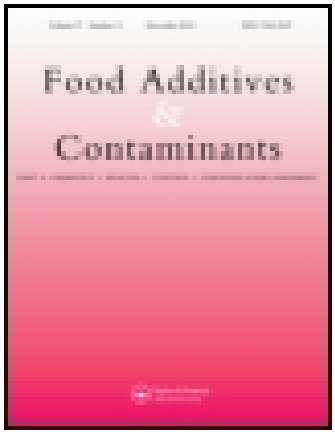

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Biological control as a strategy to reduce the impact of mycotoxins in peanuts, grapes and cereals in Argentina

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Biological control as a strategy to reduce the impact of mycotoxins in peanuts, grapes and cereals in Argentina

S.N. Chulze[a,b]*, J.M. Palazzini[a,b], A. M. Torres[a,b], G. Barros[a,b], M.L. Ponsone[b,c], R. Geisen[d], M. Schmidt-Heydt[d] and J. Köhl[e]

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Mycotoxins including aflatoxins, deoxynivalenol, fumonisins and ochratoxin A are among the main fungal secondary metabolites detected as natural contaminants in South America in different commodities such as peanuts (aflatoxins), cereals (deoxynivalenol and fumonisins) or grapes (ochratoxin A). Different strategies including crop rotation, tillage practices, fungicide application and planting less susceptible cultivars are used in order to reduce the impact of these mycotoxins in both food and feed chains. The development of fungicide resistance in many fungal pathogens as well as rising of public concern on the risks associated with pesticide use led to the search for alternative environmentally friendly methods. Biological control of plant pathogens and toxigenic fungi offers an alternative that can complement chemical control in the frame of an integrated pest management to reduce the impact of mycotoxins in the food and feed chains. The advances made in Argentina on reducing the impact of toxigenic fungi and mycotoxins in peanut, grapes and cereals using the biocontrol strategy are summarised. Native bacteria, yeasts and filamentous fungi have been selected to evaluate them as potential biocontrol agents. Field trials showed that *Bacillus subtilis* RC 218 and *Brevibacillus* sp. RC 263 were effective at reducing deoxynivalenol accumulation in wheat. The application of *Clonostachys rosea* isolates on wheat stubble reduced *Fusarium* colonisation on the stubble. *Bacillus amyloliquefaciens* and *Microbacterium oleovorans* showed good activity to control both *Fusarium verticillioides* growth and the accumulation of fumonisins at pre-harvest stage in maize. Control of toxigenic *Aspergillus flavus* and aflatoxin accumulation in peanuts was achieved using a native atoxigenic *Aspergillus flavus* strain based on competitive exclusion of the toxigenic strains. *Kluyveromyces thermotolerans* strains were used as biocontrol agents to reduce the impact of *Aspergillus* section *Nigri* and ochratoxin A accumulation in grapes.

Keywords: biocontrol; *Aspergillus*; *Fusarium*; mycotoxins

Introduction

Mycotoxins, secondary metabolites produced by filamentous fungi grown on several crops, have a strong impact on the quality and safety of food and feed products worldwide. Among them aflatoxins, deoxynivalenol, fumonisins and ochratoxin A are the main mycotoxins detected as natural contaminants in South America in different commodities such as peanuts (aflatoxins), cereals (deoxynivalenol and fumonisins), and grapes (ochratoxin A) (Garrido et al. 2012; Rodrigues & Naehrer 2012; Schatzmayr & Streit 2013). Different strategies including crop rotation, tillage practices, fungicide application and planting less susceptible cultivars are used to reduce the impact of these mycotoxins in both food and feed chains.

The development of fungicide resistance in many fungal pathogens as well as rising of public concern on the risks associated with pesticides use led to the challenge for researchers for developing environmentally friendly alternatives for combating crop diseases. Biological control of plant pathogen and toxigenic fungi offers an alternative that can complement chemical control in the frame of an integrated pest management (IPM) to reduce the impact of mycotoxins in food and feed chains.

Microorganisms as biocontrol agents have a narrow spectrum of activity compared with the chemical fungicides and are considered as plant protection products in most countries.

Peanut (*Arachis hypogaea* L.) is an economically important crop in Argentina. The exports of edible peanuts from the country are around 400 000 tonnes, ranking it first as a peanut exporter in the world. Around 65% of the Argentine peanut exports go to the European Union (mainly the Netherlands, Germany, the UK, France, Greece and Poland); other consistent importers are the United States and Canada (Cámara Argentina del Maní 2012). In Argentina, around 90% of peanut production is localised in Córdoba province where the contamination cycles with aflatoxins are sporadic. However, in some

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years aflatoxin contamination can occur and the levels detected may exceed the maximum levels established by Mercosur and European Commission regulations. This situation represents great economic losses for the peanut industry. Aflatoxin control in peanuts relies on several approaches both pre- and post-harvest such as good cultural practices, irrigation, use of drought resistant cultivars and post-harvest sorting by electronic devices and blanching (Dorner 2008; Torres et al. 2014).

Wheat (*Triticum aestivum* L.) is the most important small-grain cereal crop in the world, with an estimated production of 716 million tonnes in 2013 (FAO 2014). *Fusarium* head blight (FHB) of wheat caused by species within the *Fusarium graminearum* complex is a devastating disease that causes extensive yield and quality losses to wheat in humid and semi-humid regions of the world. Besides the economic losses due to reduction in grain yield, the main problem is the potential mycotoxin contamination of wheat mainly with deoxynivalenol (DON) (McMullen et al. 2012). During the last 50 years, several epidemics of FHB of varying degrees of severity have occurred in Argentina, *F. graminearum* being '*sensu stricto*' the main pathogen associated with FHB (Ramirez et al. 2007; Kikot et al. 2011; Palazzini, Fumero, et al. 2013).

