



Progress Report 2004 and Application for Continuation in 2005

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

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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4. Participating institutes

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Technical University of Denmark (DTU)

BioCentrum-DTU

Center for Microbial Biotechnology (CMB) (formerly Centre for Process Biotechnology)
Søltofts Plads, Building 221
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5. Other project staff

6. Project period (month, year)

Start of project: July 1st 2000

End of project: December 31st 2004 (some money transferred to 2005 according to Enclosure 1)

7. Midterm description of the project, its results and progress, and application for continuation in 2005

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

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” have been listed and discussed (Elmholt, 2003; Elmholt & Mortensen, 2003); Kristensen, 2003; 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 our present recommendations have been summarised (Elmholt *et al.*, 2003). These conclusions have been disseminated to farmers and extension service at field days, meetings and seminars) as well as in short, popular publications.

WP2: No financed activities in 2004

WP3: No financed activities in 2004.

WP4: Comparative drying tests using different drying techniques have been finished. The results were described in the midterm report for 2003. The only point remaining to be done is the work related to *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. Concerning species identification of *Eurotium*, the strains isolated and cultivated at Foulum were transported to DTU and a number of characteristic species identified (e.g. *Eurotium amstellodami*, *A. flavus*).

WP5: Our field experiment did not indicate that infested seed leads to contamination of the heads while plants are still rooted, 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. In outlined in the midterm report of 2003, no field work has therefore been performed in 2004, and resources instead put into laboratory experiments.

Based on previous work (Elmholt, 2003 and Haase, 2003), including OTA analyses on a range of our samples, a laboratory experiment was set up in September 2003. The main experiment and four sub-experiments were described in detail last year’s midterm report:

- Main experiment with damaged grain at three different moisture contents
- Sub-experiment with grain, grown at “Low level of fertilizer”
- Sub-experiment at three temperature-regimes and two moisture contents
- Sub-experiment with 30 temperature-regimes and one moisture content
- Sub-experiment with un-winnowed grain at two moisture contents

The experiments are almost finished except for some samples remaining incubated at low temperature for a few more months. The following variables have been measured: Moisture content, *P. verrucosum* (dilution plating), ergosterol and OTA, the latter using ELISA test kits from R-biopharm, a method which was implemented at Dept. for Agroecology in April 2004. Verifications of OTA measurements are being performed at the Danish Institute for food and Veterinary Research. The results clearly indicate that grain moisture content is much more crucial to OTA formation than temperature as *P. verrucosum* grows well at both 2, 10, 15 and 20°C.

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), and a draft of a collaborative scientific paper has been produced.

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*
<i>Deliverables</i>		
WP1-D1-D4: 1 st to 4 th annual report 2000-2003	2000 - 2003	OK
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		Video substituted by other sources 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 03 and 04 cancelled
WP1-M6: Mid-term conclusions on critical control points and their implementation into WP4 and WP5	2001	OK
WP1-M7: Collection of material for use in the video presentation on how to prevent mycotoxin problems		Cf. WP1-D7

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.

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	Scientific paper submitted July 2004
<i>Milestones</i>		
WP3-M1: Technical modifications completed	2000	OK
WP3-M2: Analyses of baking quality completed	2000/2001	OK

WP3-M3: Mycological analyses completed	2000/2001	Extended with <i>P. verrucosum</i> contaminated samples from commercial farmer CCC analysis not delivered
WP3-M4: Drum drying procedure for use in 2002 in WP4 established	2001	OK

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)	2004	
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>	2005	
<i>Milestones</i>		
WP4-M1: Grain samples from different drying procedures distributed to RCF and DTU	2002	OK
WP4-M2: Mycological analyses completed	2003	<i>Eurotium</i> analyses not completed
WP4-M3: Analyses for OTA and DON completed	2003	Will not be performed

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>		
WP5-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 Paper submitted to Mycopathologia
<i>Milestones</i>		
WP5-M1: Obtaining naturally or artificially contaminated seed for use in 2002 and 2003	2001-2002	OK

