazard Analysis of Critical Control Points' concept). The experiments are designed on the basis of four hypotheses on where to look for the critical points. These were established at project initial. The relevance of the project and the hypotheses are unchanged since the start and PREMYTOX is proceeding according to the intentions in the application.

The intention of PREMYTOX is to merge knowledge on current practice in Danish organic farming with knowledge on the life cycle of relevant toxin producing fungi. We have put a large effort into elucidating the general practice of post harvest grain handling by organic farmers. The aim was primarily to assure that planned experiments were relevant to organic farmers. This work (WP2) has been completed in cooperation with the Danish Agricultural Advisory Centre and with the processing industries that use organically grown grain (Aurion, Drabæks Mølle).

Regarding the scientific approach we have followed our plans closely in WP3 and WP4 (the post harvest part of the project) and can by now present results, which show drum drying to be extremely quick and efficient in reducing the number of fungal conidia on the grain without losing baking quality. The latter has been a major concern of the milling and baking industry. Supplementary experiments have also demonstrated that conidia of the OTA producing *Penicillium verrucosum* can be killed by this technique. Such a quick and efficient reduction in *P. verrucosum* will minimize the storage risk of OTA contamination as compared to platform drying, where the drying process is much slower and much less efficient. In earlier projects we have demonstrated that poor platform drying may lead to large increases in grain contamination by *P. verrucosum.* We have continued these studies in PREMYTOX (WP5) and shown large spatial variations in contaminated kernels and OTA in a platform dryer, and we have shown that *P. verrucosum* can give rise to OTA concentrations far beyond established limits. Because we are talking fungal contaminations that cannot be seen with the naked eye we find these results extremely important in communicating the relevance and importance of an efficient drying process to farmers and grain processors.

Drum drying is a new technique regarding bread grain. Our results have been presented at an agricultural and engineering conference (AgEng, Budapest; Kristensen & Elmholt, 2002) and they gave rise to much interest and many questions. At the time of writing we are obtaining the first results of the experiments that compare drum drying with continuous drying and platform drying (WP4). These results will be important in the final conclusions as to how drum drying competes with these commonly used techniques for drying of organically cultivated grain regarding both mycological and baking quality.

Mycological analyses at Foulum and DTU as well as baking tests (Drabæks Mølle) are performed using well-implented techniques and proceed according to plans. There are, however, two major adjustments in PREMYTOX that are relevant to mention:

The abolishment of the Dept. of Analytical Chemistry at DIAS has implied that quantitative analyses of mycotoxins have to be performed as required work and consequently more expensive than expected. Having consulted the secretariat of FØJO we decided to allocate a larger proportion of our grant to these analyses. The reason is that although the presence of the OTA producing fungus is a potential risk of the production of the toxin, only the detection of OTA itself can verify a toxicological risk in consuming the grain. We have obtained a good agreement with the Danish Veterinary and Food Administration (DVFA), whose laboratory is accredited to performing these analyses and in their daily work heading national surveys of mycotoxins in cereal commodities. This agreement has the further advantage that it will strengthen our cooperation with DVFA, which plays an important role in the debate on mycotoxins in Denmark and the EU.

The other important adjustment owes to a combination of scientific and manning conditions: At the initiation of PREMYTOX weight was given to both OTA and *Fusarium* produced trichothecenes. The latter is reflected in the cooperation with DTU and the choice of Helle Hestbjerg (RCF), who is a specialist on *Fusarium*, as the head of WP5. Helle Hestbjerg has unfortunately resigned, and WP5 is now headed by Susanne Elmholt. We shall continue working with trichothecene producing *Fusarium* species in the project via DTU, who are specialists on *Fusarium* taxonomy. However the emphasis in PREMYTOX will be shifted towards OTA and *P. verrucosum*. This has scientific reasons as well: Firstly the drying experiments (WP3) were so very promising, and these results are most relevant to *P. verrusosum*, whose life cycle is more closely bound up to the drying process than the *Fusarium* producers of trichothecenes. Secondly knowledge from former work with *P. verrucosum* has been synthesized (WP1, Elmholt, 2003). This has confirmed our assumption that it is not organic farming as such, which causes OTA problems but rather specific management factors, which need improvement – and this work is judged to be highly relevant the research contents of FØJO.

During the cause of PREMYTOX we have had a direct cooperation with other researchers on mycotoxins. As mentioned above we have established a co-operation with DVFA concerning mycotoxin analyses. We have also initiated a co-operation with RISØ National Laboratory and the Royal Veterinary and Agricultural University), who are studying occurrence of naturally produced toxins from plants and fungi in the environment. In connection with the field experiments in WP5, they analysed a number of soil samples for OTA after sowing of *P. verrucosum* contaminated seed (pre-harvest part of WP5). We have furthermore exchanged grain samples and pure cultures of fungi with several researchers at DTU, Flemming Lund and Jens Frisvad (participants in the Danish part of the OTAPREV project (Prevention of ochratoxin A in cereals), headed by Monica Olsen from Sweden and part of EU's 5th framework) and Birgitte Andersen ("Prevention of fungal growth and mycotoxin production in Danish foods"). We are very exited to see how much genetic similarity we'll find among our isolates from two different Danish localities and isolates from other European localities. This work may well turn out to be quite important to future research into the ecology of *P. verrucosum*.