Different strategies are used to reduce the impact of FHB including crop rotation, tillage practices, fungicide application and planting less susceptible cultivars. None of these strategies by themselves is able to reduce the impact of this disease. Biological control offers an additional strategy and can be used as part of an integrated management of FHB (Gilbert & Haber 2013).

Grapes and grape-derived products have a significant worldwide importance. Most grapes are used for wine-making (71%), about 27% are consumed fresh and only a minor portion (2%) are consumed as dried fruits. Both grapes and grape-derived products can be contaminated by ochratoxin A (OTA). This toxin is one of the most important mycotoxins of concern for human and animal health. It is produced by a number of fungal species that can colonise a range of food products. These species include *Aspergillus* section *Nigri*, *Penicillium verrucosum, Penicillium nordicum* and *Aspergillus ochraceus* that predominantly colonise cereals, coffee, cocoa and grapes. Grapes and their derived products as grape juice, raisins and wine are frequently contaminated with OTA (Zimmerli & Dick 1996). This toxin showed nephrotoxic, immunotoxic, genotoxic, neurotoxic and teratogenic properties. The IARC has classified OTA as a possible human carcinogen, group 2B (IARC 1993). Based on the available scientific toxicological and exposure data, the European Union established 2 μg kg^{-1} as the maximum permitted level for OTA in wines (European Commission 2006).

Prevention of growth of OTA-producing fungi is the most effective strategy for controlling the entry of this mycotoxin in the food and feed chains. Biological control has been proposed as a strategy to reduce the impact of ochratoxigenic species. Among the microorganisms considered as potential biological control agents, yeasts are particularly promising due to their capacity to colonise plant surfaces or wounds for long periods under dry conditions (Bleve et al. 2006; Dimakopoulou et al. 2008), their simple nutritional requirements, the capacity to grow on inexpensive media, and the ability to survive in a wide range of environmental conditions. Furthermore, yeasts do not produce anthropotoxic compounds (Wilson & Wisniewski 1989).

Maize (*Zea mays* L.) is a major staple food worldwide and also an important feed crop including the use as forage crop for silage. The main fungal species and toxins associated to maize are *Aspergillus flavus* and aflatoxins, *Fusarium verticillioides*, *Fusarium proliferatum* and fumonisins, *Fusarium graminearum* and trichothecenes and zearalenone. *A. flavus* can infect maize pre- and post-harvest and an increase in aflatoxin content can occur if drying and storage are poorly managed. Although *Fusarium* species are predominantly considered as field fungi, it has been reported that fumonisin production may occur post-harvest when the storage conditions are inadequate (Chulze 2010). Both aflatoxins and fumonisins are relevant in maize and maize-based foods and feeds due to their widespread occurrence and co-occurrence.

This article reviews the advances achieved in Argentina to reduce the impact of *Aspergillus* and *Fusarium* species and their mycotoxins in peanuts, grapes and cereals by using biocontrol

Biological control of *Aspergillus* section *Flavi* in peanuts

Biological control achieved by applying competitive non-toxigenic strains of *A. flavus* and/or *A. parasiticus* to the soil of developing crops has been developed for reducing pre-harvest aflatoxin contamination of crops. This approach is based on the premise that spores of non-toxigenic strains compete with naturally occurring toxigenic strains for infection sites for growth on peanut and for essential nutrients. It has been demonstrated that the pre-harvest application of the biocontrol agents has a carry-over effect and also protects peanuts from contamination during storage (Dorner & Cole 2002).

Biological control using competitive exclusion of toxigenic strains by non-aflatoxigenic strains has been demonstrated under field conditions in cotton (Cotty 1994), peanuts (Dorner et al. 2003; Dorner & Lamb 2006; Pitt & Hocking 2006; Alaniz Zanon et al. 2013) and maize (Abbas et al. 2006; Atehnkeng et al. 2008, 2014).

The effectiveness of pre-harvest biocontrol strategies using atoxigenic strains is based on competition for substrate and space, the potential production of inhibitory metabolites, and on their inability to recombine with

native toxigenic strains, thus preventing the reacquisition of aflatoxigenicity (Abbas et al. 2011; Ehrlich 2014).

During the selection of atoxigenic strains as potential biocontrol agents their phenotypic and genotypic characteristics must be considered. It is preferable to select strains that have lost part or the whole aflatoxin biosynthetic cluster (Barros et al. 2007).

The efficacy of a native non-aflatoxigenic *A. flavus* AFCHG2 strain to reduce aflatoxin production in peanuts was evaluated under field trials in Argentina. The production of the non-toxigenic *A. flavus* strain by solid-state fermentation was evaluated on three substrates: autoclaved long-grain rice, wheat seeds and bran (Chiotta et al. 2007). The three substrates produced equivalent loads of *A. flavus* AFCHG2 (mean 10^6 cfu g^{-1}). Long-grain rice was chosen to prepare the final inoculum for the field trials since this substrate gave a suitable granular and dried product, which facilitated dispersion of the biopesticide using standard farm machinery as fertiliser spreaders. The inoculum application rate used in this study was 50 kg/hectare higher than other rates employed in peanuts in the United States, where the atoxigenic strains are routinely applied one per crop at 10–20 kg/hectare. In this case the biopesticides, Afla-guard was based on hulled barley coated with conidia of *A. flavus* (NRRL 218882). The strain is a not aflatoxin nor cyclopiazonic acid producer (Dorner et al. 1998; Dorner & Cole 2002; Dorner & Horn 2007) but lower than other application rates evaluated in peanuts in Australia, where the atoxigenic *A. flavus* and *Aspergillus parasiticus* on cracked barley and molasses were applied at 0.3–0.5 tonnes/hectare (Pitt & Hocking 2006).