WP5-M2: Performance of field experiment 2002 and 2003	2002-2003	Adjustments according to so-far obtained results and changes in project manning
WP5-M3: Mycological analyses completed	2003-2005	Last analyses will be performed in 2005

* Deviations are further discussed in D

D. Description of deviations and subsequent adjustments of plans

WP3-D1: A manuscript entitled “High-temperature treatment for efficient drying of bread rye and for reduction of fungal contaminants” has been submitted to Biosystems Engineering in July 2004. This manuscript includes 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 and analyses of baking quality were chosen according to a certain statistical design (CCC) to save laboratory work. The CCC analysis should have been performed at DTU and the results delivered by October 2003. Due to staff reductions at DTU they were, however, not received by June 2004 and we therefore decided to proceed without this analysis.

WP4-M2: Concerning species identification of *Eurotium*, the strains isolated and cultivated at Foulum were transported to DTU and a number of characteristic species identified (*Eurotium amstellodami*, *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 last year’s midterm report, this work was planned for November 2003 at DTU. A further study of *Eurotium* has been discussed within the Biodiversity research team at CMB/DTU; however it has been concluded that this work cannot be performed within the allocated resources from PREMYTOX and CMB.

WP4-M3: These analyses will not be performed, as it is most unlikely (due to temperature and moisture conditions as well as to fungal composition) that OTA and DON have been formed during drying.

WP5-D1: A paper on “*Penicillium verrucosum* occurrence and Ochratoxin A contents in organically cultivated grain with special reference to ancient crop types and drying practice”, authored by S. Elmholt and P.H. Rasmussen (DIFVR) has been submitted to Mycopathologia by April 2004. A second revised version will be forwarded by Oct. 2004. **WP5-M3:** The samples incubated at 2°C have to remain incubated for a long period to verify whether they become contaminated by OTA. The last analyses of these samples will be performed by the end of 2004. Regarding the collaboration with the OTAPREV project, pure cultures of *Penicillium verrucosum* from the farms included in the study by Elmholt (2003) were analysed by AFLP and a scientific paper authored by J.C. Frisvad, (CMB/DTU) F. Lund and S. Elmholt (Title: Ochratoxin A producing *Penicillium verrucosum* isolates from cereals reveal large AFLP fingerprinting variability) has been accepted with minor revisions for publication in Journal of Applied Microbiology. A revised manuscript will be re-submitted in October 2004.

E. Project publications and other products

1. Products from Organic Eprints archive

Publications from 2004 have been deposited on Sept. 23rd 2004

Number of eprints: 23.

Schjøning, P. and Elmholt, S. and Christensen, B.T., Eds. (2004) [*Managing Soil Quality: Challenges in Modern Agriculture*](#). CABI Publishing, Wallingford, UK.**

Elmholt, S. and Labouriau, R. (2004) [Fungi in Danish soils under organic and conventional farming](#). [preprint]*

Elmholt, S. and Rasmussen, P.H. (2004) [Penicillium verrucosum occurrence and Ochratoxin A contents in organically cultivated grain with special reference to ancient crop types and drying practice](#). [preprint]

Elmholt, S. and Rasmussen, P.H. (2004) [Susceptibility of spelt to ochratoxin A producing fungi](#) . *DARCOFenews*. Online at <http://www.darcof.dk/enews/june04/toxin.html>

Elmholt, Susanne (2002) [Ecology of the ochratoxin A producing Penicillium verrucosum: Occurrence in field soil and grain with special attention to farming system and on-farm drying practices](#). *Biological Agriculture and Horticulture* 20:311-337.*

Elmholt, Susanne (2002) [Penicillium - kursus i kornsvampenes biologi](#). [oral] Presentation at *Landbrugets Rådgivningscenter, kursus i Kornsvampenes Biologi*, Flakkebjerg, 2-4. okt. 2002.

Elmholt, Susanne (2002) [Svampe i kornlagre - Et forsøg som belyser succession og konkurrence under forhold der favoriserer forskellige organismer](#) . Online at <http://www.farm4u.dk/sw91.asp>>. January 2002 by Farm4U.dk**

Elmholt, Susanne (2001) [Forebyg svampegift i korn](#). In *Landsbladet*, 10. August.

