

Climate change, food security and mycotoxins: do we know enough?

Angel Medina, Asya Akbar, Alaa Baazeem, Alicia Rodriguez & Naresh Magan

Applied Mycology Group, Environment and AgriFood Theme, Cranfield University,
Cranfield, Bedford MK43 0AL, U.K.

Corresponding author: Prof. N. Magan, Applied Mycology Group, AgriFood Theme, School of Energy,
Environment and AgriFood, Cranfield MK43 0AL, U.K.

E-mail: n.magan@cranfield.ac.uk

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Abstract

Climate change (CC) scenarios are predicted to have significant effects on the security of staple commodities. A key component of this impact is the infection of such crops by mycotoxigenic moulds and contamination with mycotoxins. The impacts of CC on mycotoxigenic fungi requires examination of the impacts of the three-way interactions between elevated CO₂ (350-400 vs 650-1200 ppm), temperature increases (+2-5°C) and drought stress on growth/mycotoxin production by key spoilage fungi in cereals and nuts. This review examines the available evidence on the impacts of interacting CC factors on growth and mycotoxin production by key mycotoxigenic fungi including *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* species. *Aspergillus flavus* responsible for producing aflatoxin B₁ (AFB₁) is a class 1A carcinogen and its growth appears to be unaffected by CC factors. However, there is a significant stimulation of AFB₁ production both *in vitro* and *in vivo* in maize. In contrast, studies on *Aspergillus* section *Circumdati* and *Nigri* species responsible for ochratoxin A contamination of a range of commodities and *F. verticillioides* and fumonisins suggest that some species are more resilient than others, especially in terms of mycotoxin production. Acclimatisation of mycotoxigenic fungal pathogens to CC factors may result in increased disease and perhaps mycotoxin contamination of staple cereals. Predictive modelling approaches to help identify regions where maximum impact may occur in terms of infection by mycotoxigenic fungi and toxin contamination of staple crops is hindered by the lack of reliable inputs on effects of the interacting CC factors. The present available knowledge is discussed in the context of the resilience of staple food chains and the impact that interacting CC factors may have on the availability of food in the future.

1. Introduction

Climate change (CC) is predicted to have significant impacts on the quality and availability of staple food commodities. Food security is determined by three key components: (a) sufficient food availability, (b) access to this food and (c) quality and utilisation of the food in terms of both nutritional and cultural perspectives (FAO, 1998). With the predicted increase in the population on a global basis, the present intensive inputs into staple cereals and rice will only provide marginal increases in yields. Thus, the capacity to provide additional food of the necessary quality/quantity in the coming 25-50 years has been questioned (Battilani et al., 2012; IPCC, 2013; Battilani et al., 2016). In the context of CC impacts, especially changes in global weather patterns, could have a profound impact on the production and delivery of enough staple foods (Paterson and Lima, 2010; 2011; Wu et al., 2011).

The European Food Safety Authority (EFSA) has examined the potential impact of CC in Europe and has suggested that effects will be (a) regional and (b) detrimental or advantageous depending on geographical region (Battilani et al., 2012). This suggests that in northern Europe the effects may be positive, while the Mediterranean basin may be a hot spot where many effects will be negative, with extreme changes in rainfall/drought, elevated temperatures and CO₂ impacting on food production. Effects of CC on cereals

will be significant and detrimental as ripening in southern and central Europe will occur much earlier than at present. This will influence pests and diseases with decreasing yields and increasing mycotoxin contamination. Indeed it has been suggested that CC may be responsible for up to a 1/3 of yield variability in key staple commodities on a global basis (Ray et al., 2015). This will have profound impacts on food security in different continents.

Based on present available data, atmospheric concentrations of CO₂ are expected to double or triple (from 350-400 to 800-1200 ppb) in the next 25-50 years. Thus, different regions in Europe mentioned previously will be impacted by the increases in temperature of 2-5°C coupled with elevated CO₂ (800-1200 ppm) and drought episodes. This will have profound impacts on pests and diseases and ultimately yields (Gregory et al., 2009; Bebbber et al., 2013; Bebbber et al., 2014; Bebbber and Gurr, 2015). Similar impacts have been predicted in other areas of the world, especially parts of Asia, and Central and South America which are important producers of wheat, maize and soya beans for food and feed uses on a global basis (IPCC, 2013).

Historically, concerns about the contamination of food and feed with mycotoxins originally arose in the 1960s when a significant number of turkey poultts died from eating feed found to be contaminated with *Aspergillus flavus* and aflatoxin B₁ (AFB₁), later found to be a Class 1A carcinogen by the International Agency for Research on Cancer (IARC) (IARC, 2012). More information on the background, history and importance of mycotoxins in food/feed chains can be found in a timely review by Pitt and Miller (2017). The focus on mycotoxins has been a high priority by the FAO and WHO because of their significant toxicological impacts on both human and animal health. This has resulted in strict legislative limits for mycotoxins in many parts of the world in a wide range of foodstuffs with the strictest limits in the EU (European Commission, 2006). However, in many African countries where legislation is often applied to export crops only, consumption of mycotoxin contaminated staple foods is a significant risk, especially in rural populations and sub-groups such as children and immunocompromised people.

The most important mycotoxins are aflatoxins (produced by *Aspergillus* section *Flavi* species), ochratoxin A (OTA) (*Aspergillus* section *Circumdati* species, *Aspergillus* section *Nigri* species, *Penicillium nordicum*, *P. verrucosum*), fumonisins (*Fusarium verticillioides*, *F. sporotrichioides*), type A trichothecenes, T-2 and HT-2 toxin (*F. langsethiae*, *F. sporotrichioides*), and type B trichothecenes (*F. graminearum* and related species) and patulin (*P. expansum*). EFSA are now examining the relative hazard posed by mycotoxins produced by *Alternaria* species across the EU to obtain information of the levels in different food products to make decisions on whether limits should be considered. The ecophysiology and toxicology of *Alternaria* toxins have been recently extensively reviewed (Lee et al., 2015).

