Catching cereal killers: a multi-omics approach to disentangle yeast-Fusarium interactions in the phyllosphere

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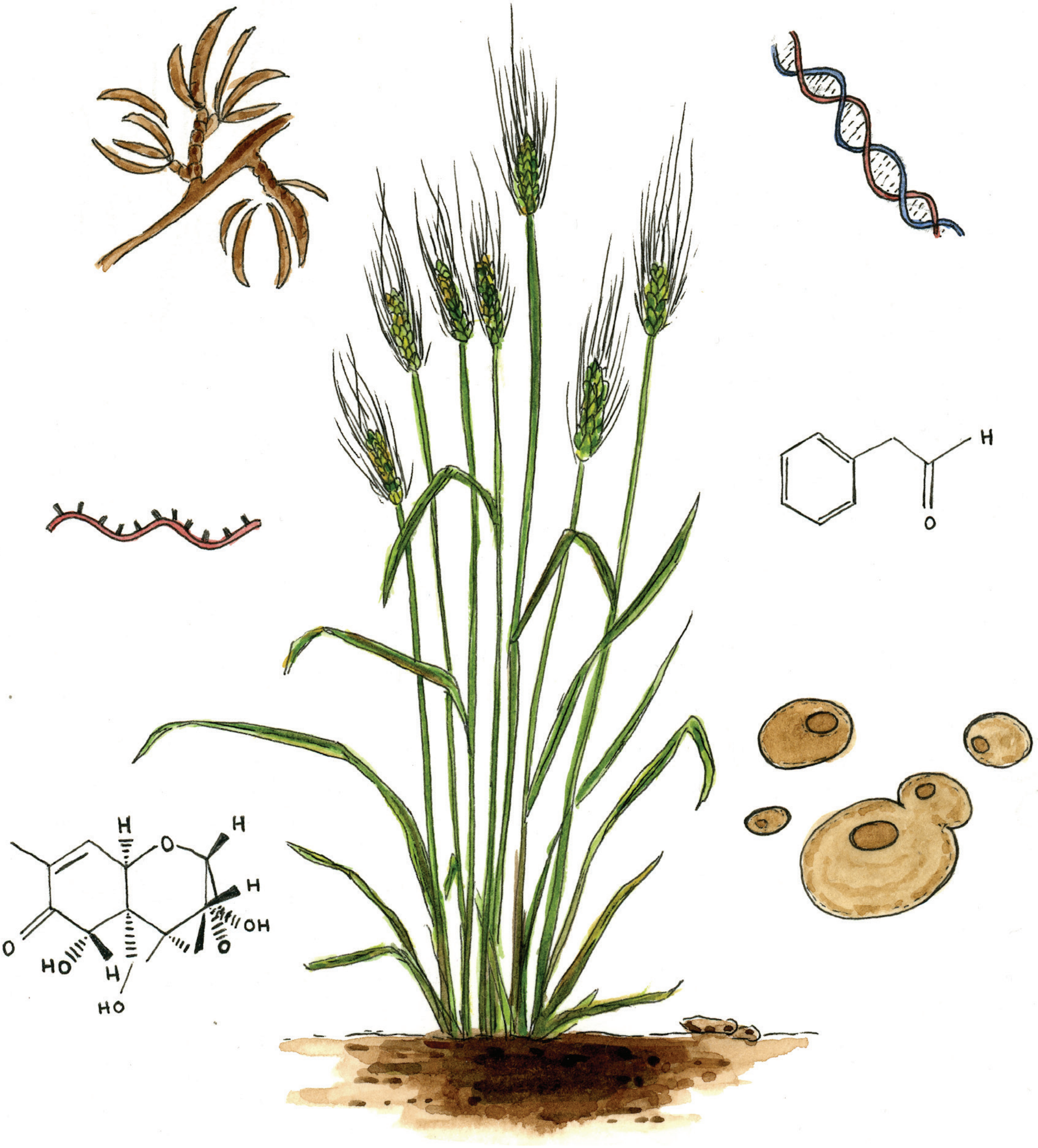
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Chapter 1

