



Midterm Status Report 2003 and Application for Continuation in 2004

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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)

2. Project title and number

I.12 Preventing Mycotoxin Problems (PREMYTOX)

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.*
- Hypothesis 2 *Harvest practice is an important control point in organic farming in the prevention of mycotoxin problems.*
- Hypothesis 3 *Drum drying at high temperatures will reduce the number of surface dwelling fungal spores and prevent mycotoxin problems.*
- Hypothesis 4 *The drying practice in commercial organic farming needs improvement to prevent mycotoxin problems.*

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	<u>SE</u> , HEH, EFK, UT	1078	2000	2004	WP1-D1-D8
2	General practice in organic farming regarding sowing, harvest, transportation, drying and storage of cereals	<u>SE</u> , 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	<u>EFK</u> , <u>SE</u> , UT, Drabæks Mølle	201	2000	2001	WP3-D1
4	Effect of drying practice on OTA- and trichothecene-producing fungi	<u>EFK</u> , <u>SE</u> , 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	<u>HEH</u> , 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: Relating to synthesis and dissemination of knowledge (Task WP1-2), a number of “Critical control points” were listed and their importance discussed in Elmholt (2003). During 2003 these points have been elaborated, incorporating results from the field study with contaminated seed (Elmholt & Mortensen, 2003), the studies on different drying techniques (Kristensen, 2003) and the study of post-harvest spore dispersal via grain dryers (Haase, 2003). Improper grain handling may lead to OTA levels far beyond the Danish limit of 5 µg/kg grain (Haase, 2003). We are, however, still lacking knowledge on why *P. verrucosum* - under apparently similar conditions - produces OTA in some cases and not in others. This emphasizes the need to prevent grain from being contaminated with *P. verrucosum* if at all possible. Based on so-far obtained knowledge we have summarised our present recommendations (Elmholt *et al.*, 2003):

- *P. verrucosum* is present in some soils and apparently more frequently occurring in organically than conventionally cultivated soils. Therefore, seek to avoid soil contamination as much as possible during harvest (Elmholt, 2003)
- Field trials do not indicate that *P. verrucosum* contamination of seed constitute a risk of grain contamination in the field (Elmholt, 2003; Elmholt & Mortensen, 2003). Yet we often find *P. verrucosum* on newly harvested grain (Elmholt, 2003), and especially damaged kernels are supposed to be vulnerable to infection. Assure that harvest equipment is properly cleaned and adjust the combine harvester so kernels are minimally damaged. The effect of damaged grain awaits further studies.
- Rapid drying is very important (Kristensen & Elmholt, 2002; Kristensen, 2003; Elmholt, 2003; Haase, 2003). Earlier studies of *P. verrucosum* at three organic farms with on-farm drying showed major differences (Elmholt, 2003). At two farms with natural air-drying and in-bin drying, respectively, we found high contaminations by *P. verrucosum* after drying, while the third farm with re-circulation drying had no contamination. This pattern was reproduced during three consecutive years. Problems were obviously not related to farming system but rather to improper drying practice.
- Winnowing the grain is often necessary - especially in organic farming – in order to reduce the risk of establishing humid ‘pockets’ of weeds, unripe grain, straw, soil etc. during drying and storage. If such pockets contain *P. verrucosum* this will probably increase the risk of OTA-formation perhaps even at very low temperatures. These aspects await further studies.
- Results from the Master’s Thesis by Haase (2003) show that dryers that are difficult to clean, can contaminate the grain with conidia of *P. verrucosum* - especially of course if the drying process is slow. Large amounts of conidia will not always result in high levels of OTA but they do constitute a risk if the fungus obtains suitable outer conditions for toxin formation. This was shown in our experiments – no matter whether OTA was formed during on-farm drying or during the following laboratory storage – as mentioned in Elmholt & Haase (2003a). Therefore: It is important that the drying facilities are properly cleaned and conidia actually removed and not just stirred up. Using an efficient vacuum cleaner can be recommended, preferably with a HEPA filter or similar to assure that conidia are trapped in the vacuum and not blown through the exhaust.

These conclusions have been disseminated to farmers and extension service at field days, meetings and seminars (cf. list of publications, point 4) as well as in short, popular publications (Elmholt & Kristensen, 2001; Elmholt, 2001b; Elmholt, 2002; Elmholt & Haase, 2003a,b; Elmholt & Mortensen, 2003; Elmholt *et al.*, 2003; Kristensen 2001a,b; Kristensen, 2003).

WP2: No financed activities in 2003

WP3: No financed activities in 2003. The project group is preparing a manuscript entitled “High-temperature treatment for efficient drying of bread rye and for reduction of fungal contaminants” that - according to present plans - will be submitted to Biosystems Engineering, in 2003. This manuscript will include results presented at the AgEng conference in 2002 (Elmholt & Kristensen, 2002) as well as data on *Fusarium* from DTU. The drying regimes chosen for the *Fusarium* work were chosen according to a certain statistical design (CCC) to save laboratory work. This analysis is being performed at DTU and the results to be delivered by October 2003.