The relative resilience of different staple food and feed chains has thus become important in the context of whether increased or decreased mycotoxin contamination may occur as well as impacts on the

nutritional value. The inherent value of staple grains such as maize, wheat and rice are approx. 15.1, 14.2 and 5.4 kJ/g. It has been previously shown that mycotoxigenic fungi in maize can cause significant impacts on both calorific value as well as increase contamination with mycotoxins (Marin et al., 1999; Ramos et al., 1999). Thus, the perspectives on CC impacts are related to both nutritional/quality losses and toxin exposure, especially in Low and Middle Income Countries (LMICs). It is thus important when examining potential impacts of CC scenarios, to consider interactions between elevated CO₂, temperature and drought stress together. The environmental changes occurring now are slowly but steadily shaping the relationship between plant growth and the associated fungal diseases and pest populations. Indeed, the traditional and classic balanced triangle between pathogen/pest, host plant and environment (Garrett, 2008; Grulke, 2011) is changing and becoming skewed because of the relative importance and pressure of the environmental component which may shape both plant agronomy and ultimately yield of key staple foods (Pautasso et al., 2012; Figure 1). This shift in these interactions could be in a state of flux depending on the push and pull of the interacting factors and have significant impacts on the food supply chains in terms of food quality and quantity necessary to satisfy a rapidly expanding world population. Fungal plant pathogens are predicted to move globally and change the diversity of diseases and pests invading staple crops with both economic and social costs (Bebber & Gurr, 2015; Medina et al., 2015a). Recent predictions by Bebbber et al. (2013) suggest that pests and diseases are migrating to the poles at the rate of 3-5 km/year, with the diversity of pest populations becoming significantly changed (Crespo et al., 2015). In the context of mycotoxigenic fungal pathogens this could result in a switch from so-called mycotoxin suppressive to mycotoxin conducive conditions. Environmental stress has been shown to have significant consequences for secondary metabolite production, especially mycotoxins (Schmidt-Heydt et al., 2011; Medina et al., 2015c). Pest damage of ripening crops, especially cereals, can predispose them to infection/colonisation by mycotoxigenic fungal pathogens leading to increased mycotoxin contamination. Thus, while these previous studies did not focus on mycotoxigenic fungi, this does suggest significant potential impacts on mycotoxin contamination of staple foods/crops (Battilani et al., 2012; Battilani et al., 2016a). Indeed, a recent UNEP report on “Emerging Issues of Environmental Concern” (UNEP, 2016) has included a section entitled “Poisoned chalice: Toxin accumulation in crops in an era of climate change” which refers to the impact that aflatoxin contamination is having in LMICs. Wild et al. (2015) also showed that consumption of staple foods based on maize/groundnuts which are often contaminated with high levels of AFB₁, produced by *Aspergillus* section *Flavi* species, in the diet of infants and children in LMICs is resulting in significant stunting of growth. Thus, CC factors could have impacts and consequences because of the potential impacts on food availability, levels of mycotoxin contamination and the consumption of staple foods predominantly based on cereals.

Many of the current predictions and hypotheses towards the real effect of CC on fungal diseases and mycotoxigenic fungi are based on historical or current climatic conditions datasets that predominantly

consider interactions between water availability and temperature (Battilani et al., 2016a). Very few research studies have examined the effect of 3-way interactions between these identified environmental factors (temperature, water availability and CO₂) and what changes in terms of the ecophysiology of mycotoxigenic fungi and effects on mycotoxin production might occur (Medina et al., 2014; 2015a,b; Vary et al., 2015; Vaughn et al., 2014; Akbar et al., 2016; Magan and Medina, 2016).

2. Impact of climate change factors on growth and mycotoxin production

Impact of water activity (a_w) x temperature interactions on growth and mycotoxin production

Over the last three decades work has been focused on the impact that a_w x temperature factors have on growth and mycotoxin production by a wide range of mycotoxigenic fungi (Sanchis and Magan, 2004; Magan and Aldred, 2007a; Magan et al., 2010). This has included data on the optimum/marginal conditions for growth, mycotoxin production, and the boundary conditions for germination, growth and toxin production *in vitro* and in some cases *in situ* in key relevant commodities. This has usually shown that the range of a_w x temperature for mycotoxin production is narrower than that for growth. The only exception, to our knowledge, is *Penicillium verrucosum* which grows and produces ochratoxin A (OTA) under a very similar range of a_w x temperature conditions (Cairns et al., 2005). However, extreme drought episodes, desertification and fluctuations in wet/dry cycles will have an impact on the life cycles of mycotoxigenic fungi (Magan et al., 2011).

Magan et al. (2011) reviewed some of the available ecological data on optimum and marginal interacting conditions of a_w x temperature for growth and mycotoxin production by several mycotoxigenic species which was done by examining effects of drought stress conditions and +3 or +5°C temperature change. These have now been updated to include more data which has become available (Medina et al., 2015c). This shows that mycotoxigenic fungi would normally grow slower and produce, in most cases, similar or lower amounts of mycotoxins under the temperature x a_w stress (Table 1). However, in some cases, such as for *A. flavus*, it is able to grow and produce AFB₁ under higher temperatures and efficiently colonize maize, groundnuts and tree nuts under drought conditions. Thus, in CC hot spot areas this could become an emerging problem mainly in the Mediterranean and other temperate regions. Field studies suggest that there are impacts of CC based on maize. For example in Serbia, there was no aflatoxin contamination in maize in the 2009-2011 seasons. However, prolonged hot and dry weather in 2012 resulted in 69% of samples being contaminated with aflatoxins (Kos et al., 2012). Similarly, in Hungary, it was shown that an increase in aflatoxins may be due to CC conditions (Dobolyi et al., 2013).

However, there are only a few concrete examples of incidences where CC factors have been implicated. Changes in water and temperature stress may impact on *Aspergillus* section *Nigri* species and influence OTA contamination of grapes and grape-based products. Also, there may be changes in mycotoxin

prevalence among the same species (Chiotta et al., 2014; Garcia-Cela et al., 2014; Gil-Serna et al., 2014). *Alternaria alternata* produces mycotoxins such as alternariol (AOH), alternariol monomethyl ether (AME) and altenuene (AE). While for AOH maximum production is at 21°C and 0.95 a_w , AME production was maximum at the same a_w levels but at much warmer conditions, 35°C. Thus, increases in temperature may equate to shifts from AOH to AME under the forecasted conditions (Vaquera et al., 2014). Interacting environmental stresses such as a_w x temperature may also affect ratios of related compounds. For example, *F. graminearum* produces type B trichothecenes (deoxynivalenol, 3-acetyl and 15-acetyl DON). Changing a_w x temperature affects the ratio of the former three compounds both *in vitro* and in wheat grain (Figure 2; Leite, 2014). Also for *F. verticillioides* and the production of fumonisins (fumonisin B₁, B₂, B₃, B₄) a_w x temperature conditions can significantly change the ratio of these toxins in colonised maize (Mylona, 2013; Table 2). This has implications for mycotoxin contamination of staple food crops under CC scenarios as there could be a switch from the dominant mycotoxins produced to other related compounds which could become the predominant toxic contaminants.

Furthermore, in the last few years there has been a growing interest in the relationship between quantifiable “free mycotoxins” and the so called “masked mycotoxins”. Bound mycotoxins are covalently or non-covalently attached to polymeric carbohydrate or protein matrices. For example, DON can be converted to deoxynivalenol-3-D-glucopyranoside (D3G) in wheat, and the latter is difficult to detect using conventional analytical analyses. These compounds are mainly produced by plants and have been related to detoxification and resistance mechanisms exhibited by plants to counteract pathogen infection (Lemmens et al., 2005; Cirlini et al., 2012). Although by themselves they often exert lower toxicity than the original mycotoxins, some of them have been demonstrated to partially or totally cleaved under gastrointestinal conditions, resulting in similar toxic effects as their parent compound (Dall’Erta, 2013). However, to date there has been no research to examine how different environmental conditions, particularly CC related changes, will affect the production of these compounds, relative to the mycotoxins detected. As has been pointed out earlier, marked changes in plant physiology will change the pathogen-host relationship under CC factors. Research is thus needed to study whether these changes will lead to changes in these plant protection mechanisms and whether this leads to increased or decreased contamination with these compounds. The relative amount of free and masked mycotoxins may change under CC scenarios and this may require more detailed analyses in the near future.