During the 2009/10 growing season, treatments resulted in significant reductions of the incidence of toxigenic isolates of *A. flavus/A. parasiticus* in soil and peanuts. No pre-harvest aflatoxin contamination was observed. In the 2010/11 growing season, plants were exposed to late-season drought conditions that were optimal for aflatoxin contamination. Significant reductions in aflatoxin levels averaging 71% were detected in treated plots with different inoculation treatments. This study showed the efficacy of using the strategy of competitive exclusion to reduce aflatoxin contamination in Argentinean peanuts (Alaniz Zanon et al. 2013). Although the reductions in aflatoxin were lower than those reached in peanuts in the United States, where an overall mean reduction in aflatoxin of 85.2% was obtained in Georgia and Alabama during 2004 (Dorner et al. 2004), our results are promising considering the levels of aflatoxins detected at harvest in Argentina.

Biological control of *Aspergillus* section *Nigri* and ochratoxins in grapes

Biological control has been proposed as a strategy to reduce the impact of ochratoxigenic species in grapes. *Aureobasidium pullulans* (Dimakopoulou et al. 2008) and *Metschnikowia fructicola* (Karabulut et al. 2001) have been evaluated in earlier studies. In Argentina, the effect of *Kluyveromyces thermotolerans* strains to control growth and ochratoxin A accumulation by *Aspergillus* section *Nigri* strains was demonstrated (Ponsone et al. 2011, 2012). In another study the growth and ochratoxin A accumulation by the fungal strains at the phenotypic and molecular levels were monitored in relation to *Aspergillus carbonarius* RC13I and *A niger* aggregate BFE631. Under the conditions evaluated both *K. thermotolerans* strains were able to control growth of the *Aspergillus* strains assayed. The results on the effect of *K. thermotolerans* strains on the activity of the ochratoxin polyketide synthese gene (*pks*) showed a clear correlation between the expression of mycotoxin biosynthetic genes and the phenotypic production of the mycotoxin. The external growth parameters, e.g. the presence of *K. thermotolerans* as a biological control agent, moderate ochratoxin A biosynthesis via their influence on gene transcription. It is important to remark that the correlation between the *pks* gene expression and OTA production was not directly proportional. This could be explained by a different time window between gene expression and phenotypic production of the toxin or due to post-transcriptional regulation. Another explanation could be that the toxin could be used as an alternative carbon source by either the filamentous fungi or the yeast strains in the co-culture. A further possibility is the adsorption of the toxin to the yeast cell wall.

Regarding *Aspergillus carbonarius* RC 13I data on *otapks*AC and OTA accumulation showed, that there was an over expression of the *otapksAC* gene on the interaction cultures between the yeasts and *A. carbonarius* during both incubation periods (6 and 10 days) in comparison with the *A. carbonarius* culture alone, but the OTA production was reduced at 10 days of incubation under some of the interaction treatments. The results on *A. niger aggregate* showed that at 6 days of incubation under the interaction treatments *otapks*AC expression was between similar or lower to the control treatment, but OTA accumulation was lower in all the interactions evaluated, except under the interaction with *K thermotolerans* RCKT5. At 10 days of incubation the *pks*AC expression was higher in all the interaction treatments analysed, but OTA accumulation showed an opposite behaviour. In the control treatments there were higher levels of OTA than under the interactions treatments.

Ochratoxin B (OTB) could be an intermediate compound on OTA biosynthesis pathway (Gallo et al. 2012). The effect of *K. thermotolerans* on ochratoxin production agrees with that hypothesis since the kinetic behaviour of production of both OTA and OTB in all the conditions evaluated was similar. It was demonstrated that some kind of post-transcriptional regulation of the *otapks* gene

occurred because transcript level was not completely congruent with OTA accumulation under the presence of the antagonistic *K. thermotolerans*. The production of OTA can be regarded as an adaptation to imposed biotic (presence of *K. thermotolerans*) and other stress conditions by these mycotoxigenic species (Ponsone et al. 2013).

Field trials were carried out to evaluate *L. thermotolerans* strains of potential biocontrol yeasts, field experiments in three growing seasons (2010/11 2011/12, 2012/13 vintages) were carried out in a commercial organic vineyard. The vineyard was planted with Cabernet Sauvignon cv. grapes and with natural occurrence of *A. carbonarius*. The treatments with the biocontrol agents were performed during veraison and 1 month after veraison. During the 2010/11 vintage, no conclusive results were obtained since there was low *Aspergillus* section *Nigri* incidence in the control treatments and there was no OTA detected in the harvested grapes. During 2011/12 and 2012/13 vintages there was higher diversity in the observed mycoflora and the OTA accumulation was successfully diminished by the biocontrol agents application. OTA levels were reduced by 48–100% by the yeast treatments (Chulze 2014).

Biological control of *Fusarium graminearum* and deoxynivalenol in wheat

Control of FHB using fungicides can be an effective strategy considering the type of fungicide used, timing of application and cultivar planted since host resistance plays an important role in host–pathogen–fungicide interaction. Therefore, the combined effect of growing moderately resistant cultivars with fungicide application can reduce damage caused by FHB even under high disease pressure (Mesterházy et al. 2011; Amarasinghe et al. 2013). Haidukowsky et al. (2012) showed that applying fungicides containing prothioconazole at the beginning of anthesis was effective in reducing FHB and DON accumulation. The effect was better on common wheat in comparison with durum wheat. Although these data could give some promising results for control FHB, other studies have shown that certain fungicides could increase DON content on grains (Ramirez et al. 2004) and pathogens can generate resistance (Yuan & Zhou 2005). The need to control FHB prompted a search for microorganisms able to control *Fusarium* species. Studies under greenhouse and field trials showed that species within the genera *Bacillus*, *Pseudomonas* and *Streptomyces* were able to reduce *F. graminearum* growth and disease severity (Andersen et al. 2000; Schisler et al. 2002; da Luz et al. 2003; Khan et al. 2004; Palazzini et al. 2007; Khan & Doohan 2009; Zhao et al. 2014). In Argentina, the efficacy of *Bacillus subtilis* RC 218 and *Brevibacillus* sp. RC 263, used as formulated dry products (spores or vegetative cells) in diminishing both FHB disease severity and DON accumulation under controlled field conditions was observed. Nourozian et al. (2006) showed that the application of *Streptomyces* sp. on wheat heads renders a reduction of FHB disease by approximately 50%. Lysobacter enzymogenes strain C3 was able to reduce FHB disease severity in five of eight wheat cultivars under field conditions (Jochum et al. 2006). *Cryptococcus* species were effective in controlling the disease both under greenhouse and field conditions (Sato et al. 1999; Schisler et al. 2002, 2006).

