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POST-HARVEST CONTROL STRATEGIES: MINIMIZING MYCOTOXINS IN THE FOOD CHAIN

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Abstract

Contamination of cereal commodities by moulds and mycotoxins results in dry matter, quality, and nutritional losses and represents a significant hazard to the food chain. Most grain is harvested, dried and then stored on farm or in silos for medium/long term storage. Cereal quality is influenced by a range of interacting abiotic and biotic factors. In the so-called stored grain ecosystem, factors include grain and contaminant mould respiration, insect pests, rodents and the key environmental factors of temperature, water availability and intergranular gas composition, and preservatives which are added to conserve moist grain for animal feed. Thus knowledge of the key critical control points during harvesting, drying and storage stages in the cereal production chain are essential in developing effective prevention strategies post-harvest. Studies show that very small amounts of dry matter loss due to mould activity can be tolerated. With <0.5% dry matter loss visible moulding, mycotoxin contamination and downgrading of lots can occur. The key mycotoxigenic moulds in partially dried grain are *Penicillium verrucosum* (ochratoxin) in damp cool climates of Northern Europe, and *Aspergillus flavus* (aflatoxins), *A. ochraceus* (ochratoxin) and some *Fusarium* species (fumonisins, trichothecenes) on temperate and tropical cereals. Studies on the ecology of these species has resulted in modelling of germination growth and mycotoxin minima and prediction of fungal contamination levels which may lead to mycotoxin contamination above the tolerable legislative limits (e.g. for ochratoxin). The effect of modified atmospheres and fumigation with sulphur dioxide and ammonia have been attempted to try and control mould spoilage in storeage. Elevated CO_2 of >75% are required to ensure that growth of mycotoxigenic moulds does not occur in partially dried grain. Sometimes, preservatives based on aliphatic acids have been used to prevent spoilage and mycotoxin contamination of stored commodities, especially feed. These are predominantly fungistats and attempts have been made to use alternatives such as essential oils and anti-oxidants to prevent growth and mycotoxin accumulation in partially dried grain. Interactions

between spoilage and mycotoxigenic fungi and insect pests inevitably occurs in stored grain ecosystems and this can further influence contamination with mycotoxins. Effective post-harvest management of stored commodities requires clear monitoring criteria and effective implementation in relation to abiotic and biotic factors, hygiene and monitoring to ensure that mycotoxin contamination is minimised and that stored grain can proceed through the food chain for processing.

1. INTRODUCTION

Microorganisms are ubiquitous in terrestrial ecosystems from which they are disseminated to contaminate plant communities. The ripening seed is no exception and is contaminated by a wide range of bacteria, yeasts and filamentous fungi via the air, insects, rain splash, equipment and agronomic practices. Thus, depending on climatic conditions at harvest, grain carries a wide range of microbial contaminants. Post-harvest treatment of such grain and the prevailing environmental factors are key determinants of the impact fungi may have on the grain quality including germinability. It is important to remember that harvested grain and contaminating microorganisms are alive and respiring slowly under dry, safe storage conditions.

Poor post-harvest management can lead to rapid deterioration in nutritional quality of seeds. Microbial activity can cause undesirable effects in grains including discoloration, contribute to heating and losses in dry matter through the utilization of carbohydrates as energy sources, degrade lipids and proteins or alter their digestibility, produce volatile metabolites giving off-odours, cause loss of germination and baking and malting quality; affect use as animal feed or as seed. Filamentous fungal spoilage organisms may also produce mycotoxins that can be carcinogenic or cause feed refusal and emesis (Magan et al., 2004). The spores of some fungi cause respiratory disease hazards to exposed workers (Lacey and Crook, 1988).

Estimated losses of grain, especially staple food grains in store, from all causes varies widely. They may amount to 10% worldwide (Anon, 1979) but can reach 50% in tropical regions (Hall 1970). Vasan (1980) estimated losses of high moisture rice in southern India to be about 15-25% in only 9 days, while Rohani et al. (1985) found storage losses of paddy in West Malaysia of only 1%. Spoilage of stored grain by fungi is determined by a range of factors which can be classified into four main groups including (a) intrinsic nutritional factors, (b)

extrinsic factors (c) processing factors and (d) implicit microbial factors (Sinha, 1995). Figure 1 summarises the factors which affect fungal colonization of stored grain.

Wallace and Sinha (1981) in the 1970s were the first to consider stored grain as a man-made ecosystem which needed to be examined in a more holistic, ecological manner to enable a proper understanding of the processes occurring and to improve post-harvest management of stored food commodities of all types. This approach has enabled prevention strategies to be developed and implemented to avoid microbial and pest infestation from damaging stored grain-based commodities. Since most cereals are stored dry, bacteria seldom cause spoilage. At intermediate moisture content levels fungal spoilage and pests are of major concern. The development of prevention strategies today has been predominantly based on using the HACCP approach and to identify the critical control points in the pre- and post-harvest food chain. This has been examined for some food chains (e.g. *Fusarium* and trichothecenes) in temperate cereals (Aldred and Magan, 2004) and the HACCP approach to mycotoxin control has been reviewed by Aldred et al. (2004. This review will examine some important mycotoxins and the post-harvest control strategies which have been developed for effective management to minimize entry of mycotoxins into the food chain. In some cases, pre-harvest decisions can significantly impact the capability for subsequent post-harvest control.

