I.12 Preventing Mycotoxin Problems

Acronym: PREMYTOX

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1. 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 cereals, ochratoxin A (OA) and trichothecenes are considered to be the most important mycotoxins. Opposed to compounds like pesticides and antibiotics, which are excluded from organic farming, the mycotoxin problems cannot be totally eliminated. They can, however, be reduced very much if fungal growth and mycotoxin formation is 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 two major objectives of the project 'PREMYTOX' are to increase our knowledge on the ecology of mycotoxin producing fungi and to 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 will focus on management practices, which are relevant to the general practice in organic farming and which are known or assumed to affect the OA producing *P. verrucosum* and the trichothecene producing species of *Fusarium*. Our work will address both preharvest and postharvest 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. During the course of the project, information on how to obviate mycotoxin problems will be made available to farmers and extension service in the form of popular papers and a video, stressing those management practices, which PREMYTOX demonstrates to be critical.

2. Research group

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3. Introduction

The successful introduction of organically grown products for human nutrition in Denmark has several reasons. Among the most important are that foods marketed with the Danish Ø-sign (organically grown) are presumed to contain no or less hazardous components than those originating from conventionally grown products. This goes for pesticides, additives, antibiotics and other compounds, which are used to increase plant and animal production. It is crucial for organic farmers to maintain the consumers' confidence in their products. This requires an on-going control to secure that the above mentioned components are actually absent or do not exceed established limits. But it also requires that organic farmers and organisations draw their attention to naturally occurring hazardous compounds and that the farmers are provided with efficient means to prevent this risk, if present.

During recent years several reports and observations indicate that organically grown cereals are more subjected to mycotoxin contamination than conventionally grown. Mycotoxins are produced by naturally occurring fungi and are toxic to humans and animals. In Danish grown small cereals, ochratoxin A (OA) and trichothecenes are considered to be the most important mycotoxins. Under Danish conditions OA is produced by *Penicillium verrucosum* and trichothecenes by different species of *Fusarium*.

In order to prevent mycotoxins problems it is important to know the ecology of the fungi, which produce these compounds. This knowledge must be related to farming practice. Here, important aspects are control of seed quality, harvesting, drying and storage practice. Infected seed, kernels damaged during treshing, slow drying and type of silo are examples of factors which may influence the risk of mycotoxin formation to varying degrees.

4. State of the art

Mycotoxin contamination of foods, feeds and agricultural crops continues to seriously affect the availability and safety of the food supply worldwide (Smith *et al.*, 1994). Mycotoxins are natural products. They are produced by certain filamentous fungi and cause a toxic response, a mycotoxicosis, when introduced by a natural route in low concentrations to higher vertebrates and other animals. Many food and feedborne fungi are able to produce one or more mycotoxins. Some of them are only produced by one or a few species, others are produced by a range of different species from several genera. More than 300 mycotoxins have been identified under laboratory conditions, but only about 20 occur naturally at significant levels and frequency to be of food safety concern (Smith *et al.*, 1994). Some mycotoxins, like aflatoxin, primarily contaminate crops in warm countries and enter the EU by importation. Others such as ochratoxin A, zearalenone and trichothe-cenes occur in crops from the temperate European countries, including Denmark. These compounds will be addressed in this project.

4.1 Ochratoxin A (OA) and its producer, *Penicillium verrucosum*

Penicillium verrucosum: OA is only produced by a few species of fungi. Based on a major research effort, taxonomists today agree that *P. verrucosum* Dierckx is the only known species of the genus to produce OA (Pitt, 1987; Frisvad and Samson, 1991). Although some species of *Aspergillus* produce OA, there are numerous indications that OA contamination in temperate regions can be ascribed solely to the activity of *P. verrucosum* (Northolt *et al.*, 1979; Häggblom, 1982; Damoglou *et al.*, 1984). *Penicillium verrucosum* belongs to the subgenus *Penicillium* and is characterised in part by its production of OA and verrucolone (Frisvad and Filtenborg, 1989). It can be divided into two physiologically distinct chemotypes (Ciegler *et al.*, 1973; Frisvad and Filtenborg, 1989). Chemotype I is found on processed meat products and produces OA. Chemotype II is found on grain and on cereal products and produces citrinin as well as OA.

Ochratoxin A: OA contamination of plant products has been reported regularly from a wide range of countries (e.g. Hokby *et al.*, 1979; Sinha *et al.*, 1986; Golinski *et al.*, 1991; Mantle and McHugh, 1993; Krogh *et al.*, 1974; Jorgensen *et al.*, 1996; Mantle and McHugh, 1993; Frank, 1999). As a result of improved analytical methods with a very low detection limit, OA consumption by humans and animals has been revealed to be widespread (Golinski *et al.*, 1991; Hald, 1991; Breitholtz *et al.*, 1993). The importance of OA as a health risk to humans and domestic animals is clearly acknowledged (Krogh *et al.*, 1974; Krogh, 1987; Boorman, 1989; Pohland *et al.*, 1992; Breitholtz *et al.*, 1993; Smith *et al.*, 1994; Frank, 1999). In order to prevent ochratoxicosis, various countries have set regulations for the OA content in food, animal feed and animal organs (Smith *et al.*, 1994). Several proposals of human tolerable daily intake (TDI) have been made (Nordic Working Group on Food Toxicology and Risk Evaluation, 1991; Joint FAO/WHO Expert Committee on Food Additives, 1991; Kuiper *et al.*, 1996). These TDI proposals are based on animal studies regarding the nephrotoxic and carcinogenic properties of OA but the toxicity of the compound also includes teratogenicity, immunosuppression and indications of genotoxicity.

Ecology of *P. verrucosum*: *P. verrucosum* is xerophilic and regarded a storage fungus. Its occurrence and behaviour under storage conditions have been widely studied with the focus on its production of OA. A Dan-ish survey on the OA content of cereals and cereal products showed that in years with wet harvest conditions, there is a high risk of consuming cereals that contain more OA than the proposed TDI values (Jørgensen *et al.*, 1996). Several reports from the Danish Veterinary and Food Administration indicate that OA contamina-tions occur more frequently in organically than in conventionally grown cereal products, especially for rye. Jacobsen and Jørgensen (1999) found OA in 27 of 30 organic rye flour samples (90%) compared with 19 of 29 conventional rye flour samples (66%). These results confirm earlier findings that organically cultivated rye seems to have OA problems (Jorgensen *et al.*, 1996; Anonymous,1997).In their review on OA contami-nation of agricultural commodities, Lillehøj and Elling (1983) drew the attention to the missing knowledge on the fungal cycle from soil to grain and back to soil. There has been practically no focus on the presence of *P. verrucosum* in the field ecosystem. Lillehøj and Göransson (1980) reported OA-producing *Penicillium* spp. on Danish pre-harvest barley but their results have been questioned according to later advances in *Peni-cillium* taxonomy (Frisvad, 1989). In only one survey made in Sweden of the OA content in grain, pre-harvest samples were included. Two samples, taken two weeks before harvest, contained OA (Hökby *et al.*, 1979).

