Limiting mycotoxins in stored wheat

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Complete List of Authors:	Magan, Naresh; Cranfield University, Applied Mycology Aldred, David; Cranfield University, Applied Mycology Group Mylona, Kalliopi; Cranfield University, Applied Mycology Group Lambert, Ronald; Cranfield University, Applied Mycology Group
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Limiting mycotoxins in stored wheat – a review

Naresh Magan, David Aldred, Kalliopi Mylona and Ronald J.W. Lambert

Applied Mycology Group, Cranfield Health, Cranfield University, Bedford MK43 0AL, U.K.

Corresponding author: Prof. N. Magan, Applied Mycology Group, Cranfield Health, Cranfield University, Bedford MK43 0AL, U.K. Tel: +44 1234 758308; fax: +44 1234 758380; E.mail: n.magan@cranfield.ac.uk

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Abstract

The quality of harvested wheat grain can deteriorate markedly during the post-harvest management stages. The biotic factors such as grain type and ripeness, coupled with the prevailing abiotic factors such as water content and temperature, and also preservative concentration will influence the safe storage life and the level of contamination with mycotoxins such as deoxynivalenol (DON) produced pre-harvest and zearalenone (ZEA) produced post-harvest by *Fusarium graminearum and Fusarium poae* respectively, ochratoxin (OTA) produced by *Penicillium verrucosum* post-harvest in cool damp northern European climates, and perhaps T-2 and HT-2 toxins produced by *Fusarium langsethiae*. This review presents recent data on the relationship between dry matter losses caused by *F. graminearum* under different environmental regimes (water activities, temperatures) and the level of contamination with DON. This is important as poor post-harvest drying and storage management may exacerbate DON contamination already present pre-harvest. It is thus critical to relate the environmental factors in stored wheat grain during storage, especially of intergranular relative humidity (RH) and temperature, to safe storage periods without spoilage or risk from increased DON contamination. The growth/no growth and DON/no DON (*F. graminearum)* and OTA/no toxin production (*P. verrucosum*) have been used to build a model with a simple interface to link the temperature and RH values to the potential risk level which may allow growth or toxin production. This paper also considers the use of modified atmospheres, preservatives and biocontrol to minimise DON and OTA in moist wheat grain. These approaches together with clear monitoring criteria and hygiene could contribute to better post-harvest management of stored temperate cereals and ensure that mycotoxin contamination is minimised during this key phase in the food/feed chain.

Introduction

In Europe the key approach to minimising natural contaminants in the food chain has been by developing minimisation strategies within a HACCP framework. Thus, the cereal food chain for different end products has been studied in detail (Aldred and Magan, 2004; Aldred et al., 2004; Magan et al., 2008). This has included the monitoring of critical control points (CCPs) both pre- and post-harvest. With regard to mycotoxins as a hazard in temperate cereals (DON, OTA) the main drivers have included EU legislative limits, customer specifications and consumer perceptions. A prevention strategy must aim to minimise the occurrence of these mycotoxins below the legislative limits based on best practice, both pre- and post-harvest. The key CCPs post-harvest have been identified for the wheat food/feed chain. In temperate cereals the main mycotoxins of concern are ochratoxin OTA (*Penicillium verrucosum*), deoxynivalenol (DON; *Fusarium graminearum*, *Fusarium culmorum*), zearalenone (*F. graminearum, Fusarium poae*), and T-2 and HT-2 toxins (*Fusarium langsethiae*). There are legislative limits for the first three of these and for the latter two, legislation is imminent.

Cereal grain, whether effectively dried or slightly moist, is alive and respiring and thus has to be treated with care during harvesting, drying, storage and subsequent downstream processing along the food chain. The concept of considering stored grain as an ecosystem was originally developed by Wallace and Sinha (1971). This was an important milestone as they included all the key biotic and abiotic interactions which might occur during storage including grain respiration, insect pests, mycoflora, insect/fungal interactions, pest immigration and emigration, heterogeneity of moisture and moisture migration, temperature and O_2/CO_2, and for animal feed the presence of preservatives. This holistic approach was

instrumental in facilitating some of the advances made during the last decade (Magan & Aldred, 2007).

