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Low temperature handling will delay but not hinder ochratoxin A formation in wet grain

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Insufficient drying of grain increases the risk of fungal growth and formation of mycotoxins. This applies especially to natural air-drying where the drying process may last for several weeks. Difficult harvest conditions increase the demand for grain drying, especially if the grain contains many impurities.

The consequences of difficult harvest conditions are known from the screenings of swine kidneys at Danish slaughterhouses. Specific kidney lesions and colour changes are signs of 'Mycotoxic Porcine Nephropathy', a syndrome induced by the mycotoxin ochratoxin A (OTA). These kidney damages are most prevalent in years and geographical areas with wet harvest conditions. If the kidney contains more than 25 µg OTA per kg the swine is condemned.

The Danish Veterinary and Food Administration perform OTA surveys of cereal commodities for human consumption. Their results show a positive correlation between OTA problems and difficult weather conditions around harvest. So did our study of grain samples from 2001 **(see DARCOFenews June 2004)**, which also pointed to the most severe OTA problems in late harvested crops with high moisture contents such as oats (Figure 1), spring wheat and spring spelt.

Figures 1-3 (left to right).
Left: *P. verrucosum* on oats. This kernel originates from an in-bin silo, in which the grain had been dried and stored for about two months at a moisture content of 18-19%.
Middle: Microphotograph of *Penicillium verrucosum* (ca. 1000x).
Right: Growth of *P. verrucosum* in rye after 100 days at 2°C and 22% moisture content. The rye had been mechanically damaged.

OTA is formed by the fungus *Penicillium verrucosum*

It is the general assumption that OTA in Danish grain is produced by only one species, i.e. *Penicillium verrucosum* (Figure 2). But the fungus may well be present without forming OTA. Why is that so and what will trigger its OTA production? These are questions addressed in the DARCOF II-project **PREMYTOX**.

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P. verrucosum found on much recently harvested grain

We know from earlier studies that *P. verrucosum* can be **found in soil** though not abundantly compared with many other species. We have also found **few heads in the field** with *P. verrucosum*, but this seems rather exceptional. Still we find many recently harvested samples to be contaminated by *P. verrucosum*. The most likely sources of infestation are soil raised by the combine harvester and grain remnants from the combine itself. Grain remnants in **poorly cleaned drying facilities** most likely contribute to early contamination.

Low temperature will delay but not hinder OTA formation

Early contamination increases the need of handling the grain in a way that makes the fungus unable to grow and produce OTA during drying and storage. Laboratory trials have shown *P. verrucosum* to depend on both temperature and moisture.

In PREMYTOX, we have studied its growth and OTA-production at different temperatures and grain moisture contents. The experiment was conducted with rye, partly because rye is important in the diet of most Danes and partly because **rye is more sensitive to OTA contamination than wheat**. The rye was inoculated with conidia of *P. verrucosum*, and we studied fungal growth over time at four temperatures (2, 10, 15 and 20°C) and three moisture contents (14, 18 and 22%). The time span between each sampling depended on storage temperature – the lower the temperature the longer the time of incubation – to ensure that the fungus had ample opportunity to develop at low temperatures too.

The experiment is not fully finished. It is, however, certain that *P. verrucosum* grows well and forms OTA at all investigated temperatures including 2°C (Figure 3)! It takes more time but it will happen eventually – if just grain moisture is sufficiently high.

Low moisture content can hinder formation of OTA

The results show that grain moisture is more important for OTA formation than temperature. *P. verrucosum* grew much better at 22% than 18% moisture (Figure 4), and at 14% there was no fungal growth – even though the fungus was abundantly present. At moisture contents of 22%, OTA was formed in amounts far exceeding the legislative limits for grain at all four temperatures (limits listed below). OTA also exceeded this limit at 18% moisture (only tested at 15°C) though the contents were lower than at 22%. We found no OTA after incubation of inoculated samples at 14% moisture.

Figure 4. *P. verrucosum* on rye with (A) 18% moisture after six weeks and (B) 22% moisture after four weeks. The rye had not been mechanically damaged.

A recent Swedish study confirms that grain moisture is more important than temperature **(Lindblad et al., 2004)**. The Swedish study points to 17-18% moisture as a critical lower limit for OTA production. Further studies within the critical area of 16 to 19% are needed.

Impurities increase the risk of 'wet pockets' in the grain

Impurities in the grain often hold more water than the grain itself. This makes drying difficult and may give rise to fungal growth. Especially weeds (Figure 5) but also immature kernels may cause problems. If impurities are not removed by threshing, winnowing the grain is recommendable especially where drying is slow and the technical facilities conducive to the formation of wet pockets.

Figure 5.

Impurities in the form of soil, weeds and immature kernels might lead to insufficient and inhomogeneous drying and increased risk of wet pockets in the grain.

Inoculum density

In the present experiment, the added conidia (5000 per g) were not visible by the naked eye and *P. verrucosum* conidia may be present on the grain in considerable amounts without notice. The Swedish study mentioned above suggests a threshold of 1000 conidia of *P. verrucosum* per g grain to predict whether or not there is a substantial risk of exceeding the legislative limit for OTA.

Monitor moisture and temperature regularly

Our results stress the importance of fast drying of wet grain and illustrate that wet pockets constitute a high risk of OTA formation if the fungus is present. Cooling the grain is not sufficient to hinder OTA formation! Cooling of grain has many well-established advantages relating to the prevention of grain pests and grain respiration. It will also inhibit the growth of many fungi and slow down the growth rate of *P. verrucosum*. But it is not sufficient to prevent *P. verrucosum* from growing and forming OTA if the grain is wet and time sufficient! A proper monitoring of grain moisture and temperature during drying and storage will help the farmer and others engaged in post-harvest grain handling to recognise incipient problems.

Legislative limits for OTA in grain

OTA has a number of detrimental effects. It is nephrotoxic, and it is considered teratogenic and carcinogenic. In the EU, the limit for grain used for human consumption is 5 µg OTA/kg and for flour 3 µg OTA/kg **(see PDF_1)**. In 2004 a limit of 0.5 µg OTA/kg was introduced for cereal-based

baby and infant food **(see PDF_2)**. There are no limits for feed grain.

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