Three way water activity (a_w) x temperature x CO₂ interactions on growth and mycotoxin production

The interactions of elevated temperature x CO₂ and extreme fluxes in drought/flooding conditions are the research areas which are now being addressed. This is because it has become clear that ecophysiological effects on plant growth and the interface with fungal disease/pest infestation could be profoundly changed.

Thus the doubling of current CO₂ levels by the 2050s' and tripling around 2100s, will have profound effects on all living species globally, with plants and fungal species being no exception. In general, photosynthesis, leaf area, plant height, total biomass and crop yield, sugar and starch content, water-use efficiency, growth, and yield have been shown to increase in the presence of higher levels of CO₂, especially in cereals (Eastburne et al., 2010; Vaughan et al., 2014; Ray et al., 2015; Vary et al., 2015). While fungi are tolerant of elevated CO₂ alone, when combined with other environmental factors they are less tolerant (Magan and Aldred, 2007b). Thus, tolerance of interacting CC factors where perhaps a doubling of existing CO₂ is predicted (350-400 to 700-800 ppm) to occur may not be problematic for the growth of many mycotoxigenic fungi. However, the growth patterns of mycotoxigenic fungi are affected by the three-way interacting conditions of temperature x water stress x elevated CO₂ (350 vs 1000 ppm). This was shown to affect growth of *F. graminearum* and *F. verticillioides* (Medina et al., 2015c). This is supported by the predicted effects of CC conditions on fungal diseases of staple grains (Chakraborty and Newton, 2011; Juroszek et al., 2011; Medina et al., 2015a) which all suggest changes in the host/pathogen interface impacting on food security. Increases or decreases in fungal disease symptoms, infection and/or pest fecundity will also contribute to the impacts on crop agronomy, yield and quality (Ziska et al., 2004; Dixon, 2012). CO₂ may also influence resilience of cereal crops and plant resistance to plant diseases (Plazek et al., 2001). A recent study also suggest that pest control using entomogenous fungi may be less effective under CC conditions which could further impact on levels of crop damage and influence mycotoxin contamination (Borisade and Magan, 2015).

Only a few studies have tried to obtain scientific data to expose economically important crops to CC conditions and examine effects on the plant, fungal infection and mycotoxin contamination. Vaughan et al. (2014) investigated the impact of elevated CO₂ on the interactions between maize and *F. verticillioides*. They found that elevated CO₂ of approx. 800 ppm CO₂ (approx. 2 x current CO₂) increased maize susceptibility to *F. verticillioides* colonisation. Interestingly, fumonisin B₁ (FB₁) production was unaffected by these interactions. They found that FB₁ contamination was not proportional to the increase in the biomass of *F. verticillioides*, and the amount per unit pathogen was reduced in elevated CO₂. This suggested that there were some physiological effects on maize agronomy under CC treatments, especially during silking, which impacted on *F. verticillioides* infection and the rate of contamination with fumonisins. However, drought stress was not included in the experimental design. Similar physiological effects were observed in wheat and FHB and STB diseases when CO₂ was doubled (Vary et al., 2015). Previous studies have certainly suggested that a key gene in the fumonisin biosynthetic pathway (*FUM 1*) is significantly affected by changes in environmental stress (Marin et al., 2010).

Medina et al. (2015a) studied the impact of CC scenarios on growth and AFB₁ production by *A. flavus* (NRRL type strain) on maize-based media and subsequently on stored maize grain. The treatments included:

(a) 34 and 37°C; (b) imposed drought stress – 0.97 to 0.95 and 0.91 a_w; and (c) CO₂ was increased from 350 to 650 and 1000 ppm. The effects on growth of *A. flavus* and expression of some aflatoxin cluster genes (*aflD*, *aflR*) and phenotypic AFB₁ production were examined. This was the first time a combination of expected CC factors were used to establish the potential effects of these scenarios on the ecophysiology of mycotoxigenic fungi. This showed that growth of *A. flavus* was relatively unaffected. In contrast, the three-way interacting conditions had a profound, statistically significant stimulatory effect on AFB₁ production (70 x the control in *in vitro* studies), especially under drought stress at 37°C and 650 and 1000 ppm CO₂ exposure. Studies of the relative expression of the biosynthetic genes in the aflatoxin pathway also corroborated these findings (Medina et al., 2015a). Research on maize grain suggested that AFB₁ production was increased but in a lower range than *in vitro* on a conducive medium. Also, *aflD* and *aflR* expression were increased by the interacting CC treatments. Table 3 compares some of the results obtained *in vitro* and on maize grain with regard to some aflatoxin cluster genes and AFB₁ production. Payne et al. (2016) have suggested that RNA sequencing of the *A. flavus* genome when infecting maize kernels under interacting CC conditions would provide insights into the key groups of genes involved in the observed changes. Medina et al. (2017) have now used a transcriptomic approach and examined the impact on overall up and down regulated genes in relation to a_w x temperature conditions and found that there are changes in relation to the secondary metabolite gene clusters (aflatoxin, cyclopiazonic acid), universal regulators, sugar transporters, other stress-related pathways. These are indicative of changes in relation to the three way interaction between CC factors. It is important to identify whether any switches in biosynthetic pathways may occur resulting in other secondary metabolites being produced rather than aflatoxins or cyclopiazonic acid by *A. flavus* under such environmental stresses.

Other recent studies with *Aspergillus* section *Circumdati* and Section *Nigri* species (*A. wetserdijkiae*, *A. ochraceus*, *A. steynii*; *A. carbonarius*) *in vitro* and on stored coffee beans in relation to OTA production under CC change conditions have been carried out (Akbar, 2015; Akbar et al., 2016). These suggest that for some species there may be a stimulation of OTA production (e.g. *A. wetserdijkiae*) while for other species (*A. carbonarius*) there was a reduction in toxin contamination (Figure 3). Table 4 shows the statistical effect of the individual and interacting CC factors on all the strains and species tested (Akbar et al., 2016). This suggests that differential effects may occur for individual mycotoxigenic species and that in depth knowledge of potential impacts are needed on strains of a specific species before making predictions of the impact of CC factors in relation to an increase or decrease in mycotoxin production.