Respiration, dry matter losses and mycotoxin contamination

Harvested grain must be dried to <14.5% moisture content (m.c., wet weight basis) to ensure that no mould spoilage or any pre-harvest contamination with mycotoxins is exacerbated. Often, grain is harvested at 16-20% m.c. because of the better efficiency of modern combine harvesters. However, this does subsequently require heated or ambient drying to be used to conserve grain quality. Where drying has not been carried out effectively, the temperature will rise quickly and a succession of spoilage moulds may develop resulting in spontaneous heating with temperatures of 60-70^{o}C being reached, ending with thermophilic actinomycetes and complete loss of quality. This is accompanied by a significant increase in the chance for mycotoxin contamination.

Studies have been carried out to examine the relationship between grain respiration, mould activity and quality loss (dry matter) during these critical periods between harvesting, drying and storage. These have shown that in wheat and barley, dry matter losses of only 0.22 – 0.44% at 20-25^{o}C and 0.90 water activity (a_w = 19-21 % m.c.) results in visible moulding already being present (Lacey et al., 1999). Indeed, studies in the USA suggested that in other cereals such as maize, losses of 0.5% dry matter can lead to rejection of the grain for human consumption and that this was accompanied by a significant chance of aflatoxin contamination (Seitz et al., 1982). Fleurat-Lessard (2002) extensively reviewed data on general spoilage and dry matter losses and suggested that the rate of CO_2 production in cereals can be effectively used to model and predict global changes in cereal quality.

Recent studies at Cranfield University have tried to relate the activity of specific mycotoxigenic species under different environmental factors which might contribute to both dry matter losses and the chances of exceeding the EU legislative limits for DON. Thus experiments were carried out with stored winter wheat inoculated with spores of *F. graminearum* at 0.86, 0.93 and 0.95 a_w and different temperatures (15-30^oC) in chambers with the same equilibrium relative humidity atmosphere and measuring the respiration using GC analyses over periods of 4-5 days. The final *F. graminearum* populations and DON contamination after 10 days were also analysed. The effect of temperature x a_w on total calculated dry matter losses over periods of 4 days storage are shown in Figure 1. This shows the effect that these two parameters have on colonisation rates and quality loss. DON concentrations were found to be above the legislative limits (>1250 ppb) at 30^oC and 0.95 and 0.93 a_w in only 10 days, while at 15-20^oC and all a_w levels the concentrations were less than the legislative limits over these short periods. Similar data on dry matter losses caused by *P. verrucosum* over 7-14 days showed that dry matter losses were also between 1-1.5% at 0.95 a_w, and lower at 0.85-0.90 a_w and 25^oC (Magan and Aldred, 2007). This approach may be beneficial for combining dry matter losses in relation to m.c.s during drying and initial storage with the range of potential risk conditions for OTA and DON, relative to the EU legislative limits. Previous comprehensive respiration data sets in relation to a_w and temperature of stored wheat, have been used to develop the possible relationship between the variables. Thus, the results obtained as O_2 d^{-1} kg^{-1} dry grain (*R*) was divided by the incubation temperature (□) to give units of O_2 d^{-1} kg^{-1} dry matter $^oC^{-1}$. There was a linear relationship (r^2=0.9594) between *R*/□ and a_w (Figure 2, Lacey et al., 1997). This is based on O2 consumption, while our new data is based on CO2 production. Thus some conversion will be required to relate our new data to the previous data. However, it may be possible to relate our present data to the risk of exceeding the EU legislative limits for mycotoxigenic fungi. This

could then be a valuable practical aid if developed for different grains and nuts prone to contamination with different mycotoxins.

Boundary models of growth/no growth and mycotoxin/no mycotoxin and stored grain management

It is essential that grain is effectively dried immediately after harvest. Often heated air drying is used to achieve the target safe moisture content of about 14.5%. However, sometimes in the harvesting season grain is left for short periods of time before drying because of logistical issues. In some European countries ambient air drying is always used. Where heated drying is used the grain is less affected by the prevailing weather conditions, especially in damp autumn seasons in northern Europe. In the UK, where ambient drying is often used, this can result in slow drying with a moist front moving vertically up through the grain bed, especially in flat bed dryers/stores. This can result in grain at the top of the store being rewetted, while grain at the bottom can become over dried. This can lead to layers of mouldy grain with the potential for increased DON or OTA contamination.