WP4: Comparative drying tests using different drying techniques are almost finished. Four drying regimes were compared: 1) Continuous drying (CD); 2) Drum drying (DD); 3) In-bin drying with non-stop operation of the drying fan and optimal supply of heat (IBDF) and 4) In-bin drying with ambient air and no supply of heat (IBDA). The drying process of IBDA was deliberately delayed in order to simulate a ‘worst case’ situation. Organically grown rye, cv. Dominator, harvested at August 14th 2002 with a moisture content of 17.7%. During unloading, three reference samples (R1, R2, R3) were taken and dried in small batch driers. The continuous flow dryer was operated at a maximum temperature of 65°C (drying air) and 45°C (grain). The drum drying treatment was carried out at fixed constant Maximum Grain Temperature (MGT) of 64°C and a retention time of 10.5 min. The drying air temperature was on average 224 °C. Three representative samples of CD grain (CD2, CD3, CD4) and DD grain (DD4, DD5, DD6) were taken at appropriate intervals during the drying process. The in-bin drying was performed in two small-scale experimental dryers. One bin had the drying fan operating non-stop (IBDF), and the grain was supplied with sufficient heat to ensure low air humidity in the drying air. The other bin had no supply of heat and the drying fan was only operated when the humidity of the ambient air was sufficiently low for drying (IBDA). In-bin sampling was performed during the drying period, three times in IBDF (Aug 19th, Aug 22nd, and Oct 9th) and nine times in IBDA (Aug 19th, Aug 22nd, Aug 29th, Sept 4th, Sept 11th, Sept 18th, Sept 25th, Oct 2nd, and October 9th). Samples were taken in three horizontal levels, bottom layer (BL), middle layer (ML) and top layer (TL) using a cylindrical grain sampling tube. Nine samples of approx. 10 gram were taken in each level according to 9-points grid. These samples were kept un-pooled at 2°C and used for microbiological analyses. Furthermore one sample was taken in each level for moisture analysis.

Samples of the treated grain have been analysed. **Direct plating of kernels:** 360 kernels were drawn representatively from the R, CD and DD samples, placing 10 kernels/plate on DYSG agar (no surface disinfection). Four plates with 10 kernels were prepared from each of the bin samples, *i.e.* 360 kernels (40 x 9) from each horizontal level at each sampling date. The following fungi were assessed: *P. verrucosum*, penicillia with crème reverse, penicillia with bright yellow reverse, *Alternaria infectoria*, *Cladosporium* spp., *Aspergillus niger*, *A. flavus*, *Eurotium amstellodami* and *Eurotium* spp. The remaining fungi were grouped as ‘Others’. Furthermore the number of kernels without visible growth and the number of kernels with growth of only one species were recorded. **Direct plating for *Fusarium* analyses (performed at DTU):** Kernels were plated directly *Fusarium* selective agar medium, Czapek-Dox Iprodione Dichloran (CZID) agar using the same plating strategy as above for the R, CD and DD samples. For the in-bin samples, however, only samples from Aug 19th and Oct 9th were analysed. All plates were incubated for six days at 23 ± 3 C under 12 hr on/off light regime using UV “black light” and cold daylight tubes. After incubation all plates were inspected and *Fusarium* cultures were identified and calculated. In most cases the *Fusarium* cultures were identified directly by microscopy, the remaining cultures were isolated and purified before identification. **Dilution plating:** Viable propagules of fungi were enumerated by dilution plating on MEA and DYSG agar. Three subsamples from the R, CD and DD sam-

ples were analysed. From the IBDA and IBDF regimes, samples from Aug. 22nd and Oct. 9th and from all three layers were analysed. All enumerations are given as cfu g⁻¹ dried grain (drying time: 2 h at 130°C, according to ISO 712:1998). **Ergosterol analyses:** Five subsamples from each of the R, CD and DD samples were analysed. From the IBDA and IBDF regimes, samples from Oct. 9th were analysed as an estimate of fungal biomass. ***Eurotium* / *Aspergillus* analyses:** Approx. 180 strains of *Eurotium* were isolated at Foulum from the kernels used for direct plating. They were cultivated on CYA agar with 40% sucrose and incubated at 25°C and 37°C to study whether the drying regimes had any influence on these fungi, among which a number of species are thermotolerant.

Below we present some of our results, but it should be noted that data from WP4 are preliminary and not yet published. The results of specific relevance to the baking quality are shown in Figure 1. No evident differences were found in the tested quality parameters. A statistical analysis (principal component analysis) based on falling number and amylograph values for max. viscosity and max. temperature shows no significant difference in the baking quality for the three drying methods compared.

