Annex B – Pre- and post-harvest strategies to mitigate mycotoxin contamination in small grains

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1. Mycotoxin producing fungi in main crops
   1. Ecology of the main *Fusarium* spp. infecting small grains

The *Fusarium* species that may infect small grain cultivars can produce a range of mycotoxins that endanger the health of both humans and animals. *Fusarium* *graminearum*, *Fusarium* *culmorum*, *Fusarium* *poae*, *Fusarium* *avenaceum*, *Fusarium langsethiae* and *Microdochium* *nivale* (formerly known as *Fusarium* *nivale*) predominantly cause *Fusarium* diseases of small-grain cereals. These fungi occur as a complex with each other and with other Fusaria and other fungal genera. Climatic conditions will influence competition between, and the predominance of, different fungi within this complex (Doohan, Brennan, & Cooke, 2003).

The ecophysiological needs of these species vary between species and cultivars, but a common trend may be withdrawn. **Table B.1** presents the ecological needs of the main *Fusarium* species: *F. equiseti*, *F. graminearum*, *F. langsethiae,* *F. proliferatum* and *F. sporotrichioides*.

**Table B.1** presents mainly *in vitro* information from fungal growth in barley, oat or wheat based media. Although temperature and water activity for growth and mycotoxin production are presented separately, the two factors should always be considered since a significant interaction between these two parameters is always observed. Nevertheless, the reported optimum temperature for growth was between 20 °C and 30 °C, independently of fungal species and growing media (**Table B.1**). Also for water activity, the optimum conditions for growth are above 0.98, independently of fungal species and growing media.

Different species of *Fusarium* are spread all over the world, surviving in the field as saprophytes and as plant pathogens associated with many cereal crops. Their presence in the environment affects cereal commodities in different ways: reduced yield, quality and nutritional value. Of major importance is the vast array of mycotoxins that most of these species can produce in crops (e.g., DON, FB, NIV, HT-2 and T-2, ZEN). To understand the ecology of these species, the interaction of temperature and water activity has been extensively studied, being water activity reported as the main factor governing mould development (Mylona & Magan, 2011; Wawrzyniak, Ryniecki, & Gawrysiak-Witulska, 2013).

Besides temperature and water activity, two other factors affect growth, light and CO2. While studying the correlation of CO2 production and fungal contamination during cereals storage, Mylona and Magan (Mylona & Magan, 2011) reported for *F. langsethiae* in oat grains the optimum conditions for growth and for mycotoxins production (T-2 and HT-2 toxins). However, at marginal water activity conditions, only microscopic growth will occur. For this species in oat, visible growth of the fungus may not be used as a criterion of grain quality, since apparently healthy grains may carry large amounts of toxins.

**Table B.1**: Summary of the effect of temperature and water activity on the ecological needs of *Fusarium* spp., growing on small grains, reported by different authors

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Topic | | Range considered | Tmin | Topt | Tmax | Commodity | Reference(s) |
|  | **TEMPERATURE (T in °C)** | | | | | | |
|  | **Fungal growth** | | | | | | |
| *F*. *equiseti* |  | 15-40 | 15 | 20-30 | <40 | Wheat medium | (Marin, Jurado, & Gonzalez-Jaen, 2015) |
| *F*. *equiseti* |  | 15-40 | 15 | 20-30 | <40 | Barley medium | (Marin et al., 2015) |
| *F*. *graminearum* |  | 15-35 | 15 | 25 | 30-35 | Trichothecene-inducing solid  agar medium | (Marin, Jurado, Magan, Vazquez, & Gonzalez-Jaen Maria, 2010) |
| *F*. *graminearum* |  | 15-30 | 20 | 30 | 30 | Wheat | (Mylona, Sulyok, & Magan, 2012) |
| *F*. *langsethiae* |  | 10-37 | 10 | 25 | 37 | Oat medium | (Medina & Magan, 2010) |
| *F*. *langsethiae* |  | 15-30 | 15 | 25 | 30 | Oat grain | (Mylona & Magan, 2011) |
| *F*. *langsethiae* |  | 5-35 | 22 | 28 | 30 | Barley | (Strub, Pocaznoi, Lebrihi, Fournier, & Mathieu, 2010) |
| *F*. *langsethiae* |  | 5-40 | 5 | 20-25 | 30 | Durum wheat medium | (Nazari, Pattori, Terzi, Morcia, & Rossi, 2014) |
| *F*. *proliferatum* |  | 15-30 | 15 | 30 (0.995 aw) | 30 | Wheat medium | (Cendoya, Farnochi, Chulze, & Ramirez, 2014) |
| *F*. *sporotrichioides* |  | 5-40 | 10 | 25-30 | 35 | Durum wheat medium | (Nazari et al., 2014) |
| *F*. *culmorum* |  | 10-30 | 10 | 25 | 30 | PDA | (Brennan, Fagan, van Maanen, Cooke, & Doohan, 2003) |
| *F*. *culmorum* |  | 10-30 | 10 | 20-25 | 30 | Wheat seedlings | (Brennan et al., 2003) |
|  | **Mycotoxin production** | | | | | | |
| *F*. *graminearum* | DON | 15-30 | 15 | 30 | 30 | Wheat | (Mylona et al., 2012) |
| *F*. *graminearum* | ZEN | 15-30 | 15 | 25 | 30 | Wheat | (Mylona et al., 2012) |
| *F*. *langsethiae* | T-2+ HT-2 | 15-30 | 15 | 25 | 30 | Oat grain | (Mylona & Magan, 2011) |
| *F*. *langsethiae* | T-2 and HT-2 | 5-35 | 22 | 28 | 30 | Barley | (Strub et al., 2010) |
| *F*. *langsethiae* | T-2 | 5-40 | 10 | 15 | 30 | Durum wheat medium | (Nazari et al., 2014) |
| *F*. *langsethiae* | HT-2 | 5-40 | 10 | 15 | 35 | Durum wheat medium | (Nazari et al., 2014) |
| *F*. *proliferatum* | FB | 5-30 | 15 | 15-25 (0.98-0.99 aw) | 30 | Wheat medium | (Cendoya et al., 2014) |
| *F*. *sporotrichioides* | T-2 | 5-40 | 5 | 10 | 30 | Durum wheat medium | (Nazari et al., 2014) |
| *F*. *sporotrichioides* | HT-2 | 5-40 | 10 | 10 | 30 | Durum wheat medium | (Nazari et al., 2014) |
|  | **Gene expression** | | | | | | |
| *F*. *equiseti* | TRI5 | 15-35 | 15 | 35 | 35 | Wheat medium | (Marin et al., 2015) |
| *F*. *equiseti* | TRI5 | 15-35 | 15 | 35 | 35 | Barley medium | (Marin et al., 2015) |
| *F*. *graminearum* | TRI5 | 15-35 | 20 | 25 (0.955aw) | 30 | Trichothecene-inducing solid  agar medium | (Marin et al., 2010) |
|  | **In planta infection** | | | | | | |
| *F*. *langsethiae* | Spike colonization | 5-40 | 10 | 25 | 35 | Durum wheat | (Nazari et al., 2014) |
| *F*. *langsethiae* | T-2 and HT-2 | 5-40 | 10 | 20 | 35 | Durum wheat | (Nazari et al., 2014) |
| *F*. *sporotrichioides* | Spike colonization | 5-40 | 10 | 30 | 40 | Durum wheat | (Nazari et al., 2014) |
| *F*. *sporotrichioides* | T-2 and HT-2 | 5-40 | 10 | 30 | 40 | Durum wheat | (Nazari et al., 2014) |
| Topic |  | Range considered | aw min | aw opt | aw max | Commodity | Reference(s) |
|  | **WATER ACTIVITY (aw)** | | | | | | |
|  | **Fungal growth** | | | | | | |
| *F*. *equiseti* |  | 0.93-0.99 | 0.93 | 0.99 | 0.99 | Wheat medium | (Marin et al., 2015) |
| *F*. *equiseti* |  | 0.93-0.99 | 0.93 | 0.99 | 0.99 | Barley medium | (Marin et al., 2015) |
| *F*. *graminearum* |  | 0.955-0.995 | 0.955 | 0.955 (25 °C) | 0.995 | Trichothecene-inducing solid  agar medium | (Marin et al., 2010) |
| *F*. *langsethiae* |  | 0.90-0.995 | 0.92-0.93 (10 °C) | 0.98-0.995 (25 °C) | 0.995 | Oat medium | (Medina & Magan, 2010) |
| *F*. *langsethiae* |  | 0.89-0.97 | 0,89 | 0.97 | 0.97 | Oat grain | (Mylona & Magan, 2011) |
| *F*. *langsethiae* |  | 0.63-0.997 | 0.845 | 0.992 | 0.997 | Barley | (Strub et al., 2010) |
| *F*. *proliferatum* |  | 0.90-0.995 | 0.90 | 0.995 | 0.995 | Wheat medium | (Cendoya et al., 2014) |
|  | **Mycotoxin production** | | | | | | |
| *F*. *langsethiae* | T-2 and HT-2 | 0.63-0.997 | 0.845 | 0.997 | 0.997 | Barley | (Strub et al., 2010) |
| *F*. *langsethiae* | T-2+ HT-2 | 0.89-0.97 | 0.89 | 0.97 | 0.97 | Oat grain | (Mylona & Magan, 2011) |
| *F*. *graminearum* | DON | 0.89-0.97 | 0.89 | 0.97 | 0.97 | Wheat | (Mylona et al., 2012) |
| *F*. *graminearum* | ZEN | 0.89-0.97 | 0.89 | 0.97 | 0.97 | Wheat | (Mylona et al., 2012) |
| *F*. *proliferatum* | FB | 0.90-0.995 | 0.92 | 0.98-0.99 (15-25 °C) | 0.995 | Wheat medium | (Cendoya et al., 2014) |
|  | **Gene expression** | | | | | | |
| *F*. *equiseti* | TRI5 | 0.93-0.99 | 0.95 | 0.98 (35 °C) | 0.99 | Wheat medium | (Marin et al., 2015) |
| *F*. *equiseti* | TRI5 | 0.93-0.99 | 0.95 | 0.98 (35 °C) | 0.99 | Barley medium | (Marin et al., 2015) |

Regarding mycotoxin production, slightly lower optimum temperature needs have been reported for T-2 and HT-2 toxin production in wheat based medium (Nazari et al., 2014). In most other cases, optimum temperature ranges for mycotoxin production are close to the optimum range for growth. Gene expression (mostly TRI5) is stable over a wider range of temperatures, but with a trend towards an induction of expression at higher temperature than the ones for optimum growth (Scherm et al., 2011).

Two-dimensional profiles for water activity and temperature interactions have been developed from collected data to identify areas where conditions indicate a higher risk from fungal growth and mycotoxin accumulation (Cendoya et al., 2014).

* 1. Ecology of the main *Claviceps* spp. infecting small grains

*Claviceps purpurea* is morphologically a highly variable species with respect to sclerotial length and shape, color of the stromata, conidial size and shape, and alkaloid production spectra (Esser & Tudzynaki, 1978). Within the set of isolates from cereals, Pažoutová and coworkers reported that two isolates with the same molecular fingerprint were not found (Menzies & Turkington, 2015).

