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Limiting mycotoxins in stored wheat

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13 5 **Limiting mycotoxins in stored wheat – a review**
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20 8 Naresh Magan, David Aldred, Kalliopi Mylona and Ronald J.W. Lambert
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23
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48 20 Key words: spoilage fungi, mycotoxins, dry matter losses, boundary models, modified
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50 21 atmospheres, preservatives, biocontrol, post-harvest management
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3 26 **Abstract**
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6 27 The quality of harvested wheat grain can deteriorate markedly during the post-harvest
7
8 28 management stages. The biotic factors such as grain type and ripeness, coupled with the
9
10 29 prevailing abiotic factors such as water content and temperature, and also preservative
11
12 30 concentration will influence the safe storage life and the level of contamination with
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14 31 mycotoxins such as deoxynivalenol (DON) produced pre-harvest and zearalenone (ZEA)
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16 32 produced post-harvest by *Fusarium graminearum* and *Fusarium poae* respectively,
17
18 33 ochratoxin (OTA) produced by *Penicillium verrucosum* post-harvest in cool damp northern
19
20 34 European climates, and perhaps T-2 and HT-2 toxins produced by *Fusarium langsethiae*.
21
22 35 This review presents recent data on the relationship between dry matter losses caused by *F.*
23
24 36 *graminearum* under different environmental regimes (water activities, temperatures) and the
25
26 37 level of contamination with DON. This is important as poor post-harvest drying and storage
27
28 38 management may exacerbate DON contamination already present pre-harvest. It is thus
29
30 39 critical to relate the environmental factors in stored wheat grain during storage, especially of
31
32 40 intergranular relative humidity (RH) and temperature, to safe storage periods without
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34 41 spoilage or risk from increased DON contamination. The growth/no growth and DON/no
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36 42 DON (*F. graminearum*) and OTA/no toxin production (*P. verrucosum*) have been used to
37
38 43 build a model with a simple interface to link the temperature and RH values to the potential
39
40 44 risk level which may allow growth or toxin production. This paper also considers the use of
41
42 45 modified atmospheres, preservatives and biocontrol to minimise DON and OTA in moist
43
44 46 wheat grain. These approaches together with clear monitoring criteria and hygiene could
45
46 47 contribute to better post-harvest management of stored temperate cereals and ensure that
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48 48 mycotoxin contamination is minimised during this key phase in the food/feed chain.
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51 Introduction

52 In Europe the key approach to minimising natural contaminants in the food chain has been by
53 developing minimisation strategies within a HACCP framework. Thus, the cereal food chain
54 for different end products has been studied in detail (Aldred and Magan, 2004; Aldred et al.,
55 2004; Magan et al., 2008). This has included the monitoring of critical control points (CCPs)
56 both pre- and post-harvest. With regard to mycotoxins as a hazard in temperate cereals
57 (DON, OTA) the main drivers have included EU legislative limits, customer specifications
58 and consumer perceptions. A prevention strategy must aim to minimise the occurrence of
59 these mycotoxins below the legislative limits based on best practice, both pre- and post-
60 harvest. The key CCPs post-harvest have been identified for the wheat food/feed chain. In
61 temperate cereals the main mycotoxins of concern are ochratoxin OTA (*Penicillium*
62 *verrucosum*), deoxynivalenol (DON; *Fusarium graminearum*, *Fusarium culmorum*),
63 zearalenone (*F. graminearum*, *Fusarium poae*), and T-2 and HT-2 toxins (*Fusarium*
64 *langsethiae*). There are legislative limits for the first three of these and for the latter two,
65 legislation is imminent.

66
67 Cereal grain, whether effectively dried or slightly moist, is alive and respiring and thus has to
68 be treated with care during harvesting, drying, storage and subsequent downstream
69 processing along the food chain. The concept of considering stored grain as an ecosystem
70 was originally developed by Wallace and Sinha (1971). This was an important milestone as
71 they included all the key biotic and abiotic interactions which might occur during storage
72 including grain respiration, insect pests, mycoflora, insect/fungal interactions, pest
73 immigration and emigration, heterogeneity of moisture and moisture migration, temperature
74 and O₂/CO₂, and for animal feed the presence of preservatives. This holistic approach was