Achievements by the PREMYTOX research group: Although *P. verrucosum* grows readily on many agar media, it was not found in Danish arable soils using general media for isolation (Elmholt and Kjøller, 1989; Elmholt *et al.*, 1993). One reason could be that this fungus occurs in very low propagule concentrations, implying that a more sensitive method of detection is required. The Mycology Group at DTU has developed a range of selective and diagnostic media for isolating xerophilic fungi from stored cereal products, including 'PRYES agar' and 'DRYES agar' (Frisvad, 1983; Frisvad, 1986). Preliminary studies indicated that neither the selective nor the diagnostic properties of these media were sufficient to detect and enumerate *P. verrucosum* (Chemotype II) in a population of soil fungi (Elmholt and Hestbjerg, 1996). They did, however, point to another medium, which was introduced by Frisvad *et al.* (1992) for the detection of *P. verrucosum* (Chemotype II) on grain. It is a modified DRYES agar with a very low a_w, called 'dichloran yeast extract sucrose 18% glycerol agar' (DYSG). In a recent paper, we demonstrate that this medium is absolutely excellent for the detection and estimation of conidial abundance of *P. verrucosum* in soil (Elmholt *et al.*, 1999). We could detect and enumerate *P. verrucosum* in conidial concentrations below 200 cfu g^{-1} soil even when it constituted no more than 0.3% of the total viable counts.

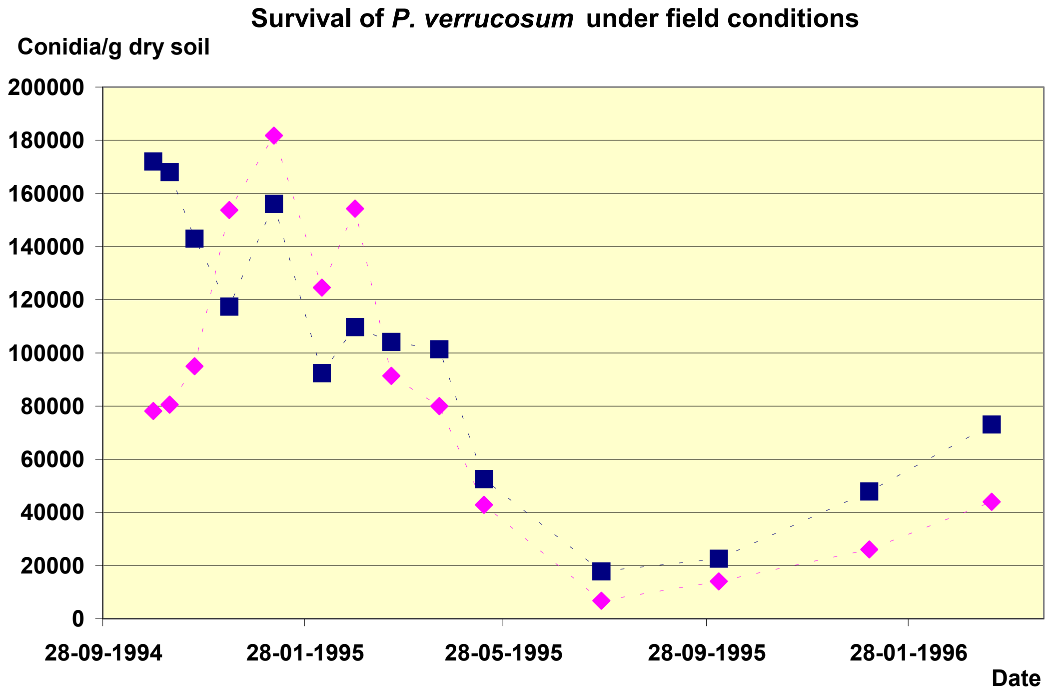

Figure 1. Abundance of *P. verrucosum* in soil, infested with either conidia of the fungus plus damaged grain (υ) or with conidia only (∝). The field trial was started 28/10 1994 and finished 18/3 1996. During this time, 14 samplings were performed in each of the two treatments. The results are means of four replications and the curves are only drawn to ease the reading.

Although normally regarded a storage fungus, we have performed field experiments showing that *P. verrucosum* can survive for many months in bulk soil, proliferate on soil organic matter and probably become an integral part of the soil ecosystem (Elmholt and Hestbjerg, *in press*), cf. Figure 1. That *P. verrucosum* does in fact exist in the soil ecosystem was verified in a project on soil fertility in organically and conventionally cultivated field soils using DYSG to detect *P. verrucosum*. We found the fungus in 8 of 25 organically cultivated fields (32%) compared with 3 of 43 conventionally cultivated fields (7%). In most of the soils, the species was rare but two of the organically cultivated soils had high frequencies (Elmholt, unpublished).

Towards an HACCP for the production of cereals: In summary the results achieved by the research group show that *P. verrucosum* is present in many arable soils and maybe more so in organically cultivated soils. The more frequent finding of the fungus in organic fields is in accordance with surveys by the Danish Veterinary and Food Administration. They all point to especially organically cultivated rye as being sensitive to OA contamination. In a attempt to elucidate the reasons for this, we have initiated a preliminary project between Drabæks Mill (the largest organic mill in Denmark) and Research Centre Foulum. We have implemented equipment for OA analysis at the mill and performed case studies at three commercially driven organic farms. The purpose of this is to study the occurrence of *P. verrucosum* at all levels: the soil, the seed, the growing crop, the harvested grain, the dried grain and the stored grain. The study also includes samples from the storage facilities. These investigations will not be finished until the end of 2000. However, they have in combination with the former findings revealed a number of 'points' in the primary production of cereals, which seem to be important in relation to the risk of OA formation. These points need further experimental elucidation with the aim to provide organic farmers (and conventional farmers as well) with knowledge and tools of how to prevent the contamination of grain with *P. verrucosum* in order to avoid OA problems. A similar Hazard Analysis of Critical Control Points (HACCP) is currently being approached with the aim to prevent OA contamination of coffee (Frank, 1999).