1. The crops

* 1. Small grains
     1. Small grains phenology

The phenological development process of small grains is well known and described by the BBCH scale (2001) as well as the Zadoks scale. According to the latter, the principal growth stage are: (0) germination, (1) seeding growth, (2) tillering, (3) stem elongation, (4) booting, (5) awn emergence, (6) flowering (anthesis), (7) milk development, (8) dough development and (9) ripening (Zadoks, Chang, & Konzak, 1974).

* + 1. Infection cycle of *Fusarium* spp. in small grains and plant pathogen interaction
       - 1. Infection cycle

Understanding the infection of grains with *Fusarium* species is crucial to develop better ways to control and mitigate the impacts of this occurrence. In fact, the study of the interaction between the pathogen and the host needs *in vivo* assessments. It is demonstrated that, for example, in aggressiveness studies in wheat, a correlation cannot be made between *in vitro* and *in field* results (Korn, Mueller, Ulrich, & Mueller, 2011).

The infection of wheat, barley and other small grains with *Fusarium* species, namely *F*. *graminearum*, *Fusarium pseudograminearum* and *F*. *culmorum*, is of major concern. In fact, these pathogens can have different impacts in plants, depending on weather at different plant development stages, causing diseases like Crown Rot and Fusarium Head Blight (FHB) (Tunali et al., 2012). FHB occurs in a wide range of small grains, raising concern not only in wheat and barley, but also in oat, rye and triticale (Audenaert, Troch, Landschoot, & Haesaert, 2014). *F. graminearum*, *F*.*avenaceum* and *F*.*poae* are often associated to this disease, mainly when it spreads in triticale (Audenaert et al., 2014). Increasingly throughout Europe, Asia and the Americas, FHB disease leads to the severe loss of wheat grain yield and quality, besides the putative occurrence of a range of mycotoxins.

*F*. *graminearum* is an opportunistic pathogen of cereals where it causes severe yield losses and concomitant mycotoxin contamination of the grains. This pathogen has mixed biotrophic and necrotrophic (saprophytic) growth phases during infection and the regulatory networks associated with these phases have so far always been analyzed together. Living plant actively suppressed fungal growth and promoted much higher toxin production in comparison to the identical plant tissue without metabolism, suggesting that molecules signaling secondary metabolite induction are not pre-existing or not stable in the plant in sufficient amounts before infection (Boedi et al., 2016). A detailed microscopic investigation has revealed how wild-type fungal hyphae colonized susceptible wheat ears and spread from spikelet to spikelet (Brown, Urban, Van De Meene, & Hammond-Kosack, 2010). *F. graminearum* genes expressed during the infection of wheat and barley have been compared [(Lysoe, Seong, & Kistler, 2011) in (Kazan, Gardiner Donald, & Manners John, 2012)] and, surprisingly, found to be substantially different.

The analysis of crown infection by *F. graminearum* identified at least three distinct stages of infection (spore germination, infection and initial colonization of leaf epidermis and extensive colonization of crown) (Kazan et al., 2012). *F. graminearum* uses the same pathogenicity genes required for FHB to infect vegetative tissues, such as crowns and roots, or similar strategies to infect head, root and crown tissue. Crown Rot establishment on wheat was proved to be associated to a high production of deoxynivalenol (DON) in stem-based tissue, while in FHB this production occurs mainly in grains, being *F. pseudograminearum* more associated in the first scenario and *F. graminearum* and *F. culmorum* in the second (Tunali et al., 2012).

Trichothecenes do not appear to be required to cause visible disease symptoms during crown infection in bread wheat, but do contribute to full fungal biomass production and stem colonization (in: (Kazan et al., 2012)). The involvement of trichothecene as a virulence factor in *F*. *culmorum*-mediated crown rot disease on durum wheat has also been demonstrated. The silencing of TRI6, a transcription factor known to regulate the expression of toxin biosynthesis genes in *F*. *culmorum*, led to reduced DON production and crown rot symptom development. In contrast, some of the transformants that showed unexpectedly high DON and TRI6 transcript levels, despite being transformed with a TRI6 silencing construct, showed increased symptom development, suggesting that TRI6 and DON play a role in the infection of the crown/stem tissue during Crown Rot disease development.

Crown rot of wheat caused by *F*. *pseudograminearum* is known to be a residue-borne disease that increases in incidence over the years if no special measures to reduce inoculum are established. The most effective preventive management practice is rotation with a non-cereal crop, and no-till, stubble-retained cropping systems are the most prone to infection (Backhouse, 2014). In the latter conditions, crown rot incidence increase over successive crops from first detection to a maximum incidence in about 5 years. In order for the infection to spread, infected residues in the field must be very close to the wheat plant (less than 2 mm), being not clear if other conditions (e.g., wind, rain splash or insects) could spread the disease over a higher distance (Backhouse, 2014; Moretti et al., 2014).

In spring barley, infection with *F. graminearum* decreases with a later flowering period and for *F. langsethiae* it occurs more often in spring barley than in winter barley because of the earlier flowering season that induces a higher inoculum dispersal (Linkmeyer et al., 2016). Besides these differences, it is also worth mentioning that different fungal species have different impacts on crops. Studying *Fusarium* spp. infection on barley, it was observed that inoculums caused diverse symptoms with consequent different yield reductions, and that these facts are not indicative of mycotoxin biosynthesis (Linkmeyer et al., 2016). Other important aspect related to mycotoxin accumulation in grains resultant from fungal infection is the general idea that when a co-occurrence of fungal species happens, the associated mycotoxin content will be higher. In fact, it has been demonstrated that the opposite occurs when wheat grains were inoculated with different combinations of fungal species, with a positive or null impact on development detected in combinations with the most aggressive isolates and a negative one related with the remaining (Siou et al., 2015).

The biotic stress occurred during FHB disease caused by *F*. *graminearum* in small grains did not seemed to have major impact in grains development, although it is deeply dependent on the ontogeny. Even so, key changes triggered by *F. graminearum* occur specially during cell differentiation, where the influence on transport, translation, DNA processing and metabolic pathways is clear, impacting proteins regulation (Chetouhi et al., 2015). Besides that, there are also modifications on energy production and on the cell wall of the hosts, which becomes thicker (Chetouhi et al., 2015).

In case of FHB establishment, yield losses are due to changes on grain filling and death of spiklets (Audenaert et al., 2014). *F*. *graminearum* can start infection by leafs or, most often, takes advantage of lesions in the host to infect plants, and FHB disease is a result of a progressive growth when the seed is germinating. The infection process initiates at an extracellular level, with macroconidia arriving to the ear, being extended to intracellular by the progressive infection of host tissues (Scherm et al., 2013). A comprehensive histological study of the infection process in a modern wheat cultivar possessing a semi-dwarfing gene was performed (Brown et al., 2010). This investigation explored the infection processes deployed by *F*. *graminearum* to colonise susceptible wheat ears and has linked the macroscopic appearance of disease symptoms with the underlying cellular colonisation pathways. Post floral invasion intercellular hyphae spread throughout the spikelet, down into the rachis node and subsequently up and down the ear in the rachis. The advancing front of infection in the rachis was found to be solely in the apoplast of the cortex and not the vasculature (Brown et al., 2010). At the infection front, the hyphae adopted the form of the intercellular spaces, taking on a triangular shape rather than disrupting the surrounding host cells. Differences on inoculation step can be observed in different geographical regions, for example, *F graminearum* initial inoculation of wheat in Australia was proven to occur by macroconidia, but in other regions of the world FHB caused by this fungus results of ascopores’ inoculation, which highlights the populations’ differences between distinct areas (Chakraborty et al., 2006). In fact, these two reproductive structures are crucial in plant infection and represent the beginning of infection cycle of *F. graminearum* (Audenaert, Vanheule, Haesaert, & Hofte, 2013).

Behind the advancing from of infection, host cell death occurs, being not clear the role of DON, in fact, this mycotoxin does not seem to play an active role in the beginning of plant infection (Audenaert et al., 2013; Brown et al., 2010; Kazan et al., 2012). Even so, DON increases virulence of infection when the fungus reaches intracellular medium, since it promotes cell damages and enables spread through the rachis from the infected to the adjacent spikelet, and may initiate programmed cell death (PCD) of the wheat host cells after reaching a certain concentration (Audenaert et al., 2013; Brown et al., 2010; Kazan et al., 2012). Through its function as translation inhibitor, DON suppresses the establishment of cell wall thickenings in the rachis node and thus inhibits this important defense response of the host. As reviewed by Kazan and coworkers (Kazan et al., 2012), during the spread of the pathogen from inoculated to non-inoculated spikelets (4–7 days post-inoculation), extensive reporter gene expression was detected in the rachis node. This suggests that the rachis tissue elicits DON biosynthesis in the pathogen, corroborating the idea that, at least in wheat, the rachis constitutes a formidable barrier to the spread of *F*. *graminearum*, and that DON biosynthesis in the pathogen is required to overcome this major obstacle. The presence of DON was also proven to negatively affect photosynthesis, as well as carbohydrate and nitrogen metabolisms (Warth et al., 2015).

As with crown rot, with FHB the main source of inoculum is the fungus that survive overwinter on wheat residues and on the soil; however, the contribution of contaminated seeds may not be excluded. (Moretti et al., 2014) showed that *F. graminearum* originated from seeds could grow systemically in the plant tissues, except in kernels and heads, and contribute to DON and DON-3G contamination. Although the fungus could not be detected in the heads, DON and DON-3G were detected in heads and kernels, reinforcing previous evidences that DON and DON-3G could be systemically translocated from stems (Kang & Buchenauer, 2002). Also, Moretti et al (Moretti et al., 2014) reported higher ratios of DON-3G to DON in the heads, indicating a much higher glycosylation in the heads compared to straw.

*F*. *graminearum* pathogenicity processes has been extensively studied by gene knock-out mutant analysis and different genes affecting different steps (from blocking the penetration of the epidermal cells to the synthesis of DON) in the pathogenicity processes have been identified (Bormann, Boenisch, Brueckner, Firat, & Schaefer, 2014). Although most gene knock-outs described so far have reduced toxin biosynthesis and attenuated virulence, mutants that show increased toxin biosynthesis and virulence have also been identified (Kazan et al., 2012), demonstrating the relation between virulence and toxin biosynthesis by this species in wheat.

When the production of secondary metabolites was analysed in dead and living plant cells, it was found that DON (and its acetylated 15-ADON and 3-ADON as well as glycosylated DON-3-glucoside derivatives), butenolide and culmorin only accumulated in the living plant tissue, remaining mycotoxin levels very low in the dead plant samples (Boedi et al., 2016). This demonstrates that the elevated mycotoxin levels are derived from a genetic induction event and cannot only be based on biochemical changes, e.g., precursor availabilities (Boedi et al., 2016; Kazan et al., 2012). However, secondary metabolism is not exclusive to grow in living plant cells, since other genes involved in secondary metabolisms (although not assigned to a classical known mycotoxin of *F*. *graminearum*) have been identified (Boedi et al., 2016).