Comparative drying test, Rye for bread

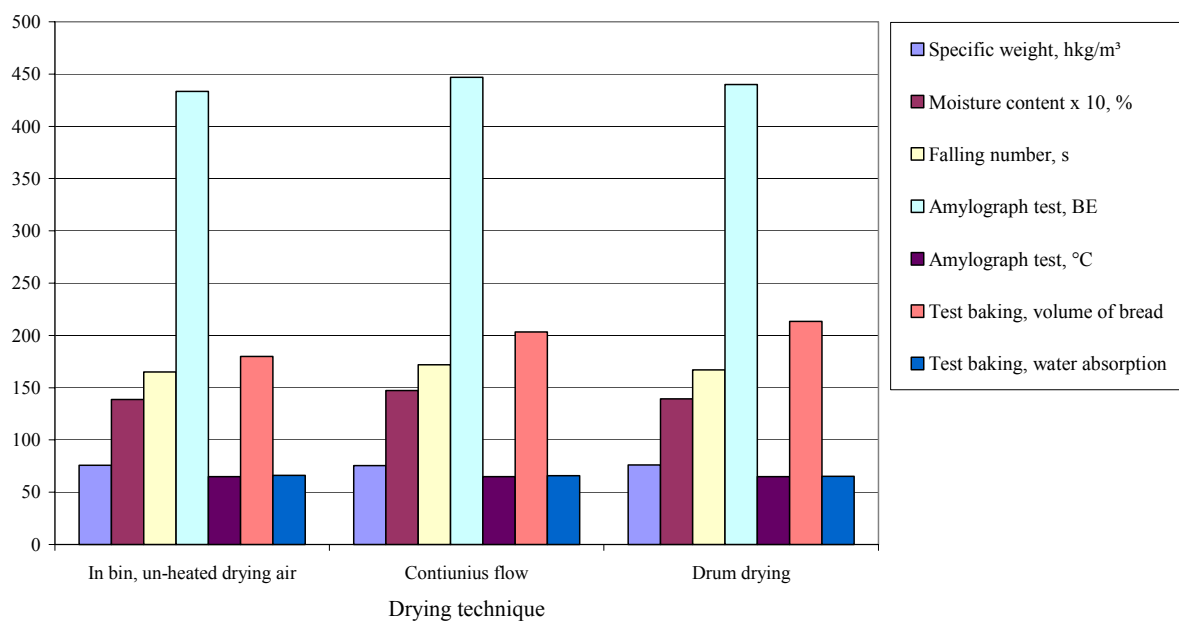


Figure 1. The effect of different drying methods on the quality when drying rye for bread.

The ergosterol analyses are not shown here but in accordance with similar results from WP3, this variable is rather insensitive to different drying regimes. Ergosterol is an estimate of fungal biomass and apparently measures total standing crop, *i.e.* both live and dead biomass. Analyses that assess living fungi, like direct plating and dilution plating seem better suited to evaluate different drying techniques as indicated in WP3 and confirmed below.

The results of kernel platings are shown in Table 1. Both continuous drying (CD) and drum drying (DD) have increased the number of kernels with one species very much primarily because the reductions in the “field fungi”, *Alternaria* and *Cladosporium*. These fungi were hardly reduced by the IBD regimes. The dominating *Fusarium* species in the grain was *F. avenaceum*, which was present in 41% (average of three samples 38-43-42 %) of the kernels before any treatment. Other species present was *F. culmorum* 6%, *F. tricinctum* 2% and *F. poae*. Drum-drying was very efficient

as the infection of *F. avenaceum* was lowered to 2.5 %, whereas continuous drying lowered the *F. avenaceum* to about 25%. In both cases a few other species were detected in very low numbers. In-bin drying with heat from August 19th to October 9th reduced *F. avenaceum* from 38-41-43 % (top-middle-bottom) to 19-16-20 % and *F. culmorum* from 8-8-5 % to 4-5-5 %. In-bin drying without heat reduced the *F. avenaceum* infection from 35-26-50 % (top-middle-bottom) to 14-10-15 %. In conclusion, drum-drying was much more efficient in reducing *Fusarium* counts in grain than continuous drying and in-bin drying.

Opposed to the reduction in field fungi, including *Fusarium*, in the CD and DD regimes, the number of kernels with *Eurotium* spp./*Aspergillus* spp. increased, especially in the CD samples but also in the DD samples. It should be noted that the *Eurotium* percentage in the reference samples is surprisingly high and it cannot be excluded that the samples have been contaminated by the batch dryer. This hypothesis is supported by numbers of *Eurotium* in the IBD samples at the first samplings being much lower than in the reference samples. *Eurotium* spp. also show distinct differences between grain layers with distinctly low percentages in the ML and the highest percentages in the TL from the start of September. *P. verrucosum* is present but below 1% in all samples.

Concerning species identification of *Eurotium*, the strains isolated and cultivated at Foulum were transported to DTU and a number of characteristic species identified (*Eurotium amstelodami*, *A. flavus*). However, some strains need to be further studied, especially those resembling *E. repens*. These are by far the most numerous on the kernels and very relevant as they seem quite tolerant to the high temperatures of the drum drying. According to DTU, this work will be performed in November 2003.

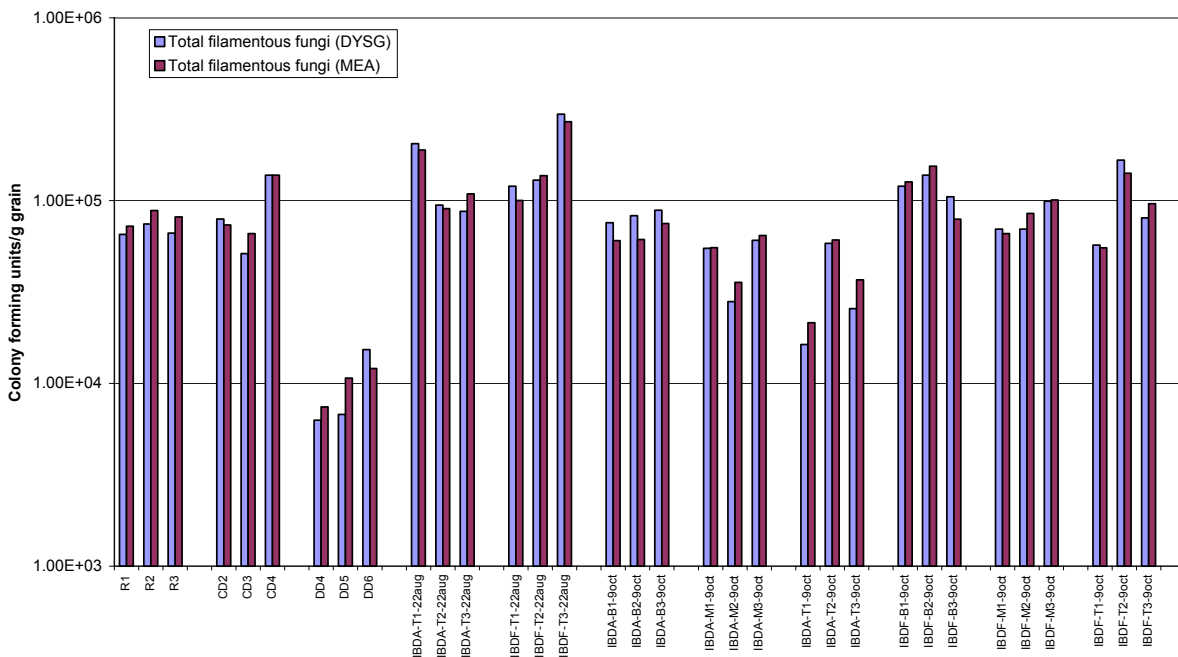


Figure 2. Dilution platings of grain from WP4. Legends as in Table 1. Results of three replicate subsamples are shown. Results have not been corrected for differences in moisture contents.