Elmholt, Susanne (2001) [Mykotoksinproducerende svampe på korn](#). [oral] Presentation at *Markvandring*, Forskningscenter Foulum, 14. juni 2001.

Elmholt, Susanne and Haase, Maiken S. and Kristensen, Erik F. (August 2003) [Uhensigtsmæssig kornhåndtering kan give store ochratoksin forekomster - risikoen kan bl.a. forebygges ved tromletørring af kornet](#) . Online at <http://www.foejo.dk/enyt2/enyt/aug03/myco.html>>. Accessed 21. August 2003

- Elmholt, Susanne and Haase, Maiken (2003) [Gifftige svampe i tørringsanlæg](#). In *Økologisk Jordbrug*, April, No 287, page 13. Økologisk Landsforening.
- Elmholt, Susanne and Krogh Mortensen, Gerda (2003) [Kan OTA-dannende lager-svampe inficere kornet i marken?](#). *Forskningsnytt om økologisk landbrug i Norden* 2003(1):18-20.
- Elmholt, Susanne and Schioldann Haase, Maiken (2003) [Improper handling of grain may result in high levels of Ochratoxin A](#). Online at <http://www.darcof.dk/enews/jun03/mycoto.html>. Accessed June 2003. DARCOF-enews
- Elmholt, Senior Scientist Susanne (2002) [Pas godt på kornet – både under og efter høst!](#). In *Økologisk Jordbrug*, 9. August, page 6.
- Elmholt, Senior Scientist Susanne and Kristensen, Scientist Erik F. (2001) [Korn uden mykotoksiner \(Grain without mycotoxins\)](#). DJF report no. 53, Department of Crop Physiology and Soil Science and Department of Agricultural Engineering, Danish Institute of Agricultural Sciences.
- Haase, Maiken S. (2003) [Forebyggelse af mykotoksindannelse i økologisk brødkorn - med fokus på *Penicillium verrucosum* og dannelse af ochratoksin A](#) [*Prevention of Mycotoxin Problems in Organically Grown Bread Grain - with Special Focus on Penicillium verrucosum and its formation of Ochratoxin A*]. Master Thesis, Afd. for Mikrobiel Økologi, Biologisk Institut.
- Hestbjerg, Helle and Nielsen, Kristian F. and Thrane, Ulf and Elmholt, Susanne (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 of Agricultural and Food Chemistry* 50(26):7593-7599.**
- Kristensen, E. F. and Elmholt, S. and Thrane, U. (2004) [High-temperature treatment for efficient drying of bread rye and reduction of fungal contamination](#). [preprint]
- Kristensen, Erik F. and Elmholt, Susanne (2002) [High-temperature drying of organically grown bread rye](#). Paper presented at International Conference on Agricultural Engineering, Budapest, 30 June - 4 July.
- Kristensen, Erik Fløjgaard (2003) [Tromletørring skal sikre kvalitetskorn](#). In *Økologisk Jordbrug*, 8. August, Volume 23, No 293, page 6. Økologisk Landsforening.

Kristensen, Erik Fløjgaard (2001) [Ny tørringsteknik kan gøre økologisk korn bedre](#). In *JordbrugsForskning*, May, Volume 5, page 12. Danmarks JordbrugsForskning.

Kristensen, Erik Fløjgaard (2001) [Tromletørring god til øko-korn](#). In *Økologisk Jordbrug*, 15. June, page 9. Økologisk Landsforening.

Kristensen, Scientist Erik Fløjgaard (2003) [Tromletørring skal sikre kvalitetskorn](#) [Drum drying, a method to ensure high-quality grain]. In *Økologisk Jordbrug*, August, No 293, page 6-6. Økologisk Landsforening.

This list was generated on **Mon Oct 4 00:18:29 CEST 2004**.

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

Elmholt, S. (2004) Gode råd om ochratoxin forebyggelse. Bilag uddelt ved Økologisk Landsforenings Generalforsamling 28-29. februar.