It should be noted that fungi have the ability to adapt to change, especially mycotoxigenic fungal pathogens, and may thus become of primary concern in the coming 20-25 years. Because fungi have a high degree of plasticity and are able to adapt to interacting environmental and other stresses, e.g. fungicides, they may rapidly evolve to adapt to and dominate crop ecosystems under CC conditions (Vaughan et al.,

2014; Vary et al., 2015; Battilani et al., 2016).

3. Acclimatisation of fungal pathogens and impacts on colonisation and mycotoxin production

There have been practically no studies on the impact that acclimatisation of crops or fungal pathogens and pests may have on the plant/disease pest interface. A recent study by Vary et al. (2015) showed that CC factors does affect the physiology of wheat with comparison of exposure to existing (390 ppm) and elevated CO₂ (780 ppm) caused changes in leaf physiology and stomatal production on adaxial and abaxial surfaces. Of particular interest was the acclimatisation of *Septoria tritici* blotch (STB) disease and *Fusarium* Head Blight (FHB; *F. graminearum*) for 10-20 generations in elevated CO₂. These acclimatised strains of the pathogens caused significantly increased disease progression in terms of foliar symptoms of STB and FHB and for the latter this was more pronounced and also confirmed by molecular analysis of fungal biomass. This study did not examine effects on type B trichothecenes (e.g. deoxynivalenol; 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol) which would have been interesting.

Work at Cranfield has been examining the relationship between *A. flavus* and contamination of pistachio nuts with AFB₁. This is a major problem in the industry in meeting the EU legislative limits for this mycotoxin, especially from Iran and the Mediterranean region. Strains of *A. flavus* isolated from pistachio nuts grow optimally at 35°C and 0.98-0.95 a_w on a pistachio nut-based medium and on pistachio nuts. Strains were acclimatised at 37°C and 0.98 a_w for 5 generations at 1000 pm CO₂ on a pistachio nut-based medium. These were then subsequently compared with the non-acclimatised strains in terms of growth and AFB₁ production in control conditions (350 ppm CO₂/35°C/0.98 a_w) and under CC scenarios (1000 ppm/37°C/0.98 a_w) on layers of pistachio nuts. Figure 4a shows the effects on growth of two strains of *A. flavus*. This shows that for 1 strain acclimatisation influenced growth while for the other strain there was no significant effect on colonisation of pistachio nuts. For this strain AFB₁ production was significantly stimulated, especially after 10 days colonisation after acclimatisation, while for the other strain there was no significant increase (Figure 4b; Baazeem, Medina and Magan, unpublished data). This certainly suggests that there may be intra-strain differences in effects of acclimatisation and this may influence mycotoxin production on such commodities as mixed populations of contaminant fungi often occurs. More focus needs to be on acclimatisation of fungal pathogens and their interactions with other mycobiota and the interface with the plant to obtain more accurate data on implications for mycotoxin contamination, especially of staple crops under CC scenarios.

Increases in pest reproduction rates under acclimatisation scenarios could increase damage to ripening crops (during anthesis in wheat; silking in maize) and facilitate increased infection by mycotoxigenic fungal pathogens with potential for increased contamination with mycotoxins. Previous studies of the bioenergetics and pest utilisation of cereals suggests that associated calorific losses may be significant under present climatic conditions (Demianyk and Sinha, 1988; Campbell and Sinha, 1990). However, with increased

fecundity under CC conditions, especially acclimatisation, will these impacts be significantly greater? The area of acclimatisation needs further investigation and could have significant implications for the prediction of impacts of CC on mycotoxigenic species of *Aspergillus*, *Penicillium* and *Fusarium*.

4. Climate change and predictions of mycotoxin contamination

Previously, modelling the potential impacts of CC scenarios on mycotoxins have focused on DON contamination of wheat in northern Europe (Van Der Fels-Klerx et al., 2012a,b) and a another study focused on aflatoxins contamination of maize, wheat and rice grown in Europe (Battilani et al., 2012; 2016). They both applied multidisciplinary inputs of information obtained from climate projections, crop phenology and fungal/mycotoxin production. Recently, Van Der Fels-Klerx et al. (2016) also examined *Alternaria* and its mycotoxins in tomatoes. Previous studies have also examined on a single country basis CC scenarios on FHB prediction and the change in the relative risk of contamination with mycotoxigenic species. However, many of these models have included an increase in temperature of +2 and +5°C, or increased CO₂ levels. For the DON in wheat predictions two empirical models were integrated to include wheat phenology and DON contamination in the regions of Europe of interest. In addition climate model projections for 2031-2050 were included from various available data bases. This included projected temperature fluctuations and rainfall patterns, solar radiation and estimated were based on 50 x 50 km grids across the area of interest. However, CO₂ changes were not included in these data bases. These results showed that under CC was predicted a 1-2 weeks earlier date for flowering and maturation of winter wheat, with DON contamination expected to increase in most of the region examined based on the simulations carried out.

Battilani et al. (2012; 2016) used IPCC CC models based on an increase in +2°C and +5°C to examine impacts on AFB₁ in maize wheat and rice in Europe. Again meteorological data sets were obtained from the LARS weather generator and they also used a 50 x 50 grid system for evaluating impacts across Europe. The aflatoxin (AFLA-maize) model was a mechanistic one based on the prediction of *A. flavus* infection and AFB₁ production on a daily basis (Battilani et al., 2013). This produced an Aflatoxin Risk Index (AFI), linked to AFB₁ contamination observed in field measurements. The inputs into the model showed that the areas in which maize would be cultivated would be larger across Europe. However, the AFI index would cover a wider area of Europe with a significantly higher risk of AFB₁ contamination. The hot spots for this were central Spain, Italy, and the Balkans for the +2°C scenario, and a much wider range of risk regions in the +5°C scenario, although in the latter case the relative AFB₁ contamination would be less.

Recently a mechanistic study has examined *Alternaria* infection of tomatoes in two regions of Europe, in Spain (Extramadura region) and in Poland (Krobia). Using the weather inputs under different CC scenarios (Van der Perre et al., 2015; Van Der Fels-Klerx et al., 2016). These results which modelled different levels of industrial development showed that in Spain the temperature changes (18.2-38.2°C) would minimize growth

of *Alternaria* and mycotoxin contamination because at the higher temperatures growth and mycotoxin production are minimal (Lee et al., 2014); while in Poland under the projected temperatures (14.2-28.4°C) there would be an increase in *Alternaria* contamination with the range being within the optimum range for production of alternariol, its monomethyl esters, altenuene and tenuazonic acid (Lee et al., 2014).