Measurements can be made within stored grain silos to measure the changes in temperature and relative humidity in the inter-granular air spaces in the grain during drying and storage. This has been done at the top and in the middle of a flat bed store. Generally, any leaks or even small openings via damage to the structure can lead to an increase in temperature and intergranular m.c. This can often occur during the early periods after drying and storage phase (5-10 days) where both these parameters can increase, especially in the top layers which can increase the risk from spoilage and mycotoxin contamination (Magan et al., 2008).

We have previously developed boundary layer contour maps for growth and DON and OTA production by *F. culmorum* and *F. graminearum* and for *P. verrucosum* (Hope and Magan, 2003; Hope et al., 2004; Cairns et al., 2005). This data is useful as a guide to conditions where there may be a risk of growth and toxin production. A polynomial equation has been used to model these growth/no growth and toxin/no toxin boundaries. Figure 3 shows an example of the data for *F.graminearum* in relation to minimal growth rates (0.1 mm day^{-1}) and for DON production (0.01 ppm).

It is possible to link the temperature and RH levels monitored in the intergranular atmosphere to these boundary models. Thus, by inputting the data into the model one can check directly whether there is a risk of conditions conducive to growth or to DON production. The inset in Figure 3 shows the simple front end diagram which can be used for this purpose. This would enable dials related to temperature and RH to be moved to determine a low (green colour) or high (red colour) risk of growth or mycotoxin (DON, OTA) in store. The model could easily be modified to include the legislative limits for specific mycotoxins and applied to a range of mycotoxigenic fungi, and food commodities. We have developed the growth and toxin models for *A. flavus* (aflatoxin), *F. verticillioides* (fumonisins), *F. culmorum* (DON) and *P. verrucosum* (OTA) and for the new *A.ochraceus* grouping (Abdel-Hadi & Magan, 2009).

Other models have also been developed for *P.verrucosum* and OTA production by Lindblad et al. (2004). They developed very useful models which relate the potential for exceeding the EU legislative limit for OTA in cereals to the CFUs isolated from grain. They showed that $>10^3$ CFUS g^{-1} on a selective medium for *P. verrucosum* would represent a risk of OTA contamination >5 µg kg^{-1} stored grain which is the EU legislative limit. They also developed models of toxin free storage times under different temperature and moisture content

conditions. These are very useful practically for the cereal industry. Studies by Jonsson et al. (2000) suggest that the maximum storage time without any visible moulding was probably reduced by 50% if the mc at harvest was decreased by 1-3% (=0.05 a_w) or if the storage temperature was increased by 5°C, for example in a mild autumn year, in temperate cereals.

Modified atmosphere storage, chemical preservation systems and biocontrol

Moist grain, especially for animal feed use is sometimes stored in sealed silos to enable the natural respiration of the grain to increase the CO_2 concentration and inhibit spoilage moulds and the potential for mycotoxin formation post-harvest. However, if there are any leaks or the conditions remain micro-aerophilic then there is potential for both growth and mycotoxin formation. Studies suggest that increasing concentrations of CO_2 to 25 or 50% has little effect on growth or OTA production by *P. verrucosum* or indeed *Aspergillus ochraceus* (=*Aspergillus westerdijkiae*). Spore germination of these two fungi is unaffected, although germ tube extension and growth can be affected by up to 75% CO_2 (Cairns-Fuller, 2004; Cairns-Fuller et al., 2005). However, this does not mean that mycotoxin production is significantly affected. While inhibition of OTA did occur when a_w and CO_2 were increased, the effect was not synergistic and sometimes inhibition was not to a level below the legislative limits.

The effect of modified atmosphere on *F. culmorum* and other species has been examined and showed that they are micro-aerophilic and thus very tolerant of very low O_2 concentrations (Magan & Lacey, 1984). Paster (2000) has reviewed the effect of modified atmospheres on mycotoxigenic and other moulds and this has shown that there is very little information on mycotoxigenic Fusaria. Work has been carried out with *Fusarium sporotrichioides* and this demonstrated that T-2 toxin was reduced by 80% in the presence of 50% CO_2/20%O_2 but that

mycelial colonisation was not affected by <60% CO_2 (Paster et al., 1986; Paster and Menasherov, 1988).