2. RESPIRATION AND DRY MATTER LOSSES

Grain itself and the microbial contaminants respire slowly when stored dry. However, if the water availability is increased to 15-19% moisture content (=0.75-0.85 water activity (a_w), wheat) spoilage fungi, particularly *Eurotium* spp., *Aspergillus* and *Penicillium* species grow, resulting in a significant increase in respiratory activity. This can result in an increase in temperature and sometimes spontaneous heating from the colonisation by a succession of fungi

resulting in colonisation by thermophilic fungi and actinomycetes (Fleurat-Lessard, 2002; Magan et al., 2004). The chemical process involved in heat generation is predominantly aerobic oxidation of carbohydrates such as starch. The energy is released by the following equation:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 2835\ kJ$$

Heating occurs when this energy is released faster than it can escape from the cereal substrate. The requirement for oxygen increases with temperature, to a maximum of 40^{o}C, but does not decrease greatly until the temperature exceeds 65^{o}C. At this temperature, microbial growth is largely inhibited and heating results from exothermic chemical oxidation. Thus the respiratory quotient (RQ) may be 0.7 to 0.9 up to 65^{o}C but <0.5 at higher temperatures.

By utilizing the RQ, CO_2 production can be translated into dry matter loss. Typically, complete respiration of carbohydrates gives a RQ, i.e. ratio of O_2 consumed to CO_2 produced of about 1.0, and it has been calculated that 14.7 g $C0_2$ kg^{-1} grain will be released for every 1% loss of grain dry matter. During anaerobic fermentation, only about 0.493 g CO_2 is evolved from a kg of grain for every 1% dry matter loss. Alternatively, a RQ <1.0 may result from lipid or protein metabolism. For example, tripalmitim has a quotient of 0.7. The greater the CO_2 production, the shorter the safe storage period without dry matter loss. Studies by Jonsson et al. (2000) utilised respiratory rates over a wide range of a_w levels and temperatures to examine development of moulds such as *P.verrucosum* in stored grain and effects on germinability, fungal biomass and maximum safe storage times. They suggested that the maximum storage time without mould growth was probably halved if moisture content at harvest was increased by 1-3% (=0.05 a_w) or if storage temperature was increased by 5^{o}C in temperate cereals. Fleurat-Lessard (2002) in his comprehensive review suggested that for the modelling and prediction of global quality changes the rate of CO_2 production can be used as a "storability risk factor".

The ratio of contribution of spoilage moulds and grain to total respiration has been argued for many years. A range of studies have demonstrated that grain spoilage and by implication dry matter loss is predominantly determined by fungal activity. Wheat quality loss has been measured and models developed based on germination rates, visible mould growth or respiration of grain and microorganisms (Fleurat-Lessard 2002). Kreyger (1972) found that storage of barley at 24% water content (=0.94 a_w) at 16^oC for 10 weeks lost 2% dry matter with visible moulding. Studies with maize showed that fungal invasion and aflatoxin content could be unacceptable before the grain had lost 0.5% dry matter and mould became visible (Seitz et al., 1982). White et al. (1982) noted that 0.1% was unacceptable for first grade wheat and proposed an absolute level of 0.04%. However, when 55 days safe storage was predicted for grain stored at 18.4% mc (=0.86 a_w) visible moulding occurred after 23 days storage.

Studies have been carried out to compare the dry matter losses caused by individual mycotoxigenic fungi and the effect of a naturally contaminated mixed fungal community. Using gamma irradiated wheat grain (12kGy) which had no fungal contamination, but had retained germination capability, studies were carried out by inoculation with mycotoxigenic fungi such as *Penicillium verucosum* and *Aspergillus ochraceus*. Figure 2 shows that this is influenced by water availability. Quantification of dry matter losses based on respiration rates and gas chromatography to measure CO_2 production is a useful tool to measure this. Based on these experiments, a maximum dry matter loss by these individual species was in the range of 5-7% (Ibupoto and Magan, Cranfield University, unpublished data). However, studies with naturally contaminated cereal grain suggested that depending on aw and temperature, much lower dry matter losses can result in visible moulding. Table 1 shows data for wheat which was obtained by Hamer et al. (1991). It is important to note that visible mould was observed when only 0.2-0.4% dry matter loss had occurred. For maize, similar studies suggested that a loss of 0.5% dry

matter due to fungal spoilage would result in rejection of grain for human consumption and aflatoxin contamination.

There are problems with the use of visible moulding as a criterion of deterioration (Hamer et al., 1991; Lacey et al., 1997). A number of studies have found this to be a subjective index of the safe storability of grain (Hamer et al., 1991; Magan et al., 2004). Magan (1993) suggested that microscopic growth may be a more effective measurement of initial colonisation than visible moulding. Some attempts have also been made to relate dry matter losses to actual calorific losses due to the activity of mycotoxigenic moulds. These have been recently reviewed (Magan et al., 2004).

3. PREVENTION OF OCHRATOXIN A (OTA) CONTAMINATION

Recent surveys of cereals in Europe, especially of wheat and barley, have shown that, of OTA producing fungi, *P.verrucosum* is predominantly isolated with only the occasional presence of *A.ochraceus*, especially in the Mediterranean basin. Thus contamination with OTA has been identified as predominantly a post-harvest problem in Europe. Lund and Frisvad (2003) demonstrated that *P.verrucosum* grain contamination occurred during the harvesting process and, critically, during drying and storage. Thus, during damp harvest years in Northern Europe it is essential that effective drying regimes are employed post-harvest. Poor drying can result in *P.verrucosum* colonisation occurring, and potential for pockets of mycotoxin contaminated grain to be present in silos. Thus effective management of this phase is critical to try and prevent OTA contamination at this post-harvest stage in the food chain. Species such as *P.verrucosum* and *A.ochraceus* are very competitive and able to dominate under conducive environmental conditions in stored grain in temperate and tropical regions, respectively (Magan et al., 2003). Some recent work has suggested that the level of contamination by *P.verrucosum* may be a good

indicator of potential contamination with OTA (Lund and Frisvad, 2003; Lindblad et al., 2004). For example, Lund and Frisvad (2003) found that samples with >7% contamination of wheat grain with *P.verrucosum* indicated OTA contamination, although no linear correlation between the two factors was obtained.