In Argentina, the efficacy of *Bacillus subtilis* RC 218 and *Brevibacillus* sp. RC 263, used as formulated dry products (spores or vegetative cells), in diminishing both FHB disease severity and DON accumulation under controlled field conditions was observed. The timing of application of the BCA is an important factor to consider. The application of the biocontrol agents at the anthesis stage was more effective in reducing FHB severity and DON accumulation on wheat heads in comparison with pre-anthesis inoculations. The inoculum concentration of the BCA used (104 and 106 cfu ml^{-1}) or inoculum type (vegetative cells or spores) showed no influence on the effectiveness of the biocontrol agents. A reduction on disease severity of approximately 50% and a total inhibition of DON production from approximately 1500 μg kg^{-1} (control) to below the detection limit (50 μg kg^{-1}) was observed (Palazzini et al. 2007; Chulze 2014). It is remarkable from the economic point of view that both bacteria evaluated were effective at low inoculum levels in controlling FHB severity and DON accumulation, as suggested by Köhl et al. (2011).

Physiological improvement of the potential BCAs is a common strategy to improve the effectiveness of these agents under field experiments (Bochow et al. 2001; Teixido et al. 2005; Cañamás et al. 2008). This strategy is used since BCA may not be adapted to fluctuating environmental conditions (mainly water availability) and could render it an ineffective biocontrol activity. Schisler et al. (2002) observed that *B. subtilis* AS 43.3, without physiological improvement, was less effective against FHB in the field than under greenhouse conditions. Further research was carried out in Argentina aimed to optimise the efficacy of BCAs for field conditions. In order to select populations well adapted to osmotic stress, growth media with the compatible solutes to improve physiologically the potential biocontrol agents were used (Palazzini et al. 2009). The results y showed that *Bacillus subtilis* RC 218 and *Brevibacillus* sp. RC 263 physiologically modified by osmotic stress treatments with NaCl, glycerol and glucose rendered in the accumulation of the compatible solute betaine and maintained the effectiveness against *F. graminearum* under greenhouse experiments. Consequently, bacteria survived better during the stages of formulation, processing, storage and application.

Table 1. Biological control agents that reduce fungal growth of mycotoxigenic fungi and mycotoxin accumulation.

Targeted mycotoxigenic fungus	Commodity	Biocontrol agents	References
Aspergillus flavus, *A parasiticus*	Peanuts, rice, maize and cotton seed	Atoxigenic *A. flavus* strains AF36 and NRRL 21882 *A. flavus* La3303, La3304, La3279 and Ka16127 *A. flavus* AFCHG2[a] *Lactobacillus casei* *L. sanfrancisco CBI* *Streptococcus* *Bifidobacterium* *Trichoderma* sp. *Sporotrichum* spp. *ADA*	Dorner et al. (1999, 2003) Cotty (1994) Atehnkeng et al. (2014) Alaniz Zanon et al. (2013) Gourama and Bullerman (1997) Corsetti et al. (1998) Shantha (1999)
F. verticillioides	Maize	Endophyte *Bacillus subtilis* *Trichoderma* sp. *Bacillus amiloliquefaciens* BA-S13[a] *Kluyveromyces* spp. L14 and L16[a] *Microbacterium oleovorans* DMS 16091[a] *Enterobacter hormomaechei* EM-562T[a]	Bacon et al. (2001) Etcheverry et al. (2009)
F. graminearum, *F. culmorum*	Barley and wheat	*F. equiseti G9* *Pseudomonas fluorescens* strains MKB 158 and MKB249 Yeasts (NRRL Y-30213, NRRL Y-30214, NRRL Y-30215, and NRRL Y-30216) *Bacillus subtilis* H-08-02 *Bacillus cereus* L-07-01 *Bacillus mycoides* S-07-01 *Lysobacter enzymogenes strain C3* *Cryptococcus* spp. *Brevibacillus* sp.[a] *Bacillus subtilis*[a]	Dawson et al. (2004) Khan and Doohan (2009) Schisler et al. (2006) Fernando et al. (2002) Khan et al. (2001) Palazzini et al. (2007)
A. carbonarius, *A niger*	Grapes	Phyllosphere yeast (e.g. *Aurebasidium pullulans*) *Kluyveromyces thermotolerans*[a]	De Felice et al. (2008) Bleve et al. (2006) Castoria et al. (2001) Dimakopoulou et al. (2008) Tjamos et al. (2004) Ponsone et al. (2011)

Note: [a]Biocontrol agents developed in Argentina.

The mechanism that could explain the effect of *B. subtilis* RC 218 and *Brevibacillus* sp. RC 263 to control *F. graminearum* and DON accumulation could be antibiosis, this mechanism has been demonstrated by Edwards and Seddon (2001), although other modes of action such as lipopeptides production (iturins, fengicins, mycosubtilins) or induced resistance in wheat plants are not discarded (Ongena & Jacques 2008; Dunlap et al. 2011).

Further field trials are needed to validate the effectiveness of the formulated BCA under different environmental conditions. These selected BCAs can then be used alone or in combination with other management tools to reduce risks of FHB and DON accumulation. In no-tillage systems with higher risks for FHB, the additional application of BCAs on crops residues is also a promising tool (Luongo et al. 2005; Palazzini, Groenenboom-de Haas et al. 2013; Vogelgsang et al. 2011).