General Introduction
Thesis aim and outline

General introduction

The phyllosphere, the aboveground part of a plant, represents the largest environmental surface area with an estimated 60% of the Earth's total biomass across taxa[1,2]. The term "phyllosphere" was first introduced in the mid-1950s by Last and Ruinen to describe a unique environment that is physically, chemically and biologically distinct from the plant leaf itself or the surrounding air[3,4]. Although plant microbiome research historically focused on the rhizosphere, the belowground part of a plant, the phyllosphere microbiome, is now receiving increased attention[1,5]. This habitat is home to a diverse array of microorganisms, including bacteria, filamentous fungi, yeasts, viruses, and archaea, among others. Initially, most studies focused on pathogenic microorganisms inhabiting the phyllosphere, while lately interest has extended to all microbial inhabitants, via both cultivation-dependent and -independent approaches[5]. The phyllosphere is considered a challenging environment due to its fluctuating moisture levels, UV radiation, temperature changes, and limited nutrient availability[5]. To survive these abiotic stresses, phyllosphere microorganisms have evolved various adaptations, such as versatile metabolic capabilities, pigmentation, biofilm formation and the ability to degrade toxic compounds[6,7]. Additionally, they can inhabit both the leaf surface as epiphytes, and internal plant tissues as endophytes, leading to complex multipartite interactions involving the host plant, the microbial inhabitants, and the surrounding environment.

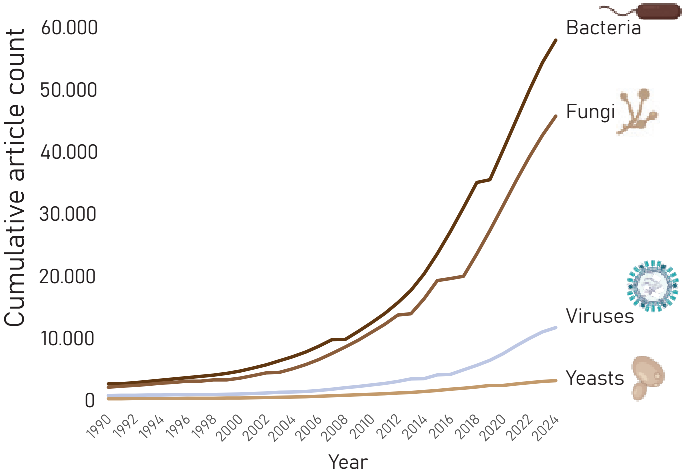

Figure 1. Cumulative number of published articles on ecology related studies. Studies are grouped by microorganism: bacteria (black), fungi (brown), viruses (blue), and yeasts (beige)

Bacteria are highly effective colonizers of the phyllosphere, typically reaching densities of 10^6 – 10^8 cells/cm[2], and numerous studies have explored and described their ecology[8]. Unlike bacteria, which colonize the phyllosphere both epi- and endophytically, filamentous fungi are predominantly known to inhabit the interior of the phyllosphere.

Although research on fungal ecology is nearly as extensive as that on bacterial ecology, yeasts have been largely neglected in these studies (Figure 1). Yeasts are single-celled eukaryotic organisms, making up an estimated 1% of the fungal kingdom[9]. Over 1.500 yeast species have been identified, spanning unrelated lineages within the ascomycetes and basidiomycetes, where they are placed based on ITS (Internal Transcribed Spacer) or whole genome sequencing, since morphological features seldom resolve their phylogenetic delineation[10,11]. Yeasts are among the most economically and scientifically significant microorganisms, in particular species such as *Saccharomyces cerevisiae*, known for its role in fermentation and as eukaryotic model organism, and *Candida albicans*, an opportunistic human pathogen. More than 2/3rd of all articles published (± 330.000) on yeasts have described one of these two species (PubMed, "Yeast", "*Saccharomyces cerevisiae*", "*Candida albicans*", Sept. 2024). Most yeasts reproduce by budding or binary fission, where a single cell divides into two equal daughter cells. Budding yeasts, such as *S. cerevisiae*, are also known as "true yeasts" and are classified within the order *Saccharomycetales*. Some yeasts, like the industrially important *Aureobasidium pullulans*, can form multicellular pseudohyphae or false hyphae displaying both yeast-like and filamentous growth; these yeasts are therefore referred to as dimorphic fungi.