Van Der Fels-Klerx et al. (2016) have suggested that there are specific gaps in relation to predictive modelling of CC impacts on mycotoxin contamination. There has been relatively limited validation of the models with the exception of the few mentioned previously. Perhaps better inputs on efficacy of a_w x temperature x CO₂ interactions on growth/mycotoxin boundary conditions are necessary to make empirical models more robust. More detailed information is necessary to input into the predictive models being developed. Van Der Fels-Klerx and Booi (2010) suggested using a geographical orientated decision support system to develop prediction on a regional or continental basis. The development of more accurate prediction really depends on the resilience of important staple food commodities under expected CC scenarios, and linking this to the life cycle for germination, growth and mycotoxin production cycle of economically important mycotoxigenic fungi especially under the three way-interacting CC variables. Furthermore, while for some fungal species growth or mycotoxin production remains similar under the forecasted CC conditions, for others, environmental changes may have significant effects, e.g., increasing toxin production or a switch in the major mycotoxin produced or the ratio of different mycotoxins. Much more data is required to enable a better understanding of the fungal and plant ecophysiology and the pathogen/host interface to improve the potential for making more accurate and relevant global predictions on the impact of mycotoxins on the food security of staple food crops.

5. Conclusions

There is clearly a need for more detailed information on the impact of CC scenarios on the germination, growth and mycotoxin production by the key mycotoxigenic species in the genera *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria*. It should be borne in mind that optimum conditions for growth are not always the same as for mycotoxin production. Phenological changes in crop production and the interface with the mycotoxigenic fungal pathogens under CC scenarios will further influence the levels of contamination with a specific mycotoxin. Key questions include whether under CC scenarios will mycotoxin production patterns change? Will other mycotoxins at present considered of secondary importance become more abundant and thus more important in the future? What is going to happen with masked mycotoxins? Are the current control/mitigation strategies going to be effective in the future? Will interactions between mycotoxigenic fungi and other microbiota in the phyllosphere and in the rhizosphere ecological niches change resulting in different community structures and dominance of different species? Will the agricultural practices, including Good Agricultural Practices and Hazard Analysis Critical Control Point management systems currently used have to change in order to minimize mycotoxin contamination when marked environmental shifts and fluxes

become the norm? Are mycotoxigenic fungal populations going to shift their location in the coming years, as has been predicted for other fungal diseases? This may of course be intimately linked to pest population shifts and changes in diversity and their movement globally. More research is required to address these key questions to effectively predict the level of risk of different mycotoxins in economically important staple food crops and to understand whether they are resilient enough to tolerate the expected CC conditions. Existing global players in the agrifood market include countries such as Brazil and Argentina and parts of Asia including China and India. These regions and parts of Africa are considered hot spots for the impacts of CC. Thus, from a food security perspective these questions need to be addressed for more accurate prediction of the impacts of CC. Without this type of information food sustainability may well be compromised in many regions of the world, with LMICs taking the brunt of the impacts possibly resulting in negative social consequences.

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Figure Legends

Figure 1. Climate change factors as drivers of change in the classic Crop/fungal disease/pest and Environment triangle which may occur due to the pressure of climate change scenarios.

Figure 2. Differential production of type B trichothecenes by *Fusarium graminearum* on wheat grain stored at 25°C for 10 days at different water activity levels (from Leite, 2014).

Figure 3. Effect of existing (30°C, 400 ppm, different water activity condition) and climate change conditions (35°C, 1000 ppm; different aw conditions on (a) strain of *Aspergillus westerdijkiae* and (b) strain of *Aspergillus carbonarius* on stored coffee. Bars indicate standard error of the mean. Note: scale ranges are different for OTA production by the two strains.

Figure 4. (a) Growth rates (mm/day) of non-acclimatised and acclimatised strains of *A. flavus*, AB3 and AB10 (5 generations) on layers of raw pistachio kernels incubated at 35°C + 400 ppm CO₂ or 37°C + 1000ppm CO₂

at 0.98 a_w ; (b) effect of treatments on aflatoxin B₁ (AFB₁) production. Different letters indicate significant difference ($p < 0.05$).

Table 1. Changes in growth and mycotoxin production by *Alternaria*, *Fusarium*, *Aspergillus* and *Penicillium* species as a result of an increase in temperature of 3 or 5°C under different water-stress conditions.

	Growth					Toxins			
	a_w	$\mu_{\max}$ range/ Temp	μ_{+3}	μ_{+5}		a_w	$\tau_{\max}$ range/ Temp	τ_{+3}	τ_{+5}
<i>Alternaria alternata</i>	0.95	2 – 1 / 25	2 - 1	1 - 0.5	Altenuene	0.95	100 - 40/25	40 - 20	20 - 5
	0.90	0.1 – 0.5 / 25	0.5 - 0.1	NG		0.90	20 - 5/25	5 - NP	NP
					Alternariol	0.95	500 - 100/25	40 - 20	20 - 5
						0.90	20 - 5/25	NP	NP
					Alternariol monomethyl ether	0.95	400 - 100/25	100 - 10	NP
<i>Alternaria tenuissima</i>						0.90	100 - 10/25	NP	NP
	0.98	13.5-14/30	8 - 9.5	7.5 - 9	Altetoxin II	0.98	50 – 150/30	100 - 250	125 - 250
	0.95	5-6/30	4.5 - 5.5	4.5 - 5.5		0.95	600 - 2100/30	500 - 1000	400 - 1300
<i>Fusarium proliferatum</i>	0.95	4 - 3/28	3 - 2	2 - 1	Fumonisin	0.95	>1000/20	1000 - 100	100 - 50
	0.90	0.5 - 0.1/28	NG	NG		0.93 ^a	50- 10/15	50 - 10	50 - 10
<i>Fusarium verticillioides</i>	0.95	4 - 3/25	4 - 3	4 - 3	Fumonisin	0.95	10000 - 1000/20	10000 - 1000	1000 - 100
	0.90	0.5 - 0.1/25	0.5 - 0.1	NG		0.93 ^a	10/15	50 - 10	50 - 10
<i>Fusarium culmorum</i>	0.95	3 - 1/20	3 - 1	3 - 1	DON	0.95	1 - 0.25/20	0.25 - 0.1	0.1 - 0.01
	0.90	1 - 0.1/20	1 - 0.1	1 - 0.1		0.93 ^a	0.25 - 0.01/20	NP	NP
<i>Fusarium graminearum</i>	0.95	>4 /20	4 - 2	2 - 1	DON	0.95	1 - 0.1/20	1 - 0.1	0.1 - 0.01
	0.90	1 - 0.1/20	1 - 0.1	1 - 0.1		0.93 ^a	NP	NP	NP
<i>Fusarium langsethiae</i>	0.98	4.6 – 5.1/25	4.6 – 4.1	3.6 – 3.1	T-2 +HT-2	0.98	10 – 11/25	13-14	14 - 15
	0.95	2.6 - 2.1/25	1.1 - 1.6	1.6 – 1.1		0.95	0 – 1/25	0 - 1	0 - 1