Biological control of fumonisins production in maize at field level

Contamination with *Fusarium* and fumonisins occurs in the field, so effective control strategies must be applied at this stage. *F. verticillioides* can be vertically transmitted in maize as an endophyte or horizontally to the next generation of plants through clonal infection of seeds and plant debris. An endophytic bacteria from maize showed activity for reducing fumonisin accumulation by *F. verticillioides* during its endophytic growth phase. Also, a *Trichoderma* sp. showed reduction of *F. verticillioides* growth and FB_1 accumulation. (Bacon et al. 2001).

Bacillus species, as a group, offer several advantages over other bacteria for protection against root pathogens because of their ability to form endospores and the broad spectrum activity of their antibiotics. In Argentina, the antagonistic activity of bacterial and yeast isolates including *Bacillus amyloliquefaciens* BA-S13, *Microbacterium oleovorans* DMS 16091, *Enterobacter hormomaechei* EM-562 T and *Kluyveromyces* spp. L14 and L16 on toxigenic *F. verticillioides* was demonstrated '*in vitro*' (Cavaglieri et al. 2005; Etcheverry et al. 2009; Pereira et al. 2010).

Field trials were carried out using the maize seeds treated with the nutrient broth where the bacteria were developed (Pereira et al. 2011). Further studies were performed in order to improve the formulations of the biocontrol agents. For this purpose, a culture medium based on molasses and soy powder was selected and physiological improvement of the bacteria with the accumulation of betaine and ectoine under media with high osmolality was evaluated (Sartori et al. 2012a, 2012b).

Field trials with freeze-dried formulated biocontrol agents based on *Bacillus amyloliquefaciens* and *Microbacterium oleovorans* showed biological control *of F. verticillioides*. FB_1 concentrations were reduced by 72–77.5% by M. *oleovorans*, while *B. amyloliquefaciens* caused a reduction by approximately 50% (Sartori et al. 2012a, 2012b, 2013). The biocontrol agents could be used with Bt maize or non-Bt maize. This is promising, since previous studies have demonstrated that lower levels of fumonisins were detected in Bt maize in comparison with non-Bt maize in Argentina (Barros et al. 2009). A summary of the successful application of biocontrol agents to reduce the toxigenic fungi growth and mycotoxin accumulation including Argentina is shown in Table 1.

Conclusions

Different strategies have been evaluated to reduce the entry of mycotoxins into cereals, peanuts and grapes. Biological control offers a promising tool to reduce mycotoxin risks. Native bacteria, filamentous fungi and yeast were successfully selected in Argentina, the biomass production was optimised and field trials were done to demonstrate their efficacy under field conditions.

Biocontrol agents can be developed for use as part of an IPM to reduce the impact of chemical fungicide on the environment. Still there is a relatively little investment in the research and development of biocontrol agents compared with that spent of the discovery of chemical pesticides. The registration procedure is expensive and time demanding. Formulations can improve the field efficacy of BCAs' physiological improvement can provide protection against desiccation and ultraviolet radiation.

More studies are needed on formulations, doses, the effect of the applied biocontrol agents on the native microflora and also on the plant response against pathogens and biocontrol agents (SAR = systemic acquired resistance).

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References

Abbas HK, Zablotowicz RM, Bruns HA, Abel CA. 2006. Biocontrol of aflatoxin in corn by inoculation with non-aflatoxigenic *Aspergillus flavus* isolates. Biocontrol Sci Techn. 16:437–449.

Abbas HK, Zablotowicz RM, Horn BW, Phillips NA, Johnson BJ, Jin X, Abel CA. 2011. Comparison of major biocontrol strains of non-aflatoxigenic *Aspergillus flavus* for the reduction of aflatoxins and cyclopiazonic acid in maize. Food Addit Contam: Part A. 28:198–208.

Alaniz Zanon MS, Chiotta ML, Giaj-Merlera G, Barros G, Chulze S. 2013. Evaluation of potential biocontrol agent for aflatoxin in Argentinean peanuts. Int J Food Microbiol. 162:220–225.

Amarasinghe CC, Lily Tamburic-Ilincic L, Gilbert J, Brûlé-Babel AL, Dilantha Fernando WG. 2013. Evaluation of different fungicides for control of *Fusarium* head blight in wheat inoculated with 3ADON and 15ADON chemotypes of

Fusarium graminearum in Canada. Can J Plant Pathol. 35:200–208.

Andersen SM, Johnsen K, Sorensen J, Nielsen P, Jacobsen CS. 2000. *Pseudomonas frederiksbergensis* sp. Nov., isolated from soil at a coal gasification site. Int J Syst Evol Microbiol. 50:1957–1964.

Atehnkeng J, Ojiambo PS, Cotty PJ, Bandyopadhyay R. 2014. Field efficacy of a mixture of atoxigenic Aspergillus flavus Link: Fr vegetative compatibility groups in preventing aflatoxin contamination in maize (Zea mays L.). Biol Control. 72:62–70.

Atehnkeng J, Ojiambo PS, Ikotun T, Sikora RA, Cotty PJ, Bandyopadhyay R. 2008. Evaluation of atoxigenic isolates of Aspergillus flavus as potential biocontrol agents for aflatoxin in maize. Food Addit Contam: Part A. 25:1264–1271.

Bacon C, Yates I, Meredith H. 2001. Biological control of *Fusarium moniliforme* in maize. Environ Health Perspect. 109:325–332.

Barros G, Chiotta M, Reynoso M, Torres A, Chulze S. 2007. Molecular characterization of *Aspergillus* section *Flavi* isolates collected from peanut fields in Argentina using AFLPs. J Appl Microbiol. 103:900–909.

Barros GG, Magnoli C, Reynoso MM, Ramirez ML, Farnochi MC, Torres A, Dalcero M, Sequeira J, Rubinstein C, Chulze S. 2009. Fungal and mycotoxin contamination in Bt maize and non-Bt maize grown in Argentina. World Mycotoxin J. 2:53–60.

