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Conditions of formation of ochratoxin A in drying, transport and in different commodities

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Abstract

The major species of fungi responsible for ochratoxin production (OTA) in a range of commodities are *Penicillium verrucosum*, *Aspergillus ochraceus* and *Aspergillus* section *Nigri*, especially *A. carbonarius. P. verrucosum* is particularly important in northern Europe where damp cooler conditions occur and where drying regimes need to be efficient and effective for preventing post-harvest contamination with OTA. *A. ochraceus* can infect cereals including barley, maize, coffee, cocoa and edible nuts. *A. carbonarius* has been identified as the key species responsible for OTA contamination of grapes, wine and vine fruits. Recent studies have identified the environmental regimes, especially of temperature and humidity, which are conducive to growth and OTA production by these species pre- and post-harvest and during transport. The optimum and marginal interacting conditions for growth and OTA contamination often vary considerably. This has to be borne in mind when effective preventative management strategies are being implemented. Recent studies with cereals have suggested that isolation frequency can be related to OTA contamination of cereals. A logistical model has been developed and identified that 1000 CFUs g^{-1} grain of *P. verrucosum* (CFUs) is the threshold limit at which the probability of exceeding the EU legislative limit of 5 µg kg^{-1} in cereal grain can be predicted under different storage regimes. Controlled atmospheres >50% $C0_2$ is required to effectively prevent OTA accumulation in damp cereals. With regard to grapes, preharvest contamination with *A. carbonarius* has been shown to be a good indicator for regional risk in southern Europe from OTA contamination. The ecological conditions for optimum growth and OTA production have been shown to differ with the optima being 30–35°C and 15–25°C and 0.98–0.99 and 0.93–0.95 water activity, respectively. Studies on vine fruits (drying currants) suggest that OTA contamination and increase contamination levels occur during this drying process of 7–14 days. This will be influenced by prevailing weather conditions and drying rates. Minimizing OTA contamination in these and other commodities including coffee and cocoa require clear guidelines on safe moisture and temperature regimes pre- and post-harvest for the development of effective management strategies based on ecological criteria.

Keywords: *Environmental factors, ochratoxin A, water activity, temperature, gas composition, growth, food commodities*

Introduction

Ochratoxin A (OTA) is one of the most important mycotoxins of concern for human health. It is produced by a number of fungal species that can colonize a range of food products. These species include *Penicillium verrucosum* and *Aspergillus ochraceus* that predominantly colonize cereals, coffee and cocoa. The former is more important in cool damp conditions of northern Europe and the latter in warmer climatic regions of the world. More recently, members of the *Aspergillus niger* section *nigri*, especially *A. carbonarius* has been found to colonize grapes, wine and vine fruits. Generally, of these products cereals are considered to represent 50–80% of the intake of OTA (SCOOP 2002).

The question arises as to how prevention strategies can be effectively instituted in these widely differing food chains in the framework of a HACCP framework to minimize consumer exposure to such natural toxins. Recently, Aldred et al. (2004) reviewed the available information that has enabled the identification of the critical control points (CCPs) in the cereal food chain. It is important that these are considered for the different commodities as they have very different production, harvesting, drying, transport and processing stages. The conditions which are conducive to growth of the fungal species and OTA production need to effectively understood from ''farm to fork'' to enable such strategies to be implemented. This paper will examine the

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conditions that are marginal and optimum for growth and toxin production by these mycotoxigenic species in relation to cereals, grapes/vine fruits and coffee.

Cereals and OTA contamination

Recent surveys of cereals from Europe, especially wheat and barley, have shown that *P. verrucosum* is predominantly isolated from such cereals with only occasional presence of *A. ochraceus*. Thus contamination with OTA is a post-harvest problem. Lund and Frisvad (2003) showed that *P. verrucosum* contaminated grain during the harvesting process and during drying and storage. Indeed, in damp harvest years in northern Europe it is essential that effective drying regimes are employed after harvest, to prevent *P. verrucosum* from becoming established. Thus effective management of this phase is critical to try and prevent OTA contamination at this stage in the food chain. Ambient drying systems in damp autumns require longer drying and this can result in layers of drying grain reabsorbing moisture and allowing the mycotoxigenic mould to become established. It is also a very competitive species and able to dominate under conducive environmental conditions in stored grain (Magan et al. 2003). Some work has suggested that the level of contamination by *P. verrucosum* is a good indication of potential contamination with OTA (Ramakrishna et al. 1996; Lund and Frisvad 2003; Lindblat et al. 2004). For example, Lund and Frisvad (2003) found that samples with more than 7% contamination of grain with *P. verrucosum* indicated OTA contamination, although no linear correlation between the two factors was obtained.