Yeast dynamics in the phyllosphere

The composition and activity of the phyllosphere microbial communities change considerably due to the open environment and the dynamic physiology and chemistry of leaves throughout plant development[1]. Additionally, the phyllosphere is also considered a 'short-lived' environment for microbes inhabiting flowering and shedding plants, while newly formed leaves provide new territory for colonization[12]. Besides providing a home to a diverse beneficial or neutral microbiome, the phyllosphere is also an important entry point for many pathogens[12]. As a result, increased attention is devoted to exploring the factors that influence and shape the phyllosphere microbiome, the multipartite interactions among its members, and the potential impact on pathogen invasion and disease development. By employing metabarcoding of the ITS region, researchers aim to predict potential interactions in the phyllosphere through co-occurrence network analysis. This approach is particularly interesting when considering the role of important pathogens, providing candidate strains for further evaluation in the development of biocontrol strategies[13].

The wheat phyllosphere fungal community was one of the first phyllosphere communities studied[4]. Wheat is a critical crop for global food security, contributing to one-fifth of the world's total caloric intake. The study by Sapkota et al. (2017) investigated fungal communities within the wheat canopy across different growth stages and host backgrounds[12]. They found that yeast genera such as *Cryptococcus*, *Sporobolomyces*, *Dioszegia*, and the pathogen *Zymoseptoria tritici* were particularly dominant in the wheat phyllosphere. The type of cultivar played a significant role in shaping the fungal community on older leaves, while geographical location was more influential for younger leaves such as flag leaves. A negative co-occurrence was observed between *Z. tritici* and the yeast genera *Dioszegia*, *Sporobolomyces* and *Cystofiliobasidiaceae*. Chen et al. (2022) reported on the fungal community composition in the presence of two pathogens; *Puccinia striiformis* f. sp. *tritici*, the causal agent of wheat stripe rust, and *Blumeria graminis* f. sp. *tritici*, which causes powdery mildew[14]. A decrease in α-diversity of the phyllosphere microbiome was observed upon disease progression. Notably, the abundance of yeast genera such as *Aureobasidium*, *Rhodotorula* and *Sporobolomyces*, among others, increased at the early stages of pathogen invasion, but declined as the disease progressed, possibly due to competition for nutrients leaking from the infected tissue[15].

Several studies have been conducted to examine variations in fungal communities and which factors influence and shape the wheat phyllosphere microbiome. Karlsson et al. (2014 & 2017) explored the impact of fungicides and organic farming on fungal community composition[16,17]. Organically managed fields showed an increased species richness compared to conventional cultivation, which was best supported by the increased weed biomass[16]. While foliar fungicides are routinely used in agricultural practices to control specific pathogens, they can also disrupt the diversity and density of non-target fungi, potentially increasing susceptibility of the host plant to other pathogens like *Fusarium* spp.[18]. An overall reduction in fungal diversity could be observed after fungicide-treatment. Additionally, their study revealed that the abundance of the yeasts *Dioszegia* spp., *Aureobasidium pullulans*, and *Leucosporidium golubevii* was lower in fungicide-treated plots compared to the controls. Notably, the reduction in abundance of *A. pullulans*, a yeast extensively described for its biocontrol potential, raises questions whether this reduction has an impact on overall antagonistic capacity of the phyllosphere fungal community[17]. Following broad spectrum fungicide treatment, an increase in *Cryptococcus* and *Sporobolomyces* was observed, likely due to their rapid growth rate allowing them to quickly exploit the available vacuum space[19,20]. However, the foliar wheat pathogen *Z. tritici* also showed an increase, as it re-infected the wheat after early fungicide treatment.

Microbial interactions in the phyllosphere

Multiple strategies have been described to play a role in the resilience of yeasts in the phyllosphere. These include competition for space and nutrients, secretion of enzymes, mycoparasitism, induction of plant resistance and production of toxins or volatile organic compounds (VOCs)[21]. Competition for space and nutrients is a widespread phenomenon not restricted to the phyllosphere environment. However, mechanistic understanding is difficult to gain since its role in natural environments is probably more pronounced due to the limited resources and the co-occurrence with other (pathogenic) microbes, compared to laboratory-based studies[21]. One well-studied competitive strategy is biofilm formation which involves yeast cells in various stages, giving it a thick, structured appearance that protects against chemical and physical damage. Biofilm formation is not restricted to human pathogens; yet, research on beneficial plant-associated yeasts lags significantly behind studies on medically important yeasts like *Candida* species. For example, biofilm formation in *Candida albicans* allows it to rapidly proliferate and cause infection[22]. The biocontrol yeast *Pichia fermentans* forms biofilms on apple wounds, protecting against the postharvest pathogen *Monilinia fructicola*. This is proposed to be linked to the yeast's pseudohyphal growth, which also functions as a virulence factor that potentially transforms this biological agent into a destructive pathogen[23].