During this infection, not only mycotoxin, but also enzyme production rates can get higher (Scherm et al., 2013). The production of peptidases to allow nutrient uptake and assist breaking of cell walls is also part of infection mechanism of *F*. *graminearum* (Lowe et al., 2015). In fact, the infection process is accompanied by the formation of infection cushions (Boenisch and Schäfer, 2011), an agglomeration of fungal hyphae which secrete various hydrolyzing enzymes able to degrade components of the epidermal plant cuticle and the plant cell wall. From around 70 hours after inoculation (hai) onwards, plant tissue necrosis occurs which is triggered by mycotoxins and intracellular growth of the pathogen (in (Boedi et al., 2016)).

These transcriptome comparisons revealed that specific subsets of *F*. *graminearum* genes are exclusively expressed *in planta*, and based on these observations were designated as “pathogenicity- related” or “virulence” genes (Boedi et al., 2016). However, some of the fungal genes expressed only *in planta* may not be related to pathogenic processes but simply responding to the specific substrate (floral tissue) while others may be directly involved in the pathogenic process (overcoming resistance).

Brown et al. (Brown et al., 2010) concluded that, similarly to crown infection, during floral infection, *F*. *graminearum* seems to follow a hemibiotrophic lifestyle, characterized by a relatively long symptomless period, followed by massive tissue necrosis and a rapid increase in fungal biomass.

*Fusarium* infection of emmer grains lead to changes on protein expression, with less N and S sources available and more globulins, that overcomes the depletion on α-gliadin content observed (Eggert, Zoerb, Muehling, & Pawelzik, 2011). Allantoin was suggested as a potential source of nitrogen used by *F*. *graminearum* during the infection process in wheat (Lysoe etal, 2011, in (Walkowiak & Subramaniam, 2014)). Overall, for a given gene, the results from 22 different nitrogen compounds indicated that expression was lower in media appended with preferred nitrogen sources, such as glutamine, and greater in non-preferred nitrogen sources, such as cysteine and ammonium phosphate dibasic (NH4)2HPO4. Also, the expression of most TRI genes increases in the presence of non-preferred nitrogen sources (Walkowiak & Subramaniam, 2014). Identification and characterization of genes responsive to nitrogen signals that both positively and negatively affect virulence emphasizes the complex relationship between environmental signals and pathogenesis (Walkowiak & Subramaniam, 2014).

* + - * 1. Pests

The action of insects in crops leads to damages that enable fungal contaminations to spread into the plants. In small grains this action is mainly due to aphids and, particularly in wheat, *F*. *graminearum* spread can be double when plants host these insects (Drakulic, Bruce, & Ray, 2017; Ferrigo, Raiola, & Causin, 2016).

A recent review on the on the role of arthropods in disease epidemiology (Drakulic et al., 2017) explores how insects and mites may directly or indirectly facilitate *Fusarium* diseases. Most of the studies were done with maize, and a few in small grain cereals. Nevertheless, a consistent positive correlation between the incidence of insects and *Fusarium* species was reported. Studies done with maize report that insecticides were more effective than fungicides in reducing fumonisin levels (Blandino et al., 2008).

The review of (Drakulic et al., 2017) concluded that this small amount of research supports the hypothesis that insect and mite activity can impact the progress of FHB disease in such a way as to increase the hosts’ susceptibility, impacting yield, grain quality, and mycotoxin accumulation.

* + - * 1. Plant pathogen interaction: *Fusarium* spp.

The infection of plants, particularly small grains, with *F*. *graminearum* induces a diversity of responses that are specific of each infected species (Chetouhi et al., 2015).

Wheat plants have been proven able to partially detoxify DON by a glycosylation process that transforms it in DON-3G, as a defensing mechanism. (Moretti et al., 2014) reported the presence of DON-3G at levels 23 times higher than DON in the heads at milk stage without the occurrence of *F. graminearum*, indicating the simultaneous occurrence of two events: the translocation of DON from stems to heads, and the glycosylation of DON in the head tissues. The high levels of DON-3G detected in the stems, at two stages of the wheat plants (milky and vitreous ripening of heads), and in the heads show a robust plant response to DON, confirming that DON plays an active role in the plant/*F. graminearum* interaction.

Role of environmental conditions/climate change

As said before, environmental conditions play a key role on fungal infection of small grains, since weather will determine if one or another species will prevail and be able to infect the plant. Major difficulties in relating environmental conditions to disease spread and mycotoxin accumulations in grains relay in the need to consider different variables and their influences (Kriss et al., 2012). In a general way, drought has more influence on the susceptibility of the plant than the fungus itself (Scherm et al., 2013). Even so, it is well established that for FHB, high moisture and hot conditions are favorable, but the needs of each of the causal species can slightly differ, and the same is truth for mycotoxin production (Scherm et al., 2013; Tunali et al., 2012). In fact, on a field study of infection of wheat with *F*. *graminearum* and *F*. *culmorum*, it was observed that no development of these pathogens occurred during a dry year, even though their aggressiveness had already been demonstrated on *in vitro* results (Korn et al., 2011). Impact of weather conditions differs according to the plant’s stage of development. This is proved by the observance that at anthesis the occurrence of rain induces FHB since fungal pathogens’ dispersal is facilitated (Audenaert et al., 2014). Wheat is highly susceptible to FHB infection during the time period of anthesis, where temperatures ranging from 15 °C up to 29 °C and high humidity represent favorable environmental conditions (in (Boedi et al., 2016)). In addition, there is strong evidence that rain is important in the dispersal of *F*. *culmorum* and *F*. *graminearum*. For *F*. *culmorum*, macroconidia that are produced at ground level are splashed onto the wheat heads during rainfall (Edwards, 2011).

*In planta* experiments in durum wheat have shown that *F*. *langsethiae* and *F*. *sporotrichioides* optimum temperatures ranges for floret infection are, respectively, 10 °C - 35 °C and 10 °C - 40 °C, with optimal values of 25 °C and 30 °C (Nazari et al., 2014). Also, mycotoxin production was studied, and observations shown that T-2 and HT-2 biosynthesis were higher in ranges of 15 °C - 35 °C and 20 °C - 25 °C, for *F*. *langsethiae* and *F*. *sporotrichioides*, respectively (Nazari et al., 2014).

For spring barley, it has been demonstrated that dry and hot climate at flowering benefit the infection by *F*. *langsethiae* and consequently the development of FHB (Linkmeyer et al., 2016). Also in emmer, there was a higher development of *Fusarium* isolates when exposed to favorable weather conditions, where wind and drought were minimized (Eggert et al., 2011).

How farming practices and environmental shifts, such as climate change, may affect the evolution of virulence and toxin biosynthesis in *F*. *graminearum* is another area of growing interest. Recent research has shown that larger numbers of *F*. *graminearum* isolates with 3-ADON chemotype, higher aggressiveness and greater levels of grain DON content can be found in current *F*. *graminearum* isolates than those collected between 1980 and 2000 in North Dakota (Kazan et al., 2012).

The understanding of climate change effects comprises knowledge on impact of the combination of precipitation and drought events, and increased levels of CO2 and temperature, which represent high stresses to crops and can increase their susceptibility to fungal infections (Medina, Rodriguez, & Naresh, 2015). In wheat, FHB and Crown Rot incidences are expected to be amplified by higher amounts of atmospheric CO2 (Chakraborty & Newton, 2011). Besides direct impacts, increased levels of CO2 and temperature lead to quality losses by decreasing nutritional parameters such as protein and micronutrient amounts, favoring fungal development and mycotoxin biosynthesis (Chakraborty & Newton, 2011). Drought effect on fungal infection is dependent on crop development stage and, as said before, especially in anthesis, development of *Fusarium* inoculum is triggered by rainfall (Chakraborty & Newton, 2011; Scala et al., 2016). In durum wheat, it has been found that high humidity enhanced *Fusarium* contamination, as well as DON content, even if optimal conditions for both incidences are not exactly the same (Chandelier, Nimal, Andre, Planchon, & Oger, 2011; Scala et al., 2016). Even so, this event was also been discovered to cause leaching of DON after its production in plant (Gautam & Dill-Macky, 2012a).

Climate is expected to change the environment in which crops will be grown in the next 10–20 years, with predicted yearly atmospheric CO2 concentrations rise of 1.5 micromol (European Commission, 2007). These changes will possibly affect harder the Mediterranean region, with extreme changes in temperature, CO2 and rain patterns (N. Magan, Medina, & Aldred, 2011). The impact of these changes will be dependent on the adaptation of each species to the changing conditions, being known that in FHB infection and DON production *F. graminearum* is increasing his incidence over *F. culmorum* (N. Magan et al., 2011). In addition, environmental changes might cause different impacts in different plant pathogen system. For oats, a strong negative relationship between harvest rainfall and HT2+T2 were reported, indicating that concentrations are higher when weather is drier in July and August, while the opposite was found for ZEN concentration in wheat and barley (Edwards, 2012). The effect of climate change will also have an impact at a post-harvest stage.

* + 1. Infection cycle of *Claviceps* spp. in small grains and plant pathogen interaction

The genus *Claviceps* consists of a unique group of species, characterized by infecting only the ovaries of grasses and leading to a disease named ergot. There are many species of *Claviceps*, but just a few are known to cause the disease with significant severity (Miedaner & Geiger, 2015). The species of greatest concern is *Claviceps purpurea*, which is distributed across temperate regions of the world, affecting mainly rye, and at a lesser extent, wheat and barley. Other species occur mainly in tropical and sub-tropical areas, but with a narrower set of susceptible crops, or even a single host genus.

The greatest concern from ergot is the contamination by heavily toxic alkaloids, and not the reduction in yield. In field growing areas, the yield reduction due to the disease is usually below 10% (Wegulo & Carlson, 2011). *Claviceps purpurea* produces all three major groups of ergot alkaloids: Clavine alkaloids, D-lysergic acid and its derivatives, and ergopeptines. Also in these respect, the other *Claviceps* species produce a narrower set of toxic alkaloids. These alkaloids can cause severe health problems in both humans and animals, and are associated to outbreaks since the Middle Ages.

Ergot disease has sporadic incidence, but a devastating power on people and livestock.

* + - * 1. Infection cycle

The infection cycle of *Claviceps purpurea* starts with sclerotia laying down on the field from the previous crop. Sclerotia may survive on soil for up to one year (Menzies & Turkington, 2015) and, under appropriate weather conditions (cool and wet weather), may germinate, release ascospores and infect forage or wild grass in the Spring (Miedaner & Geiger, 2015). Infected grasses act as inoculum for the cereal infection. Ascospores are spread by the wind or rain splash, and are trapped by a stigma. Germination and colonization of the host ovary is quick, and the evolution of the infection takes place in two stages. Initially, the growing fungus produces an exudate, called honeydew, which attracts insects that spread the disease to other nearby flowers. At a second stage, the production of honeydew is replaced by the formation of sclerotia, also known as ergot bodies. This ergot bodies on the host crop take the place of the seed. Mature sclerotia drop on the soil and complete the disease cycle (Menzies & Turkington, 2015; Miedaner & Geiger, 2015). Because *Claviceps* species are not able to penetrate through closed glumes, cross-pollinated crops are most threatened.

* + 1. Cropping system

Adopting an integrated cropping approach to reduce mycotoxins risks needs to start with pre-harvest management. In small cereals, soil tillage and crop rotation managements are considered to be of major importance on mycotoxins control, being followed by hybrid selection and fungicide treatment (Ferrigo et al., 2016). It should also be taken into account practices like weeding, balanced fertilization and harvest, that can significantly impact contamination of grains, as well as other factors with reduced impact (planting date, seed density, irrigation and insecticide treatment) (Ferrigo et al., 2016).