The most important abiotic factors which influence the 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 colonize 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 ($\geq$27–30% m.c.) over the temperature range 10–25°C and is almost completely inhibited at about 0.80–0.83 a_w (=17.5–18% m.c.).

Figure 1 shows the effect of these interacting factors on OTA production at different water activities and temperatures. No OTA was produced at 0.80 a_w, although some was produced at 0.85 (=19%) at 15 and 20°C. Optimum conditions were at 0.93–0.98 a_w (23.5–27.5%) at 10–25°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 legislative 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). The limits for growth and OTA production are shown in Figure 2.

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 contents 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.

The potential of using controlled atmospheres has been examined for OTA control in cereal grain. Studies have suggested that while spore germination is not affected, germ tube length is significantly inhibited by 50% CO_2, especially at 0.90–0.995 a_w for both *P. verrucosum* and *A. ochraceus* (Cairns-Fuller 2004). Growth and OTA production were highest in air, followed by 25 and 50% CO_2 regardless of the a_w level tested. Generally, CO_2 and a_w together cause an enhanced inhibitory effect, although this was not synergistic. No other studies have been carried out on controlled atmosphere effects on growth and OTA production by *P. verrucosum*, although information does exist for some *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 achieve rapidly to prevent OTA contamination in store or during transport.

Recently, Lindblat et al. (2004) developed a logistical model to relate populations of *P. verrucosum* (CFUs) to probability of exceeding the European

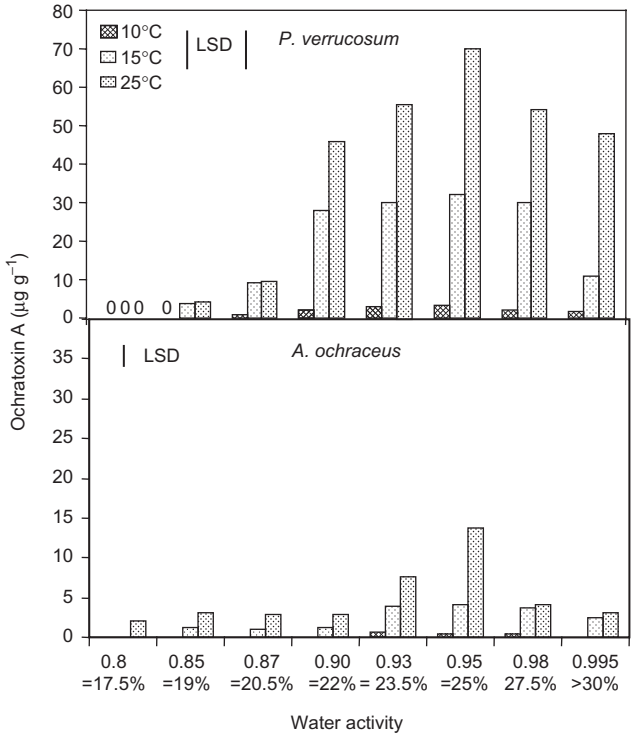

Figure 1. Effect of temperature and water activity on production of ochratoxin (μg/g) by *Penicillium verrucosum* (isolate OTA11) and *A. ochraceus* on wheat grain stored for 56 days under different temperature and moisture conditions. Bars represent LSD ($p = 0.05$) between treatments. Please note the scale on the y-axis differs.

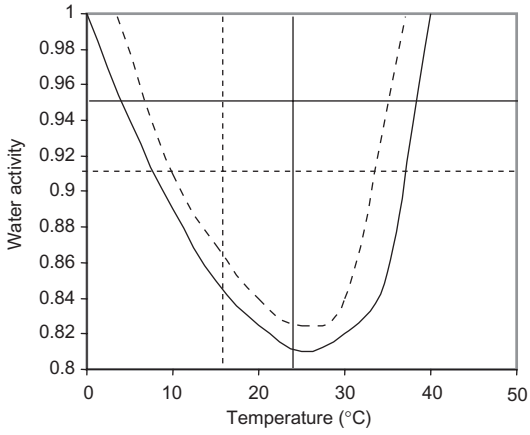

Figure 2. Comparison of limits for for growth (0.1 mm/day) and ochratoxin A production (10 ppb; - - -) by a strain of *P. verrucosum* grown on irradiated wheat grain for 35 days. Straight lines indicate optimum conditions of temperature and water activity (adapted from Sanchis and Magan (2004)).

legislative limit of $5\,\mu g\,kg^{-1}$ in cereal grain under different a_w and 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 greater than 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 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 Lindblat 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.