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

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

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

Elmholt, S. (2001c) Hvad ødelægger kvaliteten af økologisk korn? Oral presentation at seminar on Grain Quality for dlq 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.

* 25-75% financed by DARCOF

** 5-25% financed by DARCOF

F. Scientific education

Maiken S. Haase completed her M.Sc. thesis in 2003.

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, Drabøks 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 2003-2004 we conduct 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. Our results so far point to moisture content being much more important to OTA formation than temperature, a conclusion that has recently (September 2004) been drawn also in a paper produced in the EU project OTAPREV.

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.

There are 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 Institute of Food and Veterinary Research, whose laboratory is accredited to performing these analyses and in their daily work heading national surveys of mycotoxins in cereal commodities. We have had a very good collaboration, which has resulted in a paper, submitted for publication in *Mycopathologia*. In the spring of 2004, an ELISA method for OTA determination has been set up at the Dept. of Agroecology: This method is faster and cheaper than the standard HPLC, and in collaboration with the Danish Institute of Food and Veterinary Research (P.H. Rasmussen) we are currently comparing results obtained by these two methods using some of the samples from the laboratory study in WP5.

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 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"). Our cooperation with OTAPREV has resulted in a collaborative draft for a paper, which hopefully will be submitted. 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 emphasising 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	Consumption 2003	Expected consumption 2004	Budget 2005	Total
Man-months					
Scientific personnel	26.8	9.7	9.4	3.8	50
Technical personnel	22.46	7.07	0.67	0.34	31

Year:	Consumption before 2003	Consumption 2003	Expected consumption 2004	Budget 2005	Total
Salaries					
Scientific personnel	1064	402	409.5	166	2042
Technical personnel	534	182	18.5	7	742
Other operational costs	225	78	13.5	5	322
Equipment					
Others (please specify)	75		50		125
Direct costs	1898	662	491.5	178	3230
Indirect costs (20% of direct costs)	364	132.6	88.9	35	621
Total	2262	794.6	580.4	213	3850

Comments:

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project Susanne Elmholt, Senior Scientist, Ph.D	Danish Institute of Agricultural Sciences Department of Crop Physiology and Soil Science Research Centre Foulum (RCF)	October 1 st 2004	

Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)

Name of Institute and department: RCF, DIAS, Department of Agroecology

Year:	Consumption before 2003	Consumption 2003	Expected consumption 2004	Budget 2005	Total
Man-months					
Scientific personnel	16.5	8.7	9.4	3.8	38.4
Technical personnel	11.26	6.07	0.67	0.34	18.34

Year:	Consumption before 2003	Consumption 2003	Expected consumption 2004	Budget 2005	Total
Salaries					
Scientific personnel	662	361	410	166	1599
Technical personnel	273	157	19	7	456
Other operational costs	124	68	14	5	211
Equipment					
Others (please specify)	75		50		125
Direct costs	1134	586	492	178	2390
Indirect costs (20% of direct costs)	212	117	88	35	452
Total	1346	703	580	213	2842

Comments:

PREMYTOX applies DFFE for the transfer of 213 KKR from 2004 to 2005, including 25 KKR which the CMB/DTU has not used in 2004. Letters concerning this matter have been forwarded to DFFE on Sept. 6th and October 4th

B. Budget for each participating department (1.000 DKK)

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

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

Year:	Consumption before 2003	Consumption 2003	Expected consumption 2004	Budget 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 and department: Center for Microbial Biotechnology (CMB/DTU)

Year:	Consumption before 2003	Consumption 2003	Expected consumption 2004	Budget 2005	Total
Man-months					
Scientific personnel	1.8	1			2.8
Technical personnel	3.7	1			4.7

Year:	Consumption before 2003	Consumption 2003	Expected consumption 2004	Budget 2005	Total
Salaries					
Scientific personnel	72	41			113
Technical personnel	87	25			112
Other operational costs	25	10			35
Equipment					
Others (please specify)					
Direct costs	184	76			260
Indirect costs (20% of direct costs)	37	15			52
Total	221	91			312

Comments:

Regarding expected consumption 2004, please cf. comments for the Dept. of Agroecology above