* + - * 1. Soil management

The type of soil is an important aspect when fungal and mycotoxin contaminations are of concern. Sandy soils has been associated to higher *F*. *graminearum* occurrence in a study comprising barley, oat and wheat, when compared with clay and silty soils (Bernhoft, Torp, Clasen, Loes, & Kristoffersen, 2012).

* + - * 1. Seeds treatment and seedlings insecticide defence

The aim of the application of fungicides in infected seeds is to prevent fungal disease development through the entire plant. However, the efficacy of this method in controlling the spread of FHB in barley and wheat seeds have not being yet confirmed (Fernandez, May, & Lafond, 2010)**.**

To prevent the spread of *F. graminearum* to regions were the incidence is still limited; seed treatment with fungicides is applied. Fernandez et al (2010) studied seed treatments (mainly azole group fungicides) in Canada in field conditions, and concluded that none of the treatments was consistently effective in preventing the disease (Fernandez et al., 2010).

* + - * 1. Managing crop residues and crop rotations

The continuous cropping of a land may lead to an increase on fungal content in soil. In fact, for *F*. *culmorum* and the development of Foot Root Rot, it is known that despite of the intrinsic proprieties of each cereal, that can be exploited to minimize this impact, all of them favor the survival of the inoculum (Scherm et al., 2013). Even so, it is worth to mention that the rotation of small cereals with crops other than cereals leaded to reduced *Fusarium* and mycotoxin contents (Bernhoft et al., 2012). For this reason, there is a need to adopt an effective tillage system and appropriate crop rotations, which can help reducing the presence of harmful crop residues (Audenaert et al., 2014; Ferrigo et al., 2016).

Crop residues in soil samples of a wheat field, as a consequence of reduced tillage, were confirmed to have *Fusarium* inoculums, proving the association between continuous cropping and higher fungal population (Gomez, Aulicino, Monaco, Kripelz, & Cordo, 2015; Hofgaard et al., 2016). Most probably due to the importance of crop debris in the epidemiology of head blight infection, a significant interaction between previous crop and the occurrence of DON incidence was observed. Highest predicted DON concentration occurred in wheat following maize, which is a known alternate host for *Fusarium* species. Ploughing generally reduced DON concentration; this reduction was greatest for crops following maize then for crops following wheat (Edwards, 2011). The risk is greater for crops following grain maize compared to forage maize, probably due to the greater amount of crop debris remaining.

Plow seems to be an effective practice to prevent *Fusarium* infection resultant from soil contamination (Hofgaard et al., 2016). The effect of tillage practices is also observed in crop yields. In wheat, moldboard plow tillage lead to higher yields, compared with chisel plow or no-till (Munger, Vanasse, Rioux, & Legere, 2014). However, the effect of tillage practices is not entirely clear, since it affects soil microbial population, which is known to be associated to antagonism relations with pathogens like *Fusarium* spp. (Ferrigo et al., 2016). In a wheat experiment, under low-input practices regarding weed control and organic fertilization, no tillage conducted to less *F*. *graminearum* (Munger et al., 2014). In addition, nitrogen and carbon levels in soil might be reduced with aggressive tillage; in fact, reduced tillage practices were proven to increase carbon levels in soil, having minimal effects on microbial populations in soil, without confirming higher fungal biomass (van Groenigen et al., 2010).

Studying the effect not only on fungal content, but also DON contamination, it was found that either moldboard plow, chisel plow or no-till had effect on mycotoxin accumulation in wheat (Munger et al., 2014). Comparing conventional and no tillage, the same was reported for DON, ZEN and T-2 toxin in wheat, barley and triticale, even with no tillage practice leading to higher *Fusarium* spp. in soil, cereal seeds, seedlings and harvested grains (Baliukoniene, Bakutis, Januskeviciene, & Miseikiene, 2011). Contrarily, in a field study in Italy, DON levels in durum wheat were higher in minimum tillage, compared with conventional tillage, and the same was observed for FHB incidence (Scala et al., 2016).

Selvaraj et al (Selvaraj et al., 2015) studied different crops in rotation with wheat in Yangtze-Huaihe river basin of PRChina - an highly susceptible area for *Fusarium* infection and DON contamination. Although conclusions were based on one-year data, wheat rotation with rice was more prone to *Fusarium* infection and DON accumulation, followed by maize, soya bean and cotton. Rotation with cotton exhibit the lowest average DON content, which could be due to the lower water requirement of this crop.

* + - * 1. Hybrid selection

In order to control mycotoxins in crops, selection of more resistant cultivars appears as a factor with a high impact (Ferrigo et al., 2016). In moderately resistant cultivars fungicide treatments can lower DON to the desirable level; however, in susceptible cultivars most fungicides fail to reduce mycotoxins levels to a desirable value (Mesterhazy et al., 2011).

* + - * 1. Sowing period

The date in which sowing occurs is related to the weather conditions that might affect crop in its different development stages, with all the impacts that this has on fungal growth and mycotoxin production (Ferrigo et al., 2016).

* + - * 1. Sowing density

Besides sowing period, also its intensity is an aspect to consider. An intense sowing can leads to a lack of water to the plant, which boosts pathogen development (Scherm et al., 2013).

* + - * 1. Weeds control

The need to control weeds in the field is related to the additional stress to small cereals, resulting from the competition for vital resources, with a negative impact in the crop production rate (Ferrigo et al., 2016). Even so, the use of herbicides in barley, oat and wheat has been studied, being found an increase in *F*. *graminearum* and *F*. *langsethiae* infestations with that use (Bernhoft et al., 2012). On the other hand, weeds may be a source of other pathogens, such as *Claviceps* spp.

* + - * 1. Irrigation

Even when irrigation as a mean of avoiding drought and consequent yield losses is required, its impact on *Fusarium* development needs to be taken into account, as irrigation leads to an increased moisture that favors fungal growth. For this reason, this practice should be minimized specially during anthesis, which is the most susceptible phase for mycotoxin accumulation, mainly DON and ZEN (Ferrigo et al., 2016; Scala et al., 2016).

This was confirmed in wheat experiments, with less FHB development observed when less irrigation was applied (Gautam & Dill-Macky, 2012b). Also, an observational study of wheat fields in Washington State showed that FHB was much more prevalent in fields with irrigation compared to fields with no irrigation (Edwards, 2011). Besides that, the impact on mycotoxins reduction was proven to be maximized by late-season irrigation, suggesting the leaching of these substances (Gautam & Dill-Macky, 2012b).

* + - * 1. Sowing fertilization

Nitrogen fertilization intensity is an aspect to consider when mycotoxin control is intended. Even if high fertilizations are related with disease spread, it has been reported that at the minimum necessary level to achieve quality of wheat grains, nitrogen fertilization at the studied levels have no effect on Crown Rot development caused by *Fusarium* spp. (Davis, Huggins, Cook, & Paulitz, 2009). Contrarily, in a FHB infected barley, it was found that lower levels of nitrogen were associated to a higher disease development (Yang et al., 2010). Also, other authors reported that the absence of mineral fertilization in barley, oat and wheat experiments led to higher *Fusarium* contaminations (Bernhoft et al., 2012). Lysoe et al. (2011) indicated that genes involved in nutrient acquisition and secondary metabolism are expressed at the early stages of infection (Lysoe et al., 2011; Walkowiak & Subramaniam, 2014).

* + - * 1. Fungicide treatments

Fungicide treatments enable direct control of fungal diseases and its efficacy has been demonstrated, for example, in controlling infection of triticale with *Fusarium* (Audenaert et al., 2014). To have a proper protection of barley grains, tillering is the best stage to start the application of fungicides (Bingham, Young, Smith, Spink, & Paveley, 2010). Opposite results were obtained from a barley, oat and wheat study, where reductions on *Fusarium* infestations were achieved by not using fungicides (Bernhoft et al., 2012). Even so, considering the overall efficacy of fungicides in reducing fungal infections, the effect in controlling mycotoxin contents is not so clear, and a correlation between both factors cannot be made (Audenaert et al., 2014; Ferrigo et al., 2016). In case of *Fusarium* infection, azoles are the natural choice, but it should be mentioned that this is not a complete control and adaption of fungi to azole fungicides may occur, and even result in higher mycotoxin production (Audenaert et al., 2013; Ferrigo et al., 2016).

Azole group fungicides are known to control *F. graminearum* and *F. culmorum* FHB and DON, but have little effect of *Microdochium nivale* causing FHB. Azoxystrobin controls mainly *M. nivale*, but has less effect on the other pathogens. For this reason, the selection of the fungicide is crucial. Controlling just *M. nivale* will potentially increase incidence of DON (Mateo, Valle-Algarra, Jimenez, & Magan, 2013; Pirgozliev, Ray, Edwards, Hare, & Jenkinson, 2012), but controlling both groups of pathogens may open the gate to other less competitive species - as *F. langsethiae* - not involved in FHB, but a T-2 and HT-2 producer (Mateo et al., 2013).

A novel and promising strategy to be used for the chemical control of pathogens is Spray Induced Gene Silencing (SIGS). This method is based on the host induced gene silencing, that is also of great potential, but requires a transgenic crop expressing an inhibitory noncoding ds-RNA. Recently, (Koch et al., 2016) reported the direct application of ds-RNA by spraying as an effective way to inhibit *F. graminearum* in barley leaves. According to these authors, the use of target specific dsRNA as an antifungal agent is simple to design, highly specific, applicable to many pathogens, and a potentially new plant protection strategy.

Besides the selection of the fungicide, its application mode will also affect efficacy. Sres et al (Sres, Trdan, Leskosek, Vidrih, & Vucajnk, 2015) tested the spraying speed of azole type fungicides in wheat, concluding that increasing spraying speed decreased the coverage of wheat head with consequent increase on FHB incidence. Nevertheless, DON occurrence was not significantly affected. Obviously, this conclusion is dependent on other variables as the type of injector nozzle and conditions of application, but emphasizes that not only the amount of fungicide but also how it is applied plays a role.

When relying on fungicides, resistance development risk needs to be considered. Assessment of resistance risk is a requirement but current tools have a limited predictive value (Grimmer, van den Bosch, Powers, & Paveley, 2014), due to the introduction of fungicides with new modes of action, targeting newly prevalent pathogen, where there is insufficient knowledge on resistance development.

It is clear that the decision of fungicide application is complex. It involves the cultivar used, the cropping practices (crop rotation, tillage, ...), the choice of fungicide and its application. By combining knowledge of fungicides mode of action and their absorption and movement in the plant, it is possible to construct a toll to predict the best timings to use for better crop protection. In wheat crops, there are typically three key fungicide timings: start of stem elongation (GS31-32), flag leaf emergence (GS39) and head emergence (GS59). In barley, there are two fungicide timings: start of stem elongation (GS30-31) and flag leaf to booting (GS39-49). However, under disease pressure, these timings need to be integrated with other factors, as host resistance or environmental issues (Poole & Arnaudin, 2014).