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3 75 instrumental in facilitating some of the advances made during the last decade (Magan &
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5 76 Aldred, 2007).
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10 78 **Respiration, dry matter losses and mycotoxin contamination**
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15 80 Harvested grain must be dried to <14.5% moisture content (m.c., wet weight basis) to ensure
16
17 81 that no mould spoilage or any pre-harvest contamination with mycotoxins is exacerbated.
18
19 82 Often, grain is harvested at 16-20% m.c. because of the better efficiency of modern combine
20
21 83 harvesters. However, this does subsequently require heated or ambient drying to be used to
22
23 84 conserve grain quality. Where drying has not been carried out effectively, the temperature
24
25 85 will rise quickly and a succession of spoilage moulds may develop resulting in spontaneous
26
27 86 heating with temperatures of 60-70°C being reached, ending with thermophilic actinomycetes
28
29 87 and complete loss of quality. This is accompanied by a significant increase in the chance for
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31 88 mycotoxin contamination.
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38 90 Studies have been carried out to examine the relationship between grain respiration, mould
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40 91 activity and quality loss (dry matter) during these critical periods between harvesting, drying
41
42 92 and storage. These have shown that in wheat and barley, dry matter losses of only 0.22 –
43
44 93 0.44% at 20-25°C and 0.90 water activity ($a_w = 19-21$ % m.c.) results in visible moulding
45
46 94 already being present (Lacey et al., 1999). Indeed, studies in the USA suggested that in other
47
48 95 cereals such as maize, losses of 0.5% dry matter can lead to rejection of the grain for human
49
50 96 consumption and that this was accompanied by a significant chance of aflatoxin
51
52 97 contamination (Seitz et al., 1982). Fleurat-Lessard (2002) extensively reviewed data on
53
54 98 general spoilage and dry matter losses and suggested that the rate of CO₂ production in
55
56 99 cereals can be effectively used to model and predict global changes in cereal quality.
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3 100 Recent studies at Cranfield University have tried to relate the activity of specific
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5 101 mycotoxigenic species under different environmental factors which might contribute to both
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8 102 dry matter losses and the chances of exceeding the EU legislative limits for DON. Thus
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10 103 experiments were carried out with stored winter wheat inoculated with spores of *F.*
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12 104 *graminearum* at 0.86, 0.93 and 0.95 a_w and different temperatures (15-30°C) in chambers
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14
15 105 with the same equilibrium relative humidity atmosphere and measuring the respiration using
16
17 106 GC analyses over periods of 4-5 days. The final *F. graminearum* populations and DON
18
19 107 contamination after 10 days were also analysed. The effect of temperature x a_w on total
20
21 108 calculated dry matter losses over periods of 4 days storage are shown in Figure 1. This shows
22
23 109 the effect that these two parameters have on colonisation rates and quality loss. DON
24
25 110 concentrations were found to be above the legislative limits (>1250 ppb) at 30°C and 0.95
26
27 111 and 0.93 a_w in only 10 days, while at 15-20°C and all a_w levels the concentrations were less
28
29 112 than the legislative limits over these short periods. Similar data on dry matter losses caused
30
31 113 by *P. verrucosum* over 7-14 days showed that dry matter losses were also between 1-1.5% at
32
33 114 0.95 a_w , and lower at 0.85-0.90 a_w and 25°C (Magan and Aldred, 2007). This approach may
34
35 115 be beneficial for combining dry matter losses in relation to m.c.s during drying and initial
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37 116 storage with the range of potential risk conditions for OTA and DON, relative to the EU
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39 117 legislative limits. Previous comprehensive respiration data sets in relation to a_w and
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41 118 temperature of stored wheat, have been used to develop the possible relationship between the
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43 119 variables. Thus, the results obtained as O_2 d⁻¹ kg⁻¹ dry grain (R) was divided by the incubation
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45 120 temperature (\square) to give units of O_2 d⁻¹ kg⁻¹ dry matter °C⁻¹. There was a linear relationship
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47 121 ($r^2=0.9594$) between R/\square and a_w (Figure 2, Lacey et al., 1997). This is based on O_2
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49 122 consumption, while our new data is based on CO_2 production. Thus some conversion will be
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51 123 required to relate our new data to the previous data. However, it may be possible to relate our
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53 124 present data to the risk of exceeding the EU legislative limits for mycotoxigenic fungi. This
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3 125 could then be a valuable practical aid if developed for different grains and nuts prone to
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5 126 contamination with different mycotoxins.
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10 128 **Boundary models of growth/no growth and mycotoxin/no mycotoxin and stored grain**
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12 **management**
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17 131 It is essential that grain is effectively dried immediately after harvest. Often heated air drying
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19 132 is used to achieve the target safe moisture content of about 14.5%. However, sometimes in