4.2 Trichothecene producing species of *Fusarium*

Fusarium: Many *Fusarium* species are known to produce trichothecenes. The main producers of the most abundant trichothecenes are shown in the Table 1.

Table 1. The main *Fusarium* species from the temperate zone producing the most abundant trichothecenes (based on Marasas *et al.*, 1984; Miller *et al.*, 1991; Thrane and Hansen, 1995; Thrane, *in press*).

Trichothecene	*Fusarium* species
Deoxynivalenol (DON)	*F. culmorum*, *F. graminearum*
Nivalenol (NIV)	*F. culmorum*, *F. graminearum*, *F. crookwellense, F. poae, F. equiseti*
Diacetoxyscirpenol (DAS)	*F. equiseti*, *F. poae*, *F. venenatum*, *F. sambucinum*
T-2 toxin	*F. sporotrichioides*, *F. poae*

In addition to trichothecenes, *F. culmorum*, *F. crookwellense* and *F. graminearum* in particular (but also *F. equiseti*) can produce zearalenone, a mycotoxin with oestrogenic properties (Mirocha and Christensen, 1974). Of the trichothecene producers, *F. culmorum* is believed to be the main cause of cereal head blight in the North western part of Europe, whereas F. *graminearum* is prevalent in warmer regions (Parry *et al.*, 1995). Accordingly, the main producer of DON under Danish conditions is presumably *F. culmorum*.

Trichothecenes: The trichothecenes are a large group of compounds – more than 60 are known to day. The commonly encountered compounds in cereals can be devided into two groups: type A and type B, differing in the presence or absence of a ketone group (Ishii, 1983). DAS and T-2 toxin are type A trichothecenes while DON and NIV belong to the type B trichothecenes. The analytical procedure for detection of the two groups is different. By far, the most abundant trichothecene in agricultural commodities is DON (Eriksen and Alexander, 1998; Smith *et al.*, 1994). It is generally detected in more than 50% of the analysed samples. In a Danish survey DON was detected in 29 out of 55 wheat samples (average 20 µg/kg) and in 18 out of 41 rye samples (average 50 µg/kg) (Berg *et al.*, 1994). DAS and T-2 toxin were not detected. Swedish and Norwegian surveys revealed oats to be more contaminated than wheat, barley and rye. 76% of 1221 oat samples contained DON (Eriksen and Alexander, 1998). The average concentration in the contaminated samples was 513 µg/kg. There are large differences among the regulations that exist on *Fusarium* toxins in food and so far only a few countries have official regulations. In Scandinavia a level of 0-1 µg DON/kg bodyweight has been proposed as the level of tolerable daily intake. This is to be held against a calculated mean daily intake of 0.3 µg DON/kg bodyweight for a 60 kg person. The toxic effect of trichothecenes relates to inhibition of protein synthesis and lipid peroxidation (Bamburg, 1976; Rizzo *et al.*, 1994). The toxicity, however, differs between the compounds. Measured as LD_{50} values in rodents, T-2-toxin is 5-15 times more toxic and NIV is

2-8 times more toxic than DON (Eriksen and Alexander, 1998). The symptoms of acute intoxication include feed refusal, vomiting, diarrhoea, and haemorrhages whereas immunotoxicity and possibly carcinogenicity are listed as chronic or subchronic effects (Rotter *et al.*, 1996; Eriksen and Alexander, 1998).

Ecology of *Fusarium*:
Fusarium spp. are abundant soil fungi. The distribution of species varies very much from field to field (Elmholt 1996; Hestbjerg, 1999). Most trichothecene producing *Fusarium* species are facultative plant pathogens, i.e. they are capable of living on dead organic material in the soil but can switch to a pathogenic mode of existence when suitable host plants appear (Parry *et al.*, 1995). Several species, e.g. *F. culmorum*, can cause seedling blight and – later in the season – foot rot and head blight. Infection may be due to inoculum present in the soil or seed borne inoculum – the relative importance of these is not elucidated. The production of trichothecenes has been shown to play a role in the pathogenicity of several *Fusarium* species (Desjardins and Hohn, 1997; Proctor *et al.*, 1995). At harvest the grain may therefore already contain considerable levels of these toxins. If the conditions are suitable for further fungal activity (adequate moisture, appropriate temperatures) during storage, additional trichothecene production may occur. The dominant *Fusarium* species on Danish malt barley were found to be *F. culmorum, F. equiseti, F. poae, F. avenaceum, and F. tricinctum* (Andersen *et al.*, 1996). Of these, the first three are known trichothecene producers.

Achievements by the research group: We have performed a range of investigations on the occurrence of *Fusarium* in agricultural soil (Elmholt and Kjoller, 1989; Elmholt *et al.*, 1993; Knudsen *et al.*, 1995; Elmholt, 1996; Hestbjerg, 1999; Hestbjerg *et al.* 1999). In a study of 10 European wheat field soils, the occurrence varied from practically missing to the most dominating part of the soil mycobiota (Hestbjerg, 1999). These differences could not be related to either climate or soil chemical or physical conditions and must be attributed to biological factors. Elmholt and Kjøller (1989) and Elmholt (1996) showed a high abundance of *Fusarium* in organically cultivated soils. *F. culmorum* is able to cause seedling blight as well as foot rot and head blight. Our studies of *Fusarium* in an organically and a conventionally cultivated soil revealed that *F. culmorum* was more abundant in the organically cultivated soil (Knudsen *et al.*, 1995). We have tested the variation in pathogenicity among 70 isolates of *F. culmorum* derived from organic soil particles (Hestbjerg, 1999). Every isolate was able to cause seedling blight of barley but with a great variation in aggressiveness. This variation was shown to correlate to the DON content of the barley seedlings. However, this correlation was blurred by isolates that were able to produce NIV. Within *F. culmorum* two chemotypes exist: one which produces DON and one which produces NIV and Fusarenon-X. The ecology of these two chemotypes seems to differ in that the trichothecenes influence the pathogenicity. From a toxicological point of view this is very relevant as NIV is far more toxic than DON.