Sampling date	Drying regime	Sample no.	Grain layer	Kernels plated	No fungal growth	One species only	<i>Aspergillus niger</i>	<i>Cladosporium</i> spp.	<i>P. verrucosum</i>	<i>P. spp. (creme reveris)</i>	<i>P. spp. (yellow reveris)</i>	<i>F. avenaceum</i>	<i>F. culmorum</i>	<i>Aspergillus niger</i>	<i>Eurotium</i> spp.	Others
	R	1		360	0	8	215	301	1	115	43	138	18	0	127	2
	R	2		360	0	8	214	290	1	81	22	153	28	0	78	19
	R	3		360	0	4	192	279	0	75	22	150	19	0	101	8
	CD	2		360	0	31	132	231	0	39	30	102	7	0	165	11
	CD	3		360	0	32	83	200	0	62	11	98	0	2	208	7
	CD	4		360	0	62	80	181	0	53	6	87	5	1	215	7
	DD	4		360	1	139	3	23	1	53	1	9	0	0	176	39
	DD	5		360	0	88	27	38	2	113	3	13	1	0	116	21
	DD	6		360	0	80	36	43	0	82	2	6	1	0	114	19
19-Aug	IBDA		BL	360	0	5	257	339	2	78	24	178	11	2	33	60
	IBDA		ML	360	0	8	290	340	0	89	20	93	15	1	1	63
	IBDA		TL	360	0	14	299	338	1	74	22	126	7	0	5	44
	IBDF		BL	360	0	17	293	339	1	44	20	156	17	0	14	47
	IBDF		ML	360	0	9	267	328	0	56	10	148	30	0	19	75
	IBDF		TL	360	0	10	306	348	0	73	15	138	30	0	7	41
22-Aug	IBDA		BL	360	0	9	270	330	0	63	21			1	35	49
	IBDA		ML	360	0	13	310	340	0	83	22			0	1	50
	IBDA		TL	360	1	6	286	319	1	53	12			0	11	55
	IBDF		BL	360	0	7	288	332	2	47	51			2	24	62
	IBDF		ML	360	0	10	300	344	0	66	25			0	10	41
	IBDF		TL	360	0	11	303	347	0	75	13			0	6	47
29-Aug	IBDA		TL	360	0	30	245	312	0	21	13			1	112	32
04-Sep	IBDA		BL	360	0	13	264	334	2	80	17			1	47	51
	IBDA		ML	360	0	18	313	336	0	81	11			1	4	42
	IBDA		TL	360 ¹⁾	0	20	193	291	0	60	4			3	142	23
18-Sep	IBDA		BL	360	2	21	205	293	1	72	9			0	82	45
	IBDA		ML	360	0	10	298	324	0	98	12			2	3	45
	IBDA		TL	360	0	27	120	266	0	45	4			0	207	29
02-Oct	IBDA		BL	360	0	7	265	333	1	108	24			1	63	46
	IBDA		ML	360	0	6	317	341	1	65	10			0	6	57
	IBDA		TL	360	0	48	182	257	1	45	4			1	169	31
09-Oct	IBDA		BL	360	0	7	264	312	2	84	40	53	9	1	50	30
	IBDA		ML	360	1	18	277	306	0	70	18	37	3	0	7	57
	IBDA		TL	360	0	37	224	268	1	43	5	49	9	2	114	26
	IBDF		BL	360	0	21	217	315	0	101	51	71	17	0	84	32
	IBDF		ML	360	0	16	274	327	0	66	17	59	17	0	17	51
	IBDF		TL	360	0	31	158	281	0	46	6	67	16	3	191	23
¹⁾ One Petri dish discarded																

Table 1. Fungal colonisation of kernels from different drying regimes: 1) Continuous drying (CD); 2) Drum drying (DD); 3) In-bin drying with non-stop operation of the drying fan and optimal supply of heat to ensure sufficient low air humidity in the drying air (IBDF); 4) In-bin drying with ambient air and no supply of heat (IBDA).

Figure 2 (please note logarithmic scale) shows the result of the dilution plating in which the total abundance of filamentous fungi has been assessed on two different agar substrates. The results confirm that drum drying is much more efficient than the other drying methods in reducing the number of fungal propagules in grain. Surprisingly the “worst-case regime” (IBDA) has lower numbers of propagules by the end of the drying period than the IBDF regime.