* + - 1. Host resistance

In a general way, defense mechanisms of plants against pathogens include salicylic and jasmonic acid-types responses, according to the specific type of pathogen infecting the plant (Audenaert et al., 2013). In the first scenario reactive oxygen species are induced and host prevent nutrient intake by the infector thought apoptosis and hypersensitive responses, and the two responses often co-occur when it is a *Fusarium* infection (Audenaert et al., 2013). In wheat infected with FHB, immunity is triggered and cell walls are reinforced (Chetouhi et al., 2015).

Priming strategies appear as a way to improve crops response to fungal attacks, since after an initial sensitization, defense will be greater and in less time, minimizing losses (Ameye et al., 2015). In a study with FHB infected wheat, Z-3-hexenyl acetate priming induced jasmonic acid-type responses, decreasing lesions caused by *F*. *graminearum* (Ameye et al., 2015).

Mycotoxin biosynthesis is influenced by the acidity of the culture medium, which promotes DON biosynthesis in the pathogen (Kazan et al., 2012). In response to mycotoxin biosynthesis, plants show its ability to glycosylate the compounds, as result of a defense mechanism already detected in wheat, durum wheat and barley that aims to decrease their impact on infected tissues by chemical transformation (Cirlini et al., 2014; Scherm et al., 2013). In fact, this might be an efficient mechanism through plant protection, but an ineffective one when considering consumption of infected plants, since mycotoxins can return to the original compound during processing or digestion (Scherm et al., 2013). Besides detoxification of DON, wheat responses to the presence of this mycotoxin include cell wall thickening mechanisms and incremented amino acids amounts (Warth et al., 2015).

The production of secondary metabolites, namely phenolic compounds, represents other way of plant response to fungal infection and mycotoxin production (Scherm et al., 2013). A study with *F*. *graminearum* infected barley showed its ability to produce natural compounds, secreted through the roots, as a defense mechanism against this soil pathogen (Lanoue et al., 2010). Higher levels of cinnamic acid and ferulic acid have been found in resistant wheats compared with susceptible genotypes; these compounds induce toxin biosynthesis, but also show antifungal activity on *F*. *graminearum* (in (Kazan et al., 2012)). In addition, the enzyme activity can be trigger by fungal infection. In an inoculation study of *Fusarium* in wheat, it was observed that extracellular invertase increased after infection, as a response of the infected plant (Korn et al., 2011).

After *Fusarium* infection of emmer grains the expression of protease inhibitors increases as a way to protect the host from fungal action against storage proteins, since the secretion of proteases by the fungus is a part of the infection cycle that enables its nutrient uptake and the attack of host cell walls (Eggert et al., 2011; Lowe et al., 2015). Besides that, as a defense response to DON accumulation in grains, emmer produced UDP-glucosyltransferase (Eggert et al., 2011).

Host resistance is a result of qualitative, single gene, and quantitative, multiple genes, sets (Audenaert et al., 2014). Genetic strategies to obtain resistant crops still have constrains because of the consumers’ acceptation and regulatory affairs (Mullins, 2015). In case of FHB, many Quantitative Trait Locus (QTL) are involved and obtaining resistant crops can be achieved through the incorporation of genes from resistant cultivars in the new one, or by controlled crosses to introduce those genes (Audenaert et al., 2014).

One mechanism of resistance detected in case of *F*. *graminearum* inoculation, but also triggered by the presence of DON, relies on the action of pleiotropic drug-resistant proteins, from ABCG transport group, that enable plant to expel pathogens’ compounds (Muhovski, Jacquemin, & Batoko, 2014).

* + - 1. Pest and disease control
         1. Chemical control

Aphids represent an important class of insects that requires control in order to reduce its impact on fungal infection (Ferrigo et al., 2016). Since insect damage enables *Fusarium* to reach interior parts of plant, and insects can be themselves the carriers of the inoculum, insecticide application emerges as a strategy to overcome this issue (Ferrigo et al., 2016; Scherm et al., 2013).

* + - * 1. Biologically based methods

The use of antagonistic microorganisms to control fungal infection, which can lead to the production of mycotoxins, lays on the competition between the different species in terms of nutrient uptake and attack through the production of antifungal compounds. Besides that, the inoculation of crops with biological control agents (BCAs) can promote defense mechanisms and prepare the plant to defend itself from the mycotoxigenic fungi, being the term also applied when a control of weeds is the main objective (Ferrigo et al., 2016; Heydari & Pessarakli, 2010). These methods can be applied by spraying techniques or seed dressing, with proven effectiveness on FHB and Foot and Root Rot (also called *Fusarium* Crown Rot) control in wheat, particularly caused by *F*. *culmorum* infection (Scherm et al., 2013). It is of concern the practical application of biological control under field conditions, since even when great results are achieved in laboratory and greenhouse scales, the same efficacy is rarely achieved in the field (Heydari & Pessarakli, 2010).

**Table C.2** presents data on biological microorganisms used to control target fungal species involved in small grains’ contamination, along with the respective type of assay. As it can be seen, *Bacillus* and *Streptomyces* species are predominant bacteria with reported antagonistic activity against pathogenic fungi, while regarding fungal agents, *Clonostachys* and *Trichoderma* genera appear as effective options.

When assessing biological control potential of *Bacillus* spp., *in* *vitro* inhibition of both *F*. *graminearum* and *F*. *culmorum*, main fungi responsible for FHB in wheat, was observed (Grosu et al., 2015). Also, the utilization of *Bacillus* *subtilis* has successfully controlled FHB development caused by *F*. *graminearum* and DON accumulation under field conditions, being in line with Argentine studies that report also the efficacy of *Brevibacillus* sp, as BCA and highlight the importance of assessing the time of application (Chulze et al., 2015; Juan M. Palazzini, Dunlap, Bowman, & Chulze, 2016). In fact, *Bacillus* species are often associated to their ability to inhibit fungal development, with efforts on identifying the metabolites that can be associated to this antagonistic activity. Bacillomycin D, a metabolite produced by *B. subtilis*, is an example, being reported its inhibition potential of *A. ochraceus* growth and OTA production, at a concentration of 90 µg/g in rice and oat matrixes (Ferrigo et al., 2016; Qian et al., 2016). Application of *Bacillus* species to control mycotoxin risk is being studied simultaneously because of their ability to degrade it. In a study on *B*. *amyloliquefaciens* effect on ZEN content in wheat flour, a reduction of 62,1 % was obtained after 24 h, which can indicate an even higher potential of these bacteria on biological control of fungal contaminations (Jianhong et al., 2016).

The combined use of *B*. *amyloliquefaciens* and *Lactobacillus* *plantarum* have shown not only the individual ability to *in* *vitro* inhibit *Fusarium* spp., but also reductions on FHB in wheat field with applications from the heading to anthesis (Baffoni et al., 2015).

*Streptomyces* sp. was also studied as a potential BCA against *F*. *graminearum*, aiming to control FHB development in wheat, showing that disease severity can be reduced after spraying of wheat heads (Jung, Park, Lee, & Lee, 2013). Also, *in* *vitro* assessments have shown that *Streptomyces* spp. and, more significantly, *Cellulosimicrobium* sp. inhibit *Botrytis* *cinerea*, *Fusarium* *oxysporum* and *Verticillium* *dahlia*, with advantages for barley seeds, observed *in* *vitro* and in soil pots experiments, associated to higher germination and growth (Nabti et al., 2014).

Assessments of *Pseudomonas* ability to control FHB caused by *F*. *graminearum* were also made. In field experiments, *Pseudomonas* *chlororaphis* subsp. *Aurantiaca* inhibit the development of the pathogen, and at *in* *vitro* scale it was shown that a produced metabolite, phenazine-1-carboxamide, not only acts against conidial germination and mycelial growth, but also can inhibit DON production (W. Hu et al., 2014). These results indicate that the potential of this bacterial strain can be greatly dependent on the antifungal activity of the studied metabolite.

Among fungal BCAs, *Trichoderma spp* have a great potential of usage, being detected that *T*. *viride* can inhibit AFB1 and AFG1 production by *A*. *flavus*, on ranges of 90,15 % and 100 %, respectively (Misra, Dixit, & Singh, 2010). Despite being associated to a high antagonistic activity, a specie of *Trichoderma* genera, *T*. *gamsii*, was unable to inhibit *F*. *culmorum* and *F*. *graminearum* on wheat haulms, highlighting the need to perform assess on different substrates to ensure the efficacy of microorganisms as BCAs (Matarese, Sarrocco, Gruber, Seidl-Seiboth, & Vannacci, 2012).

In addition, studies on biocontrol potential of *Clonostachys* *rosea* have shown its potential on FHB control and DON accumulation in wheat, and when conidial suspensions were applied a reduction in *Fusarium* spp. contamination of naturally infected wheat stalks was observed (J. M. Palazzini, Groenenboom-de Haas, Torres, Köhl, & Chulze, 2013; Xue et al., 2014). Also, *Aureobasidium* *pullulans* was found to be an antagonist for *Fusarium* species, including *F*. *culmorum*, in wheat grains, avoiding the penetration of the kernels and consequent damage (Wachowska, Tanska, & Konopka, 2016).

Besides using other species to control mycotoxigenic fungi, the application of strains without the potential to produce mycotoxins is an alternative. An *in* *vitro* investigation on the possibility of a atoxigenic *A*. *flavus* to reduce AFB1 and AFG1 production by toxigenic *A*. *flavus* have shown that in wheat grains inhibitions of up to 80,7 % and 100 %, respectively, can be achieved (Misra et al., 2010).

Processing

Apart from the biological control in field, alternative cultures can be applied during processing. Data on these microorganisms can also be found at **Table C.3** (underlined). At bread making, the application of microbial cultures to inhibit fungal growth is also a field of study, with assessments on the use of different species to perform the fermentation while having antifungal effects. Lactic acid bacteria (LAB), besides the already mentioned efficacy *in* *vitro* and at field scale, appear as an alternative, with special relevance on fermented products, since a proper selection of cultures to perform this step may provide not only the desirable final characteristics to the final production, but also an additional control of fungal spoilage (Varsha & Nampoothiri, 2016). *Lactobacillus* *plantarum*, *Lactobacillus* *amylovorus*, *Lactobacillus* *reuteri*, *Lactobacillus* *rossiae* and *Lactobacillus* *paralimentarius* are among the studied species with potential to provide additional control of fungal contamination of breads when applied in formulations (Coda et al., 2011; Garofalo et al., 2012; Jonkuviene, Vaiciulyte-Funk, Salomskiene, Alencikiene, & Miezeliene, 2016; Ryan Liam et al., 2011).

LAB may be applied in combination with other microorganisms with antagonistic activity against pathogenic fungi in order to increase control. The fermentation of sourdough with *Lactobacillus* *plantarum* and *Wickerhamomyces* *anomalus* inhibited *Penicillium*, *Eurotium* and *Aspergillus* species, being this inhibitory activity in some cases higher than the obtained by the application of a single biocontrol microorganism (Coda et al., 2011). The results of this method were very closed to the obtained with calcium propionate, allowing a preservation of bread slices for 28 days. Similarly, the production of breads with *Lactobacillus* *amylovorus* fermented sourdough have shown inhibition of *Fusarium* *culmorum*, *Aspergillus* *niger*, *Penicillium* *expansum* and *Penicillium* *roqueforti*, with an extension on calcium propionate preservation time (Ryan Liam et al., 2011). *Lactobacillus* *rossiae* and *Lactobacillus* *paralimentarius* were also applied in sourdough preparation with positive results on fungal development in bread and *in* *vitro* inhibition of *Aspergillus* *japonicus*, *Eurotium* *repens* and *Penicillium* *roseopurpureum* (Garofalo et al., 2012).