Towards a HACCP for the production of cereals:
Because trichothecenes are largely produced in the field, elucidation of the field ecology of the important trichothecene producing *Fusarium* species is crucial to the development of HACCP in order to obviate or prevent high levels of trichothecenes in foods and feeds. One very important issue is the significance of the soil borne compared to the seed borne *Fusarium*. Another important issue is the competition between isolates of the two chemotypes under natural conditions.

4.3 Drying techniques for cereals

Cereal grains are both exceedingly durable and highly perishable. If harvested in good quality, dried and kept at low moisture content and low temperature, they may retain their processing quality and their germinability for years. But if not sufficiently dry or stored at to high temperatures they will be highly susceptible to damage from fungi, insects and mites (Christensen and Kaufmann, 1968; Beattie *et al.*, 1998; Landskontoret for Bygninger og Maskiner, 1995).

In Denmark grain is often harvested at too high moisture contents for safe storage and has to be dried prior to storage. In the Fifties on-farm drying of grain became common. At that time, mainly batch dryers were used.

The temperatures of the drying air for that type of grain dryer is 30 – 45°C. Due to a relatively low capacity and a high labour input to ensure an efficient operation of the dryers other types were introduced. In the Sixties many on-farm continuous dryers were established , and later especially platform dryers, became common. At forage drying stations industrial drum drying plants for grass and alfalfa were established. This type of plants has a very high water evaporation capacity, and at the end of the Seventies tests with grain drying were carried out at these plants. In 1988 the first commercially drum dryer, especially designed for grain drying, was established in Denmark.

Today, platform dryers are the most common used type of grain dryer on farms. The advantages of platform dryers are their great intake capacity at harvest, and that the plant may be used for both drying, storage and aeration and cooling throughout the storage period. The disadvantages are the risk of non-uniform drying in case of a badly operated drying process and a long drying time and thereby risk of deterioration due to growth of fungi and mycotoxin formation. Finally the plants need much space (Landskontoret for Bygninger og Maskiner 1995). Continuous dryers are also common at traditional farms. At large plant producing farms, estates and grain dealers continuous dryers are the most common type of dryers used. The advantages are there high capacity due to high air temperatures (60 – 120 °C), there uniform drying and high degree of automation of the drying process. The disadvantages are the need of continuous transport of grain into and from the dryer. Later, there may be a need for cooling of the grain. At the highest air temperature the grain quality, especially the germination ability, may be damaged (Oxley, 1948; Thoftdahl-Olesen, 1982).

Until now, drum dryers have only been used by grain dealers and at industrial plants and almost exclusively for drying of grain for feed. New techniques and operation systems have been introduced in order to optimise the process with respect to grain quality, degree of drying and energy consumption (Jacobsen, 1979). The advantages are high capacity due to very high air temperatures (up to 1000°C). Experimental results show that it is possible to reduce dormancy in barley and to reduce the content of fungi without deterioration of the grain (Kristensen, 1998). The disadvantages are the lack of technical knowledge and experience together with the need for the development of farm size dryers.

5. 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.

An important achievement of PREMYTOX is that most of the obtained results will also be of use in conventional farming, which is also subjected to mycotoxin problems.

PREMYTOX will contribute to the general knowledge on the ecology of important mycotoxin producing fungi. This is needed to develop a general HACCP procedure for cereal production. The Mycology Group at DTU will secure a close contact between PREMYTOX and a currently starting EU project on OA, in which they are participating. The EU project aims to implement a general HACCP for cereal production in the European Union. As the HACCP concept focuses on critical control points, our results on critical points which are of special importance to organic farmers in Denmark, can contribute to the value of the EU-project.

Finally, one of the most important achievements 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. This knowledge will be made available to both farmers and extension service in the form of popular papers and a video, showing the importance of the control points, which are demonstrated to be critical.

6. Description of work packages including methods

In the following, we shortly present the working hypotheses and methods, which we plan to use in PREMYTOX. These plans will of course be modified during the course of the project in accordance with obtained experience from the literature and from our own results.

6.1 Working hypotheses for experimental testing

Based on previous results and experience, the following working hypotheses are defined with the aim to test their value experimentally in the present project. Focus will be put on rye and on the elucidation and evaluation of the control points, which at the moment appear to be the most relevant for organic farmers.

Hypothesis 1 *Exclusion of seed-treatment fungicides in organic farming favours the dissemination and maintenance of* P. verrucosum *and* Fusarium *in the field environment*
This hypothesis is based on the fact that organic farmers often use their own grain for next year's crop and that the farming system does not allow the use of seed-treatment with fungicides. As outlined above, the OA-producing *P. verrucosum* as well as the trichothecene-producing species of *Fusarium* may survive storage and be present on the seed and they are known to survive well in the field environment. In combination, the exclusion of seed-treatment and the ecology of the mycotoxin producing fungi would seem to favour the dissemination and maintenance of *P. verrucosum* and *Fusarium* in organically cultivated soil.
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Hypothesis 2 *Harvest practice is an important control point in organic farming in the prevention of mycotoxin problems*
This hypothesis is based on the fact that many mycotoxin-producing fungi, are saprophytes or facultative pathogens, which will be favoured by damaged or broken kernels. As harvest time and harvest practice are known to affect the exposure of the grain to damage, these practices are very likely to constitute important control points in the prevention of mycotoxin problems during drying and storage.

Hypothesis 3 *Drum drying at high temperatures will reduce the number of surface dwelling fungal spores and prevent mycotoxin problems*
This hypothesis is based on results of a new technique of drum drying grain. The technique is not fully implemented for bread grain but preliminary results indicate that fungal colonisation of the grain surface is reduced very much. This may significantly reduce the risk that surface-dwelling fungal spores of *e.g. Penicillium* and *Fusarium* cause mycotoxin problems if the grain is also subjected to the proper environmental conditions during the proceeding storage.

Hypothesis 4 *The drying practice in commercial organic farming needs improvement to prevent mycotoxin problems*
This hypothesis is based on the well-known fact that drying of grain should be fast and well controlled and that the grain has to be sufficiently cooled afterwards. Nevertheless we know that many organic farmers (and many conventional farmers too) have drying facilities, which do not meet these demands. There is, however, a lack of documentation to show how the important mycotoxin producing fungi react to differences in the prevailing drying techniques.