WP5: In spring and summer 2002, a field experiment was performed at Foulumgaard with *P. verrucosum* contaminated seed (Task WP5-2). The purpose was to test if contaminated seed presents a risk of contaminating the grain before harvest. This is very relevant because organic farmers don't treat their seed with fungicides. The results presented in Elmholt (2003) indicated that farmers with insufficient drying and storage facilities, who use home-grown contaminated seed will risk OTA contamination of the soil and higher occurrence of *P. verrucosum* in the field ecosystem. In April 2002, eight small organically cultivated plots were established at Foulumgård, four with oats and four with spring wheat. All seed samples were organically cultivated and unbaited, originating from different farms and naturally contaminated with *P. verrucosum*. Some of the samples had high contents of OTA as well indicating that the fungus had not only infested the grain (surface contamination) but also infected the kernels. Seed germination and number of plants/m were satisfactory and by mid June, soil and root samples were taken in two of the plots and the OTA content of the rhizosphere soil determined. No samples exceeded the limit of detection (0.1 µg/kg dry soil). In the beginning of August, 30 heads were sampled in each plot. The heads were numbered and kept apart and the still rooted stems were numbered correspondingly enabling us to trace the plants with contaminated kernels. From each head, both glumes and kernel+bracts were tested (a total of 2400 glumes and 2400 kernels+bracts). We found no infested kernels and only one (spring wheat) glume with *P. verrucosum*. Analysing the straw on which the contaminated glume had been sitting, we recovered *P. verrucosum* from one of the root pieces but not from the above ground plant parts. The results of the field experiment have been published in Elmholt & Mortensen (2003) and the conclusions outlined in popular papers on *P. verrucosum* prevention (Elmholt & Haase, 2003a,b; Elmholt *et al*, 2003). In conclusion, our results do not confirm that infested seed may result in contamination of the heads while the plants are still rooted. When the heads had been analysed, the crop was harvested and 300 kernels from each plot were tested for occurrence of *P. verrucosum*. We found the fungus but on less than 1% of the kernels. So when we occasionally have batches of recently harvested grain with high contents of *P. verrucosum* (Elmholt, 2003), it is probably due to contamination during harvest rather than in the field.

Relating to Task WP5-4, a M.Sc. project (Maiken Haase (MH), biology, University of Århus) was finished May 2003 (Haase, 2003). The thesis is entitled: “Prevention of mycotoxin formation in organically cultivated bread grain – with focus on *Penicillium verrucosum* and formation of ochratoxin A”. It consists of a theoretical part on mycotoxins in general and mycotoxin producing fungi with special regard to *P. verrucosum*, on how the fungi and toxins are detected and which ecological factors will affect their growth and toxin production, and on how post-harvest treatment of grain will affect these fungi. The major part of the thesis reports the results of two sub-projects. The first subproject is a case study at one of the farms with drying problems, also included in Elmholt (2003). Samples were taken in rye and oats in 2000 and 2001 and analysed for occurrence of *P. verrucosum*. The farm had a home-made natural air-drying system (7.5 kW). The main duct was made of chipboard, internally covered by plastic. The side-ducts were made of boards with bottoms of plywood and covered by old grain sacks made of hessian, some of which being 20 years old. During the drying process, a statistically significant increase was found in the number of both rye and oats kernels that were contaminated with *P. verrucosum*. As the bottom layer of the rye contained more contaminated kernels than the top layer, it is most likely that contamination has taken place via the dryer as both the ducts and the hessian sacks contained large

amounts of *P. verrucosum* conidia. These will inevitably be dispersed into the grain. When the fungal analyses were completed, the remaining grain was placed at 2°C. The relative humidity in the room was high, and because the packaging was not completely air tight, the moisture content of the grain increased. After 18 months it was approx. 23% in all samples of rye. At this time, The Veterinary and Food Administration analysed the OTA content in some of the samples. Both top- and bottom samples of rye from the first four samplings were below the accepted limit of grain for human consumption (5µg OTA/kg). So were all bottom samples of rye from the fifth sampling while all top samples from the fifth sampling exceeded this limit by 10-70 times! We do not know for sure whether OTA was produced during drying or during the following cool storage – or perhaps both. After a further six months' storage in these conditions, however, some of the top samples from the fifth sampling were analysed again and the same level of OTA was found this time, indicating that no OTA had been produced during these six months. It is known, however, that *P. verrucosum* is able to grow at 2°C, and German studies have shown that the fungus can produce OTA at 4°C in grain with water contents above 20%. Since publication of the thesis, we have drawn attention to these results in relation to cleaning of drying and storage facilities as being very relevant post-harvest precautions to be taken by the farmer (Elmholt & Haase, 2003a,b; Elmholt *et al.*, 2003). The second subproject was a semi-controlled experiment in which damaged barley was infested with *P. verrucosum* and mixed into rye, oats, spelt and wheat. Infested grain constituted 5% of the grain volume and the grain was placed in metal tins (4 L), simulating small silos. Sampling was performed initially and after 11, 32 and 52 weeks, respectively. The last two samplings were postponed according to the original plans because the growth of *P. verrucosum* from the "hot spot" barley kernels was much slower than expected. The reasons are discussed in detail in Haase (2003) but are most probably related to the moisture content of the grain being too low (14-15%) for the development of *P. verrucosum* – although the infested barley had an initial moisture content of app. 20%. The wish to obtain results under conditions close to ordinary practice (most grain is traded with moisture contents around 15%) in this case, however, left us with a trial, from which nothing much could be concluded.

In collaboration with the EU funded OTAPREV project pure cultures of *Penicillium verrucosum* from the farms included in the study by Elmholt (2003) were analysed by molecular methods (AFLP; for methodological details, please consult last year's mid-term report). The results show that there is a great genetic variability among *P. verrucosum* strains from the Northern hemisphere (*e.g.* Denmark, Sweden, U.K.). The large amount of molecular data is still under detailed evaluation, but more than 40 different genetic clones have been observed and in some cases more than 20 clones within one farm have been observed. The overall conclusions from the joint EU project and the specific conclusions concerning the PREMYTOX strains are to be drawn in the very near future, as soon as all participants have finalised their work. A common scientific paper will be submitted on these results.