Besides bread production, LAB species seem to be effective antagonistic agents with a potential broad application. On an oat-based beverage, application of *Lactobacillus* *plantarum* strains was able to assure a full preservation of the product against *F*. *culmorum*, and *in* *vitro* efficacy was also observed for *A*. *niger*, *A*. *flavus*, *Penicillium* spp. and *Cladosporium* spp. (Russo et al., 2017).

The application of protective cultures in malting process is also of interest, with particular relevance on the control of *Fusarium* spp. growth that compromises not only the safety of the final product, but also its quality because of the gushing phenomenon. After the optimization of wort conditions, *Lactobacillus* *reuteri* use on malting led to a reduction of *F*. *culmorum* of 23 % and of DON production by more than 80 % (P. Oliveira et al., 2015). Similarly, *Wickerhamomyces* *anomalus* showed inhibition potential against *Fusarium* species during malting, being a potential yeast to be used in the control of fungal spoilage of beer (Laitila et al., 2011).

When fermenting barley in order to ensure a safe storage until it is used to feed cattle, the addition of the yeast *Pichia* *anomala* to the already used LAB cultures, has led to reductions on the final content of filamentous fungi (Olstorpe, Borling, Schnurer, & Passoth, 2010).

**Table B.2**: Biocontrol microorganisms with inhibitory potential on mycotoxin-producing fungi

| Biocontrol microorganism | Fungal species | Type of assay | Reference(s) |
| --- | --- | --- | --- |
| *Bacillus* spp. | *F*. *graminearum* and *F*. *culmorum* | *In* *vitro* | (Grosu et al., 2015) |
| *B*. *subtilis* | *F*. *graminearum* | Wheat field | (Juan M. Palazzini et al., 2016) |
| *B*. *amyloliquefaciens* and *Lactobacillus* *plantarum* | *Fusarium* spp. | *In* *vitro* and wheat field | (Baffoni et al., 2015) |
| *Streptomyces* sp. | *F*. *graminearum* | *In* *vitro* and *in* *vivo* (wheat seedlings and heads) | (Jung et al., 2013) |
| *Streptomyces* spp. | *Botrytis* *cinerea*, *Fusarium* *oxysporum*, *Verticillium* *dahliae* | *In* *vitro* | (Nabti et al., 2014) |
| *Cellulosimicrobium* sp. | *Botrytis* *cinerea*, *Fusarium* *oxysporum*, *Verticillium* *dahliae* | *In* *vitro* | (Nabti et al., 2014) |
| *Pseudomonas* *chlororaphis* subsp. *aurantiaca* | *F*. *graminearum* | Wheat field | (W. Hu et al., 2014) |
| *Talaromyces* *purpurogenus*, *E*. *nidulans*, *A*. *fumigatus*, *Alternaria* sp. and *F*. *solani* | *A*. *ochraceus* and *F*. *graminearum* | *In* *vitro* | (Madbouly, 2016) |
| *Trichoderma* *harzianum* and *Eppicoccum* *nigrum* | *Alternaria* *arborecens* | *In* *vitro* (wheat grains) | (Perello, Lampugnani, Abramoff, Slusarenko, & Bello, 2017) |
| *Trichoderma* *viride* | *A*. *flavus* | *In* *vitro* (wheat grains) | (Misra et al., 2010) |
| *Clonostachys* *rosea* | *Gibberella* *zeae* (Schwein.)  Petch (anamorph *Fusarium* *graminearum* Schwabe) | Greenhouse and field trials (wheat) | (Xue et al., 2014) |
| *Clonostachys* *rosea* | *Fusarium* spp. | Wheat stalks naturally infected (field conditions) | (J. M. Palazzini et al., 2013) |
| Atoxigenic *A*. *flavus* | *A*. *flavus* | *In* *vitro* (wheat grains) | (Misra et al., 2010) |
| *Aureobasidium* *pullulans* | *Fusarium* spp. (including *F*. *culmorum*) | Wheat grains (field and storage) | (Wachowska et al., 2016) |
| *Lactobacillus* *rossia* and *Lactobacillus* *paralimentarius* | *Aspergillus* *japonicas*, *Eurotium* *repens* and *Penicillium* *roseopurpureum* | *In* *vitro* | (Garofalo et al., 2012) |
| *Lactobacillus* *plantarum* | *A*. *niger*, *A*. *flavus*, *Penicillium* spp. and *Cladosporium* spp. | *In* *vitro* | (Russo et al., 2017) |
| *Lactobacillus* *plantarum* and *Wickerhamomyces* *anomalus* | *Penicillium* spp., *Eurotium* spp. and *Aspergillus* spp. | Sourdough fermentation | (Coda et al., 2011) |
| *Lactobacillus* *amylovoru*s | *Fusarium* *culmorum*, *Aspergillus* *niger*, *Penicillium* *expansum* and *Penicillium* *roqueforti* | Sourdough fermentation | (Ryan Liam et al., 2011) |
| *Lactobacillus* *plantarum* | *F*. *culmorum* | Oat-based beverage | (Russo et al., 2017) |
| *Lactobacillus* *reuteri* | *F*. *culmorum* | Malting | (P. Oliveira et al., 2015) |
| *Wickerhamomyces* *anomalus* | *Fusarium* spp. | Malting | (Laitila et al., 2011) |
| *Pichia* *anomala* + LAB | Filamentous fungi | Barley fermentation (feed) | (Olstorpe et al., 2010) |

* + - * 1. Natural compounds

Investigations on bioactive compounds that can ensure the safety of products are of growing interest, because of consumers’ demand for more natural products as an alternative to the usual use of synthetic additives. Particular attention is being given to antioxidants, phenolic compounds and essential oils, but other alternatives are emerging. An overview of some studies on inhibition potential of natural compounds, with reference to the target microorganisms can be found in **Table B.3**.

Besides the effect of natural compounds on fungal growth, its efficacy can also be observed on mycotoxins levels. A study on inhibition of fungal growth and OTA production in wheat grains, revealed that extracts from grape pomace and grape seeds can effectively decrease both these parameters, at an extent comparable to the obtained when using the synthetic phenolic antioxidant butylated hydroxytoluene (Alexa, Poiana, & Sumalan, 2012). Efficacy of *Cuminum* *cyminum* seed, *Coleus* *aromaticus*, *Hyptis* *suaveolens*, *Ageratum* *conyzoides* and *Trachyspermum* *ammi* fruit essential oils on inhibition of both *A*. *flavus* growth and aflatoxin biosynthesis *in* *vitro*, on stored wheat and on wheat seeds has also been observed (Jaya & Dubey, 2011; Kedia, Prakash, Mishra, Dwivedy, & Dubey, 2015; Kedia, Prakash, Mishra Prashant, & Dubey, 2014). Essential oils of lemon balm, garden sage, coriander, thyme, peppermint and cinnamon showed its *in vitro* efficacy on inhibiting DON and fumonisins biosynthesis by *Fusarium* spp., with evidences that a greater fungal inhibition is not necessarily associated to a high reduction on mycotoxins levels (Sumalan, Alexa, & Poiana, 2013). Also, with the use of small pieces of clove, *in* *vitro* inhibition of both *P*. *citrinum* growth and citrinin production was obtained (Aiko & Alka, 2013).

Regarding essential oils inhibition of fungal development, the mode of action seems to lay on membrane modifications, with mineral leaks, changes on organelle organization and reduction in ergosterol synthesis (Gill, Scofield, Li, & Saenger, 2016; Kedia et al., 2015). Particularly for phenolic fractions, the target looks the same, with detected degradation of cell walls (Scherm et al., 2013).

Fungal development and mycotoxin production can also be inhibited by the application of the whole plant material, as is the case of leaves. Neem and kikar leaves were found to inhibit *A*. *parasiticus* and aflatoxins biosynthesis in wheat, maize and rice, with the first presenting the highest protection (Sultana, Naseer, & Nigam, 2015).

Important aspects to consider when assessing the activity of antifungals, also for natural compounds, are the differences between *in vitro* and field results. This is particularly relevant because of the interactions that occur during plant infection, since the studied compounds may drive a greater or lesser defense response of the plant to the invasive fungi. An example of these differences was obtained during an investigation on dried bark of *Frangula* *alnus* efficacy in controlling FHB causal agents, with no significant reduction observed *in* *vitro*, but large inhibition of fungal development and DON production on field inoculations assays (Forrer et al., 2014).

Research interest is driven a more and more diversified investigation on new fungal inhibitors from natural origin. A study on the potential of intracellular and extracellular compounds obtained from microorganisms’ fermentation of cereals or sugars (in case, cultured corn syrup solids and citric acid, wheat solids and dextrose) have shown that inhibition of *P*. *chrysogenum* and *P*. *paneum* can be achieved, especially at low pH (Samapundo, Devlieghere, Vroman, & Eeckhout, 2017). Similarly, pea (*Pisum* *sativum*) hydrolysate inhibition of *Penicillium* spp., *Aspergillus* spp. and *Eurotium* spp. was found *in* *vitro*, and of *P*. *roqueforti* in inoculated bread, at an extent close to the obtained with calcium propionate (Rizzello Carlo, Lavecchia, Gobbetti, & Gramaglia, 2015).

The utilization of LAB as protective cultures in processed products, e.g. for fermentation, is, as stated before, a field of growing interest. Their efficacy is reported, with commercial products already available, but the application can also rely on the bioactive compounds produced by these cultures, which can show antifungal activity (Varsha & Nampoothiri, 2016). The use of antimicrobial peptides produced by *Lactobacillus* *plantarum*, has revealed their potential against *A*. *niger*, *Rhizopus* *stolonifer*, *Mucor* *racemosus* and *Penicillium* *chrysogenum* at *in* *vitro* conditions, and against natural flora in wheat grains, however causing a delay on the germination phase (Gupta & Srivastava, 2014).

Also, other natural products like the obtained from enzyme activity on glucosinolates, the isothiocyanates, have been studied for their antimicrobial properties. In fact, these compounds have shown antifungal activity against *Gibberella moniliformis* and some mycotoxigenic *Fusarium* strains, as well as FB2 and AFs production in bread and in wheat tortillas, respectively (Azaiez, Meca, Manyes, & Fernandez-Franzon, 2013; Quiles, Manyes, Luciano, Maes, & Meca, 2015).

Use of acivicin, a natural product that results from changes on amino acid structure, has demonstrated to be effective on decreasing the biosynthesis of trichothecenes produced by *F*. *graminearum*, although having less impact on fungal growth of *F*. *sporotrichioides* and *F*. *graminearum* (Maeda et al., 2014).

In barley processing, the application of a protein synthesized by *A*. *giganteus* - AFP - to different stages of the malting process resulted in promising effects, with a complete inhibition of fungi, including *Fusarium* genera, and reductions on DON contents (Barakat et al., 2010).

To reach effective fungal protection, research not only in new natural compounds, but also on the stability of the ones discovered when exposed to processing conditions is of major relevance, since it is known that the applied parameters may have impact on the structure of these bioactive compounds and, therefore, affect their antifungal activity (Ferrigo et al., 2016).