Niche and nutrient competition, including priority effects that lead to niche pre-emption, have been well studied in environmental yeasts[24–26]. Advances in genomic studies, along with other 'omics' approaches like transcriptomics and metabolomics, have greatly enhanced our ability to unravel these molecular processes. One of the first studies to identify genes involved in resource preemption focused on nutrient competition by the nectar yeast *Metschnikowia reukaufii*[24]. This study revealed that extensive genome expansion, particularly in the high-capacity amino acid transporter genes GAP1 and PUT4, favors rapid depletion of amino acids. This competitive effect was diminished by adding a surplus of amino acids, while no impact was observed with the addition of sucrose[24]. Another yeast commonly found in the phyllosphere, *Metschnikowia pulcherrima*, produces the red pigment pulcherriminic acid, an iron chelating compound. Although iron competition is suggested as a biocontrol mechanism, pigmentless colonies still showed antifungal activity, indicating that antagonism is not solely reliant on this strategy[27]. Biocontrol products like Botector® and Blossom protect®, with *A. pullulans* as the active yeast, are thought to suppress plant pathogens partly by competing for space and nutrients[28]. However, it is more likely that a combination of mechanisms contributes to pathogen suppression.

Yeasts can secrete various enzymes into their surroundings, influencing not only their host plant but also other nearby microbes. Additionally, they can trigger the expression of genes involved in the biosynthesis of lytic enzymes in plants. The cell wall of filamentous fungi is mainly composed of glucans (50 – 60%), chitin (± 20%) and proteins. Consequently, lytic enzymes like chitinases, glucanases and lipases greatly influence fungal membrane composition and integrity. *A. pullulans* has been shown to induce chitinase and β-1,3-glucanase production, either within apple fruits or through its own activity, affecting the postharvest pathogens *Botrytis cinerea* and *Penicillium expansum*[29]. Cloning and overexpression studies have enhanced our understanding of the genes encoding chitinases, thereby confirming their role in biocontrol activity. For example, overexpression studies in *P. pastoris* of the MfChi gene, encoding a chitinase in *M. fructicola*, demonstrated that MfChi is required for the yeast to inhibit the fruit and pear pathogens *M. fructicola* and *M. laxa*[30]. The biocontrol product Aspire, based on *Candida oleophila*, was initially believed to act through competition for space and nutrients[31]. However, knock-out and overexpression experiments have revealed the critical role of the gene CoEXG1, encoding exo-β-1,3-glucanase, in suppression of *Penicillium digitatum*[32]. Furthermore, the addition of cell wall fragments from *P. digitatum* increased the production of exo-β-1,3-glucanase, chitinase and protease in *C. oleophila*.

Mycoparasitism by yeasts has been described since 1997, but the underlying molecular mechanisms remain poorly understood[21,33]. Using time-lapse microscopy combined with fluorescent dye, Junker et al. (2018) demonstrated that the predatory yeast *Saccharomycopsis schoenii* can kill various *Candida* species, including the multidrug resistant human pathogen *Candida auris*. This process involves penetration pegs that induce vacuolization and ultimately lead to cell death[33]. The epiphytic yeast *Pseudozyma aphidis* suppresses powdery mildew partially through mycoparasitism, though the exact contribution of this mode of action remains unclear as it may operate in combination with antibiosis[34]. Therefore, further research is needed to elucidate how wide-spread this intriguing mechanism of microbial interactions in phyllosphere environments is.