<i>Aspergillus westerdijkiae</i>	0.95	5 - 4.5/30	4.45 - 3.95	4.25 - 3.75	Ochratoxin A	0.95	1065.7 - 1014.9/30	719.4 - 685.1	488.5 - 465.3
	0.90	1.85 - 1.35/30	1.85 - 1.35	1.80 - 1.30		0.90	54.1 - 51.6/30	51.6 - 49.2	49.9 - 47.6
<i>Aspergillus carbonarius</i>	0.95	>6/30	>6	6 - 5	Ochratoxin A	0.95	2000 - 1500 /20	1000 - 500	500 - NP
	0.90	4 - 3/30	4 - 3	4 - 3		0.90	1000 - 500 /20	500 - NP	500 - NP
<i>Aspergillus flavus</i>	0.95	6.9 /35	5.6	5.0	Aflatoxin B1	0.95	3082 - 2278 / 37	102 - 138	6.1 - NP
	0.90	2.9 /37	1.4	0.7		0.90	448.5 - 331.5 / 37	1- NP	NP
<i>Penicillium verrucosum</i>	0.97	2.2- 2.4/25	1.8- 1.9	0.9-1	Ochratoxin A	0.97	60-50 /20	75-70	> 80
	0.95	3-4 /25	4 - 3	3 - 2		0.95	>50 / 20	>50	50 - 25
	0.90	2.5 - 1/25	2 - 1	2 - 0.5		0.90	50 - 30 /20	50 - 30	50 - 30 (5-3 ^a)
<i>Penicillium nordicum</i>	0.97	1.4- 1.5/20	1.5	2-1	Ochratoxin A	0.97	7-6 /20	6-5	4-3
	0.94	1.8- 2.0/20	1.8- 1.7	1.4- 1.7		0.94	7-6 /20	7-6	7-6
	0.90	1.2- 1.3/20	1.2- 1.3	1.2- 1.4		0.90	8-7 /20	9-8	9-8
<i>Penicillium expansum</i>	0.98	9.1/20			Patulin	0.98	3.5-0.55/20	NS	NS
	0.95	6.9 - 5.5 ^a / 25	6.9 - 5.5	6.9 - 5.5		0.95	NS	NS	NS
	0.90	2.9 - 1.6 ^a / 25	2.9 - 1.6	2.9 - 1.6		0.90	NS	NS	NS

$\mu_{\max}$: Maximum growth rate (mm day⁻¹); μ_{+3} : Growth rate increasing 3 centigrade degrees; μ_{+5} : Growth rate increasing 5 centigrade degrees; $\tau_{\max}$: Maximum toxin production ($\mu\text{g ml}^{-1}$); τ_{+3} : Predicted toxin increasing 3 centigrade degrees; τ_{+5} : Predicted toxin increasing 5 centigrade degrees; NG, no growth; NP, no toxin production; NS, not studied.

For *Aspergillus* and *Penicillium* species: $\tau_{\max}$: Maximum toxin production (ng g⁻¹); τ_{+3} , predicted toxin production with 3°C increase; τ_{+5} , predicted toxin production with 5°C increase.

^a Minimum water availability for toxin production.

Table 2. The ratio of the four fumonisins (FB₁, FB₂, FB₃, FB₄) produced by *Fusarium verticillioides* when colonising maize at different temperatures and water activities for 10 days. The ratios in bold indicate changes in relative amounts of the two mycotoxins.

(a) Ratio of FB₁/FB₂

Temperature (°C)	15	20	25	30
Water activity (a _w)				
0.97	5.86	4.28	4.95	7.61
0.95	6.80	4.69	5.33	5.81
0.91	5.79	7.67	5.07	6.67

(b) Ratio FB₁/FB₃

0.97	15.16	6.30	9.07	6.51
0.95	11.00	7.61	6.41	6.22

0.91	10.31	14.33	11.14	12.11
(c)	Ratio FB ₂ /FB ₃			
0.97	2.78	1.48	1.82	0.86
0.95	1.62	1.65	1.24	1.07
0.91	2.14	1.83	2.51	1.97
(d)	Ratio FB ₂ /FB ₄			
0.97	11.53	5.22	8.46	9.06
0.95	16.16	6.31	7.24	7.67
0.91	10.05	13.29	13.55	14.17

Table 3. Relative effect of interacting climate change environmental factors (+3°C; 1000 ppm CO₂; drought stress) on *A. flavus* growth, a regulatory gene (*aflR*) in the biosynthetic pathway for aflatoxin production and aflatoxin B₁ production (a) *in vitro* and (b) on stored maize grain. Numbers refer to fold variation relative to the controls at 30°C and 350 ppm CO₂ and the same a_w levels (data for (a) from Medina et al., 2015a; (b) unpublished data; Medina, Rodriguez & Magan).

(a) *A. flavus* growth on an *In vitro* on a conducive medium (10 days)

Temperature (°C)	a _w	CO ₂ (ppm)	<i>aflR</i>	Aflatoxin B ₁
34	0.97	650	=	=
		1000	=	=
	0.95	650	=	=
		1000	↑(x 3.6)=	
	0.92	650	↑(x 24.4)	↑(x 2.8)
		1000	↑(x 2.0)	(x 2.0)↑
37	0.97	650	=	↑↑(x 30.7)
		1000	=	↑↑(x 23.8)
	0.95	650	↑(x 14.6)	↑↑(x 79.2)
		1000	↑↑(x 43.9)	↑↑(x 78.5)
	0.92	650	↑↑(x 40.4)	↑↑(x 15.1)
		1000	↑↑(x 1680)	↑↑(x 23.8)

(b) *A. flavus* colonisation of stored maize grain (10 days)

Temperature (°C)	a _w	CO ₂ (ppm)	<i>aflR</i>	Aflatoxin B ₁
30	0.99	650	=	=
		1000	=	=
	0.91	650	↑(x 2.0)	↑(x 2.7)
		1000	=	=
37	0.99	650	↑(x 2.0)	↑(x 3.6)
		1000	↑(x 3.0)	↑(x 5.0)
	0.91	650	↑(x 2.0)	↑(x 1.8)
		1000	=	↑(x 1.5)

Table 4. Summary statistical table of effect of climate change treatments on ochratoxin A production by *Aspergillus* section *Circumdati* and *Nigri* species and strains at different water activity (a_w) and temperatures using Kruskal-allis Test (non-normality data) and ANOVA (normality data; from Akbar et al., 2016). Numbers in parentheses refer to strain numbers).