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21 133 the harvesting season grain is left for short periods of time before drying because of logistical
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23 134 issues. In some European countries ambient air drying is always used. Where heated drying is
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25 135 used the grain is less affected by the prevailing weather conditions, especially in damp
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27 136 autumn seasons in northern Europe. In the UK, where ambient drying is often used, this can
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29 137 result in slow drying with a moist front moving vertically up through the grain bed, especially
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31 138 in flat bed dryers/stores. This can result in grain at the top of the store being rewetted, while
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33 139 grain at the bottom can become over dried. This can lead to layers of mouldy grain with the
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35 140 potential for increased DON or OTA contamination.
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43 142 Measurements can be made within stored grain silos to measure the changes in temperature
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45 143 and relative humidity in the inter-granular air spaces in the grain during drying and storage.
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47 144 This has been done at the top and in the middle of a flat bed store. Generally, any leaks or
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49 145 even small openings via damage to the structure can lead to an increase in temperature and
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51 146 intergranular m.c. This can often occur during the early periods after drying and storage
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53 147 phase (5-10 days) where both these parameters can increase, especially in the top layers
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55 148 which can increase the risk from spoilage and mycotoxin contamination (Magan et al., 2008).
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3 150 We have previously developed boundary layer contour maps for growth and DON and OTA
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6 151 production by *F. culmorum* and *F. graminearum* and for *P. verrucosum* (Hope and Magan,
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8 152 2003; Hope et al., 2004; Cairns et al., 2005). This data is useful as a guide to conditions
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10 153 where there may be a risk of growth and toxin production. A polynomial equation has been
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12 154 used to model these growth/no growth and toxin/no toxin boundaries. Figure 3 shows an
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14 155 example of the data for *F.graminearum* in relation to minimal growth rates (0.1 mm day^{-1})
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16 156 and for DON production (0.01 ppm).
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22 158 It is possible to link the temperature and RH levels monitored in the intergranular atmosphere
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24 159 to these boundary models. Thus, by inputting the data into the model one can check directly
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26 160 whether there is a risk of conditions conducive to growth or to DON production. The inset in
27
28 161 Figure 3 shows the simple front end diagram which can be used for this purpose. This would
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30 162 enable dials related to temperature and RH to be moved to determine a low (green colour) or
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32 163 high (red colour) risk of growth or mycotoxin (DON, OTA) in store. The model could easily
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34 164 be modified to include the legislative limits for specific mycotoxins and applied to a range of
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36 165 mycotoxigenic fungi, and food commodities. We have developed the growth and toxin
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38 166 models for *A. flavus* (aflatoxin), *F. verticillioides* (fumonisins), *F. culmorum* (DON) and *P.*
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40 167 *verrucosum* (OTA) and for the new *A.ochraceus* grouping (Abdel-Hadi & Magan, 2009).
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48 169 Other models have also been developed for *P.verrucosum* and OTA production by Lindblad
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50 170 et al. (2004). They developed very useful models which relate the potential for exceeding the
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52 171 EU legislative limit for OTA in cereals to the CFUs isolated from grain. They showed that
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54 172 $>10^3 \text{ CFUS g}^{-1}$ on a selective medium for *P. verrucosum* would represent a risk of OTA
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56 173 contamination $>5 \mu\text{g kg}^{-1}$ stored grain which is the EU legislative limit. They also developed
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58 174 models of toxin free storage times under different temperature and moisture content
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3 175 conditions. These are very useful practically for the cereal industry. Studies by Jonsson et al.
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5 176 (2000) suggest that the maximum storage time without any visible moulding was probably
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8 177 reduced by 50% if the mc at harvest was decreased by 1-3% (=0.05 a_w) or if the storage
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10 178 temperature was increased by 5°C, for example in a mild autumn year, in temperate cereals.
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14 15 180 **Modified atmosphere storage, chemical preservation systems and biocontrol**

