Summary

In the framework of the MycoKey project, this PhD thesis was mainly directed to search for new biocontrol agents (BCAs) that can be used to control *Fusarium graminearum* during the pre-harvest stage. MycoKey is a European Horizon 2020 project under the grant agreement No. 678781 which aims at developing smart, integrated, sustainable solutions and innovative tool kits to reduce the major mycotoxins in economically important food and feed chains. One of the main MycoKey approaches is to develop novel preventive measures and to improve, combine and fine-tune existing measures.

Moreover, this PhD work was achieved through the Association Research Platform at Ghent University called MYTOX which strives to solve current mycotoxin problems endemic in human food and animal feed by incorporating it into a global research framework, based on four main pillars. This includes: (i) identification, characterization and transcriptional engineering of toxigenic fungi; (ii) prevention and control of toxigenic fungi in the food and feed chain; (iii) targeted and non-targeted mycotoxin analysis and (iv) health impact upon toxins exposure. The Centre of Excellence in Mycotoxicology and Public Heath (CoEMPH) together with the Laboratory of Applied Mycology and Phenomics (LAMP) were involved in the work through an intra-university collaboration.

Chapter 1 presents a brief introduction to the reported pre-and post-harvest strategies to control the common toxigenic fungi and to reduce their associated mycotoxins with an updated overview of the documented BCAs that were used in the last 30 years in the pre-harvest stage for management of toxigenic fungi and their associated mycotoxins *in vitro* and/or in the field. This also includes a focus on the different modes of action of the most frequently studied BCAs as well as a comprehensive overview on their ability to suppress mycotoxin biosynthesis.

Chapter 2 states a general outline of the work and the main objectives aimed to achieve during the PhD work. In the first part of chapter 3, we screened for beneficial fungal endophytes, a polyphyletic group of highly diverse fungi that are defined functionally by their occurrence within tissues of plants without causing any obvious harmful effects, in stubbles/straw residues from Belgian maize field. Screening was conducted from the residues as they represent a matrix expected to contain primary inoculum of the pathogen in addition to several antagonistic fungi adapted to local environmental conditions, which can be used as BCAs to effectively prevent fungal infection through reduction of the pathogen inoculum.

Several fungal endophytes from various crop residues and soil samples were isolated and identified. Numerous fungal strains mainly of *Epicoccum* and *Sordaria* genera were tested

against *F. graminearum* and compared with previously known BCAs such as *Piriformospora* spp., an elusive endophytic genus, both *in vitro* and *in planta* (maize pot experiments). This part was performed at LAMP (UGent) where several protocols for screening, identification and testing of BCAs are established.

In the second part of this research, we investigated the effect of these endophytes on zearalenone, deoxynivalenol, and 15-acetyldeoxynivalenol levels as they are major mycotoxins produced by *F. graminearum*. As plants are endowed with several detoxification mechanisms comprising e.g., glucosylation of trichothecenes, the effect of the isolated fungal endophytes on the deoxynivalenol-3-glucoside level was also assessed. With state-of-the-art facilities such as liquid chromatography−tandem mass spectrometry at the CoEMPH (UGent), it was feasible to achieve this part through determination and quantification of the secondary metabolites of *F. graminearum* in one single run.

The obtained results showed a considerable variability in the antifungal activity, both among species and among isolates within one species. Additionally, the effect on mycotoxin levels was variable, and not necessarily related to the antifungal activity except for zearalenone levels which were consistently reduced by the endophytes. These results highlight the great potential of certain endophytic fungal strains as new BCAs in agricultural science.

In chapter 4, untargeted analysis using liquid chromatography−high resolution mass spectrometry was applied to investigate the effect of exo-metabolites of the two selected endophytic fungi *E. nigrum* and *S. fimicola* on the *F. graminearum* metabolism under an optimized *in vitro* assay. The growth of *F. graminearum* was inhibited after the exposure to the exo-metabolites. Furthermore, the color of the mycelium was changed suggesting an change in the secondary metabolism of the pathogen. Univariate and multivariate analysis showed several interesting metabolomic features that were significantly different between the treatment and control samples. However, the identification of these features was not achieved after using the available database for metabolite identification. This work indicates that the exo-metabolites exert a biocontrol effect *i.e.* the selected endophytes have more than one mode of action.

As a post-harvest control strategy, the effect of supercritical carbon dioxide ($ScCO_2$) and ultraviolet light type C (UV-C) treatments to inactivate or inhibit the germination of *Aspergillus flavus* in fresh pistachio and *F. graminearum* in wheat and maize was evaluated in chapter 5. The results indicated that $ScCO_2$ might have a potential to mitigate toxigenic fungi in agricultural products. Complete inhibition of the fungal growth and spore germination of *F. graminearum* was achieved with this technology. Furthermore, a reduction in the microbial

count for *A. flavus* was achieved after applying the $ScCO_2$ at 45 °C. However, no inhibitory effect on mycotoxin production was observed. The work suggested that longer treatments coupled with drying could be an effective way to inactivate the germination of *A. flavus* spores, fungal growth and aflatoxin production.

Chapter 6 gives a general conclusion and broader international context and relevance. The chapter starts with a brief introduction on the history of some mycotoxins and their producing fungi and discoveries. Furthermore, the effect of the current global issues such as climate change and ongoing research to address the potential effect of the climate change on the mycotoxin problem are discussed. As general future perspectives, food safety and the implementation of the BCAs in food industry is also covered. Exposomics which are a hot topic nowadays is also discussed and the current challenges for using this research in the mycotoxin field is presented.