6.2 Methods

Drying of cereals: During the grain drying tests, a final moisture content of the grain of about 14% is aimed at, because at this level grain will traditionally be considered sufficiently dry for storage. During the drying process the moisture content (ISO Standard 712, International Standard Org., 1980) of the grain before and after drying as well as the temperature and the humidity of the inlet air will be recorded. In all essentials, the comparative drying tests will be made in accordance with the Danish Standard for grain drying plants (Dansk Standardiseringsråd/R 6016). However, only one test per drying plant will be made, and not three repetitions of the test as prescribed in the standard. The methods described in "Grain dryer testing in theory and practice" by Nellest and Bruce (1982) will be used to form the basis for the drying technical result statement.
Baking quality: The baking laboratory at Drabæks Mill will determine the quality. Grain samples will be tested by amylograph test including maximum viscosity (maximum Brabender unit measured at the amylograph, BE) and temperature corresponding to maximum viscosity T_{max} and Falling Number (ICC Standard 107, Int. Ass. for Cereal Sci. & Tech.,.1998).
Screenings for *P. verrucoum* and *Fusarium* spp. and determination of their potential for mycotoxin formation: Contamination of soil and kernels by *P. verrucosum* will be assessed by plating grain on DYSG agar (Frisvad *et al.*, 1992; Elmholt *et al.*, 1999). Infection of kernels can be assessed following surface desinfection. Contamination of kernels with *Fusarium* will be assessed by plating on SNA (Nirenberg, 1981), using the method of Hestbjerg *et al.* (1999) or surface desinfected kernels on CZID (Abildgren *et al.*, 1987). For verification of species identity and assessment of potential mycotoxin production, selected strains will be chemically characterised. Pure CYA and YES cultures of *P. verrucosum* and PSA and YES cultures of *Fusarium* spp. will be analysed qualitatively by HPLC-DAD using agar-plug extraction (Smedsgaard, 1997).
Mycotoxin formation
The Central Laboratory at Research Centre Foulum will implement methods for the quantification of trichothecenes in cereals, applying GC/EC according to Møller and Gustavsson (1992). OA will be determined using HPLC with a fluorescence detector and immunoaffinity column clean-up (Scudamore and MacDonald, 1998), possibly supplemented with a semi-quantitative method (Ochra-Scan®)

Table 1: Work package list

Work-package No	Work package title	Responsible participant	Budget kkr	Start	End	Deliverable No.
1	Project co-ordination, synthesis and dissemination of existing knowledge and PREMYTOX results to farmers and extension service	SE	1128	2000	2004	WP1-D1-D8
2	General practice in organic farming regarding sowing, harvest, transportation, drying and storage of cereals	SE	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 OA- and trichothecene-producing species	EFK and SE	221	2000	2001	WP3-D1
4	Effect of drying practice on OA- and trichothecene-producing fungi	EFK and SE	964	2001	2003	WP4-D1-D2
5	Effect of seed quality, harvest practice and other critical control points on OA- and trichothecene-producing fungi	HEH	1508	2001	2004	WP4-D1

Table 2: Description of work packages

VP1: Project co-ordination, synthesis and dissemination of existing knowledge and REMYTOX results to farmers and extension service

Work package number:	1
Start date or starting event:	Begin of project
Responsible person:	SE
Contributing persons:	Inputs from research group and subcontractors
Person-months:	19,5

Objectives of WP1

- to co-ordinate work within PREMYTOX
- to disseminate information to farmers and extension service on the importance of mycotoxin producing fungi and to provide farmers with practical means to reduce the risk of grain contamination with mycotoxin producing fungi

Description of work

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

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

Deliverables
WP1-D1-D4: 1st to 4th annual report 2000-2003
WP1-D5: Final report 2005
WP1-D6: DARCOF report, which summarises the so-far obtained results on the ecology of the OA-producing *P. verrucosum*
WP1-D7: Video on the prevention of mycotoxin problems
WP1-D8: Popular paper summarising the contents of the video

Milestones
WP1-M1-M5: Annual project meeting 2000-2003
WP1-M6: Mid-term conclusions on critical control points and their implementation into WP4 and WP5
WP1-M7: Collection of material for use in the video presentation on how to prevent mycotoxin problems

VP2: General practice in organic farming regarding sowing, harvest, transportation, drying nd storage of cereals

Work package number:	2
Start date or starting event:	2000
Responsible person:	SE
Contributing persons:	The Danish Agricultural Advisory Centre (subcontractor)
Person-months:	-

Objectives of WP2

- to gain information on the general practice in organic farming regarding seed control, harvest, drying and storage of cereals

Description of work

Task WP2-1: The Danish Agricultural Advisory Centre (DAAC) will function as a subcontractor on this work package. In co-operation with the research group of PREMYTOX, a questionnaire will be made. It will address those farming practices, which are supposed at the moment to be critical control points in the dissemination and proliferation of mycotoxin producing *Penicillium* and *Fusarium* species in organic farming. Among these are sowing practice (use of home grown seed, storage of seed, seed treatment if any), harvest practice (time, combine harvesting technique), transportation of grain and the drying and storage practice. DAAC will distribute the questionnaires to representatives among the organic farmers' local advisors and be responsible for the collection of the questionnaires. As evident from the amount of money to be used for WP2, the report will give an exhaustive answer to the raised questions. It is rather meant to secure that the control points, which we'll agree to focus on in the experimental part of the project, are actually relevant to general practice in organic farming

Deliverables

WP2-D1: Report on the results drawn from the questionnaire

Milestones

WP2-M1: Questionnaire prepared and distributed to local advisors
WP2-M2: Questionnaires filled in and returned

VP3: Implementation of drum dryer facilities for bread grain and the effect of drum drying on he grain mycobiota with special regard to OA- and trichothecene-producing species

Work package number:	3
Start date or starting event:	2000
Responsible persons:	EFK (field experiment) and SE (mycological analyses)
Contributing persons:	UT and Bent Thomsen, Drabæks Mill (subcontractor)
Person-months:	5

Objectives of WP3

- to modify existing drum dryer pilot plant for bread grain
- to implement the drum dryer for rye
- to elucidate the effect of drum drying on the grain mycobiota with special regard to OA- and trichothecene-producing species of *Penicillium* and *Fusarium*

Description of work

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

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

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

Deliverables

WP3-D1: Report and/or popular paper

Milestones

WP3-M1: Technical modifications completed
WP3-M2: Analyses of baking quality completed
WP3-M3: Mycological analyses completed
WP3-M4: Drum drying procedure for use in 2002 in WP4 established

WP4: Effect of drying practice on OA- and trichothecene-producing fungi

Work package number:	4
Start date or starting event:	2002
Responsible persons:	EFK (field experiment) and SE (mycological analyses)
Contributing persons:	HEH, UT & B. Thomsen, Drabæks Mill (subcontractor)
Person-months:	23

Objectives of WP4

- to produce grain, which has been subjected to different drying practices
- to elucidate the effects of drying practice on the baking quality
- to elucidate the effect of drying practice on the grain mycobiota with special regard to OA- and trichothecene-producing species of *Penicillium* and *Fusarium*

Description of work

Task WP4-1: The objective is to study the effect of platform drying, continuous drying and drum drying on mycotoxin producing fungi. At the moment it seems possible to obtain rye from the Ecological Research Station, Rugballegaard. Following harvest, the grain will be processed in the different pilot plants: platform fryer, continuous dryer and drum dryer.