Preservatives are often added to moist grain, especially for grain destined for animal feed. Commercial products are mainly based on mixtures of salts of aliphatic acids such as propionic, sorbic and benzoic acids. However, these are all fungistats and require effective contact with the product. Thus mixing is very important to ensure that inhibition of spoilage is maintained. Work has been carried out to try and identify alternative compounds which may be used to replace these aliphatic acid based products. Since *P. verrucosum* often causes problems post-harvest then the use of natural and novel preservatives may have an impact on OTA contamination. Aldred et al. (2008) screened a number of essential oils and antioxidants for control of both growth and OTA production by both *P. verrucosum* and *A. ochraceus*. They found that while essential oils such as clove, cinnamon and thyme were effective in vitro, in situ on grain they were relatively ineffective. This may perhaps be due to binding of the essential oil components to grain constituents. However, they did find that anti-oxidants, such as butylhydroxyanisole (BHA), propyl paraben, and especially resveratrol, were effective on naturally contaminated and grain artificially inoculated with *P. verrucosum*.

A range of essential oils (23) and 6 antioxidants were screened for control of growth and DON and NIV production by *F. culmorum,* DON production *by F. graminearum* and T-2 and HT-2 by *F. langsethiae* (Hope, 2004). Of these, three essential oils (bay, clove, cinnamon oil) and two antioxidants (propyl paraben and hydroxymethylanisole) were found to be effective in controlling growth of these species in the range 50-200 ppm at 15 and 25°C at 0.995 and 0.955 a_w in vitro on wheat-based media. More detailed studies on stored wheat grain at 15 and 25°C and essential oil/antioxidants treatments (100ppm; 500ppm) were carried out. This

showed that 500 ppm significantly reduced or inhibited growth at all the environmental combinations examined. However, at 100 ppm the essential oils/antioxidants stimulated growth under some experimental condition. Cinnamon and clove essential oils were the most effective inhibitors of growth regardless of temperature, a_w or species. The essential oils and antioxidants had a variable effect on inhibition of mycotoxin production. BHA and clove oil inhibited DON at both 100 and 500 ppm. However, propyl paraben and cinnamon oil enhanced NIV production at 100 ppm and intermediate a_w levels. Table 1 shows the efficacy of 500 ppm of these potential preservatives on control of DON and T-2 and HT-2 by *F. graminearum* and *F. langsethiae*, respectively, when inoculated onto wheat grain and stored for 28 days.

Studies have been carried out to use inoculants such as yeasts or lactic acid bacteria to control stored moist cereals destined for animal feed. The applied inoculants need to be able to become established and dominate the stored grain ecosystem to minimise the contamination with mycotoxins. A significant amount of work has been carried out to use xerotolerant yeasts such as *Pichia anomala* to control spoilage of feed grain, especially in airtight storage. It has been shown that this competitive yeast can prevent spoilage by micro-aerophilic fungi such as *Penicillium roqueforti* (Druverfors et al., 2002). Recently, different fluidised bed dried formulations of this yeast has been examined for the specific control of *P. verrucosum* and OTA contamination of stored wheat (Mokiou and Magan, 2008). These studies showed that formulation had an impact on the efficacy of control of both *P. verrucosum* populations and the relative effectiveness of controlling OTA in stored wheat grain.

Many lactic acid bacteria (e.g. *Lactobacillus acidophilus* strains) have been screened for the ability to either degrade or bind different mycotoxins (Fuchs et al., 2008). They have become

attractive inoculants because of the indication that they can give beneficial health effects. Studies have suggested that they can detoxify aflatoxin B1. Others studies suggest that they may bind or degrade zearalenone, trichothecenes and fumonisins (El-Nezami et al., 2002). However, less work has been carried out in situ under realistic storage conditions, especially intermediate moisture and temperature conditions. Potential does exist for this approach to pay dividends in relation to preservation of moist cereals specifically for animal feed purposes.