Temperature 30°C

Factors

Strains	CO ₂	a _w	a _w x CO ₂
<i>A. westerdijkiae</i> (B 2)	S ^b	S ^b	S ^b
<i>A. westerdijkiae</i> (CBS 121986)	S ^{*b}	NS ^b	NS ^b
<i>A. niger</i> (A 1911)	S ^{*b}	S ^b	S ^b
<i>A. carbonarius</i> (ITAL 204)	NS ^b	S ^b	S ^b
<i>A. ochraceus</i> (ITAL 14)	NS ^a	S ^a	N/A

Temperature 35°C

<i>A. westerdijkiae</i> (B 2)	S ^a	S ^a	N/A
<i>A. westerdijkiae</i> (CBS 121986)	S ^a	NS ^a	N/A
<i>A. niger</i> (A 1911)	NS ^b	S ^b	NS ^b
<i>A. carbonarius</i> (ITAL 204)	NS ^a	S ^a	N/A
<i>A. ochraceus</i> (ITAL 14)	NS ^a	S ^a	N/A

Temperature 35°C

	CO ₂ (1000ppm)	a _w	Temp 30+35°C
<i>A. westerdijkiae</i> (B 2)	NS ^a	NS ^a	S ^a
<i>A. westerdijkiae</i> (CBS 121986)	S ^a	NS ^a	S ^a
<i>A. niger</i> (A 1911)	NS ^a	NS ^a	S ^a
<i>A. carbonarius</i> (ITAL 204)	NS ^a	S ^a	NS ^a
<i>A. ochraceus</i> (ITAL 14)	NS ^a	NS ^a	S ^a

S significant ($P < 0.05$)

NS not significant ($P > 0.05$)

^a Kruskal-Wallis test

^b ANOVA

N/A Not Applicable

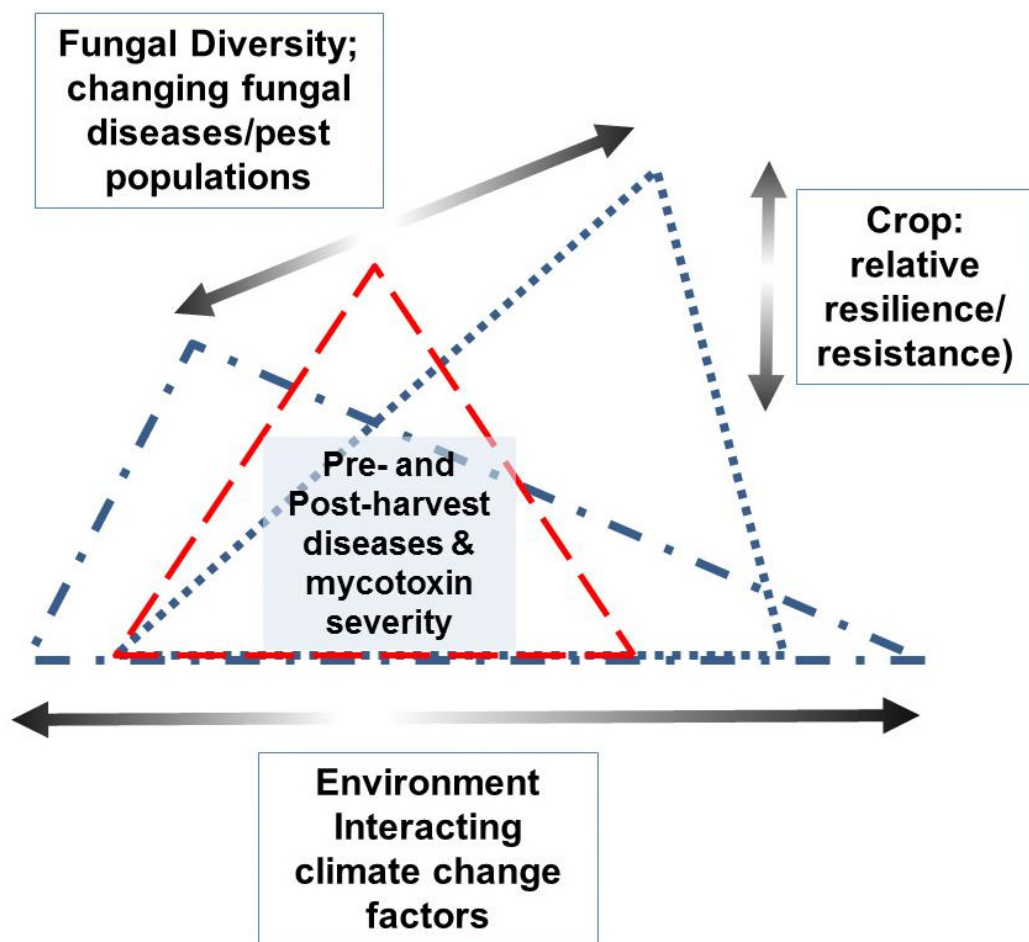


Figure 1. Medina et al.

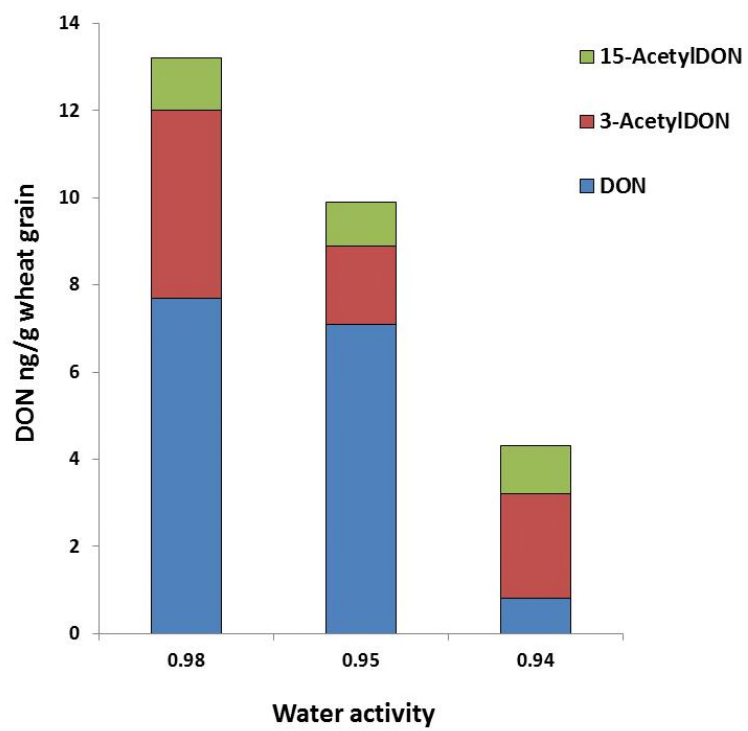


Figure 2. Medina et al.