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17 181 Moist grain, especially for animal feed use is sometimes stored in sealed silos to enable the
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19 182 natural respiration of the grain to increase the CO₂ concentration and inhibit spoilage moulds
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21 183 and the potential for mycotoxin formation post-harvest. However, if there are any leaks or the
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23 184 conditions remain micro-aerophilic then there is potential for both growth and mycotoxin
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25 185 formation. Studies suggest that increasing concentrations of CO₂ to 25 or 50% has little effect
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27 186 on growth or OTA production by *P. verrucosum* or indeed *Aspergillus ochraceus*
28
29 187 (= *Aspergillus westerdijkiae*). Spore germination of these two fungi is unaffected, although
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31 188 germ tube extension and growth can be affected by up to 75% CO₂ (Cairns-Fuller, 2004;
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33 189 Cairns-Fuller et al., 2005). However, this does not mean that mycotoxin production is
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35 190 significantly affected. While inhibition of OTA did occur when a_w and CO₂ were increased,
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37 191 the effect was not synergistic and sometimes inhibition was not to a level below the
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39 192 legislative limits.
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48 194 The effect of modified atmosphere on *F. culmorum* and other species has been examined and
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50 195 showed that they are micro-aerophilic and thus very tolerant of very low O₂ concentrations
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52 196 (Magan & Lacey, 1984). Paster (2000) has reviewed the effect of modified atmospheres on
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54 197 mycotoxigenic and other moulds and this has shown that there is very little information on
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56 198 mycotoxigenic Fusaria. Work has been carried out with *Fusarium sporotrichioides* and this
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58 199 demonstrated that T-2 toxin was reduced by 80% in the presence of 50% CO₂/20%O₂ but that
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3 200 mycelial colonisation was not affected by <60% CO₂ (Paster et al., 1986; Paster and
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5 201 Menasherov, 1988).
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10 203 Preservatives are often added to moist grain, especially for grain destined for animal feed.
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12 204 Commercial products are mainly based on mixtures of salts of aliphatic acids such as
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14 205 propionic, sorbic and benzoic acids. However, these are all fungistats and require effective
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16 206 contact with the product. Thus mixing is very important to ensure that inhibition of spoilage
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18 207 is maintained. Work has been carried out to try and identify alternative compounds which
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20 208 may be used to replace these aliphatic acid based products. Since *P. verrucosum* often causes
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22 209 problems post-harvest then the use of natural and novel preservatives may have an impact on
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24 210 OTA contamination. Aldred et al. (2008) screened a number of essential oils and antioxidants
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26 211 for control of both growth and OTA production by both *P. verrucosum* and *A. ochraceus*.
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28 212 They found that while essential oils such as clove, cinnamon and thyme were effective in
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30 213 vitro, in situ on grain they were relatively ineffective. This may perhaps be due to binding of
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32 214 the essential oil components to grain constituents. However, they did find that anti-oxidants,
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34 215 such as butylhydroxyanisole (BHA), propyl paraben, and especially resveratrol, were
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36 216 effective on naturally contaminated and grain artificially inoculated with *P. verrucosum*.
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44 218 A range of essential oils (23) and 6 antioxidants were screened for control of growth and
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46 219 DON and NIV production by *F. culmorum*, DON production by *F. graminearum* and T-2 and
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48 220 HT-2 by *F. langsethiae* (Hope, 2004). Of these, three essential oils (bay, clove, cinnamon oil)
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50 221 and two antioxidants (propyl paraben and hydroxymethylanisole) were found to be effective
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52 222 in controlling growth of these species in the range 50-200 ppm at 15 and 25°C at 0.995 and
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54 223 0.955 a_w in vitro on wheat-based media. More detailed studies on stored wheat grain at 15
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56 224 and 25°C and essential oil/antioxidants treatments (100ppm; 500ppm) were carried out. This
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3 225 showed that 500 ppm significantly reduced or inhibited growth at all the environmental
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5 226 combinations examined. However, at 100 ppm the essential oils/antioxidants stimulated
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8 227 growth under some experimental condition. Cinnamon and clove essential oils were the most
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10 228 effective inhibitors of growth regardless of temperature, a_w or species. The essential oils and
11
12 229 antioxidants had a variable effect on inhibition of mycotoxin production. BHA and clove oil
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14 230 inhibited DON at both 100 and 500 ppm. However, propyl paraben and cinnamon oil
15
16 231 enhanced NIV production at 100 ppm and intermediate a_w levels. Table 1 shows the efficacy
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18 232 of 500 ppm of these potential preservatives on control of DON and T-2 and HT-2 by *F.*
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20 233 *graminearum* and *F. langsethiae*, respectively, when inoculated onto wheat grain and stored
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23 234 for 28 days.