C2 Fulfilment of deliverables and milestones

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

WP1	Time schedule according to application	Deviations, if any*
<i>Deliverables</i>		
WP1-D1-D4: 1 st to 4 th 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 <i>P. verrucosum</i>	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	
<i>Milestones</i>		
WP1-M1-M5: Annual project meeting 2000-2003	2000 - 2003	Annual meeting of 2003 cancelled
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	

Annual meetings 2001-2002 (**WP1-M1 and M2**) were held according to plan. The annual meeting in spring 2003 (**WP1-M3**) was cancelled as explained below. Annual reports (**WP1-D1-D4**) have been delivered according to plan. A synthesis of the so-far obtained results on *P. verrucosum* ecology has been published in *Biological Agriculture and Horticulture* (Elmholt, 2003).

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 as outlined above. Some of these critical control points are being studied in WP4 and WP5.

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

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*
<i>Deliverables</i>		
WP3-D1: Report and/or popular paper	2002	
<i>Milestones</i>		
WP3-M1: Technical modifications completed	2000	
WP3-M2: Analyses of baking quality completed	2000/2001	

WP3-M3: Mycological analyses completed	2000/2001	Extended with <i>P. verrucosum</i> contaminated samples from commercial farmer CCC analysis yet to be completed
WP3-M4: Drum drying procedure for use in 2002 in WP4 established	2001	

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

WP4	Time schedule according to application	Deviations, if any*
<i>Deliverables</i>		
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 <i>Penicillium</i> and <i>Fusarium</i>	2004	
<i>Milestones</i>		
WP4-M1: Grain samples from different drying procedures distributed to RCF and DTU	2002	
WP4-M2: Mycological analyses completed	2003	<i>Eurotium</i> analyses yet to be completed
WP4-M3: Analyses for OTA and DON completed	2003	

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*
<i>Deliverables</i>		
WG5-D1: Scientific paper(s) on the effect of seed quality, harvest practice and 'hot-spot' formation on <i>P. verrucosum</i> and <i>Fusarium</i>	2004	No scientific paper on contamination from seed
<i>Milestones</i>		
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	

D. Description of deviations and subsequent adjustments of plans

WP1: Relating to Task WP1-1 (Project co-ordination), it was decided to skip the annual project meeting and plan common activities via mail and phone. One reason was that the planned field experiment for 2003 was substituted by an intensified program of analyses in WP4 autumn 2002 and by a large laboratory trial in WP5.

WP3 and WP4: The statistical analysis of the reduced experimental design (the CCC analysis) and the results on *Eurotium* composition will be completed at DTU during Oct and Nov. 2003.

WP5: As announced in the Mid-term report from 2002, the project manning has been changed with Helle Hestbjerg's resignation and Susanne Elmholt's taking over as head of WP5. In accordance with this and so far obtained results, project focus on *P. verrucosum* was increased. Our field experiment from 2002 did not confirm that seed contamination with *P. verrucosum* is a significant risk of grain contamination in the field. No scientific paper will be published on this exclusively on this theme, instead the results were reported in a popular paper (Elmholt & Mortensen, 2003). It was furthermore decided to substitute the planned field experiment for 2003 with laboratory experiments to elucidate the effect of grain damage and impurities on the risk of OTA contamination. Ultimo September we set up a rather large laboratory experiment to. The experiment consists of a main experiment and four sub-experiments:

Main experiment with damaged grain at three different moisture contents:

This experiment is designed to elucidate the effect of mechanical damage and thus facilitated fungal access to the kernels at 14%, 18% and 22% moisture (w.b.), respectively. The grain (rye, cv. Hacada) originates from the DARCOF crop rotation trial at Jyndevad. Grain from "high level of fertilizer" has been chosen to increase the degree of generalisation and because a Swedish study indicates that increasing OTA contents are correlated with increasing levels of fertilization. All grain has been winnowed because focus in this part of the experiment is on grain damage. Half of the grain is inoculated with *Penicillium verrucosum*, the other half is not to secure a "healthy" control. Approx. 5000 viable conidia g⁻¹ dry grain is aimed at.

1. Sub-experiment with grain, grown at "Low level of fertilizer":

This sub-experiment is included because the low fertilizer level is relevant to organic farmers and because of lack of knowledge concerning the correlation between fertilizer status and the growth and OTA production by *P. verrucosum*. This sub-experiment is designed so that results can be compared with those of the main experiment. Sub-experiment 1 is conducted at two moisture contents (14% og 22%) with non-damaged, winnowed grain.

2. Sub-experiment at three temperature-regimes and two moisture contents:

The aim of this experiment is to elucidate the conclusions of the main experiment by studying OTA development at two lower and one higher temperature than the 15°C from the main experiment. 2°C has been chosen because OTA is not regarded to be a problem at these very low temperatures. However, our conclusions till now indicate that this should be studied in more details, e.g. by incubating the grain samples for a longer time than most often used in experimental work (Haase, 2003; Elmholt & Haase, 2003a,b). 10°C has been chosen to enable a comparison with similar German studies. 20°C has been chosen because *P. verrucosum* is probably more subjected to competition by e.g. *Aspergillus* species at higher temperatures (e.g. Haase, 2003). This sub-experiment is conducted at 14% and 22% moisture with both damaged and undamaged grain. All grain is inoculated and winnowed.