The most important abiotic factors which influence growth and OTA production by such spoilage fungi include water availability, temperature, and when grain is moist, gas composition (Magan et al., 2004). The interaction between these variables primarily determine whether mould growth will occur and if so the relative development of the fungal community. An accurate determination of the marginal conditions for growth and OTA production by species such as *P.verrucosum* and *A.ochraceus* is important as it can be used to provide guidelines of the level of risk of contamination of the grain through the food chain. However, this requires detailed information on the ability of isolates of these species to colonise grain matrices over a range of interacting conditions.

Recent studies by Cairns-Fuller (2004) and Cairns-Fuller et al. (2005) have shown the general relationship between water availability (water activity, a_w), temperature, growth and OTA production for *P.verrucosum* and *A.ochraceus*. For example, rapid growth occurs at 0.98-0.99 a_w (= >27-30% m.c.) over the temperature range 10-25^{o}C and is almost completely inhibited at about 0.80-0.83 a_w (=17.5-18% m.c.). No OTA was produced at 0.80 a_w, although some was produced at 0.85 (=19%) at 15 and 20^{o}C. Optimum conditions were at 0.93-0.98 a_w (23.5-27.5%) at 10-25^{o}C on wheat grain incubated for up to 56 days. The temporal production of OTA by strains of *P.verrucosum* showed that on wheat grain between 7-14 days was required for significant OTA to be produced at levels above the EU tolerable limit (Cairns-Fuller et al., 2005). Contour maps of the optimum and marginal conditions of water and temperature for growth and OTA production have been constructed (Cairns-Fuller et al., 2005). This study

showed that approx. 17-18% moisture content (ca. 0.80-0.83 a_w) is the limit for any potential growth or OTA production in wheat grain. Thus, it is essential that grain is dried to lower moisture content as quickly as possible regardless of the drying system employed. To avoid initiation of moulding by xerophilic *Eurotium* species, drying to <14.5% m.c. (=0.70 a_w; Magan et al. 2004) is essential. This has to be maintained during storage and transport to effectively prevent contamination with OTA.

Recently, Lindblad et al. (2004) developed a logistical model to relate populations of *P.verrucosum* (CFUs) to probability of exceeding the European legislative limit of 5 µg kg^{-1} in cereal grain under different a_w x temperature storage regimes. They suggested a threshold of 1000 CFU of *P.verrucosum* g^{-1} grain as a threshold limit for the probability of risk from contamination with OTA at above the legislative limit. They found that *P.verrucosum* populations increased at 0.80 a_w, based on spore production (serial dilution counts) but without forming OTA. However, spore production may not always be an accurate measure of fungal growth, but gives an indication of sporulation capacity. Previously, Frisvad and Samson (1991) estimated that the threshold for growth and OTA production by *P.verrucosum* would be approx. at 0.81-0.83 a_w and 0.83-0.90 a_w, respectively. The modelling of Lindblad et al. (2004) and those of Cairns-Fuller et al. (2005) suggest that growth can certainly occur under some conditions at 0.80 a_w, although OTA production may be limited to about 0.83 a_w. Thus the so-called "zone of uncertainty" which exists for OTA contamination is between 15-17.5% moisture content which is critical in determining whether a high risk from OTA can occur.

Key post-harvest critical control points which are important include:

1. Regular and accurate moisture measurement determinations

2. Efficient and prompt drying of wet cereal grain. This will be directly related to the buffer storage time and temperature prior to drying as well as the actual drying conditions (e.g. ambient, heated air drying) to target safe moisture contents. Wheat/Barley/Oats, 14-14.5%; Maize, 14%; Rice, 13-14%; Canola (rape seed) 7-8%.
3. Infrastructure for quick response, including provision for segregation and appropriate transportation conditions
4. Appropriate storage conditions at all stages in terms of moisture and temperature control, the general maintenance and effective hygiene of storage facilities for prevention of pests and water ingress
5. Ability to efficiently identify and reject material below specified standards in terms of both fungal contamination and, at some stages, mycotoxin levels (e.g. when passing to a third party)
6. Operation of approved supplier systems. This requires the setting of specifications for acceptance/rejection

4. PREVENTION OF MYCOTOXINS IN MAIZE

Harvesting of maize is often carried out at moisture contents which are >14-15% which requires drying to reduce the available water to <0.70 a_w (=14%) which is safe for storage. Often harvested maize is left at drying facilities during this critical part of the chain if drying facilities are working at full capacity. This can create problems with an opportunity for growth and mycotoxin contamination of maize, especially by *Fusarium* section Liseola (fumonisins by *F.verticilioides*, *F.proliferatum*), *F.graminearum* (trichothecenes; zearalenone), and *Aspergillus flavus* (aflatoxins). The ecology and physiology of germination, growth and fumonisin production on maize has been described in detail recently (Marin et al., 2004; Desjardins, 2006).

The role of mycotoxin production in competitiveness of these moulds has been considered by Magan and Aldred (2007).

For maize, the pre-harvest selection of hybrids, time of planting, plant density and insect control have all be found to have an impact on contamination of maize with these mycotoxins pre-harvest and during drying and storage. For example, late maturing hybrids (600-700 FAO classes) had ZEA and DON levels 3-4 times higher than early maturing hybrids (400-500 FAO classes). Interestingly, fumonisins were significantly correlated with other genetic traits such as kernel specific weight or starch composition. Pre-harvest sowing time also has an impact on later contamination with fumonisins. For example, late sowing times in Europe (e.g. May), were found to have 4 x higher fumonisins than earlier sowing times. A key critical control point appears to be the harvesting time. In late maturing hybrids there was an increase in fumonisins and zearalenone produced by different *Fusarium* species (*Fusarium* section Liseola; *F.graminearum*). This was found to be less significant in medium-early hybrids (Blandino et al., 2004; Reyneri, 2006).