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28 235 Studies have been carried out to use inoculants such as yeasts or lactic acid bacteria to control
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30 236 stored moist cereals destined for animal feed. The applied inoculants need to be able to
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32 237 become established and dominate the stored grain ecosystem to minimise the contamination
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34 238 with mycotoxins. A significant amount of work has been carried out to use xerotolerant
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36 239 yeasts such as *Pichia anomala* to control spoilage of feed grain, especially in airtight storage.
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38 240 It has been shown that this competitive yeast can prevent spoilage by micro-aerophilic fungi
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40 241 such as *Penicillium roqueforti* (Druverfors et al., 2002). Recently, different fluidised bed
41
42 242 dried formulations of this yeast has been examined for the specific control of *P. verrucosum*
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44 243 and OTA contamination of stored wheat (Mokiou and Magan, 2008). These studies showed
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46 244 that formulation had an impact on the efficacy of control of both *P. verrucosum* populations
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48 245 and the relative effectiveness of controlling OTA in stored wheat grain.

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58 247 Many lactic acid bacteria (e.g. *Lactobacillus acidophilus* strains) have been screened for the
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60 248 ability to either degrade or bind different mycotoxins (Fuchs et al., 2008). They have become

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3 249 attractive inoculants because of the indication that they can give beneficial health effects.
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5 250 Studies have suggested that they can detoxify aflatoxin B1. Others studies suggest that they
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8 251 may bind or degrade zearalenone, trichothecenes and fumonisins (El-Nezami et al., 2002).
9
10 252 However, less work has been carried out in situ under realistic storage conditions, especially
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12 253 intermediate moisture and temperature conditions. Potential does exist for this approach to
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14 254 pay dividends in relation to preservation of moist cereals specifically for animal feed
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17 255 purposes.
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22 23 257 **Conclusions and future strategies**

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25 258 There are a number of key points which are important post-harvest in any management
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27 259 system to minimise mycotoxin contamination especially downstream for processing
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30 260 purposes. These include

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32 261 (a) regular and accurate moisture determination,
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34 262 (b) minimising time between harvesting and drying; this will relate to holding
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36 263 time/temperature prior to drying as well as the actual drying conditions,
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38 264 (c) efficient and prompt drying of wet grain to target moisture contents (temperate cereals
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40 265 <14.5%) by heated drying where possible as ambient drying can result in spoilage problems
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42 266 (d) infrastructure for quick response, including provision for segregation and appropriate
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44 267 transportation conditions,
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46 268 (e) appropriate storage conditions at all stages in terms of moisture and temperature control,
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48 269 and general maintenance and hygiene of facilities for prevention of pest and water ingress
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50 270 which can lead to pockets of metabolic water and initiate spoilage,
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52 271 (f) ability to efficiently identify and reject material below specified standards in terms of both
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54 272 fungal diseases and, at some stages, mycotoxin levels (e.g. when passing onto a third party),
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3 273 (g) operation of approved supplier systems. This involves setting specifications for
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5 274 acceptance/rejection.
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10 276 Other important areas which need to be considered are those in relation to better
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12 277 representative sampling and early and rapid detection of mycotoxigenic fungi post-harvest.

13 278 Problems do still arise in relation to obtaining a representative sample both pre- and post-

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15 279 harvest. Whitaker (2004) and Whitaker et al. (2000) have shown that in stored cereals, nuts
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17 280 and other commodities that the error in obtaining a representative sample can be 25-60% of

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19 281 the total error. There are specific EU sampling plans (e.g. for DON; Commission Directive
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21 282 2005/38/EC) which are prescribed but have been found cumbersome to use. The question
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23 283 arises as to whether one should use a regular grid type sampling approach or use a random

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25 284 sample approach. Which type of sampling regime should be employed may further be
26
27 285 dictated by the type of mycotoxin as different contaminants may be distributed

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29 286 homogeneously or heterogeneously or spatially either in regular pockets or randomly. Recent
30
31 287 research has been undertaken to try and use geostatistics to try and unravel this complex

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33 288 issue. This approach is beneficial if you have the exact grid positions of the samples in three
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35 289 dimensions, it can then be used on any scale. Recent studies by Rivas et al. (2009 a, b)

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37 290 suggest that mycotoxins such as DON, have a spatial structure in the store and thus a regular
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39 291 grid may be more precise than using a random sampling approach. This may not be the case

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41 292 for other mycotoxins and in other commodities and thus further work is imperative to enable
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43 293 more accurate representative samples to be taken and improve the accuracy of such sampling.

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47 295 We believe that there are new technologies which need to be utilised in the future to improve
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49 296 the strategies to minimise mycotoxins in temperate cereals. As has been mentioned earlier the
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51 297 potential for using remote sensing especially in silos linked to post-harvest models of