Conclusions and future strategies

There are a number of key points which are important post-harvest in any management system to minimise mycotoxin contamination especially downstream for processing purposes. These include

(a) regular and accurate moisture determination,

(b) minimising time between harvesting and drying; this will relate to holding time/temperature prior to drying as well as the actual drying conditions,

(c) efficient and prompt drying of wet grain to target moisture contents (temperate cereals <14.5%) by heated drying where possible as ambient drying can result in spoilage problems

(d) infrastructure for quick response, including provision for segregation and appropriate transportation conditions,

(e) appropriate storage conditions at all stages in terms of moisture and temperature control, and general maintenance and hygiene of facilities for prevention of pest and water ingress which can lead to pockets of metabolic water and initiate spoilage,

(f) ability to efficiently identify and reject material below specified standards in terms of both fungal diseases and, at some stages, mycotoxin levels (e.g. when passing onto a third party),

(g) operation of approved supplier systems. This involves setting specifications for acceptance/rejection.

Other important areas which need to be considered are those in relation to better representative sampling and early and rapid detection of mycotoxigenic fungi post-harvest. Problems do still arise in relation to obtaining a representative sample both pre- and post-harvest. Whitaker (2004) and Whitaker et al. (2000) have shown that in stored cereals, nuts and other commodities that the error in obtaining a representative sample can be 25-60% of the total error. There are specific EU sampling plans (e.g. for DON; Commission Directive 2005/38/EC) which are prescribed but have been found cumbersome to use. The question arises as to whether one should use a regular grid type sampling approach or use a random sample approach. Which type of sampling regime should be employed may further be dictated by the type of mycotoxin as different contaminants may be distributed homogenously or heterogeneously or spatially either in regular pockets or randomly. Recent research has been undertaken to try and use geostatistics to try and unravel this complex issue. This approach is beneficial if you have the exact grid positions of the samples in three dimensions, it can then be used on any scale. Recent studies by Rivas et al. (2009 a, b) suggest that mycotoxins such as DON, have a spatial structure in the store and thus a regular grid may be more precise than using a random sampling approach. This may not be the case for other mycotoxins and in other commodities and thus further work is imperative to enable more accurate representative samples to be taken and improve the accuracy of such sampling.

We believe that there are new technologies which need to be utilised in the future to improve the strategies to minimise mycotoxins in temperate cereals. As has been mentioned earlier the potential for using remote sensing especially in silos linked to post-harvest models of

mycotoxigenic moulds integrated with the legislative limits could be very useful. There are now many useful molecular-based techniques which could be applied effectively post-harvest. For example, Schmidt-Heydt et al. (2000) utilised the expression of the OTA polyketide synthase (*otapks*PV) to examine temporal colonisation of stored wheat grain of different moisture contents (14-24%) and this was correlated with OTA production analysed by HPLC and populations by CFUs. Thus the RT-PCR approach could be used as a sensitive measure of the quality status with regard to OTA contamination in wheat during 3 months storage. These powerful molecular tools have been applied pre-harvest but not widely post-harvest. This is supported by the recent work by Suanthe et al. (2009) who have developed multiplex RT-PCR for detection and quantification of mycotoxigenic *Aspergillus, Penicillium* and *Fusarium* species.

In the future another important potential impact is that environmental change. This may have an impact on the relative importance of different mycotoxins in different geographical regions. This may become important and have a significant impact on which mycotoxins may become important in the next 5-10 years. There is a lack of data on the impact that twice or three times the existing CO_2 concentrations combined with 2-3^{o}C elevation in temperature might have on colonisation and mycotoxin production. A certain amount of this information can be generated by modelling approaches. However, the potential for stimulation of mycotoxin production is more difficult to predict. In conclusion, while this paper has dealt with post-harvest issues in temperate cereals it has to be seen in the context of a more holistic food chain systems approach with better and more effective management post-harvest being just one component of this.

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Figure legends

Figure 1. Comparison of dry matter losses caused by *Fusarium graminearum* when inoculated onto wheat grain stored at three different water activities and four different temperatures stored for 4 days. Respiration was measured using GC analyses.

Figure 2. Linear regression of the ratio of O_2 consumption rate/temperature at different water activity levels, corrected for temperature for wheat 7 days storage (adapted from Lacey et. al., 1997).

Figure 3. The growth/no growth and deoxinivalenol/no deoxynivalenol boundaries used to develop the polynomial based model to describe the contour diagrams for *F. graminearum*. Insert: shows the simple front end diagram which could be developed and linked to real time temperature and relative humidity data by movement of the dials to indicate low or high risk of growth and mycotoxin production.