Studies of moist maize of different moisture contents have also pointed to the importance of moisture content and efficiency of drying regimes required to control fumonisins and zearalenone contamination. For example, moist maize (25% m.c.) kept for 7 days after harvesting, and prior to drying, resulted in a siginificant increase in fumonisins (77%) and an even greater accumulation of zearalenone (Blandino et al., 2004). Overall, pre-harvest factors are critical for effective post-harvest prevention of fumonisins from contaminated maize entering the post-harvest phase of the food chain. The key factors are:

Pre-harvest

1. Proper selection of maize hybrids; prevent use of soft kernel hybrids
2. No late sowing dates (in Europe, in May) and avoid high cropping density

3. Good balanced fertilization
4. Avoid late harvesting
5. Effective control of pests such as European corn borer

Post-harvest

1. Minimize times between harvesting and drying
2. Effective cleaning of maize prior to storage
3. Efficient drying to <14% m.c.
4. Effective hygiene and management of silos
5. Absence of pests in store which can provide metabolic water and initiate heating
6. Clear specifications and traceability from field to store

5. MODIFIED ATMOSPHERES FOR MYCOTOXIN PREVENTION IN STORED GRAIN

For many years modified atmospheres or alternative gases have been examined for the medium and long term storage of cereal grain destined for food/feed. While fungi involved in biodeterioration of grain are considered to be obligate aerobes, many are actually micro-aerophilic, being able to survive and grow in niches where other species cannot grow and thus dominate specialised grain ecosystems. In many cases decreasing O_2 to < O.14% is required before growth can be substantially reduced. Increasing CO_2 to > 50% is required for inhibition of mycelial growth (Magan and Lacey 1984). Some species, e.g. *P.roqueforti,* are able to grow and infect grain at > 80% $C0_2$ provided at least 4% 0_2 is present. The use of integrated post-harvest systems for prevention of deterioration entails modifying O_2 and CO_2 simultaneously and the use of (O_2 free) N_2. The tolerance to low O_2 and high CO_2 is also influenced by interactions with grain type and water availability. The drier the grain, the more effective the treatment. Modified

atmosphere storage is used for control of both moulds and insects in moist stored grain. Regimes sufficient for moulds may not however be effective against some storage insects, which can survive and grow over a wider equilibrium relative humidity range.

Modified atmosphere storage has been examined for the storage of moist grain, especially for animal feed. Studies with *P.verrucosum* and *A.ochraceus* with up to 50% CO_2 suggest that spore germination is not markedly affected, although germ tube extension and hence colonisation is significantly inhibited by 50-75% CO_2, especially at 0.90-0.995 a_w for both *P.verrucosum* and *A.ochraceus* (Cairns-Fuller, 2004; Cairns-Fuller et al., 2005). Growth and OTA production were highest in air, followed by 25 and 50% CO_2 regardless of the a_w level tested on wheat grain (Figure 3). Generally, CO_2 and a_w together cause an enhanced inhibitory effect, although this was not synergistic.

More comprehensive studies have been carried out on mycotoxigenic *Aspergillus* and *Fusarium* species. For example, Paster et al. (1983) reported that OTA production by *A.ochraceus* was completely inhibited by >30% CO_2 on agar-based media after 14 days suggesting that there are differences between mycotoxigenic species. This suggests that for efficient storage of moist cereals >50% CO_2 concentrations needs to be achieved rapidly to prevent OTA contamination in storage or during transport.

Work with *Fusarium sporotrichioides* showed that T-2 toxin production could be reduced by 80% with 50% CO_2/20% O_2, but growth was not affected by <60% CO_2 in vitro (Paster et al., 1986; Paster and Menasherov, 1988). Contaminated maize stored at 26^{o}C for 14 days at 22% moisture content in 60% CO_2/20% O_2 reduced T-2 production completely, with 40% CO_2/5% O_2 treatment resulting in only trace amounts. Production of zearalenone by *Fusarium equiseti* was almost completely inhibited by >20%CO_2 with either 20 or 5% O_2 in grain (Paster et al., 1991). Samapundo et al. (2007) found that fumonisin production by Fusarium section Liseola species on

maize was inhibited by 30% CO2 over a range of aw levels although sealed systems were used in which final CO2 concentrations were much greater.

Diener and Davis (1977) made a systematic study of CO_2 and O_2 and how these gases affected aflatoxin production in maize. When O_2 concentration was decreased from 21 to 15 % there was no effect on aflatoxin production and a marked inhibition occurred only when the O_2 concentration was decreased < 5%. Moreover, aflatoxin production was decreased by 25% when the CO_2 elevated to 20% although it had no visible effect on growth and sporulation. These results are different from those with *P.verrucosum* and OTA production where 25% CO_2 reduced growth but resulted in a limited reduction in OTA accumulation. All these studies suggest that mycotoxigenic fungi have different responses to exposure to CO_2 in relation to growth and toxin production and factors such as water activity, temperature, nutrients and times of exposure all need to be considered in establishing effective control regimes.

5.1 Sulphur dioxide gas for post-harvest control

Sulphur dioxide (SO_2) is one of the oldest food additives and it has a long history as a disinfectant by the burning of elemental sulphur and the use of the resultant fumes. After the development of inorganic chemistry SO_2 and its salts became commonly used as preservatives, particularly of food and beverages (Magan, 1993). It is commonly used as a fungal inhibitory treatment of grapes and sometimes raisins. For example, it is used for table grape storage in order to prevent growth of *Botrytis cinerea*. It is also used in the process of wine making and it is a necessity for the storage and preservation of white wines. During processing, golden raisins are often treated with $S0_2$ in order to prevent the enzymatic browning and additionally act as an antimicrobial to prevent growth of moulds, yeasts and bacteria. While SO_2 fumigation has been

examined for medium term storage of cereals, from an engineering point of view there are problems with corrosion especially of pipework used for delivery of the SO_2 to the grain stores.