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3 298 mycotoxigenic moulds integrated with the legislative limits could be very useful. There are
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6 299 now many useful molecular-based techniques which could be applied effectively post-
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8 300 harvest. For example, Schmidt-Heydt et al. (2000) utilised the expression of the OTA
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10 301 polyketide synthase (*otapksPV*) to examine temporal colonisation of stored wheat grain of
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12 302 different moisture contents (14-24%) and this was correlated with OTA production analysed
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14
15 303 by HPLC and populations by CFUs. Thus the RT-PCR approach could be used as a sensitive
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17 304 measure of the quality status with regard to OTA contamination in wheat during 3 months
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19 305 storage. These powerful molecular tools have been applied pre-harvest but not widely post-
20
21 306 harvest. This is supported by the recent work by Suanthe et al. (2009) who have developed
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23 307 multiplex RT-PCR for detection and quantification of mycotoxigenic *Aspergillus*, *Penicillium*
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25 308 and *Fusarium* species.
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32 310 In the future another important potential impact is that environmental change. This may have
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34 311 an impact on the relative importance of different mycotoxins in different geographical
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36 312 regions. This may become important and have a significant impact on which mycotoxins may
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38 313 become important in the next 5-10 years. There is a lack of data on the impact that twice or
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40 314 three times the existing CO₂ concentrations combined with 2-3°C elevation in temperature
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42 315 might have on colonisation and mycotoxin production. A certain amount of this information
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44 316 can be generated by modelling approaches. However, the potential for stimulation of
45
46 317 mycotoxin production is more difficult to predict. In conclusion, while this paper has dealt
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48 318 with post-harvest issues in temperate cereals it has to be seen in the context of a more holistic
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50 319 food chain systems approach with better and more effective management post-harvest being
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52 320 just one component of this.
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6 420 **Figure legends**7
8 4219
10 422 Figure 1. Comparison of dry matter losses caused by *Fusarium graminearum* when
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12 423 inoculated onto wheat grain stored at three different water activities and four different
13
14 424 temperatures stored for 4 days. Respiration was measured using GC analyses.15
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17 42518
19 426 Figure 2. Linear regression of the ratio of O₂ consumption rate/temperature at different water
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21 427 activity levels, corrected for temperature for wheat 7 days storage (adapted from Lacey et. al.,
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23 428 1997).24
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27 42928
29 430 Figure 3. The growth/no growth and deoxinivalenol/no deoxynivalenol boundaries used to
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31 431 develop the polynomial based model to describe the contour diagrams for *F. graminearum*.
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33 432 Insert: shows the simple front end diagram which could be developed and linked to real time
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35 433 temperature and relative humidity data by movement of the dials to indicate low or high risk
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37 434 of growth and mycotoxin production.38
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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) <i>F.graminearum</i>		DON (PPM)			
Temperature (°C)	15		25		
	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) <i>F.langsethiae</i>		Mycotoxin			
Temperature (°C)	15		25		
	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