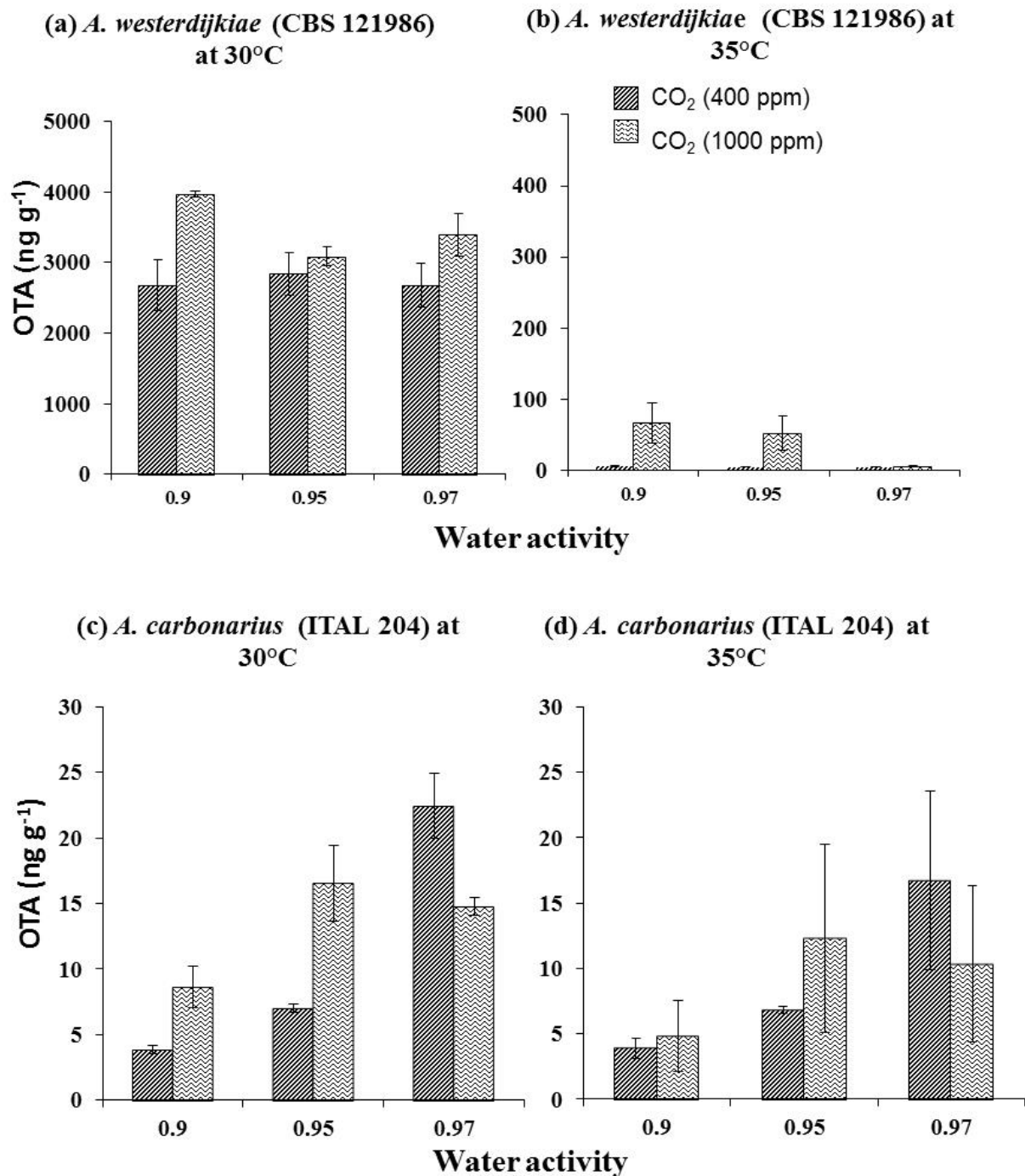


Figure 3. Medina et al.

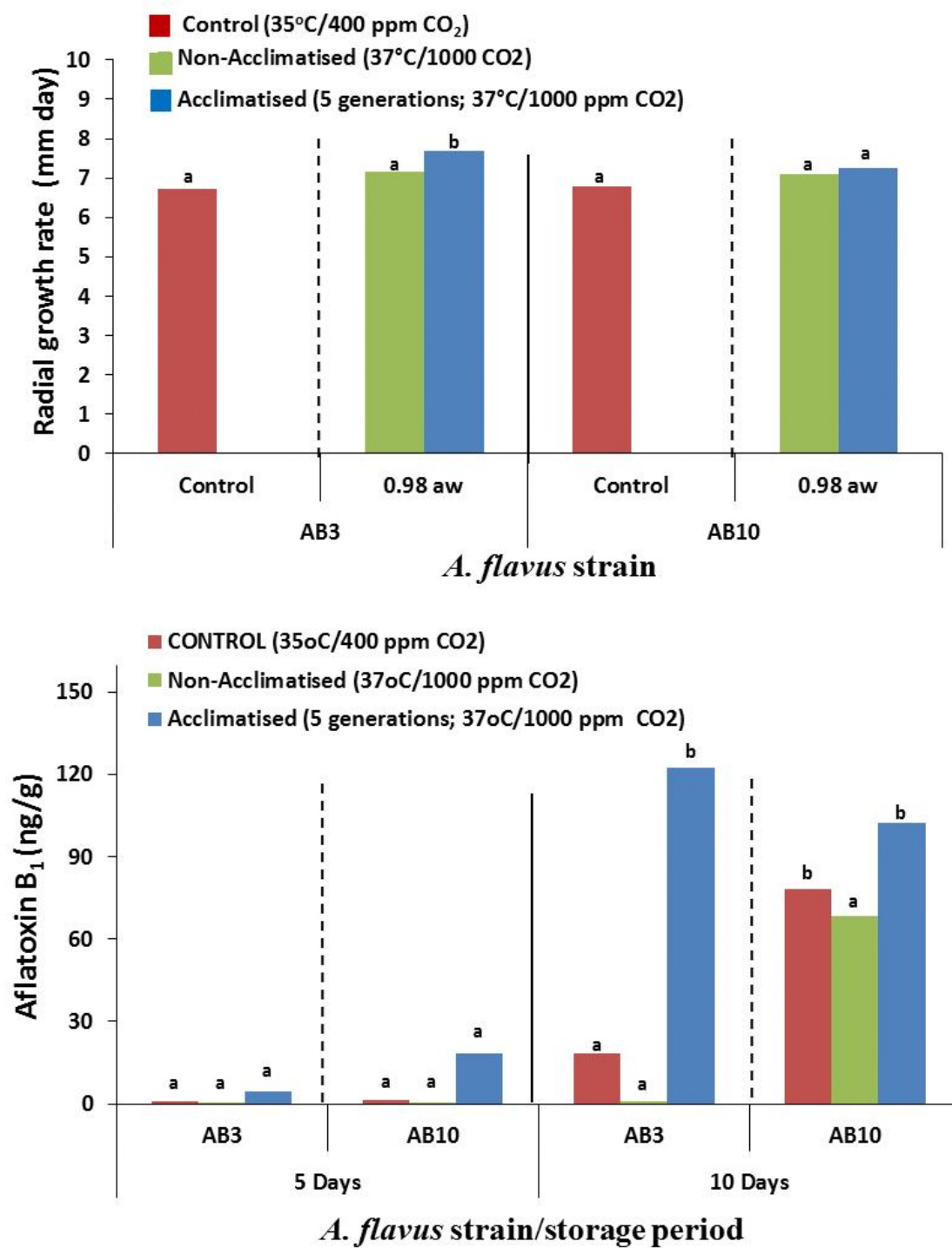


Figure 4. Medina et al.



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