3. Sub-experiment with 30 temperature-regimes and one moisture content:

This sub-experiment aims to elucidate if there is a critical temperature for OTA development. It is conducted in a temperature gradient block, set at the interval of 2.5°C - 31.5°C. The results will be compared with the main experiment (15°C) and sub-experiment 2 (2°, 10° and 20°C). The experiment is conducted at moisture content 22% with inoculated, winnowed grain. Main emphasis

on damaged grain but in six evenly distributed temperature stations, we include non-damaged grain.

4. Sub-experiment with un-winnowed grain at two moisture contents

This sub-experiment is included because impurities – others than damaged kernels – are assumed to increase the risk of fungal growth and OTA production in the grain. The purpose is to study OTA contents in un-winnowed grain and compare the results with the results in the winnowed grain of the main experiment. “Un-winnowed” grain was obtained artificially by adding impurities (1-2 mm) to the winnowed grain. The impurities consist primarily of small pieces of straw and weed plants. The experiment is conducted with both inoculated and non-inoculated grain. All grain is un-damaged in order to avoid interaction between damaged grain and impurities. Incubation at 15°C and 22% moisture.

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**, 311-337. **Available in Orgprints**

****Hestbjerg, H., Nielsen, K.F., Thrane, U. & Elmholt, S. (2002)** Production of trichothecenes and other secondary metabolites by *Fusarium culmorum* and *F. equiseti* on common laboratory media and a soil organic matter agar: An ecological interpretation. *Journal Agricultural and Food Chemistry*, **50**, 7593-7599. **Available in Orgprints**

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>. **Available in Orgprints**

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. **Available in Orgprints**

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

Elmholt, S. & Haase, M.S. (2003b) Improper handling of grain may result in high levels of Ochratoxin A. DARCOFenews. Newsletter from Danish Centre for Organic Farming. Jun03. No. 2. <http://www.darcof.dk/enews/jun03/mycoto.html>. **Available in Orgprints**

Elmholt, S. & Haase, M.S. (2003a) Giftige svampe i tørringsanlæg. En u hensigtsmæssig håndtering af korn kan føre til dannelse af ochratoxin. Økologisk Jordbrug, nr. 287, 18. april, p. 13. **Available in Orgprints**

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. *Available in Orgprints*

Elmholt, S. & Mortensen, G.K. (2003) Kan OTA-dannende lagersvampe inficere kornet i marken? Forskningsnytt nr. 1, 18-20. *Available in Orgprints*

Elmholt, S. Haase, M.S. & Kristensen, E.F. (2003) Uhensigtsmæssig kornhåndtering kan give store ochratoksin forekomster - risikoen kan bl.a. forebygges ved tromletørring af kornet. FØJOenyt. Nyhedsbrev fra Forskningscenter for Økologisk Jordbrug, Aug03, nr. 4. <http://www.foejo.dk/enyt2/enyt/aug03/myco.html>. *Available in Orgprints*

Haase, M.S. (2003). Forebyggelse af mykotoksin dannelse i økologisk brødkorn - med fokus på *Penicillium verrucosum* og dannelse af ochratoksin A (Preventing Mycotoxin Problems in Organic Farming - with Focus on *Penicillium verrucosum* and Ochratoxin A). Master's Thesis (in Danish), University of Aarhus, Aarhus. 148 sider + 3 Appendices. *Available in Orgprints*

Høy, J.J. (2001) Økologisk kornopbevaring (Organic handling of grain). Intern rapport fra spørgeskemaundersøgelse (Report from questionnaire) (in Danish). Landbrugets Rådgivningstjeneste, Skejby. 2 pp. *Not available in Orgprints*

Kristensen, E.F. (2003) Tromletørring anvendt til at sikre kvaliteten af korn. Økologisk Jordbrug, nr. 293, 6. *Available in Orgprints*

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. *Available in Orgprints*

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

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

Elmholt, S. (2003) Forebyggelse af mykotoksinproblemer – en status (abstract). Idéforum for planteavlsudvikling og –forskning, Skejby 5. maj 2003. *Not available in Orgprints*

Elmholt, S. (2003) Undervisning ved Kursus i "Kornsvampenes Biologi, 2-4. okt. Landbrugets Rådgivningstjeneste, Skælskør/Flakkebjerg: Foredragstittel: "Penicillium" 4/10 kl. 13-14. *Available in Orgprints*

Elmholt, S. (2003) Forebyggelse af mykotoksinproblemer. Poster præsenteret ved generalforsamlingen i Økologisk Landsforening den 8. - 9. marts på Den Økologiske Landbrugsskole.

Elmholt, S. (2002 and 2003) Svampe i kornlagre (Storage fungi). Project Farm4U 'Researcher for a day' (please cf. <http://www.farm4u.dk/sw91.asp>). Five student teams in 2002: January 30th, February 5th, February 27th, August 13th, August 14th. One team in 2003 (February 25th). *Available in Orgprints*

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

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

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.

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

Kristensen, E.F. (2003) Heat treatment for disease control. Research Centre Flakkebjerg. 22 January 2003.

Kristensen, E.F. (2002) Varmebehandling til kvalitetsforbedring af frø. Workshop: Frø- og kornkvalitet. DIAS, Research Centre Flakkebjerg. 18 December 2002.

F. Scientific education

Susanne Elmholt has been a Member of the Evaluation Committee at Janne Lager's Ph.D. (Nov. 15th 2002): Soil-borne Clover Diseases in Intensive Legume Cropping, SLU Department of Plant Pathology and Biocontrol Unit.

In April 2003, the Master's Thesis by Maiken S. Haase (2003) was defended at Aarhus University with Professor J.C. Frisvad (participant in the EU OTAPREV project) as opponent.