Bleve G, Grieco F, Cozzi G, Logrieco A, Visconti A. 2006. Isolation of epiphytic yeasts with potential for biocontrol of *Aspergillus carbonarius* and *A. niger* on grape. Int J Food Microbiol. 108:204–209.

Bochow H, El-Sayed S, Junge H, Stavropoulou A, Schmiedeknecht G. 2001. Use of *Bacillus subtilis* as biocontrol agent. IV. Salt-stress tolerance induction *by Bacillus subtilis* FZB24 seed treatment in tropical vegetable field crops, and its mode of action. J Plant Dis Prot. 108:21–30.

Cámara Argentina del Maní [Internet]. 2014. [cited 2014 May 3]. Available from: http://www.camaradelmani.com.ar/en

Cañamás TP, Viñas I, Usall J, Magan N, Solsona C, Teixidó N. 2008. Impact of mild heat treatments on induction of thermotolerance in the biocontrol yeast *Candida sake* CPA-1 and viability after spray-drying. J Appl Microbiol. 104:767–775.

Castoria R, De Curtis F, Lima G, Caputo L, Pacifico S, De Cicco V. 2001. *Aureobasidium pullulans* (LS-30) an antagonist of postharvest pathogens of fruits: study on its modes of action. Post Biol Tech. 22:7–17.

Cavaglieri L, Orlando J, Rodríguez MI, Chulze S, Etcheverry M. 2005. Biocontrol of *Bacillus subtilis* against *Fusarium verticillioides* in vitro and at the maize root level. Res Microbiol. 156:748–754.

Chiotta M, Oviedo S, Barros G, Torres A, Chulze S. 2007. Potential biocontrol agent for aflatoxins in peanuts in Argentina. Poster session presented at: XII International IUPAC Symposium on Mycotoxins and Phycotoxins. Istambul, Turkey.

Chulze SN. 2010. Strategies to reduce mycotoxin levels in maize during storage: a review. Food Addit Contam. 27:651–657.

Chulze SN. 2014. Biological control as a strategy to reduce the impact of mycotoxins in the food and feed chains. Book of abstracts ISM Conference 2014: Perspectives on the Global Prevention and Control of Mycotoxins, Beijing, RP China.

Commission Regulation (EC) No 1881. 2006. Setting maximum levels for certain contaminants in foodstuffs Off J Eur Union. L 364, 5–24, 20.12.

Corsetti A, Gobbetti MJ, Rossi J and Damiani P. 1998. Antimould activity of sourdough lactic acid bacteria: identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco* CB1. Appl Microbiol Biotech. 50:253–256.

Cotty PJ. 1994. Influence of field application of an atoxigenic strain of *Aspergillus flavus* on the populations of *A. flavus* infection cotton bolls and on aflatoxin content of cottonseed. Phytopathology. 84:1270–1277.

Da Luz WC, Stockwell CA, Bergstrom GC. 2003. Biological control of *Fusarium graminearum.* In: Leonard KJ, Bushnell WR, editors. *Fusarium* head blight of wheat and barley. St. Paul (MN): American Phytopathology Society Press; p. 381–394.

Dawson WAJM, Jestoi M, Rizzo A, Nicholson P, Bateman GL. 2004. Field evaluation of fungal competitors of *Fusarium culmorum* and *F. graminearum*, casual agents of ear blight of winter wheat, for the control of mycotoxin production in grain. Biocontrol Sci Tech. 14:783–799.

De Felice DV, Solfrizzo M, De Curtis F, Lima G, Visconti A, Castoria R. 2008. Strains of Aureobasidium pullulans can lower ochratoxin A contamination in wine grapes. Phytopathology. 98:1261–1270.

Dimakopoulou M, Tjamos SE, Antoniou PP, Pietri A, Battilani P, Avramidis N, Markakis A, Tjamos E. 2008. Phyllosphere grapevine yeast *Aureobasidium pullulans* reduces *Aspergillus carbonarius* (sour rot) incidence in wine-producing vineyards in Greece. Biol Control. 46:158–165.

Dorner JW. 2004. Biological control of aflatoxin contamination of crops. J Toxicol Toxin Rev. 23:425–450.

Dorner JW. 2008. Management and prevention of mycotoxins in peanuts. Food Addit Contam: Part A. 25:203–208.

Dorner JW, Cole RJ. 2002. Effect of application of nontoxigenic strains of *Aspergillus flavus* and *A. parasiticus* on subsequent aflatoxin contamination of peanuts in storage. J Stored Prod Res. 38:329–339.

Dorner JW, Cole RJ, Blankenship PD. 1998. Effect of inoculum rate of biological control agents on preharvest aflatoxin contamination of peanuts. Biol Control. 12:171–176.

Dorner JW, Cole RJ, Connick WJ, Daigle DJ, McGuire MR, Shasha BS. 2003. Evaluation of biological control formulations to reduce aflatoxin contamination in peanuts. Biol Control. 26:318–324.

Dorner JW, Cole RJ, Wicklow DT. 1999. Aflatoxin reduction in maize through field application of competitive fungi. J Food Prot. 62:650–656.

Dorner JW, Horn BW. 2007. Separate and combined applications of nontoxigenic *Aspergillus flavus* and *A. parasiticus* for biocontrol of aflatoxin in peanuts. Mycopathologia. 163:215–223.

Dorner JW, Lamb MC. 2006. Development and commercial use of afla-guard, an aflatoxin biocontrol agent. Mycot Res. 22:33–38.

Dunlap CA, Schisler DA, Price NP, Vaughn SF. 2011. Cyclic lipopeptide profile of three *Bacillus subtilis* strains; antagonists of *Fusarium* head blight. J Microbiol. 49:603–609.

Edwards S, Seddon B. 2001. *Mode of antagonism of Brevibacillus brevis against Botrytis cinerea in vitro*. J Appl Microbiol. 91:652–659.