**Table B.3**: Natural compounds with inhibitory potential on mycotoxin-producing fungi

| Biocontrol/Plant compound | Fungal species | Type of assay | Reference(s) |
| --- | --- | --- | --- |
| Essential oils |  |  |  |
| Mexican oregano (*Lippia berlandieri* Schauer) essential oil | *Aspergillus* spp. | Wheat-flour based medium | (Avila Sosa Sanchez, Portillo-Ruiz, Viramontes-Ramos, Munoz-Castellanos, & Nevarez-Moorillon, 2015) |
| Mexican oregano (*Lippia* *berlandieri* Schauer) essential oil | *Aspergillus* sp., *Penicillium* sp., and *Rhizopus* sp. | Wheat flour-based medium | (Portillo-Ruiz Martha, Sanchez Raul, Ramos Sabina, Munoz Jose Vinicio, & Nevarez-Moorillon Guadalupe, 2012) |
| *Cuminum cyminum* seed essential oil | *Alternaria* *alternata*, *Aspergillus* spp., *Fusarium* *oxysporum,* *Penicillium* spp. | *In vitro* (PDA) | (Akash, Bhanu, Prashant, & Dubey, 2014) |
| *Cuminum cyminum* seed essential oil | *A*. *flavus* | Stored wheat | (Akash et al., 2014) |
| Lemon balm (*Melissa* *officinalis* L.), garden sage (*Salvia* *officinalis* L.), coriander (*Coriandrum* *sativum* L.), thyme (*Thymus* *vulgaris* L.), peppermint (*Mentha* *piperita* L.) and cinnamon  (*Cinnamomum* *zeylanicum* L.) essential oils | *Apergillus* spp., *Cladosporium* spp. *Fusarium* spp. and *Alternaria* spp. | *In vitro* (wheat seeds) | (Sumalan et al., 2013) |
| *Coleus* *aromaticus*, *Hyptis* *suaveolens* and *Ageratum* *conyzoides* essential oils | *A*. *flavus*, *A*. *niger*, *A*. *terreus*, *A*. *fumigatus*, *F*. *roseum*, *Alternaria* *alternata*, *Cladosporium* *cladosporioides*, *P*. *italicum*, *Curvularia* *lunata* and *Trichoderma* *viride* | *In vitro* and stored wheat | (Jaya & Dubey, 2011) |
| *Trachyspermum* *ammi* fruit essential oil | *Absidia* *ramosa*, *Alternaria* *alternata*, *Aspergillus* spp., *Cladosporium* *cladosporioides*, *Curvularia* *lunata*, *F*. *oxysporum*, *Mucor* sp., *Mycelia* *sterilia*, *Penicillium* spp., *Rhizopus* *stolonifer* and  *Spondylocladium* *australe* | *In vitro* and wheat and chickpea  seeds | (Kedia et al., 2015) |
| Others |  |  |  |
| Neem (*Azadirachta* *indica*) and kikar (*Acacia*  *Nilotica*) leaves | *A*. *parasiticus* | *In* *vitro* (wheat grains) | (Sultana et al., 2015) |
| Olive leaf water extract | Total yeast-mould | Wheat flour bread | (Degirmencioglu et al., 2011) |
| *Astrachantha* *echinus*, *Seriphidium* *herba*-*album*, *Peganum* *harmala*, *Diplotaxis* *acris* and *Tamarix* *aphylla* extracts | *Fusarium* spp., *Aspergillus* spp., *Alternaria* *alternata*, *Macrophomina* *phaseolina* and *Epicoccum* *nigrum* | *In vitro* (PDA) | (Madbouly, Ei-Magly, Madbouly, & Ei-Magly, 2015) |
| Chlorogenic acid, caffeic acid | *F*. *graminearum* and *F*. *culmorum* | *In vitro* (PDA) | (Gauthier et al., 2016) |
| *Galla* *chinensis* powder, *Frangula* *alnus* dried bark, tannic acid powder | *F*. *graminearum* and *F*. *crookwellense* | Wheat field | (Forrer et al., 2014) |
| Fermentates from cultured corn syrup solids and citric acid, wheat solids and dextrose fermentation | *P*. *chrysogenum* and *P*. *paneum* | *In* *vitro* | (Samapundo et al., 2017) |
| Hydrolysate from a mixture of pea, lentil, and faba bean flours | *P*. *roqueforti* | Wheat bread | (Rizzello, Verni, Bordignon, Gramaglia, & Gobbetti, 2017) |
| Hydrolysate from a mixture of pea, lentil, and faba bean flours | *A*. *parasiticus*, *P*. *carneum*, *P*. *paneum*, *P*. *polonicum* | *In vitro* | (Rizzello et al., 2017) |
| Pea (*Pisum* *sativum*) hydrolysate | *Penicillium* spp., *Aspergillus* spp. and *Eurotium* spp. | *In* *vitro* | (Rizzello Carlo et al., 2015) |
| Pea (*Pisum* *sativum*) hydrolysate | *P*. *roqueforti* | Bread | (Rizzello Carlo et al., 2015) |
| Grape pomace and grape seeds extracts | *A*. *ochraceus*, *P*. *verrucosum* | Wheat grains | (Alexa et al., 2012) |
| Phenolic compounds: carvacrol, thymol, isoeugenol, eugenol, vanillin, creosol, m-cresol, o-cresol, p-cresol,  and guaiacol | *F*. *verticillioides* | *In vitro* | (Dambolena, Lopez, Meriles, Rubinstein, & Zygadlo, 2012) |
| Clove | *P*. *citrinum* | *In vitro* (YES) | (Aiko & Alka, 2013) |
| Thymol emulsions | *F*. *graminearum* | *In vitro* and wheat plants | (Gill et al., 2016) |
| Antimicrobial peptides (AMPsLR14) produced by *Lactobacillus* *plantarum* | *A*. *niger*, *Rhizopus* *stolonifer*, *Mucor* *racemosus* and *P*. *chrysogenum* | *In* *vitro* | (Gupta & Srivastava, 2014) |
| Isothiocyanates | *Gibberella* *moniliformis*; *Fusarium* strains | *In* *vitro* and bread | (Azaiez et al., 2013) |

* + 1. Harvest management

At harvest, the adopted procedures must always have in mind the potential risk of propagation of contamination. Techniques applied from cutting may cause increased levels of fungal contents, with all the undesirable consequences. Specifically, a straight cut of barley was proven to favor pathogenic fungal contamination (Chen et al., 2016).

An effective crop management should allow harvesting at the right moment to assure the intended grain development and the reduction of contamination risks. Since a later harvest period is associated to a higher pathogenically fungal load, recorded for barley, this practice should be avoided (Chen et al., 2016).

* + - * 1. Drying

Being moisture content an important factor for fungal development, drying represents an effective way of make cereals suitable for storage. The utilization of airflows has proven successful in avoiding OTA contamination by *Penicillium verrucosum*, and the necessary rate of the flow is independent from the initial amount of fungal isolate (Wontner-Smith, Bruce, Cardwell, Armitage, & Jennings, 2014).

* + - * 1. Cleaning and sorting

Cleaning and sorting intend to screen cereal grains and eliminate undesirable materials, both other than the cereal itself and damaged kernels or grains that do not met the required specifications (Tibola, Cunha Fernandes, & Guarienti, 2016).

The effect of cleaning and sorting as primary steps after pre-harvest on mycotoxin control was observed in wheat samples, leading to consecutive lower levels of contamination, with greater impact being caused by mechanical gravity separation (Tibola et al., 2016). Besides that, on oat experiments, the effect of cleaning step on reduction of T-2 and HT-2 was not significant (Schwake-Anduschus et al., 2010). One effective way to reduce mycotoxins contaminations might be the inclusion of color sorting in the cleaning step. In fact, this method was proven to be successful in reducing mycotoxins risks, since it provides an appropriate segregation of *Fusarium* species contaminated grains (Nagy, Korzenszky, & Sembery, 2016).

* + 1. Post-harvest management
       - 1. Storage

At storage, as a result of favorable temperatures and humidity, fungi can deteriorate crops and cause significant losses, with *Fusarium*, *Penicillium* and *Aspergillus* species having a main role on this matter. At this stage, besides biological approaches, modified atmospheres and preservatives, aiming for the detoxification of mycotoxins or inhibition of responsible microorganisms, are exploited and can have great impacts on the final safety of the products (Naresh Magan, Aldred, Mylona, & Lambert Ronald, 2010).

Control of temperature and humidity is one way of controlling fungal spoilage of stored crops. A experiment with barley, in unfavorable conditions of temperature (23 °C and 30 °C) and water activity (0.80 to 0.94), proved that fungal growth was mainly affected by water activity (Wawrzyniak et al., 2013). In the latter study, an aw of 0.80 at 30 °C prevented fungal growth by more than 30 days, while an aw of 0.81 even at the lower temperature (23 °C) was conducive of fungal growth.

Spoilage during storage may be monitored by assessing temperature increases or CO2 production. (Mylona & Magan, 2011) reported a procedure for avoiding mycotoxin production in stored grains by monitoring CO2 production. Based on their findings, it was possible to correlate CO2 production with H-2 and HT-2 toxins accumulation in oat.

The use of silos bags to store crops is generalized and guarantees safety regarding fungi and mycotoxins accumulations, since it has the advantage of allowing proper sealing, whatever the amount of cereal, which does not happen when the storage occurs in conventional silos or warehouses (Gregori et al., 2013). The containment of cereals inhibits the contact with outside air, causing CO2 levels to increase, as a result of respiration process of the cereal, preventing fungal growth (Gregori et al., 2013; Naresh Magan et al., 2010). Based on this knowledge, the application of modified atmospheres to control fungal contaminations is often related to the control of O2/CO2 ratio (Naresh Magan et al., 2010).

Keeping natural coating materials around cereals is a way to protect them from fungal attacks during storage periods. In spelt wheat, the effect of hulls has been demonstrated to be positive in avoiding *Alternaria* toxins (Vuckovic, Bodroza-Solarov, Vujic, Bocarov-Stancic, & Bagi, 2013). In wheat and barley, aiming to study seeds coats protection against *Aspergillus*, conclusions showed that both fungal growth and mycotoxin production were reduced when natural coats were kept (Al-Hazmi & Gomaa, 2012). In line with these results, the effect of de-hulling on T-2 and HT-2 concentrations was proven to reduce a least 90 % of the initial levels of the mycotoxins, attesting the advantage of maintaining hulls to protect the inner parts, since mycotoxins will be concentrated at the external portion (Schwake-Anduschus et al., 2010).

The type of product that is to be stored is determinant, since the transformations it has already suffered may turn it more or less prone to fungal deterioration. In cereal flours, which have low moisture, storage temperatures might not have a key role, and, in this case, the material used to package acts as the main factor, since it will determine eventual exposure to outer atmosphere (Kolmanic, Simoncic, Vajs, Cencic, & Lesnik, 2010).

In this line, continuous innovation on packaging materials with antimicrobial nanoparticles is taking part. A study on the efficacy of incorporating zinc oxide nanoparticles in package of sliced bread have shown that at a concentration of 1 % and 2 % a total inhibition of fungal growth during 15 days occurred (Nooshin, Babak, Rezaei Mokarram, & Mahdi, 2017).