SO_2 can be highly toxic to micro-organisms as it has mutagenic effects, and thus inactivates mRNA and reacts with disulphide linkages in proteins, enzyme cofactors, aldehyde and ketone structures of five and six carbon sugars; it deaminates cytosine derivatives to uracil compound, and it has deleterious effects on the membrane (Babich and Stotzky, 1980). In contrast, small quantities of SO_2 may stimulate growth as the sulphur is an essential element for the growth. Raghunathan et al. (1969) found that treatment of 13% moisture content sorghum with sodium metabisulphite (64mg L^{-1}, 48 hrs at 25^{o}C) inhibited 95% of internal grain fungi, but also significantly reduced germinability. Treatment of moist maize (24% m.c.) with 0.3% SO_2 resulted in a significant decrease in microbial colonisation and practically no deleterious effect on grain quality (Eckhoff et al., 1979). Further studies by Eckhoff et al. (1983) of moist maize (26.5%) with 0.066% SO_2 alone or combined with 0.018% NH_3 showed that after 60 mould-free days, *Penicillium* spp. were found to have colonised the to third of the bin. It has been suggested that for medium term storage of 5 months at least 4.4% SO_2 g kg^{-1} was required for effective preservation (Katangaza, 1990). However, some studies have suggested that much higher concentrations may be required because of adsorption and binding of the SO_2 to the wheat grain, reducing the antifungal activity. Both Maleque (1989) and Serre (1991) showed that depending on the wheat m.c. between 20-30% gaseous SO_2 became bound to the grain during treatment. Thus, intermediate moisture contents of grain (15-19%) may be more effectively treated than moist grain (>20%) as less of the treatment will become bound to the substrate.

There is very little detailed information on the actual tolerance and sensitivity of mycotoxigenic fungi to SO_2. Previously, Magan (1993) examined the effect of different concentration of SO_2 in relation to different temperatures (15, 25^{o}C) and a_w levels in vitro and in

situ on grain for control of *Penicillium* and *Aspergillus* spp. The growth of *Aspergillus* species (*Aspergillus flavus, Aspergillus ochraceus, Aspergillus terreus*) was inhibited by 50 mg L^{-1} dissolved SO_2 on a malt extract-based medium at 0.995 and 0.95 a_w. Some *Penicillium* species and *Aspergillus niger* were tolerant of up to 250 mg L^{-1}. In contrast, growth of *Penicillium* spp. was stimulated by 100 mg L^{-1}. Moreover, phyllosphere fungi such as *Cladosporium herbarum* and *Epicoccum nigrum* were tolerant of up to 200 mg L^{-1} while *Aureobasidium pullulans* and *Botrytis cinerea* were inhibited by this level. Overall, at 0.96 and 0.92 a_w up to 2000 mg L^{-1} was needed to obtain a 1-2 log decrease in populations of spoilage fungi on cereal grain. Furthermore, Majumber et al. (1973) reported the inhibition of both fungal growth and mycotoxin production. However, no studies have examined the effect of SO_2 alone or interactions with water activity on germination, germ tube extension and OTA production on cereals.

The difference in sensitivity between spoilage moulds could be due to the fact that several factors influence the efficacy of SO_2. The tolerance of *Penicillium* species to high concentrations of SO_2 has been suggested to be due to their ability to actively transport the SO_2 into the mycelia (Tweedie and Segal, 1970). Furthermore, King *et al*. (1981) showed that SO_2-binding substances enabled yeasts to be tolerant to higher concentration of SO_2.

Furthermore, the relationship between the concentration of available SO_2 and effects on fungi is influenced by formulation of the product and critically by pH. The solubility products of SO_2 in water vary with the different pH level (Babich and Stotzky, 1974). The significant point is that the toxicity of these products also differs, with greatest efficacy of the undissociated sulphurous acid (H_2SO_3) > bisulphite (HSO_3^-) > sulphite (SO_3^{-2}). The presence of SO_2 mainly in the bisulphite form, can have moderate toxicity of the solubility products. Recent studies with strains of *A. carbonarius* and OTA production suggested that the Effective Dose $(ED)_{90}$ values of

sodium metabisulphite (mg L^{-1}) used for generating the SO_2 for OTA control were about 650-700 at 0.985 a_w, 400-500 at 0.965 a_w and 400-450 at 0.93 a_w (Pateraki et al., 2005; Pateraki et al., 2006).

6. POST-HARVEST CONTROL USING PRESERVATIVES

Moist grain specifically destined for animal feed is often treated with aliphatic acid-based preservatives. There are a number of commercial products predominantly based on salts of propionic and sorbic acids. However, these are fungistats and thus the coverage of the grain must be efficient to prevent under-treated pockets. Poor coverage can lead to growth of spoilage fungi, especially mycotoxigenic moulds which can sometime metabolise these aliphatic acids. Studies by Marin et al. (1998, 2000) showed that growth of *Fusarium* section Liseola species and fumonisin production was relatively unaffected by different mixtures of proprionic and sorbic acids. There is thus interest in finding alternative compounds to either enhance or to replace such compounds. Research has been carried out on both essential oils and anti-oxidants (Cairns and Magan, 2003; Hope et al., 2003; Fanelli et al., 2003; Hope et al., 2005). These studies have suggested that only a few essential oils such as cinnamon and clove leaf oil have the capacity for control of mycotoxigenic *Fusarium* species, *P.verrucosum, A.ochraceus* and DON and OTA production depending on environmental conditions. However, there are many economic and technological hurdles associated with this type of approach. In tests on wheat grain, butylhydroxyanisole (BHA), propyl paraben (PP), cinnamon oil and resveratrol gave greater than 90% reduction in DON and NIV accumulation. Resveratrol has been demonstrated to have a particularly wide spectrum of mycotoxin control, although at present this is a relatively expensive product (Fanelli et al., 2003). Table 2 shows example of the ED_{50} for growth inhibition and OTA inhibition of *P.verrucosum* and *A.ochraceus* on wheat grain under different

environmental conditions. At present this type of product may be uneconomical. However, should costs decrease then it may become a viable alternative to existing preservation systems for animal feed grain.