Task WP4-2: The extent of the mycological analyses to be performed in relation to WP4-1 will be determined at the annual project meeting in the spring of 2002. At the moment they are planned to consist of dilution platings to assess the number of viable propagules on the grain surface (RCF) or measurements of ergosterol content in the grain (RCF) to elucidate the level of fungal contamination. Screenings will be performed on DYSG for *P. verrucosum* (RCF) and on SNA/CZID for *Fusarium.* spp (RCF/DTU). The work on *Fusarium* will focus on trichothecene-producing species. Isolates of *F. culmorum* will be determined with regard to chemotype (DON or NIV producer). The grain will be screened for OA and DON.

Deliverables

WP4-D1: Popular paper on the results from WP4-1 and WP4-2 (*e.g.* Grøn Viden)
WP4:D2: Scientific paper on the effect of harvest time and drying practice on the grain mycobiota with special regard to OA- and trichothecene-producing species of *Penicillium* and *Fusarium*

Milestones

WP4-M1: Grain samples from different drying procedures distributed to RCF and DTU
WP4-M2: Mycological analyses completed
WP4-M3: Analyses for OA and DON completed

VP5: Effect of seed quality, harvest practice and other critical control points on OA- and richothecene-producing fungi

Work package number:	5
Start date or starting event:	2002
Responsible person:	HEH
Contributing persons:	SE and UT
Person-months:	21,5

Objectives of WP5

- to elucidate the effect of seed quality on the mycobiota of the grain
- to elucidate the effect of threshing damage etc. on 'hot spots' of *P. verrucosum* and *Fusarium* spp.

Description of work

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

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

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

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

Deliverables

WG5-D1: Scientific paper(s) on the effect of seed quality, harvest practice and 'hot-spot' formation on *P. verrucosum* and *Fusarium*

Milestones

WG5-M1: Obtaining naturally or artificially contaminated seed for use in 2002 and 2003
WG5-M2: Performance of field experiment 2002 and 2003
WG5-M3: Mycological analyses completed (2002 and 2003)

7. Implementation and time schedule

Table 3: List of deliverables

No.	Title	Date	Meeting	Nature
WP1-D1	1st annual report	2000	x	Re
WP1-D2	2nd annual report	2001	x	Re
WP1-D3	3rd annual report	2002	x	Re
WP1-D4	4th annual report	2003	x	Re
WP1-D5	Final report	2005	x	Re
WP1-D6	DARCOF report on so-far obtained results on the ecology of the OA-producing *P. verrucosum*	2001		Re
WP1-D7	Video on the prevention of mycotoxin problems	2004		O
WP1-D8	Popular paper summarising the contents of the video	2004		Pop
WP2-D1:	Report on the results drawn from the questionnaire	2001	X	Re
WP3-D1:	Report and/or popular paper	2002	-	Re/Pop
WP4-D1:	Popular paper on the results from WP4-1 and WP4-2	2003	-	Pop
WP4:D2:	Scientific paper on the effect of harvest time and drying practice on the grain mycobiota with special regard to OA- and trichothecene-producing species of *Penicillium* and *Fusarium*	2004	-	Pu
WG5-D1:	Scientific paper(s) on the effect of seed quality, harvest practice and 'hot-spot' formation on *P. verrucosum* and *Fusarium*	2004	-	Pu

Pu = International publication
Re = Report
Pop = Popular papers
O = Others

Table 4. Time table

TITLE		2000						2001						2002						2003						2004					
TASKS	Month *	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
	WP 1: : Project co-ordination, synthesis and dissemination of existing knowledge and project results to farmers and extension service																														
WP1-1	Co-ordination of PREMYTOX																														
WP1-2	Synthesis and dissemination of results																														
WP1-M1-5	Annual meetings			x					x						x						x						x				
WP1-M6	Mid-term conclusions on critical control points											x																			
WP1-M7	Material for video																										x				
	WP 2: General practice in organic farming regarding harvest, drying and storage of cereals																														
WP2-1	Questionnaire to organic farmers in Denmark																														
WP2-M1	Questionnaire prepared and distributed			x																											
WP2-M2	Questionnaire filled in and returned					x																									
	WP 3: Implementation of drum dryer facilities for bread grain and the effect of drum drying on the grain mycoflora																														
WP3-1	Technical modifications and implementation																														
WP3-2	Optimising drum drying, incl. effect of harvest time																														
WP3-3	Analyses of fungi and mycotoxins																														
WP3-M1	Technical modifications completed						x																								
WP3-M2	Field experiment performed										x																				
WP3-M3	Analyses of baking quality completed					x						x																			
WP3-M4	Mycological analyses completed						x						x																		
WP3-M5	Drum drying procedure established												x																		
	WP 4: Effect of drying practice on OA- and trichothecene-producing fungi																														
WP4-1	Different drying practices																														
WP4-2	Analyses of fungi and mycotoxins																														
WP4-M1	Differently dried grain samples distributed																	x													
WP4-M2	Mycological analyses completed																				x										
WP4-M3	Analyses for OA and trichothecenes completed																				x										
	WP 5: Effect of seed quality and harvest practice for OA and trichothecene producing fungi																														
WP5-1	Screening for naturally contaminated seed																														
WP5-2	Performance of field experiment																														
WP5-3	Analyses of fungi and mycotoxins																														
WP5-4	Study of 'hot-spot' contaminations																														
WP5-M1	Contaminated seed obtained										x						x														
WP5-M2	Field experiments performed																x						x								
WP5-M3	Mycological analyses completed																			x						x					

* Months are given as 1-6: 1=jan-feb, 2=mar-apr, 3=may-jun, 4=jul-aug, 5=sep-oct, 6=nov-dec

8. Collaborative partners

PREMYTOX will be in close contact with The Danish Agricultural Advisory Centre (DAAC) during the project in order to assure that the problems, which we focus on, are relevant to common practice in organic farming.