Communicating results to farmers and extension service is an important element in PREMYTOX. Results and conclusions are communicated to primary producers, extension service (organic farmers' organization; DAAC, organic and conventional farmers' magazines) and the grain handling industry. At meetings and seminars we have experienced much interest from farmers and industry. Our cooperation with the organic grain industry (Drabæks Mølle; Aurion) has implied more rigorous demands on farmers appointed on a contractual basis (*e.g.* cleaning of harvesting, drying and storage facilities; air-drying with heat).

We are attempting to make it clear to the society (producers as well as consumers) that toxigenic fungi are naturally occurring and cannot be totally avoided. The aim is rather to minimize the dispersal, growth and toxin production of harmful fungi – an aim, which for that matter applies to conventionally cultivated grain as well. In the project group of PREMYTOX we are very much aware of the negative signals, mycotoxin problems may provoke in parts of the press. Large headlines could be rather detrimental to the production of organic cereals. In our communication we therefore aspire to balance our message in emphazising that proper management adjustments to a large extent seem to solve the problems (cf. Elmholt 2003).

Many international studies on OTA problems i food products have a chemical/toxicological basis. Compared with this our work is to a larger extent pointed towards an understanding of the interaction between the biology of the fungus and its environment, *i.e.* a more ecological approach. The very positive review comments on the BAH paper (Elmholt, 2003) reflects that this approach has international attention: One referee states that 'very few studies have been carried out on the occurrence of *P. verrucosum* in soil or the sources of inoculum of this fungus' and continues that the paper 'does not appear to clear up the controversy of whether organically grown cereals are more prone to mycotoxins but suggests that other factors are more important in the formation of OTA (a view that I currently share)'. The other referee states that 'The manuscript offers the finest comprehensive examination of *P. verrucosum* ecology (or any other *Penicillium* spp.) from an agroecosystem perspective', that 'The sampling effort, collection of relevant agricultural information, and interpretation of the results on a case by case basis is outstanding' and that 'This will be a difficult study for anyone to repeat and therefore will remain a classic in the field for many years and stimulate others to examine *P. verrucosum* populations in agricultural soils'.

In conclusion – and despite the unforeseen events outlined above - we are so far very satisfied with our results in PREMYTOX and – including the adjustments concerning toxin analyses and contents of WP5 - it is our intention to continue the project as planned.

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:	Consump-tion before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	13.3	14.5	11	11		49.8
Technical personnel	10.2	11.8	7.2	0.8		29.9

Year:	Consump-tion before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	524	579	459	481		2043
Technical personnel	236	286	186	21		729
Other operational costs	94	129	80	15		318
Equipment						
Others (please specify)	50	42		50		142
Direct costs	904	1036	725	567		3232
Indirect costs (20% of direct costs)	170	198.6	145.2	104		617.8
Total	1074	1235.6	870.2	671		3849.8

Comments:

Others include subcontractors, *e.g.* WP 2 (DAAC) and expenditures for mycotoxin analyses. No overhead has been included for these costs

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project Susanne Elmholt	RCF, DIAS	Oct. 1st 2002	

Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)

Name of Institute: RCF, DIAS, Department of Crop Physiology and Soil Science

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	7	10.5	10	10.5		38
Technical personnel	3.5	7.26	6.22	0.76		17.74

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	282	419	418	460		1579
Technical personnel	83	178	161	21		443
Other operational costs	28	94	70	15		207
Equipment						
Others (please specify)	50			50		100
Direct costs	443	691	649	546		2329
Indirect costs (20% of direct costs)	78	138	130	100		446
Total	521	829	779	646		2775

Comments:
Others include subcontractors, *e.g.* WP 2 (DAAC) and expenditures for mycotoxin analyses. No overhead has been included for these costs

B. Budget for each participating department (1.000 DKK)

Name of Institute: RCB, DIAS, Department of Agricultural Engineering

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	5.5	3				8.5
Technical personnel	5	2.5				7.5

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	210	120				330
Technical personnel	114	60				174
Other operational costs	56	20				76
Equipment						
Others (please specify)						
Direct costs	380	200				580
Indirect costs (20% of direct costs)	76	40				116
Total	456	241				696

Comments:

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

Name of Institute: BioCentrum-DTU, Technical University of Denmark

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	0.8	1	1	0.5		3.3
Technical personnel	1.7	2	1			4.7

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	32	40	41	21	0	134
Technical personnel	39	48	25	0	0	112
Other operational costs	10	15	10	0		35
Equipment						
Others (please specify)		42				42
Direct costs	81	145	76	21	0	323
Indirect costs (20% of direct costs)	16	20.6	15.2	4		55.8
Total	97	165.6	91.2	25	0	378.8

Comments:

The project group within PREMYTOX has decided to allocate 42 KKR to mycotoxin analyses. No overhead has been included for these costs.