Table 1. Effect of 500 ppm of three essential oils (extracts of bay leaf (Bay), cinnamon (Cin) and clove) and two anti-oxidants (butylhydroxyanisole (BHA), propyl paraben (PP) on (a) deoxynivalenol (DON, ppm) production by *F.graminearum* at 15, 25°C and 0.995 and 0.95 water activity, and (b) T-2 and HT-2 (ppm) by *F.langsethiae* on stored wheat grain at 15, 25°C and 0.95 water activity.

(a) *F.graminearum*

	DON (PPM)			
Temperature (°C)	15		25	
Water activity	0.995	0.95	0.995	0.95
Control	10.11	0.25	3.52	44.07
Bay	0.55	0.30	1.72	2.12
BHA	0.43	0.27	0.91	2.15
Cin	0.57	0.30	1.05	55.11
Clove	0.45	0.32	0.85	1.60
PP	0.75	0.40	1.05	3.10

L.S.D. (P=0.05): Temperature x water activity: 0.25 ppm

(b) F.langsethiae

Temperature (°C)	15		25	
Mycotoxin	T-2	HT-2	T-2	HT-2
Control	0.53	7.25	0.63	5.07
Bay	0.13	0	0.50	4.71
BHA	0	0	0.79	8.93
Cin	0	0	0	0
Clove	0.13	0.55	4.20	6.52
PP	0.22	0.75	0.42	2.20

