WATER AVAILABILITY AND THE OCCURRENCE OF TOXIGENIC FUNGI AND MYCOTOXINS IN STORED PRODUCTS

J. LACEY

A.F.R.C. Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ (United Kingdom).

INTRODUCTION

Water is perhaps the most important ecological determinant of mould growth in stored products. Without water, no mould can grow, regardless of temperature, intergranular gas composition or other factors. However, even when water is present, it is not all equally available to colonizing microorganisms. Some is strongly bound in chemical union with the substrate but the remainder, more weakly bound, is available for microbial growth. However, the ease with which it can be removed depends on the water content of the substrate.

Availability of water in a substrate can be expressed either as water content, water activity ($a_w$) or water potential ($\psi$). Water content is the easiest to determine but, because the degree of binding of water differs between substrates, it does not allow comparison of water availability in different substrates. Availability of water to microorganisms is better expressed by $a_w$ or $\psi$. $a_w$ is widely used in storage and food microbiology and $\psi$ in soil microbiology, but there is pressure for all to adopt $\psi$ (ref. 1). At 0.65 $a_w$, the maximum for safe long-term storage (ref. 2), the water content of wheat is about 14% and that of groundnuts about 6%.

WATER AVAILABILITY AND FUNGAL GROWTH

The availability of water in substrate determines not only which microorganisms are capable of growth but also the rate of their spore germination and subsequent growth, their interactions with other fungi, their ability to produce spores and their metabolic activity. Fungi differ in their tolerance of low $a_w$ (Table 1). Fungi common on grain before harvest mostly require $> 0.85$ $a_w$ for germination and growth while those that develop during storage of grain can often tolerate as little as 0.75-0.65 $a_w$ (ref. 3). Monascus bisporus, the most xerophilic fungus known, can even grow down to 0.60 $a_w$. Tolerance of low water availability is greatest when the temperature is close to the optimum for growth. For
many species, growth rates increase as $a_w$ increases above the minimum for
growth. However, many xerophilic fungi have optima for growth down to 0.93
$a_w$ with $M$. bisporus even growing best at 0.85 $a_w$ and not at all above 0.97
$a_w$. Generally, slightly less water is necessary for germination than for
growth and slightly more for sporulation. For instance, the minima for
germination, growth and sporulation of Alternaria alternata are, respecti-
vely, 0.85, 0.88 and 0.90 $a_w$ and for anamorph production by Eurotium
amstelodami, 0.72, 0.73 and 0.78 $a_w$ (ref. 4). However, water requirements
for anamorph and teleomorph production also differ. For instance, Emeri-
cella nidulans produces its anamorph down to 0.85 $a_w$ but its teleomorph to
0.95 $a_w$ only. For Eurotium spp., the limits are, respectively, 0.75-0.78
and 0.77-0.86 $a_w$ (ref. 5). With sufficient water in the substrate, micro-
bial respiration may produce heat at a faster rate than it can escape,
leading to spontaneous heating (ref. 6). Temperatures up to 70°C may occur
with the growth of thermophilic microorganisms. The combination of water
availability and temperature determines which organisms can colonize the
substrate and which predominate and is also important in determining
mycotoxin production.

**TABLE 1**
Temperature and water relations of 'field' and 'storage' fungi (ref. 3)

<table>
<thead>
<tr>
<th>Species</th>
<th>Temperature (°C)</th>
<th>$a_w$ (for growth)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Optimum</td>
</tr>
<tr>
<td>Cladosporium herbarum</td>
<td>-10-32</td>
<td>25</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>0-35</td>
<td>20-25</td>
</tr>
<tr>
<td>Fusarium culmorum</td>
<td>&lt; 0-37</td>
<td>25</td>
</tr>
<tr>
<td>Penicillium roquefortii</td>
<td>&lt; 5-35</td>
<td>25</td>
</tr>
<tr>
<td>P. aurantiogriseum</td>
<td>-2-32</td>
<td>23</td>
</tr>
<tr>
<td>P. brevicompactum</td>
<td>12-30</td>
<td>23</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>10-55</td>
<td>40-42</td>
</tr>
<tr>
<td>A. flavus</td>
<td>6-54</td>
<td>35-37</td>
</tr>
<tr>
<td>A. parasiticus</td>
<td>4-39</td>
<td>25-30</td>
</tr>
<tr>
<td>A. versicolor</td>
<td>3-44</td>
<td>25-32</td>
</tr>
<tr>
<td>A. restrictus</td>
<td>9-40</td>
<td>30</td>
</tr>
<tr>
<td>Eurotium repens</td>
<td>7-40</td>
<td>25-27</td>
</tr>
<tr>
<td>E. amstelodami</td>
<td>5-46</td>
<td>33-35</td>
</tr>
</tbody>
</table>

WATER AVAILABILITY AND MYCOTOXIN PRODUCTION

Mycotoxin production is usually restricted within narrower limits of
water availability and temperature than fungal growth (ref. 7). Conditions
for the production of a toxin may differ between species of fungi or
between mycotoxins produced by the same fungus (Table 2). As with growth, the minimum water availability permitting mycotoxin production increases as temperature departs from the optimum. For *Alternaria* toxins, amount produced increases with water activity to near 1.0 $a_w$ (ref. 8) but with *Aspergillus ochraceus*, the optimum for ochratoxin production is about 0.95 $a_w$ (ref. 9).