Ehrlich KC. 2014. Non-aflatoxigenic *Aspergillus flavus* to prevent aflatoxin contamination in crops: advantages and limitations. Front Microbiol. 5:1–9.

Etcheverry MG, Scandolara A, Nesci A, Boas Ribeiro MSV, Pereira P, Battilani P. 2009. Biological interactions to select biocontrol agents against toxigenic strains of *Aspergillus*

flavus and *Fusarium verticillioides* from maize. Mycopathologia. 167:287–295.
FAO. 2014. Online Archive of FAO [Internet; cited 2014 May 3]. Available from: http://www.fao.org/worldfoodsituation/csdb/en/
Fernando WGD, Chen Y, Parks P, 2002. Effect of three Bacillus sp. from wheat on FHB reduction. In: National Fusarium Head Blight Forum Proceedings. U.S. Wheat and Barley Scab Initiative, East Lansing, MI; p. 73–76.
Gallo A, Bruno KS, Solfrizzo M, Perrone G, Mule G, Visconti A, Baker SE. 2012. New insight into the ochratoxin A biosynthetic pathway through deletion of a nonribosomal peptide synthetase gene in *Aspergillus carbonarius*. Appl Environ Microbiol. 78:8208–8218.
Garrido CE, Hernández Pezzanic C, Pacin A. 2012. Mycotoxins occurrence in Argentina's maize (Zea mays L.), from 1999 to 2010. Food Contr. 25:660–665.
Gilbert J, Haber S. 2013. Overview of some recent research developments in Fusarium head blight of wheat. Can J Plant Pathol. 35:149–174.
Gourama H, Bullerman LB. 1997. Anti-aflatoxigenic activity of Lactobacillus casei pseudoplantarum. Int J Food Microbiol. 34:131–143.
Haidukowsky M, Visconti A, Perrone G, Vanadia S, Pancaldi D, Covarelli L, Ballestrazzi R, Pascale M. 2012. Effect of prothioconazole based fungicides on Fusarium head blight, grain yield and deoxynivalenol accumulation in wheat under field conditions. Phytopathol Mediterr. 51:236–246.
International Agency for Research on Cancer (IARC). 1993. Ochratoxin A. Monographs on the evaluation of carcinogenic risks to humans: some naturally occurring substances, food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 56:489–521.
Jochum CC, Osborne LE, Yuen GY. 2006. Fusarium head blight biological control with *Lysobacter enzymogenes* strain C3. Biol Control. 39:336–344.
Karabulut OA, Lurie S, Droby S. 2001. Evaluation of the use of sodium bicarbonate, potassium sorbate and yeast antagonists for decreasing postharvest decay of sweet cherries. Postharvest Biol Techn. 23:233–236.
Khan MR, Doohan F. 2009. Bacterium-mediated control of Fusarium head blight disease of wheat and barley and associated mycotoxin contamination of grain. Biol Control. 48:42–47.
Khan NI, Schisler DA, Boehm MJ, Lipps PE, Slininger PJ. 2004. Field testing of antagonists of Fusarium head blight incited by *Gibberella zeae*. Biol Control. 29:245–255.
Khan NI, Schisler DA, Boehm MJ, Slininger PJ, Bothast RJ. 2001. Selection and evaluation of microorganisms for biocontrol of *Fusarium* head blight of wheat incited by *Gibberella zeae*. Plant Dis. 85:1253–1258.
Kikot GE, Moschini R, Consolo V, Rojo R, Salerno G, Hours RA, Gasoni L, Arambarri A, Alconada T. 2011. Occurrence of different species of *Fusarium* from wheat in relation to disease levels predicted by a weather-based model in Argentina pampas region. Mycopathologia. 171:139–149.
Köhl J, Postma J, Nicot P, Ruocco M, Blum B. 2011. Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. Biol Control. 57:1–12.
Luongo L, Galli M, Corazza L, Meekes E, de Haas L, Van Der Plas C, Köhl J. 2005. Potential of fungal antagonists for biocontrol of *Fusarium* spp. in wheat and maize through competition in crop debris. Biocontrol Sci Technol. 15:229–242.
McMullen M, Bergstrom G, De Wolf E, Dill-Macky R, Hershman D, Shaner G, Van Sanford D. 2012. A unified effort to fight an enemy of wheat and barley: fusarium head blight. Plant Dis. 96:1712–1728.
Mesterházy Á., Tóth B, Varga M, Bartók T, Szabó-Hevér Á., Farády L, Lehoczki-Krsjak S. 2011. Role of fungicides, application of nozzle types, and the resistance level of wheat varieties in the control of Fusarium Head Blight and deoxynivalenol. Toxins. 3:1453–1483.
Nourozian J, Etebarian H, Khodakaramian G. 2006. Biological control of *Fusarium graminearum* on wheat by antagonistic bacteria. J Sci Technol. 28:29–38.
Ongena M, Jacques P. 2008. Bacillus lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol. 16:115–125.
Palazzini JM, Fumero V, Barros G, Cuniberti M, Sofía N, Chulze SN. 2013. *Fusarium graminearum* and deoxynivalenol in wheat spikes, grains and flour in Argentina: effect on food safety and quality of wheat grains and by- products. Paper presented at: ICFM Workshop Food Mycology in a Globalized World Challenges and Solutions to the Safety of Food, Freising; Jun 3–5.
Palazzini JM, Groenenboom-de Haas BH, Torres AM, Köhl J, Chulze SN. 2013. Biocontrol and population dynamics of *Fusarium* spp. on wheat stubble in Argentina. Plant Pathol. 62:859–866.
Palazzini JM, Ramirez ML, Alberione EJ, Torres AM, Chulze SN. 2009. Osmotic stress adaptation, compatible solutes accumulation and biocontrol efficacy of two potential biocontrol agents on *Fusarium* head blight in wheat. Biol Control. 51:370–376.
Palazzini J, Ramirez M, Torres A, Chulze S. 2007. Potential biocontrol agents for Fusarium head blight and deoxynivalenol production in wheat. Crop Prot. 26:1702–1710.
Pereira P, Nesci A, Castillo C, Etcheverry M. 2010. Impact of bacterial biological control agents on fumonisin B1 content and *Fusarium verticillioides* infection of field-grown maize. Biol Control. 53:258–266.
Pereira P, Nesci A, Castillo C, Etcheverry M. 2011. *Field Studies on the Relationship between Fusarium verticillioides* and Maize (Zea mays L.): effect of biocontrol agents on fungal infection and toxin content of grains at harvest. Int J Agr. 1:1–7.
Pitt JI, Hocking AD. 2006. Mycotoxins in Australia: biocontrol of aflatoxin in peanuts. Mycopathologia. 162:233–243.
Ponsone ML, Chiotta ML, Combina M, Dalcero A, Chulze SN. 2011. Biocontrol as a strategy to reduce the impact of ochratoxin A and *Aspergillus* section *Nigri* in grapes. Int J Food Microbiol. 151:70–77.
Ponsone ML, Chiotta ML, Palazzini JM, Combina M, Chulze S. 2012. Control of ochratoxin A production in grapes. Toxins. 4:364–372.
Ponsone ML, Kuhn Y,G, Schmidt-Heydt M, Geisen R, Chulze SN. 2013. Effect of *Kluyveromyces thermotolerans* on polyketide syntahase gene expression an ochratoxin accumulation by *Penicillium* and *Aspergillus*. World Mycotox J. 6:291–297.
Ramirez ML, Chulze S, Magan N. 2004. Impact of environmental factors and fungicides on growth and deoxynivalenol production by *Fusarium graminearum* isolates from Argentinian wheat. Crop Prot. 23:117–125.
Ramirez ML, Reynoso MM, Farnochi MC, Torres A, Leslie J, Chulze S. 2007. Population genetic structure of *Gibberella zeae* isolated from wheat in Argentina. Food Addit Contam. 24:1115–1120.