From 1998 to 2000 Susanne Elmholt collaborates with Drabæks Mill on the implementation of OA analysis at the mill and on a number of case studies at organic farms. Experiences from this project can be used in PREMYTOX and will form the basis for some of the experimental work. The co-operation with Drabæks Mill will continue in PREMYTOX, where Drabæks Mill will function as subcontractor on the analyses of baking quality.

We have agreed with The Danish Veterinary and Food Administration (Kevin Jørgensen) to initiate a co-operation with regard to analysis of some of our cereal samples for trichothecenes. At the moment they are implementing methods to analyse these compounds and they will receive some of the samples from WP4 and WP5 for analysis. This co-operation will enable an exchange of experience with regard to critical control points in Danish farming practice and with regard to methodology as we also intend to set up methods for mycotoxin analyses at Research Centre Foulum during the course of the project.

At the international level, the Mycology Group at DTU will secure a close contact to the currently starting EU project on OA, in which Jens Frisvad from DTU is participating. The overall aim of the EU-project is to implement a general HACCP for cereal production in the EU. A direct co-operation on some of our samples may be possible and advantageous to both projects.

9. Budget (kkr)

	2000	2001	2002	2003	2004	Total
Institution 1 (RCF)						
Salary (scientific)		282	419	418	460	1580
Salary (technical)		82	178	161	21	442
Operation		43	79	70	15	207
Overhead		81	135	130	99	446
Total		489	812	778	596	2675
Institution 2 (RCB)						
Salary (scientific)	94	116	120			330
Salary (technical)	44	70	60			174
Operation	30	26	20			76
Overhead	34	42	40			116
Total	201	255	241			697
Institution 3 (DTU)						
Salary (scientific)		39	60	41	21	161
Salary (technical)		47	48	25		120
Operation		10	15	10		35
Overhead		19	25	15	4	63
Total		115	148	92	26	380
Subcontractors	50				50	100
Total	251	858	1201	870	671	3851

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Appendix

Curriculum vitae: Susanne Elmholt (head of project)

Date of birth 28 06 54

Education

02/11 1984: M.Sc. biology, *University of Copenhagen*
17/01 1985: M.Sc. agronomy, *Royal Veterinary and Agricultural University, Copenhagen*
30/10 1991: Ph.D. (plant pathology), R*oyal Veterinary and Agricultural University, Copenhagen*

Affiliation

01/02 85 - 31/12 87: Scientist at Dept. of Pesticide Analysis and Ecotoxicology, Danish Institute of Plant and Soil Science
01/01 88 - 31/12 90: Ph.D. student at the Research Academy, University of Århus
14/03 89 - 17/06 89: Visiting scientist at Long Ashton Research Station (University of Bristol), UK
01/01 91 - 15/06 92: Scientist at Dept. of Pesticide Analysis and Ecotoxicology, Danish Institute of Plant and Soil Science
16/06 92 - : Senior scientist at Dept. of Crop Physiology and Soil Science, Danish Institute of Agricultural Sciences

Role in PREMYTOX

Susanne Elmholt's role in PREMYTOX is to co-ordinate and be in charge of the project. She will be responsible for the part of the project that deals with synthesis and dissemination of knowledge to the farmers and the extension service. In the experimental part of the project, she will be responsible for the results on *P. verrucosum*.

Qualifications, capacity and experience

Susanne Elmholt (SE) is a senior scientist in the research group on Microbial Ecology at the Dept. of Crop Physiology and Soil Science. The group comprises 5 senior scientists, 1 scientist and 3 technicians. The laboratory is well equipped for studies of structural and functional microbiology. SE has a master's degree in biology and agronomy, and a Ph.D. in plant pathology. Her research is related to fungi in arable soils. She has studied side-effects of fungicides under laboratory and field conditions, which was also the subject of her Ph.D. thesis. At present her main research areas are related to the ecology of mycotoxin producing fungi (*Penicillium*) and to the effects of different management systems on aspects of soil fertility. SE is a 'National delegate' in COST 835 on 'Agriculturally Important Toxigenic Fungi', Work Group 3 (Ecology and pathogenicity of toxigenic Fungi). SE is on the Editorial Board of Biological Agriculture and Horticulture. She is author or co-author on 17 scientific papers and 23 presentations at symposia/congresses.

Relevant publications

Elmholt, S. and H. Hestbjerg Field Ecology of the Ochratoxin A producing *Penicillium verrucosum* Dierckx. I: Survival and resource colonization in soil. *Mycopathologia. In press.*

Elmholt, S., R. Labouriau, H. Hestbjerg and J.M. Nielsen (1999) Detection and estimation of conidial abundance of *Penicillium verrucosum* in soil by dilution plating on a selective and diagnostic agar medium (DYSG). *Mycological Research*, **113,** 887-895.

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Elmholt, S. and A. Kjøller (1989) Comparison of the occurrence of the saprophytic soil fungi in two differently cultivated field soils. *Biological Agriculture and Horticulture,* **6**, 229-239.

Curriculum vitae: Helle Hestbjerg
Date of birth 07 08 64

Education

11/06 1992: M.Sc. biology, *University of Copenhagen*
09/04 1999: Ph.D. (mycology) *University of Copenhagen*

Affiliation

01/08 93 – 01/11 93: Scientist, Department of Soil Biology and Chemistry, Danish Institute of Plant and Soil Science
01/04 94 – 31/10 98: Ph.D. student at the Research Academy/Department of Crop Physiology and Soil Science, DIAS
01/02 96 – 15/10 96: Scientist, Department of Crop Physiology and Soil Science, DIAS
01/11 98 - Scientist, Department of Crop Physiology and Soil Science, DIAS

Role in PREMYTOX

The role of Helle Hestbjerg in PREMYTOX is to be responsible for the experiments on *Fusarium*. Further she will take part in the dissemination of knowledge to the farmers and the extension service.

Qualifications, capacity and experience

Helle Hestbjerg (HEH) is a scientist in the research group on Microbial Ecology at the Dept. of Crop Physiology and Soil Science (see the CV of Susanne Elmholt). HEH has a background in general mycology. Her Ph.D. is on the ecology of *Fusarium* with emphasis on the role of mycotoxins and other metabolites in the ecology of the fungi. At present she works with fungi having a potential for bioremediation (degradation of polycyclic aromatic hydrocarbons in polluted soil). She is author or co-author of 5 scientific papers and 4 presentations at symposia/congresses.