Grapes and vine fruits

The finding of OTA in grapes and wine in the last 5–6 years resulted in a search for the mycotoxigenic species responsible. The species responsible for OTA in cereals and other food products were not responsible for OTA contamination of grapes, wine and vine fruits. The common contaminant of these commodities was found to be *Aspergillus* section *Nigri*. They are common soil inhabitants where saprophytic colonization and survival on crop debris producing large numbers of spores which can become air-borne and contaminate ripening crops in Mediterranean, tropical and

subtropical regions. In the mid-1990s it was discovered that a member of this group, *A. carbonarius*, was responsible for the production of OTA (Abarca et al. 1994, 2001; Cabanes et al. 2002). Subsequently, *A. carbonarius* and OTA were detected in grapes, wine and other grape products (Zimmerli and Dick 1996; MacDonald et al. 1999; Majerus et al. 2000; Bragulat et al. 2001; Battilani and Pietri 2002; Stefanaki et al. 2003).

The presence of OTA as a contaminant of grapes, grape products and wine is again dependent on conducive microclimate conditions which can facilitate germination, germ tube extension, establishment and mycelial colonisation to occur. The most important factors governing these components of the life cycle of micro-organisms are water availability, temperature and their interaction with the nutrient status of the food matrix. For processing of grapes, pH is also important. There have been some studies of the effect of water and temperature on germination and growth of isolates of the *A. niger* group, prior to knowledge of the existence of *A. carbonarius*. For example, Ayerst (1969) and Marin et al. (1998) examined *A. niger* group isolates from cereal grain. More recently, Parra et al. (2004) and Para and Magan (2004) studied growth and sporulation capacity of *A. niger* wild-type and genetically-modified strains and modelled the effect of interacting water activity (a_w) × temperature conditions on growth. However, only a few studies have examined the effect of these abiotic variables on growth and OTA production by the *A. niger* Section nigri group (Bellí et al. 2004a, 2004b, Mitchell et al. 2003, 2004, Esteban et al. 2004).

Figure 3 shows the effect of temperature and water activity on germination of an *A. carbonarius* isolate from Italy. Temperature, a_w and time affected both germination and also germ tube extension of conidia in a similar way. Generally germination and initial establishment was very rapid at 25–30°C, especially under freely available water conditions. However, at 15°C germination was significantly slower with about 50% after 36 h. Furthermore, germ tube extension was just over 100 μm after 72 h, while at all other temperatures tested the germ tubes had reached about 300 μm in 24 h. Germination was rapid at between 0.99–0.90 a_w with almost 100% of spores successfully germinating within 24 h. At 0.88 a_w, there was a lag of about 18 h before germination occurred, with almost all spores germinating after about 60 h. The germ tube extension reflected this. Only in the drier conditions (e.g., 0.88 a_w) were germ tube lengths <30 μm after about 60 h. At 0.85 a_w there was no germination in the time frame of the experiments. Results were similar for isolates from both Italy and Portugal.

Optimum radial extension (approx. 10 mm day^{-1}) was at 30–35°C at 0.99–0.93 a_w. Figure 4 shows the

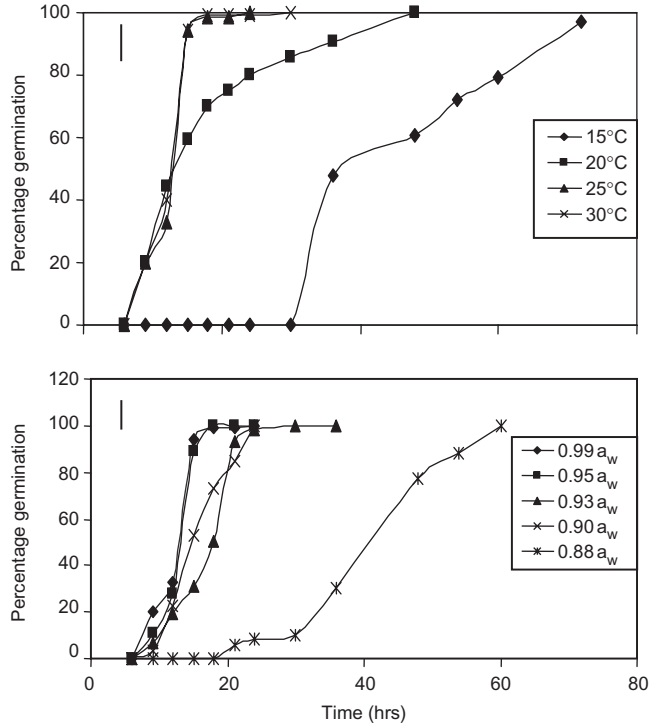

Figure 3. Effect of temperature and water activity on germination rates of an isolate of *A. carbonarius* on an artificial grape juice medium. Bar represents LSD ($p = 0.05$) between treatments.