G. National and international cooperation

National cooperation: During the project (**WP2**) there has been cooperation 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 have been studying the 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 of *Fusarium* and *eurotium* 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 DTU secures a close contact to the ongoing EU project on OTA (<http://www.mycotoxin-prevention.com/Project1.htm>), in which Jens Frisvad from DTU participates. 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. Ochratoxin 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 affect 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). A master's thesis on the subject was finished in May 2003 showing large spatial variations in contaminated kernels and OTA in a platform dryer – primarily due to inefficient cleaning of drying facilities and slow drying. The study showed that *P. verrucosum* can give rise to OTA concentrations far beyond established limits and the results were communicated to farmers in the spring

and summer of 2003 via e.g. Darcof enews and FØJOenyt. 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. In 2003 we have furthermore initiated a rather big laboratory experiment to elucidate the importance of some of the variables that are assumed to favour *P. verrucosum* growth and OTA formation, *i.e.* threshing damage and grain impurities. These factors are very poorly documented in the literature and in our opinion this documentation is very much needed.

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. By now we have obtained the results of the drying experiments that compare drum drying with continuous drying and platform drying (WP4). These results show very clearly that drum drying competes well not only with the slow platform drying but certainly also with continuous drying.

Mycological analyses at Foulum and DTU as well as baking tests (Drabæks Mølle) are performed using well-implemented 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 perform 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 resigned in 2002, and WP5 is now headed by Susanne Elmholt. We have continued working with trichothecene producing *Fusarium* species in WP4 via DTU, who are specialists on *Fusarium* taxonomy. However the emphasis in PREMYTOX WP5 was 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 in relation to *P. verrucosum*, whose life cycle is more closely bound up to the drying process than the trichothecene producers. 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 to the research within FØJO.

During the course 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. During 2002-2003 we co-operated 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) and we have produced a small paper on this common work. 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 pro-

ject (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"). Our cooperation with OTAPREV has resulted in a common paper, which will be submitted soon. The genetic variance within the species *P. verrucosum* turned out to be very big and this work will undoubtedly affect 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).

Our message to society (producers as well as consumers) is that toxigenic fungi are naturally occurring and cannot be totally avoided. The aim is rather to minimize the dispersal, growth and toxin production of these 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 emphasizing that proper management adjustments to a large extent seem to solve the problems (cf. Elmholt 2003).

Many international studies on OTA problems in food products have a chemical/toxicological basis. Compared with this, our work is to a larger extent directed 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 quite satisfied with our results in PREMYTOX and – including the adjustments concerning toxin analyses and contents of WP5 - it is our intention to finish our 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:	Consumption before 2003	Expected consumption 2003	2004	2005	Total
Man-months					
Scientific personnel	26.8	11	11		48.8
Technical personnel	22.46	7.2	0.8		30.46

Year:	Consumption before 2003	Expected consumption 2003	2004	2005	Total
Salaries					
Scientific personnel	1064	498	481		2043
Technical personnel	534	186	21		741
Other operational costs	225	80	15		320
Equipment					
Others (please specify)	75		50		125
Direct costs	1898	764	567		3229
Indirect costs (20% of direct costs)	364	153	104		621
Total	2262	917	671		3850

Comments:

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

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project			
Senior scientist Susanne Elmholt	Danish Institute of Agricultural Sciences, Dept. of Agroecology	Foulum, Sept. 30 th 2003	

Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)

Name of Institute: RCF, DIAS, Department of Agroecology

Year:	Consumption before 2003	Expected consumption 2003	2004	2005	Total
Man-months					
Scientific personnel	16.5	11	10.5		38
Technical personnel	11.26	6.22	0.76		17.74

Year:	Consumption before 2003	Expected consumption 2003	2004	2005	Total
Salaries					
Scientific personnel	662	457	460		1579
Technical personnel	273	161	21		455
Other operational costs	124	70	15		209
Equipment					
Others (please specify)	75		50		125
Direct costs	1134	688	546		2368
Indirect costs (20% of direct costs)	211	138	100		449
Total	1345	826	646		2817

Comments:

Others include subcontractors, *e.g.* WP 2 (DAAC) and expenditures for mycotoxin analyses. No overhead has been included for these costs as specified in mid-term report 2002

B. Budget for each participating department (1.000 DKK)

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

Year:	Consumption before 2003	Expected consumption 2003	2004	2005	Total
Man-months					
Scientific personnel	8.5				8.5
Technical personnel	7.5				7.5

Year:	Consumption before 2003	Expected consumption 2003	2004	2005	Total
Salaries					
Scientific personnel	330				330
Technical personnel	174				174
Other operational costs	76				76
Equipment					
Others (please specify)					
Direct costs	580				580
Indirect costs (20% of direct costs)	116				116
Total	696				696

Comments:

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

Name of Institute: Center for Process Biotechnology DTU, Technical University of Denmark

Year:	Consumption before 2003	Expected consumption 2003	2004	2005	Total
Man-months					
Scientific personnel	1.8	1	0.5		3.3
Technical personnel	3.7	1			4.7

Year:	Consumption before 2003	Expected consumption 2003	2004	2005	Total
Salaries					
Scientific personnel	72	41	21		134
Technical personnel	87	25			112
Other operational costs	25	10			35
Equipment					
Others (please specify)					
Direct costs	184	76	21		281
Indirect costs (20% of direct costs)	37	15	4		56
Total	221	91	25		337

Comments:

The project group within PREMYTOX has decided to transfer 42 KKR to *Eurotium* work at Foulum and external mycotoxin analyses (according to letter of 17th Oct 2002 to Direktoratet for Fødevareerhverv).