7. Processing for minimising entry of mycotoxins into the food/feed chain

There have been studies to examine the fate of mycotoxins if they do enter the food chain. It is important to have useful information on the relative partitioning of different mycotoxins in fractions of cereals when they are milled and used for different purposes, including human and animal consumption. Detailed studies have been carried out on the milling of wheat grain for bread and for extrusion purposes using grain contaminated at 10 and 50 initial ppb of OTA. Guy et al. (2004) showed that brown bread after cleaning and scouring contained about 40-50% of the OTA regardless of starting contamination level. The rest was predominantly in the bran fractions. In white bread production OTA was mainly found in the white flour and bread (20-30%). Again, bran contained the largest fraction of the OTA, especially when the initial contamination level was 50 ppb. Overall, the bran and offal flours which are important by-products that enter the food chains contain the highest OTA fractions.

Less information is available on deoxynivalenol. It is stable in many processes and a high pH and temperature are required for breakdown in maize. However, up to 50% survives dough fermentation and some can enter the brewing process where maize is used.

With regard to zearalenone, 60% has been found to survive in bread and 50% in noodles. The decrease during extrusion of maize grits was related to temperature as was found with DON Dry milling of maize can reduce levels by 80-90% in flour and grits but gives higher levels in the bran and germ. Wet milling leads to concentration in the gluten fraction. It can be metabolised by yeasts to produce β-zearalenol in beer and occasionally zearalenols have been found in milk

For aflatoxins, by flotation it is possible to separate contaminated maize grain. However, use of maize flour results in 50% surviving in bread. There is some redistribution during milling, and some carry over can occur in beer. Recent studies have been carried out on dry milling fractions. Table 3 shows an example of such data in relation to fumonisins, zearalenone and DON in maize (Blandino et al., 2004). This clearly shows the significant amounts of these mycotoxins which can enter the feed chain when dry milling is the technique used. This could have significant implications for effective development of prevention strategies, especially in relation to CCPs in the feed chain. More focus is needed in this area of research.

8. CONCLUSIONS

Prevention strategies post-harvest can only be effective for mycotoxins that are formed during this component of the food chain. Pre-harvest natural contamination can only be minimized post-harvest by application of processing techniques which will minimize subsequent entry into the food and feed chain where possible. There are however key management tools and traceability procedures which should be used to facilitate stored commodities to be effectively conserved with minimum loss in quality. These include accurate and regular moisture measurements to ensure safe thresholds are not breached. Efficient and prompt drying of wet cereals for medium and long term storage in hygienic silos free of insect pests and mouldy material. Traceability during silo storage and transport for processing. It is essential that Good Agricultural Practice and operation approved supplier chains are in place. This also required effective diagnostic tools which can be used to monitor and quantify mycotoxins rapidly (Magan, 2006). Representative sampling remains a problem for stored commodities. While legislation exists on sampling procedures, these are not easy to achieve and the errors in actually taking samples may be significant compared to those for actually analysing for the mycotoxin contamination level. Early

indication of changes in stored commodities due to insect or mould activity may be possible by monitoring of intergranular gas composition and the use of volatile fingerprints. The development of models on mycotoxigenic mould activity and the conditions which will prevent mycotoxin production and which can give an indication of tolerances relevant to the legislative limits are important. Combined data will enable us to realize the goal of developing realistic and accurate decision support systems for effective conservation of grain post-harvest. In the coming years we will need to build on the existing sound foundations in developing strategies for the prevention of spoiled grain and mycotoxins entering the human and animal food chains.

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Table 1. Calculated dry matter losses (%) in naturally contaminated wheat over 160 hrs at different aw and temperature levels (Hamer et al., 1991). Bold figures indicate visibly mouldy grain.

	Temperature (°C)				
Aw	*15*	*20*	*25*	*30*	*35*
0.80	0.007	0.020	0.039	0.061	**0.133**
0.85	0.018	0.027	0.130	0.161	**0.372**
0.90	0.085	**0.226**	**0.436**	**0.347**	**0.774**
0.95	**0.517**	**0.762**	**1.210**	**1.187**	**1.239**

Table 2. The concentrations of some essential oils and the antioxidant resveratrol (ppm) needed for 50% inhibition of (a) growth and ochratoxin production by *Aspergillus ochraceus* at different environmental conditions (from Cairn-Fuller, 2004).

Temperature (^{o}C)		15			25	
Water activity	0.90	0.95	0.995	0.90	0.95	0.995
(a) for control of colonisation of grain						
Treatment						
Clove	210	310	280	365	260	160
Cinnamon	210	270	190	325	220	155
Thyme	190	210	190	260	215	140
Resveratrol	60	180	190	150	110	140
(b) for control of Ochratoxin production						
Clove	225	150	275	215	200	150
Cinnamon	105	200	185	200	180	160
Thyme	60	145	120	140	150	160
Resveratrol	10	100	110	30	130	130

Table 3. Dry milling of maize has been used to exam the distribution of *Fusarium* toxins in maize (Index value of unprocessed grain = 100). Adapted from Blandino et al., 2004.

	Mycotoxin		
Product	Fumonisin B1	Zearalenone	Deoxynivalenol
Unprocessed	100	100	100
Clean grain	80	57	115
Corn meal	13	12	27
Corn flour	39	30	46
Germ	117	125	192
Animal feed	260	357	243

Figure legends

Figure 1. The interaction between intrinsic and extrinsic factors in the food chain which influences mould spoilage and mycotoxin production in stored commodities (From Magan et al., 2004).