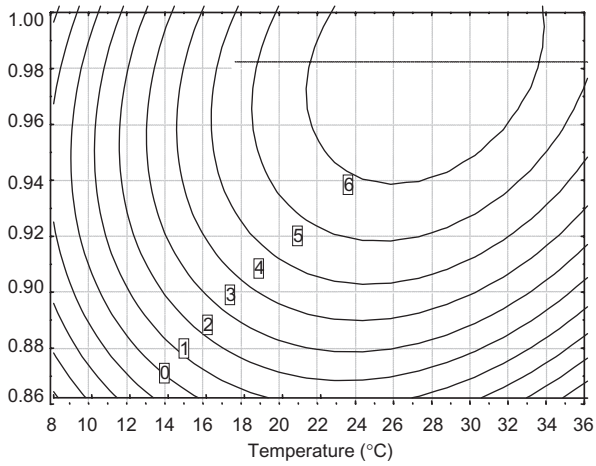

Figure 4. Contour map of the effect of water activity × tempera-temperature interactions on growth of an isolate of *A. carbonarius* on an artificial grape juice medium. The numbers on the isopleth lines refer to similar conditions of growth (mm/day). Data is adapted and modified from Mitchell et al. (2004).

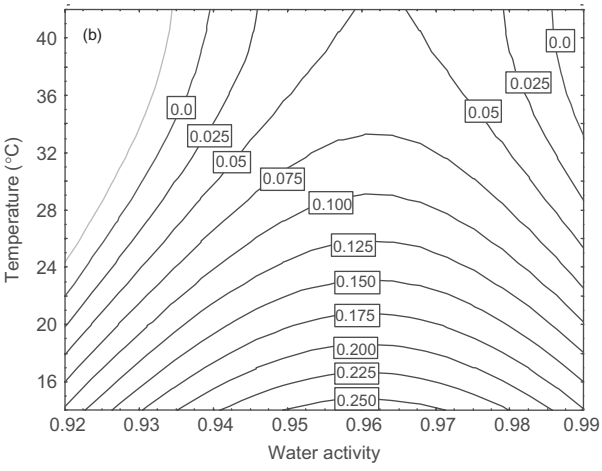

Figure 5. Contour maps of the production of ochratoxin by an isolate of *A. carbonarius* on an artificial grape juice medium (adapted from Mitchell et al. (2004)).

general trend in growth in relation to temperature at different steady state a_w levels. Recently Mitchell et al. (2004) showed that there are some intraspecific differences between isolates from a single country, and between isolates from different countries in relation to growth and these environmental factors. Data for individual isolates was subjected to statistical analysis to produce two dimensional profiles of temperature × a_w for growth and confirms the optimum conditions of a_w and temperature for growth are in the range 25–35°C, at 0.98–0.92 a_w.

The effect of environmental conditions on OTA production by strains of *A.carbonarius* has shown that these are optimum at 15–20°C and 0.95 a_w. This is very different from the conditions for growth. Figure 5 shows the contour diagram for OTA production in relation to these interacting conditions.

The range of a_w × temperature conditions for growth is often slightly wider than that for mycotoxin production and has been recently reviewed for a range of mycotoxigenic species (Sanchis and Magan 2004). For grapes pre-harvest contamination is a critical factor in determining the contamination in wine, especially of red wine where the skins are left in the initial phases of production. Studies by Battilani et al. (2005) show that there are so-called hot spots where high contamination with *A. carbonarius* occurs including parts of southern France, southern Italy and Greece. A study of wine contamination in a range of countries in Europe and surrounding regions showed that the highest OTA concentrations were in red wine, followed by rose and then white wine. Regions with highest contamination included Morocco, southern France, Italy and Greece (Battilani et al. 2004).

Drying of vine fruits. For the production of vine fruits the grapes are generally sun-dried for 7–14 days and turned regularly to ensure an even drying. They are then processed and packaged. However, during this process the sugar is concentrated as the moisture content decreases resulting in an almost selective medium for xerotolerant moulds such as *A. niger* section *nigri* species. If rain episodes occur then this can be compounded resulting is a higher risk from OTA contamination because of uneven drying.

Recent studies have been conducted to examine the population dynamics of *A. carbonarius* during the drying phase and this has showed that for Corinth currants the contamination with the mycotoxigenic *A. carbonarius* increases with drying over the 10–14 day period. At the end of the drying period before processing and packaging *A. carbonarius* can represent 50% of the contamination. In 2004, comparisons were made of fields at sea level, 500 and 1000 m elevation in Greece. This showed that contamination was higher at sea level and this was supported by the presence of higher levels of contamination at the end of drying period. The range was 2.83–11.38 and 1.81–1.62 $\mu g\,kg^{-1}$ currants (Dekanea 2005).