**TABLE 2**  
Water activities limiting mycotoxin production (ref. 7, 8).

<table>
<thead>
<tr>
<th>Species</th>
<th>Mycotoxin</th>
<th>Minimum $a_w$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alternaria alternata</em></td>
<td>Alternariol</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Alternariol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>monomethyl ether</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Altenuene</td>
<td></td>
</tr>
<tr>
<td><em>Penicillium</em></td>
<td>Ochratoxin A</td>
<td>0.89</td>
</tr>
<tr>
<td><em>aurantiogriseum</em></td>
<td>Penicillic acid</td>
<td>0.97</td>
</tr>
<tr>
<td><em>P. verrucosum</em></td>
<td>Ochratoxin A</td>
<td>0.86</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>Aflatoxin</td>
<td>0.83</td>
</tr>
<tr>
<td><em>A. ochraceus</em></td>
<td>Ochratoxin A</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>Penicillic acid</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Fungi seldom occur singly in stored products but more usually in populations of several species which may interact in different ways, some competing for the same niche or nutrient source, some producing inhibitory metabolites while others may make nutrients available to other species from substrates they are unable to degrade. Such interactions may modify the ability of a fungus to produce mycotoxins while water availability may modify both interactions (ref. 10, 11) and their effects on mycotoxin production (ref. 13, 14). Thus, *Hyphopicha burtonii* and *Bacillus amyloliquefaciens* enhanced aflatoxin production by *Aspergillus flavus* at all $a_w$ tested while filamentous fungi inhibited aflatoxin production at 1.0 $a_w$ but enhanced it at 0.95 and 0.90 $a_w$. Zearalenone production by *Fusarium graminearum* was little affected by competition with *Aspergillus flavus* at any $a_w$ at 25°C but was inhibited at all $a_w$ at 16°C.

**CONCLUSION**

Water is an important determinant of both mould growth and mycotoxin production. The only sure way to prevent both is by drying the crop sufficiently and then keeping it dry in weatherproof stores. Drying to 0.72 $a_w$ is sufficient for short-term storage (up to 3 months) without visible moulding although the extent of fungal growth and mycotoxin
production within the seed have never been determined. However, to prevent
all mould growth and allow storage for a year or longer it is necessary to
dry to 0.65 aw (ref. 2). Safe storage at higher aw requires the additional
use of other strategies, such as controlled atmospheres or chemical treat-
ment, to prevent moulding.

REFERENCES
1. P.G. Ayres and L. Boddy (Eds.), Water, fungi and plants, Cambridge
2. D. Snow, M.H.G. Crichton and M.C. Wright, Mould deterioration of
feeding-stuffs in relation to humidity of storage. Part II. The water
uptake of feeding-stuffs at different humidities, Ann. Appl. Biol., 31
(1944) 111-116.
3. J. Lacey, Ecology pre-and post-harvest of fungi causing spoilage of
foods and feeds, in: M.O. Moss and B. Jarvis (Eds.) Filamentous fungi
in food and feeds (Symposium Series No. 18), J. appl. Bact. Suppl.
71-81.
5. J. Lacey, Water availability and fungal reproduction: patterns of spore
production and liberation, in P.G. Ayres and L.Boddy (Eds.), Water,
6. J. Lacey, Colonization of damp organic substrates and spontaneous
heating, in G.W. Gould and J.E.L. Corry (Eds.) Microbial growth and
53-70.
7. M.D. Northolt and L.B. Bullerman, Prevention of mould growth and toxin
production through control of environmental conditions, J. Food Prot.
8. N. Magan, G.R. Cayley and J. Lacey, Effect of water activity and
temperature on mycotoxin production by Alternaria alternata in culture
9. C.W. Bacon, J.G. Sweeney, J.D. Robbins and D. Burdwick, Production of
penicillic acid and ochratoxin A on poultry feed by Aspergillus
ochraceus: temperatures and moisture requirements, Appl. Microbiol. 26
(1973) 155-160.
10. N. Magan and J. Lacey, Effect of water activity, temperature and
substrate on interactions between field and storage fungi, Trans. Br.
11. N. Magan and J. Lacey, Interactions between field and storage fungi on
12. R.G. Cuero, J.E. Smith and J. Lacey, Stimulation by Hyphopichia
burtonii and Bacillus amyloliquefaciens of aflatoxin production by
Aspergillus flavus in irradiated maize and rice grains, Appl. Environ.
13. R.G. Cuero, J.E. Smith and J. Lacey, Mycotoxin formation by Aspergillus
flavus and Fusarium graminearum in irradiated maize grains in the