Relevant publications

Hestbjerg, H., S. Elmholt, U. Thrane and U.B. Jensen (1999). A resource-saving method for isolation of *Fusarium* and other fungi from individual soil particles. Mycological Research, **103**, 1545-1548.

Hestbjerg, H. (1999). Mycometabolites in the ecology of Fusarium – exemplified by characterisation of F. culmorum and F. equiseti. Ph.D. thesis. University of Copenhagen.

Elmholt, S. and H. Hestbjerg. Field Ecology of the Ochratoxin A producing Penicillium verrucosum Dierckx. I: Survival and resource colonization in soil. Mycopathologia. *In press.*

Elmholt, S., R. Labouriau, H. Hestbjerg and J.M. Nielsen (1999). Detection and estimation of conidial abundance of *Penicillium verrucosum* in soil by dilution plating on a selective and diagnostic agar medium (DYSG). Mycological Research, **103,** 887-895.

Curriculum vitae: Erik Fløjgaard Kristensen
Date of birth 24 05 55

Education

24/06 1980: B.Sc. Engineering, Agro-technical line. *The Engineering College of Horsens*

Affiliation

01/07 80 - 01/10 94:	Scientist at Dept. of Farmstead Engineering, National Institute of Agricultural Engineering, Horsens
01/10 94 - 01/04 97:	Scientist at Dept. of Agricultural Engineering and Production Systems, Danish Institute of Animal Sciences
01/04 97 - :	Scientist at Dept. of Agricultural Engineering, Danish Institute of Agricultural Sciences

Role in PREMYTOX

Erik Fløjgaard Kristensen's role in PREMYTOX is to be in charge of the technical investigations and tests concerning grain drying. He will be responsible for the development and optimisation of the drum dryer for drying and heat treatment of grain for bread. He will also be responsible for the growing and harvesting of organically grown grain for the drying investigations.

Qualifications, capacity and experience

Erik Fløjgaard Kristensen is a scientist in the Mechanical Engineering Group at the Department of Agricultural Engineering. The primary goal of the group is to carry out research into the application and development of sustainable machine systems. Special allowance is made for quality, working conditions and profitability as well as for environmentally friendly production methods with special reference to preserving the fertility of the soil, minimising the input of pesticides and energy, and reducing nutrient losses. Research is carried out into the optimisation of machinery systems and the development of machines for different operations. Erik Fløjgaard Kristensen's primary research areas are grain drying techniques and techniques for harvesting and application of energy crops and non-food crops. His work concerning drying techniques includes research into and development of existing and new techniques for grain and seed crop drying as well as testing of drying plants. Results on drum drying of malting barley and bread grain were presented at the last AgEng Conference.

Relevant publications

Kristensen, E.F., Kofman, P.D., Pressure resistance to air flow during ventilation of different types of wood fuel chip. *Biomass and Bioenergy. In press.*

Guul-Simonsen, F., Kristensen, E.F. (1999), Blower for grain drying (In Danish). *Grøn Viden - Markbrug, 203*

Kristensen, E.F. (1998), New Drying Method for Improvement of the Quality of Malting Barley and Bread Grain. Proceedings of EurAgEng.

Kristensen, E.F., Nielsen, V., (1996) Harvesting and Handling of Energy Grain. Proceedings of 9th European Bioenergy Conference , 845-850.

Kristensen, E.F., Søgaard, H.T., (1995). Production of Quality Flour made from Danish Grain (In Danish),. SH, Internal Report No. 52

Curriculum vitae: Ulf Thrane
Date of Birth: 25 08 57

Education

1984: M.Sc. Food mycology and chemistry, Technical University of Denmark
1987: Ph.D. Mycology, Technical University of Denmark

Affiliation

1984-1987: Research Scientist & Ph.D. student, Dept of Biotechnology, Technical University of Denmark
1987-1988: Assistant Professor, Dept. of Biotechnology & Biotechnical Section, Technical University of Denmark
1988-1991: Post.Doc, Dept of Biotechnology, Technical University of Denmark
1991-1997: Associate Research Professor, Dept of Biotechnology, Technical University of Denmark
1998- : Associate Professor, Dept of Biotechnology, Technical University of Denmark

July-Dec 1999: Visiting Scientist, Eastern Cereal and Oilseed Research Center, Agiculture Canada, Ottawa,

Role in PREMYTOX

The role of Ulf Thrane in PREMYTOX is to be responsible for the fungal screenings to be performed at DTU and the verification of fungal identity by chemical profiling.

Qualifications, capacity and experience

Ulf Thrane (UT) is an Associate Professor in the Mycology Group at Dept. Biotechnology, DTU. The Mycology Group comprises 10 scientists, 10 technicians and 6 PhD students. The Department is well equipped for mycological studies and chemical analyses (HPLC-DAD/-MS; GCQ-MS/MS, TLC-video scanning, API-MS). UT is in charge of the IBT culture collection (22.000 strains of mainly *Penicillium, Aspergillus, Fusarium*). UT's primary research area is classification and taxonomy of *Fusarium* by multidisciplinary techniques. He is a member of ISPP Committee on *Fusarium* Systematics and is National Delegate of Management Committee of COST835 (Agriculturally important mycotoxin producing fungi). He has a wide international network to *Fusarium* and *Trichoderma* researchers. At present UT is on his second term on the Editorial Board of Applied and Environmental Microbiology. UT has authored or co-authored more than 50 scientific papers and numerous presentations at symposia/congresses.

Relevant publications

Thrane, U. Development in the taxonomy of Fusarium species based on secondary metabolites. In: *Fusarium*, edited by B. A. Summerell, L. W. Burgess, W. F. O. Marasas, and J. F. Leslie, St.Louis:APS, p. 30-50 *in press*.

Frisvad, J.C., U. Thrane, and O. Filtenborg. Role and use of secondary metabolites in fungal taxonomy. In: *Chemical fungal taxonomy*, edited by J. C. Frisvad, P. D. Bridge, and D. K. Arora, New York:Marcel Dekker, 1998, p. 289-319.

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Thrane, U. Comparison of three selective media for detecting Fusarium species in foods: a collaborative study. *Int.J.Food Microbiol.* 29:149-156, 1996.

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