Coffee

Coffee is harvested at different moisture contents varying from 50–70% in ripe cherries, 35–50% in coffee raisons and 16–30% in cherries which are dried on the plant (Kamau 1980). At the end of drying the moisture content must be <12% (approx = 0.65–0.70 a_w) to prevent mould contamination and fermentation. Drying regimes and

subsequent storage and transport environmental conditions are critical to coffee quality and to potential for OTA contamination. At <9% the coffee looses flavour, while at >13% there is an increasing risk of OTA contamination. *A. ochraceus* has been predominantly responsible for contamination of coffee. However, a recent study of green coffee from Africa, Asia and America showed that both *A. ochraceus* and *A. niger* section *Nigri* species were present in these coffee samples (Pardo et al. 2004). They found that the OTA frequency distribution was higher in African samples (5–10 μg/kg), while for Asian and American samples was often <5 μg/kg. Interestingly, the fungal contamination of African samples was not higher than other samples for both Arabica and Robusta samples.

Because coffee is a very hygroscopic material it can often reabsorb moisture from the environment during storage and transport. Palacios-Cabrera et al. (2003) showed that 12 h temperature fluctuations between 14 and 25°C at 80, 87 and 95% relative humidity (RH) affected OTA contamination levels in raw coffee. After storage for 39 and 60 days there was little OTA contamination at 80% RH. However, at 87 and 95% RH significant OTA was produced, and production was higher in the cycling environmental conditions than steady state temperature/humidity conditions. Thus cycling of environmental conditions may indirectly favour OTA production because of condensation and reabsorption of water by the coffee beans. This could be particularly important during transport where effective monitoring of conditions are crequired to maintain threshold moisture contents (12–13%) to avoid detrimental flavour and minimising risk from OTA contamination.

For example, Pardo et al. (2005a, 2005b) recently studied *A. ochraceus* colonization and OTA production on coffee based media and green coffee beans. They found that for this species 30°C and 0.95–0.99 a_w was optimum for growth; while maximal OTA was produced at 20°C and 0.99 a_w. At 10°C no OTA was produced, regardless of a_w. No OTA was produced at 0.80 a_w (=13–14% moisture content).

Cocoa

Cocoa beans are normally placed in heaps and fermented prior to drying and transport of the beans for processing. Thus during the fermentation phase the cocoa beans can become colonized by *A. ochraceus* resulting in OTA contamination. Because cocoa beans are highly hygroscopic they can absorb moisture during storage and transport. The critical moisture content range is 6–8% which is equivalent to about 0.75–0.85 aw. These conditions can allow mould growth although they are marginal for growth of *A. ochraceus* and OTA production. Since they are rich in oils they tend to deteriorate slower than some carbohydrate rich products.

A recent study has analysed cocoa beans and hand shelled (coffee nib) beans. This showed that during the shelling process there was a decrease of between 65–95% in OTA contamination (Amezqueta et al. 2005). Thus, OTA may be predominantly present in the shells and care is needed during the shelling process to minimize the contamination of cocoa-derived products with OTA. However, cocoa beans and nibs as stated earlier are also very hygroscopic and thus can absorb moisture during storage and transport. Thus management of the transport phase is critical for conserving quality and preventing OTA contamination.

Conclusions

In conclusion the following key points need to be made:

Cereals: OTA is a post-harvest problem and grain must be dried to <14–14% m.c. and maintained through the food chain for prevention of contamination (*P. verrucosum*, predominantly).

Grapes/wine: Pre-harvest climatic conditions and crop microclimate and variety are important in determining risk; relative humidity, temperature and cropping determine OTA contamination. Certain areas of Europe have climatic conditions which represent higher risk than others.

Dried Vine Fruits: This is a post-harvest problem where efficient drying and turning is required to prevent establishment of *A. carbonarius* and OTA contamination.

Coffee: There is a problem at harvest and post-harvest. Efficient drying to prevent establishment of *A. ochraceus* is required. Usually <12–13% m.c. in green coffee is recommended to avoid OTA contamination. Coffee is a hygroscopic material and thus absorbs water during storage and transport.

Cocoa: During post-harvest fermentation and drying *A. ochraceus* can become established and contaminate product with OTA. Cocoa is a very hygroscopic material and thus absorbs water during storage and transport. The moisture content must be kept at between 6–8% to minimize OTA risk.

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