L.S.D. (P=0.05): 1.25

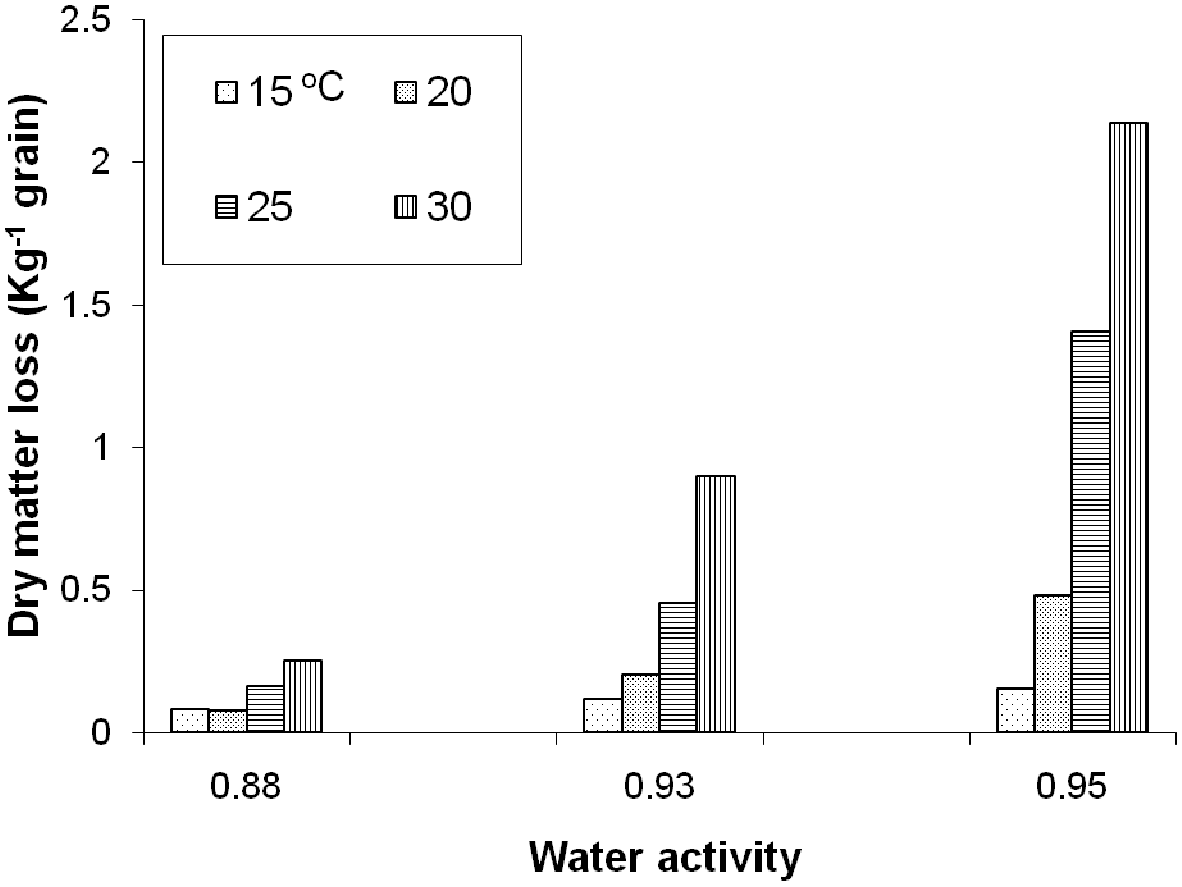

Figure 1: Magan et al.

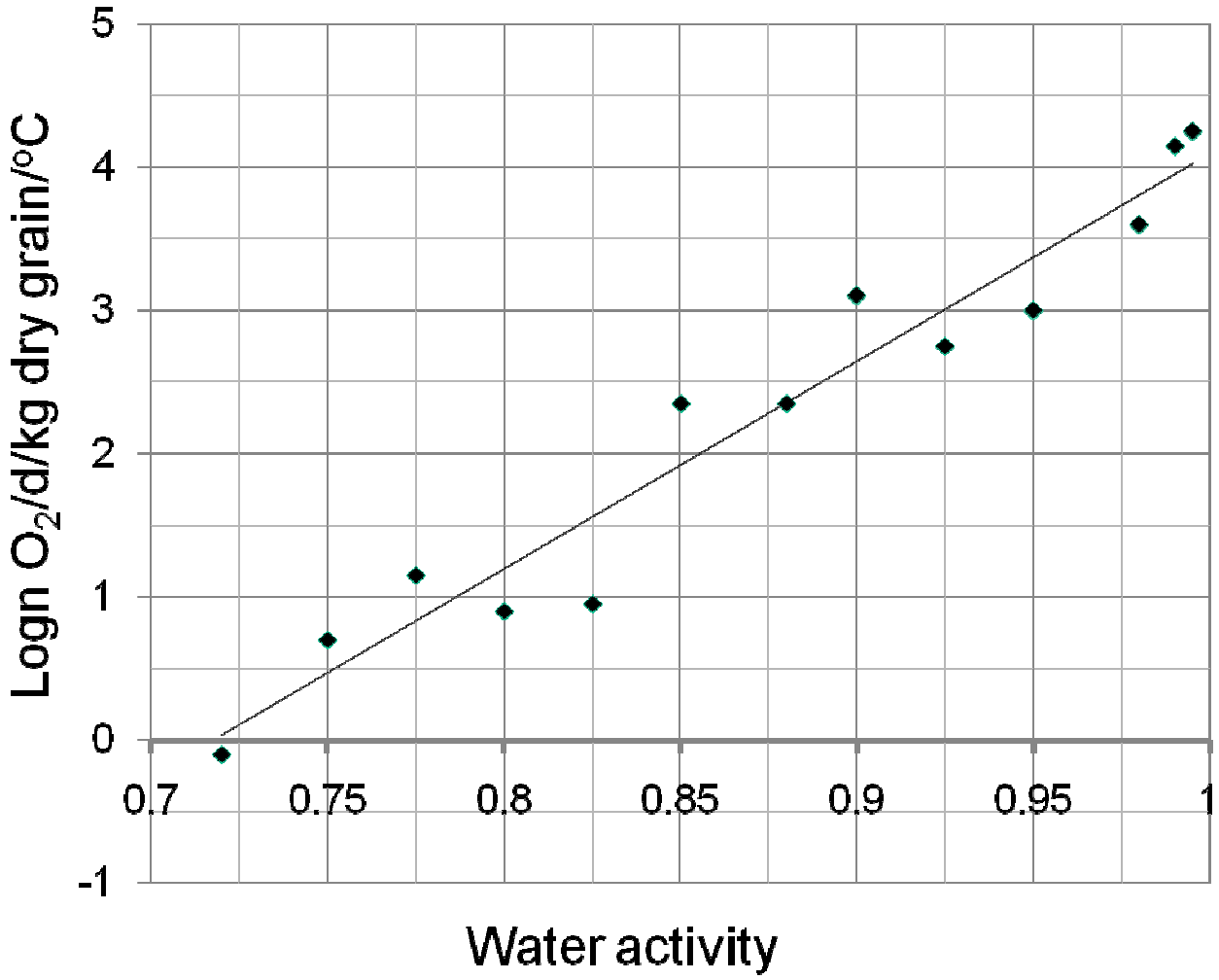

Figure 2: Magan et al.