* + - * 1. Processing

Food processing can interfere with the stability of mycotoxins present in a matrix. Parameters used may reduce, or even increase concentrations, by the different distribution on the obtained fractions. Control of temperature, time and size of the samples is essential when studying fate of mycotoxins under process conditions (Vidal, Sanchis, Ramos, & Marin, 2015).

Milling

Fungal load is usually higher on the external parts of cereals, what leads to the general assumption that the same happens with mycotoxins (Edwards et al., 2011). There are evidences that final products of wheat milling present a distribution of DON higher in bran fractions, when compared to milled and flour (Tibola, Cunha Fernandes, Guarienti, & Nicolau, 2015). Also, studying the fate of T-2 and HT-2 in durum wheat, it was observed that bran presented higher concentrations than the initial grain, and the opposite occurred for cleaned final products (Pascale et al., 2011). The same pattern was detected for enniatins, which present the higher proportion of the initial content in the final bran, compared with the wheat flour (Vaclavikova et al., 2013). Studying OTA, also a reduction of its concentration in the obtained flour was observed (Peng et al., 2015). Even knowing that, some studies have shown mycotoxins to present similar concentrations in both inner and outer layers, or even higher in flour fractions of wheat compared to bran, as a result of a higher movement of compounds through the cereal, promoted in cases of high content of free water (Edwards et al., 2011; Thammawong et al., 2011). These results prove that the association between the utilization of just a part of the entire grains and a reduction on contamination risks is not completely linear, making it essential to determine the distribution of mycotoxins in the different fractions in order to control possible contaminations. In a study aiming to assess the effect of milling on DON content in wheat, the obtained flour presented lower final DON concentrations, although in the intermediate step, differences between bran and milled wheat were not significant (Tibola et al., 2016). Similarly, DON reductions of 29 % to 40 % were observed after dry milling and sieving of naturally contaminated and spiked wheat (Israel-Roming & Avram, 2010). Studying barley and wheat samples, it was observed that reductions of up to 84,4 % were obtained on total DON, NIV and ZEN contents after milling, and of 81 % after barley washing process (Hong et al., 2014). This type of processing does not seem to impact the ratio between original mycotoxins forms and their masked ones, since it was detected that DON and DON-3G maintained their relative contents on flour and bran wheat after milling (Kostelanska et al., 2011).

Thermal processing

As cereals are often used in baking processes, it is important to study the fate of mycotoxins at this stage. Assessing DON levels during baking, an increased was observed in bread obtained from wheat flour, accompanied with a decrease on the conjugated form (DON-3G), indicating a possible return to the original form under the studied baking process conditions (Monaci, De Angelis, Monaci, Pascale, & Visconti, 2013). In case of HT-2 toxin and T-2 toxin, assessed in the same study, the opposite occurred, with both of the mycotoxins decreasing, being the decrease more relevant in T-2 (Monaci et al., 2013). Enniatin B, enniatin B1 and beauvericin were found to be reduced by 25 % to 41 % during the entire production chain from flours to wheat and rye sourdough bread (Hu, Koehler, & Rychlik, 2014).

DON is known for its high thermal stability, and for this reason is analyzed in several studies on processing at high temperatures. Assessing mycotoxins’ thermal stability has being made during the processing of bakery, being found that both OTA and DON were reduced, with the first presenting a lower reduction (Vidal et al., 2015). In particular for wheat, baking at a minimum temperature of 230 °C caused a reduction of 10 % in DON content (Israel-Roming & Avram, 2010). Prove on the thermal degradation of DON, and its glucosilate form DON-3G, was obtained by the detection of reductions of 13 % and 10 %, respectively, during wheat bread baking, with particular attention to the fact that the major reduction occurred on the crust that is where higher temperatures are achieved (Kostelanska et al., 2011). The same trend was observed on enniatins, being detected only 30 % of the initial content in final bread, and on beauvericin, with degradations of 20 % to 90 % during the production of crispy breads with different types of flours, at 160 °C to 200 °C, for 3 min to 20 min (Meca, Ritieni, & Manes, 2012; Vaclavikova et al., 2013). For sterigmatocystin, the stability during bread making have shown that there is no significant reduction in any of the mechanical or thermal processes involved, which can attest the strong stability of this mycotoxin (Versilovskis, Bartkevics, Versilovskis, & Versilovskis, 2012).

Also boiling was proven to reduce mycotoxins levels, in this case by leaching, since a reduction of 82.7 % on total DON, NIV and ZEN in barley samples boiled for 1 h have been reported (Hong et al., 2014). Because of the high temperatures in frying, the same occurred with OTA contamination, decreasing around 8 % in the final wheat bread sticks (Peng et al., 2015). In oatmeal, the effect of boiling on T-2 and HT-2 was not clear, being in line with the results of the same study that showed a maintenance on the levels during baking, confirming the high thermal stability of these mycotoxins (Schwake-Anduschus et al., 2010).

In a general way, control of the time/temperature binomial within reasonable ranges for producers may lead to reductions on mycotoxins levels that can ensure food safety. However, this is a matter of constant care, since generally the initial concentrations necessary to achieve great reductions are significantly high (Bergamini et al., 2010).

Fermentation

In case of bread, prior to baking process, fermentation is another important step on the production line. In fact, this phase induces an increase in DON, as a possible result of enzymatic activity that breaks bounds and releases masked mycotoxin forms (Bergamini et al., 2010). For OTA, the same has been reported in wheat bread (Peng et al., 2015).

For liquid fermentation, a study on possible aflatoxin decontamination during the production of *kunun-zaki*, a common Nigeria beverage obtained from wheat and sorghum, revealed that with a storage temperature of 28 °C reductions of more than 52 % of the initial amounts of toxins can be achieved (Wartu, Whong, Abdullahi, Ameh, & Musa, 2015).

Pasta production and extrusion

During the production of pasta with different compositions, the analysis of enniatins levels revealed that minimum temperatures of 45-55 °C during drying step lead to a decrease of up to 50 %, increasing to 80 % when temperatures rise to 70-90 °C; between the analyzed enniatins, ENA and ENB1 were less thermal stable than ENA1 and ENB (Serrano, Font, Manes, & Ferrer, 2016). In wheat noodles processing, reduction of OTA was also detected, being possibly a result of the associated temperature of 180 °C and alkaline medium (Peng et al., 2015).

Extrusion of wheat flour at different operational conditions, to evaluate TeA, AME and AOH showed that the reduction of the latest was more dependent on screw speed and feeding rate, while the other two were greatly influenced by moisture content and screw speed, and that the optimal mycotoxin reductions were 65.5 %, 94.5 % and 87.9 %, respectively (Janic Hajnal et al., 2016). For DON levels during the production of wheat grits, moisture content and high compression appear as main parameters on reduction rate, which increases with residence time in the equipment, under alkaline medium and at high protein contents (Wu, Lohrey, Cramer, Yuan, & Humpf, 2011).

Malting

Studying *Fusarium* spp. and *Alternaria* spp. during malting process of barley, a development promoted by this process conditions was observed, which can explain the higher DON, T-2, and HT-2 contents in the final product obtained in different studies (Chen et al., 2016; P. M. Oliveira, Mauch, Jacob, Waters, & Arendt, 2012; Strub et al., 2010). More particularly, steeping and germination seem to be crucial on fungal development and of major concern, even considering the decrease observed at drying, which was detected on *F.* *culmorum*, but not on DON levels (Krstanovic et al., 2015). These results are in line with other observations on *Fusarium* and DON levels that increase during germination and until temperatures of 49 °C or 60 °C, depending on the initial load, are reached at the kilning phase; in case of DON, levels are maintained even at these temperatures, possibly as a result of its thermal stability (Vegi, Schwarz, & Wolf-Hall, 2011). Contrarily for enniatins, a decrease on contamination levels in the obtained malt seems clear, with an apparent accumulation of the mycotoxin in the spent grains, what can be of concern when these products are used as feed ingredients (Hu, Gastl, Linkmeyer, Hess, & Rychlik, 2014; Vaclavikova et al., 2013).

* + - 1. Decontamination

The application of ozone to control fungal development and mycotoxin production during storage is of growing interest. In fact, its effectiveness has been demonstrated in *A*. *flavus* contaminated wheat, with a complete inhibition of growth and AFB1 production after a minimum of a 10 min treatment at 20 or 40 ppm (El-Desouky, Sharoba, El-Desouky, El-Mansy, & Naguib, 2012). Ozone treatment has also resulted in a complete inhibition of *A*. *flavus* and *P*. *citrinum* at 60 µmol/mol, after 180 min, as well as significant degradations of AFB1, AFB2 and citrinin (Savi, Piacentini, & Scussel, 2015). In fact, the use of ozone because of its oxidant and disinfection properties, is being studied not only at storage level, in modified atmospheres, but also at processing, by including ozonated water in wheat tempering, which proved to inhibit *A*. *parasitus* and its aflatoxin production (Mohammadi Kouchesfahani et al., 2015). Also for barley, the application of gaseous ozone, in this case during malting experiments, was proven to lead to reduced *Fusarium* spp. contamination at the end of the process (Dodd James et al., 2011).

Another strategy to detoxify cereal products may be the addition of sodium metabisulfite, which has been shown to decrease DON concentrations, when applied alone or combined with propionic acid, in wheat kernels for preservation (Daenicke et al., 2010).

An alternative method to inactivate fungal load is the application of pulsed light to allow a safe storage. In wheat grains, the effectiveness of this method was confirmed, although it causes a little reduction on germination capacity (Aron Maftei, Ramos-Villarroel Ana, Nicolau Anca, Martin-Belloso, & Soliva-Fortuny, 2014). Attention on the influence of wavelength on mycotoxins has been paid, in order to evaluate possible degradation effects resultant from de exposure to certain parts of the light spectrum. Assessments on this matter have proved the efficacy of blue light (455 nm) on degradation of OTA, OTB and citrinin, being the last completely degraded; tests on effect on a wheat matrix have shown a reduction of OTA of around 50 % compared with control (Schmidt-Heydt et al., 2012).

Also, superheated steam emerges as a method to decontaminate infected cereals. Its efficacy has been demonstrated in a study with wheat grains, at which fungal contamination was eliminated at a minimum temperature of 110 °C, for at least 30 s, indicating a possible application of this process on flour production, after milling stage (Yueming, Wei, Xinzhong, & Zaigui, 2016).

Abbreviations

|  |  |
| --- | --- |
| ADON | Acetyldeoxynivalenol |
| AFs | Aflatoxins |
| AME | Alternariol monomethyl ether |
| AOH | Alternariol |
| aw | Water activity |
| BCAs | Biological control agents |
| DON | Deoxynivalenol |
| DON-3G | Deoxynivalenol-3-glucoside |
| EN | Enniatin |
| FB | Fumonisin |
| FHB | Fusarium Head Blight |
| LAB | Lactic acid bacteria |
| NIV | Nivalenol |
| OTA | Ochratoxin A |
| OTA | Ochratoxin A |
| OTB | Ochratoxin B |
| PCD | Programmed cell death |
| QTL | Quantitative Trait Locus |
| SIGS | Spray Induced Gene Silencing |
| TeA | Tenuazonic acid |
| ZEN | Zearalenone |

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