Rodrigues I, Naehrer KA. 2012. A three-year survey on the worldwide occurrence of mycotoxins in feedstuffs and feed. Toxins. 4:663–675.

Sartori M, Nesci A, Castillo C, Etcheverry M. 2013. Biological control of fumonisins production in maize at field level. Int J Agric Pol Res. 1:188–196.

Sartori M, Nesci A, Etcheverry M. 2012a. Production of *Fusarium verticillioides* biocontrol agents, *Bacillus amyloliquefaciens* and *Microbacterium oleovorans*, using different growth media: evaluation of biomass and viability ager freeze-drying. Food Addit Contam: Part A. 29:287–292.

Sartori M, Nesci A, Etcheverry M. 2012b. Accumulation of the betaine and ectoine in osmotic stress adaptation of biocontrol agents against *Fusarium verticillioides* in maize. Agric Sci. 3:83–89.

Sato I, Kobayasi H, Hanya Y, Abe K, Murakami S, Scorzetti G, Fell JW. 1999. *Cryptococcus nodaensis* sp. nov., a yeast isolated from soil in Japan that produces salt-tolerant and thermostable glutaminase. J Ind Microbiol Biotechnol. 22:127–132.

Schatzmayr G, Streit E. 2013. Global occurrence of mycotoxins in the food and feed chain: facts and figures. World Mycotoxin J. 6:213–222.

Schisler DA, Khan NI, Boehm MJ, Lipps PE, Slininger P.J., Zhang, S. 2006. Selection and evaluation of the potential of choline-metabolizing microbial strains to reduce *Fusarium* head blight. Biol Control. 39:497–506.

Schisler DA, Khan NI, Boehm MJ, Slininger PJ. 2002. Greenhouse and field evaluation of biological control of Fusarium Head Blight on durum wheat. Plant Dis. 86:1350–1356.

Shantha T. 1999. Fungal degradation of aflatoxin B1. Nat Toxins. 7:175–178.

Teixido N, Cañamás T, Usall J, Torres R, Magan N, Viñas I. 2005. Accumulation of the compatible solutes, glycine-betaine and ectoine, in osmotic stress adaptation and heat shock cross-protection in the biocontrol agent *Pantoea agglomerans* CPA-2. Lett Appl Microbiol. 41:248–252.

Tjamos SE, Antoniou PP, Kazantzidou A, Antonopoulos DF, Papageorgiou I, Tjamos EC. 2004. *Aspergillus niger* and *Aspergillus carbonarius* in Corinth raisin and wine producing vineyards in Greece: population composition, ochratoxin A production and chemical control. J Phytopatol. 152:250–255.

Torres AM, Barros GG, Palacios SA, Chulze SN, Battilani P. 2014. Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. Food Res Int. 62:11–19.

Vogelgsang S, Hecker A, Musa T, Dorn B, Forrer H-R. 2011. On-farm experiments over five years in a grain maize/winter wheat rotation: effect on maize residue treatments on *Fusarium graminearum* infection and deoxynivalenol contamination in wheat. Mycot Res. 27:81–96.

Wilson CL, Wisniewski ME. 1989. Biological control of post-harvest diseases of fruit and vegetables: an emerging technology. Ann Rev Phytopathol. 27:425–441.

Yuan S, Zhou M. 2005. A major gene for resistance to carbendazim, in field isolates of *Gibberella zeae*. Can J Plant Pathol. 27:58–63.

Zhao Y, Selvaraj JN, Xing F, Zhou L, Wang Y, Song W, Tan X, Sun L, Sangare L, Folly YME, Liu Y. 2014. Antagonistic action of *Bacillus subtilis* strain SG6 on *Fusarium graminearum.* Plos One. 9:e92486.

Zimmerli B, Dick R. 1996. Ochratoxin A in table wine and grape juice: occurrence and risk assessment. Food Addit Contam. 13:655–668.