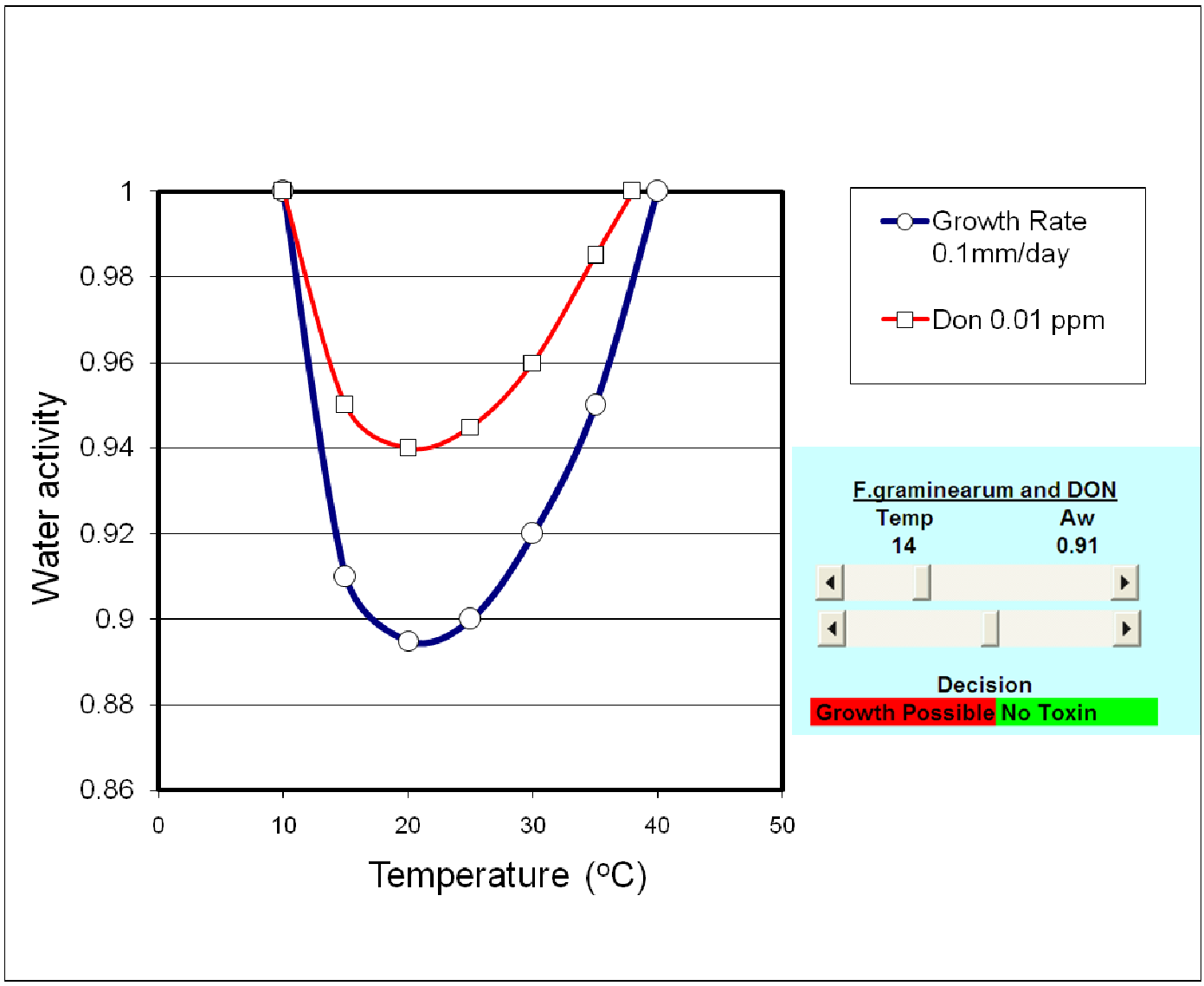

Figure 3: Magan et al.