Figure 2. The relationship between water activity, temperature and relative dry matter loss caused by *Penicillium verrucosum* and *Aspergillus ochraceus* on inoculated wheat grain. Means of five replicates per treatment. Measurements of CO_2 head space analyses were made over a 10 day period using gas chromatography.

Figure 3. Comparison of the ochratoxin produced by *P.verrucosum* and *A.ochraceus* when inoculated on wheat grain and incubated for 14 days at different water activity levels in air, or 25 and 50% CO_2 balanced with N_2 at 25^{o}C. Bars indicate Least Significant Difference (P=0.05).

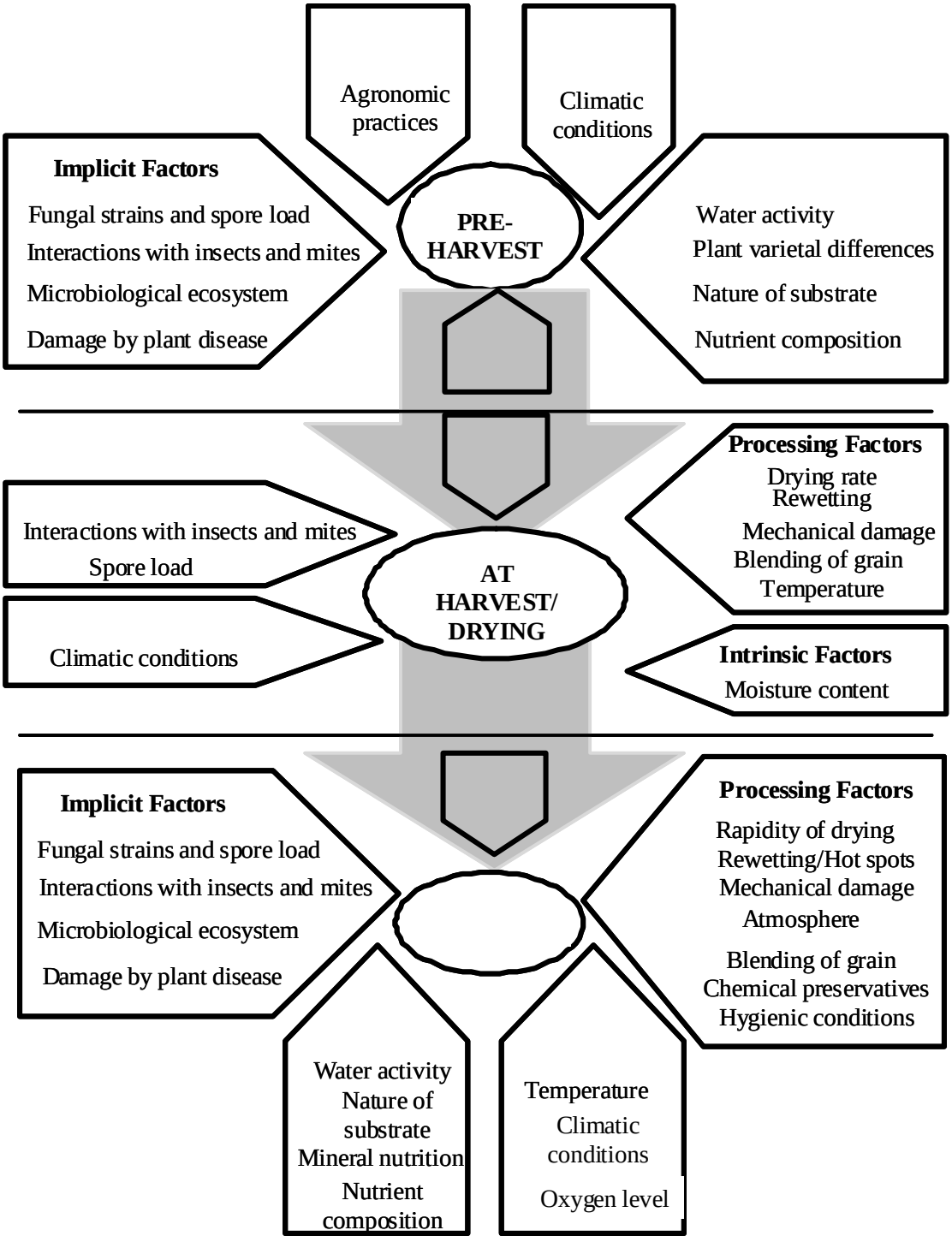

Figure 1. Magan and Aldred

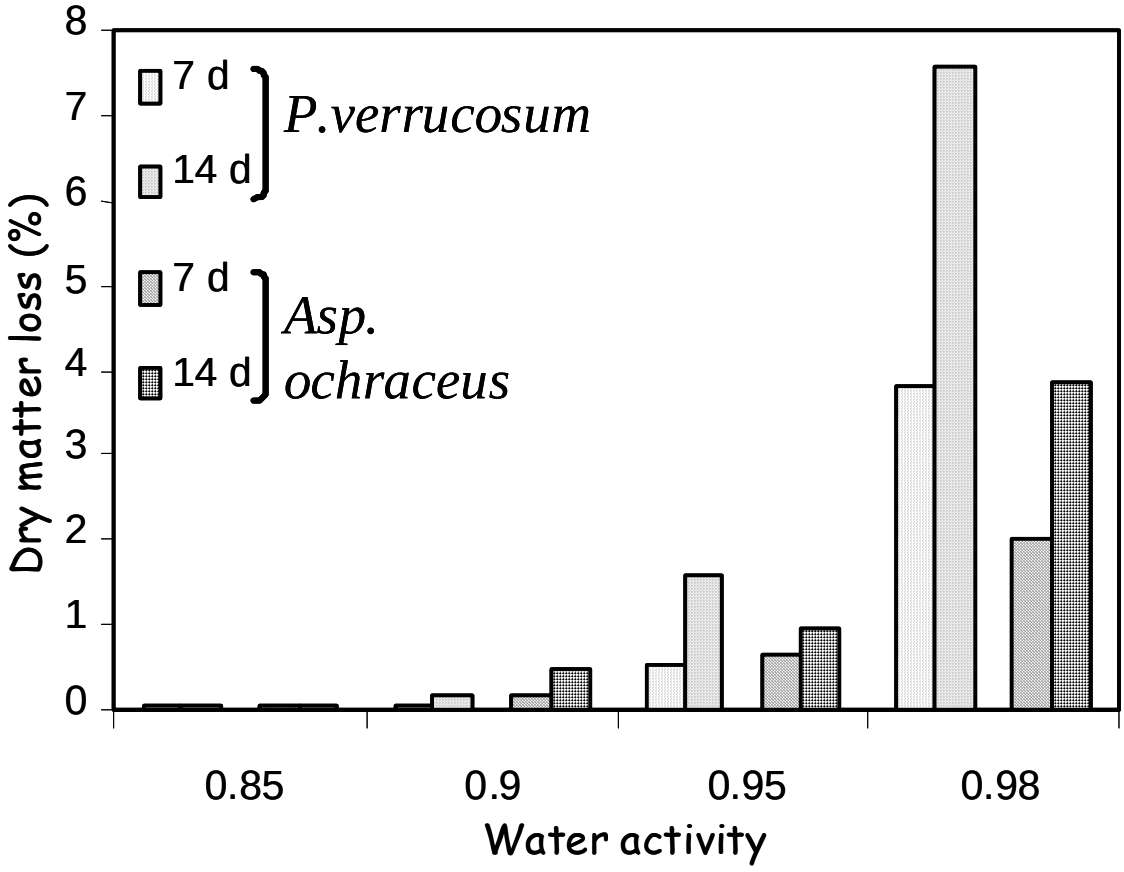

Figure 2. Magan and Aldred

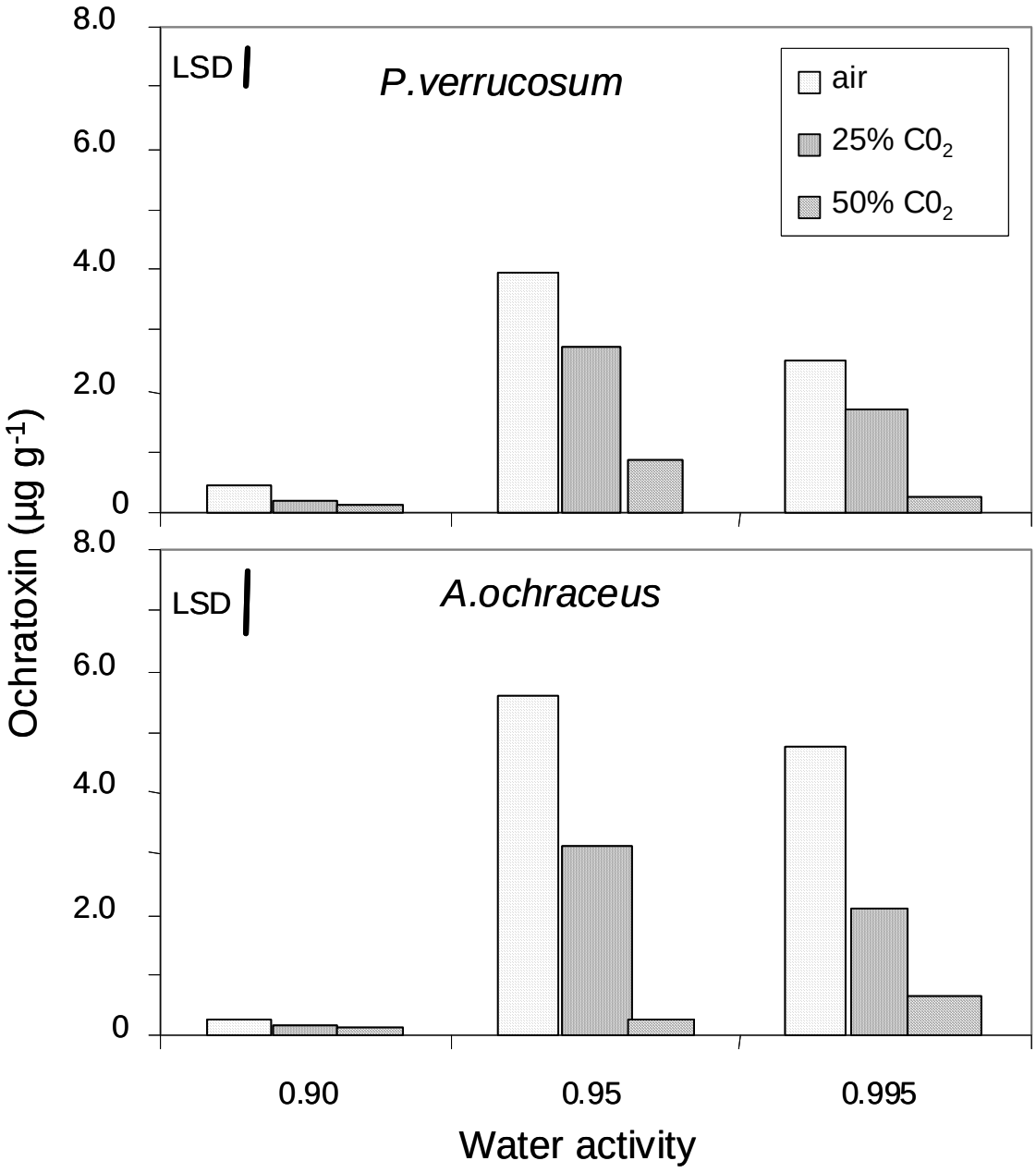

Figure 3. Magan and Aldred