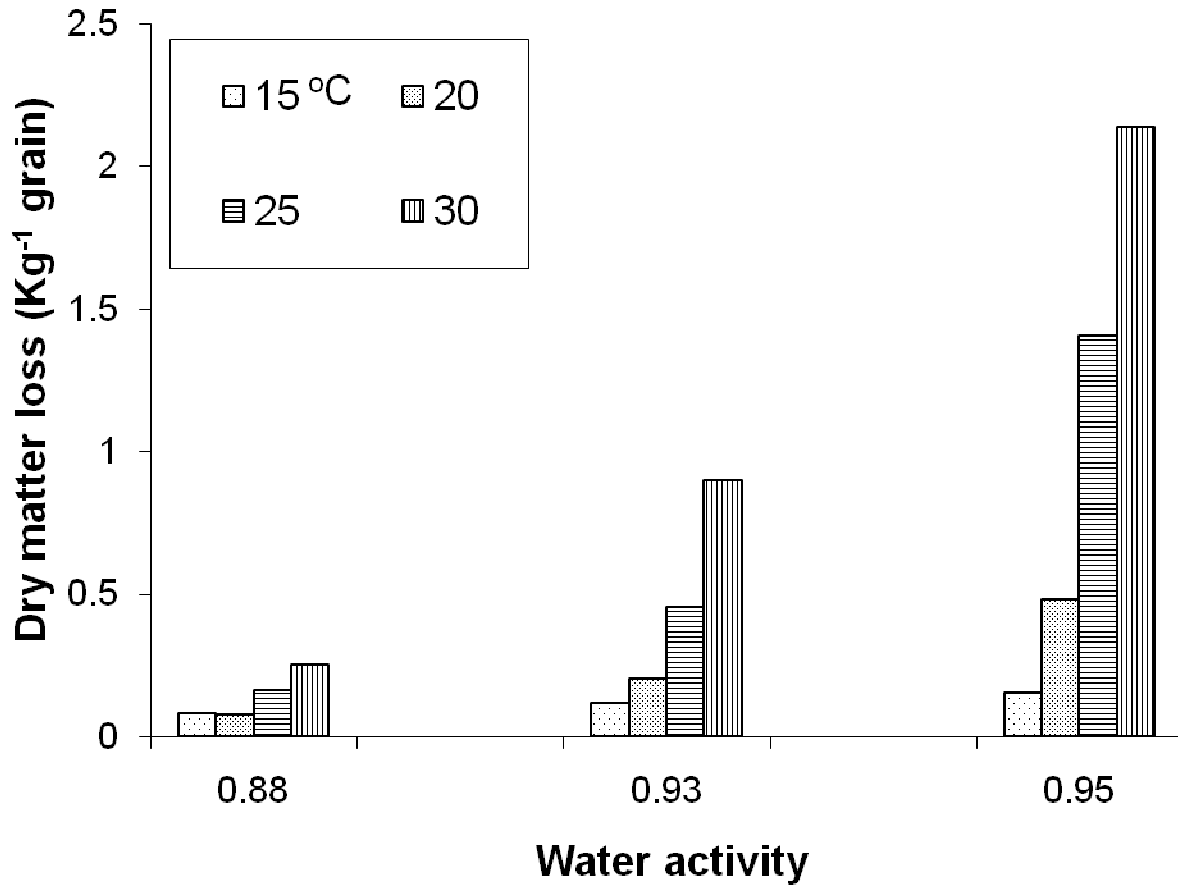


Figure 1: Magan et al.

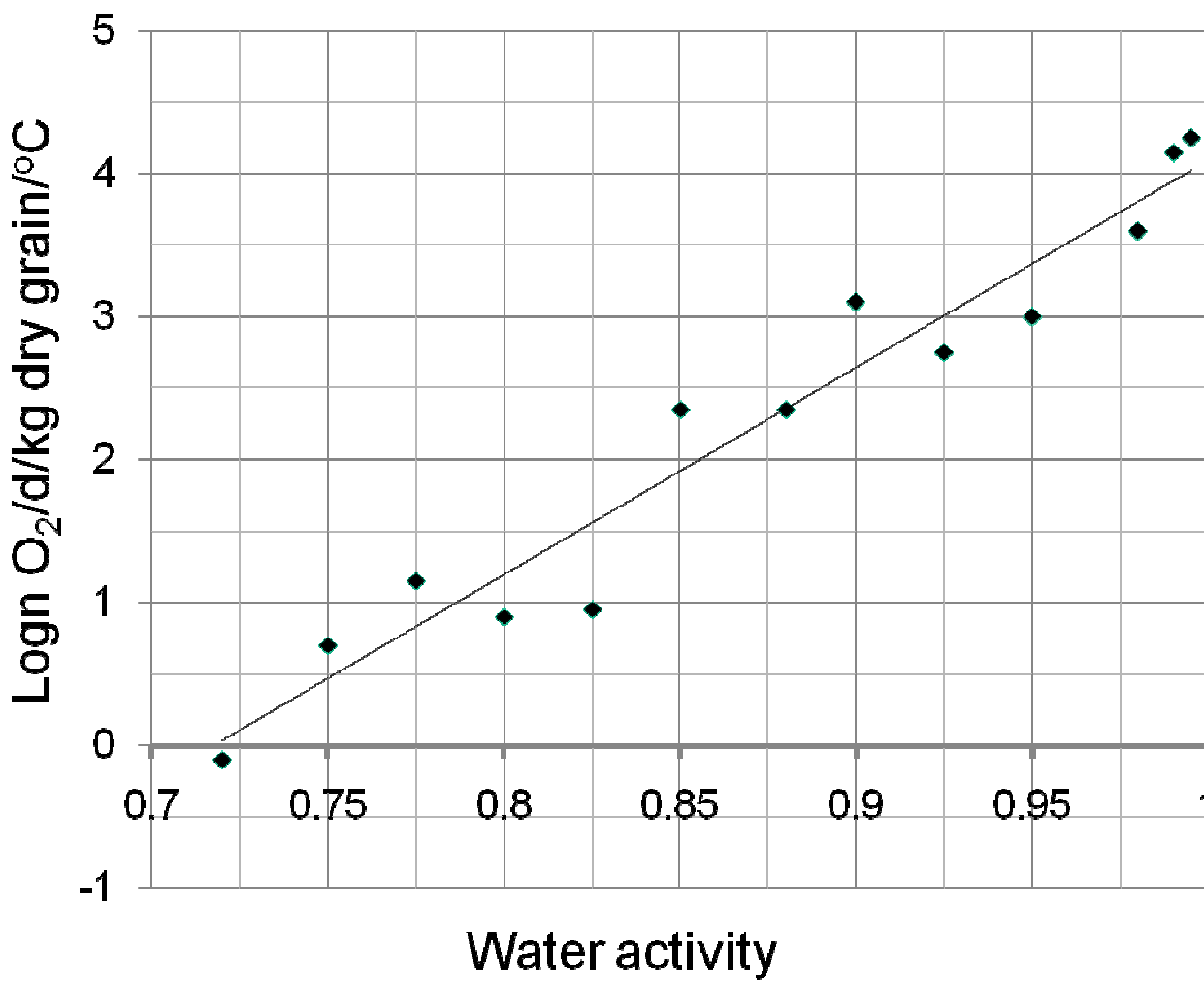


Figure 2: Magan et al.

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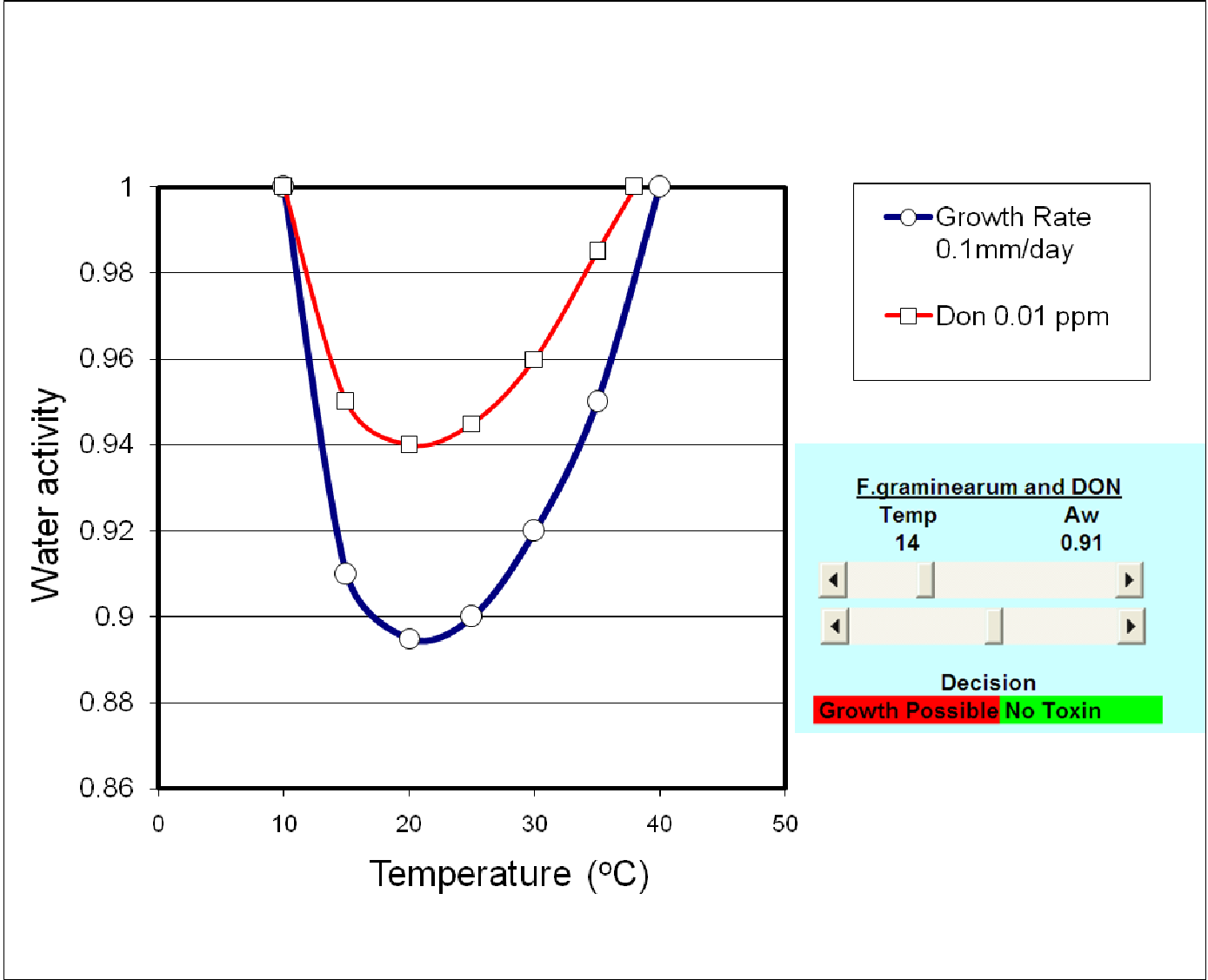


Figure 3: Magan et al.