Yeast-plant interactions

Yeasts engage in interactions with their host plants, obtaining nutrients and shelter while enhancing the plants' stress tolerance, nutrient uptake and resilience to biotic stresses. This interaction can significantly influence plant growth and health, and provide a basis for the deployment of beneficial microbes for sustainable agricultural practices[35]. Indole-3-acetic acid (IAA), a plant hormone of the auxin class, is the most commonly occurring plant growth promoting metabolite. Yeasts isolated from the phylloplane, i.e., the leaf surfaces, of various plant species grown in Thailand were shown to produce IAA[36]. This study showed that the IAA production is a widespread strain-dependent phenomenon, observed in 39 yeast strains representing 20 different species including *A. pullulans*[37]. Another study using tryptophan feeding experiments revealed the involvement of the tryptophan-dependent pathway, indole-3-pyruvic acid pathway, in the production of IAA by *Rhodosporidiobolus fluvialis*[38]. Yeasts have also been found to induce plant resistance by triggering the production of salicylic acid[39]. Foliar application of *Pseudozyma churashimaensis* led to plant defense priming and provided protection against *Xanthomonas axonopodis* as well as against several plant viruses[40]. Molecular analysis showed that the expression of the resistance marker genes CaPR4 and CaPR5 were induced in *Capsicum annuum*. Similarly, the application of *Metschnikowia pulcherrima* has been shown to induce resistance against the late blight pathogen *Phytophthora infestans* in potatoes, although no gene expression analysis was conducted to confirm this[41]. The application of the product Blossom Protect®, based on *A. pullulans*, to apple flowers stimulated the expression of PR genes, which are involved in systemic acquired resistance, suppressing infection by *Erwinia amylovora*[39].

Biotechnology of yeasts

Yeasts are well-known for their significant potential in biotechnology, producing a diverse range of metabolites, including vitamins and lipids[11]. Phyllosphere yeasts also produce a wide array of secondary metabolites, including both diffusible and volatile organic compounds (VOCs). Secondary metabolites are natural products not directly involved in the growth or development of micro-organisms or plants but play essential ecological roles[42]. For instance, functional characterization of the SNF2 gene in *Metschnikowia pulcherrima* demonstrated the involvement of pulcherriminic acid in the yeast's antifungal activity[27]. Similarly, *Pseudozyma flocculosa* has been described for its production of flocculosin, a rare antifungal glucolipid involved in the antifungal activity against powdery mildew[43]. *Debaryomyces hansenii* produces several mycocins, exotoxins against pathogenic yeasts such as *Candida albicans*[44].

Volatiles are small molecules generated through either primary or secondary metabolism[21]. Volatiles are characterized by their low molecular mass and high vapor pressure, facilitating long-distance communication and competition by diffusion through air. VOCs encompass structurally diverse molecules, including alcohols, hydrocarbons, aldehydes, ketones, esters, phenols, and terpenes, among others. The chemical composition of the volatilome varies depending on the yeast strain and growth conditions. The primary metabolite 2-phenylethanol is one of the most common yeast VOCs, and is described to inhibit mycelial growth and ochratoxin A production by *Aspergillus carbonarius*[45], and prevent spore germination, mycelial growth and aflatoxin production in *Aspergillus flavus*[46]. Furthermore, this VOC has also been shown to inhibit mycelial growth, reduce deoxynivalenol production and destroy cell membrane integrity in the wheat pathogen *Fusarium graminearum*[47]. *A. pullulans* is a well-studied producer of this and other VOCs and known to inhibit a broad range of pathogens, including *Penicillium expansum*, *P. digitatum*, *P. italicum*, *Botrytis cinerea*, *Colletotrichum acutatum* and *Alternaria alternata*[48–50]. The strong bioactivity of VOCs, combined with their minimal impact on human health and the environment, has led to an increased interest to explore and exploit these molecules as a sustainable alternative to synthetic fungicides[51]. However, limited understanding of their modes of action and the molecular mechanisms underlying yeast VOC – pathogen interactions hinder their application in agriculture.

Genomic and metabolic potential of yeasts

Saccharomyces cerevisiae is a key model organism and one of the most extensively studied eukaryotic microbes for the production of beer, bread and wine[52]. In 1996, it became the first eukaryote to have its entire genome sequenced, a project that spanned over seven years and involved collaboration among 100 different laboratories[53]. Omics techniques have been utilized to harness genomic information for natural product discovery and to identify key metabolites, primarily through the detection of biosynthetic gene clusters (BGCs)[54]. Additionally, metabolic engineering of *S. cerevisiae* has enabled the efficient biotechnological production of a wide range of metabolites, such as insulin, thanks to the comprehensive understanding of its physiology, metabolism and genetics[53]. The extensive systematic analysis of the *S. cerevisiae* genome, along with optimized tools, provides a strong foundation for exploring the genomic potential of environmental yeasts[6]. One example is the Synthetic Yeast Genome Project (Sc2.0), a global consortium aimed at constructing a synthetic eukaryote genome from scratch[55]. This effort, along with the development of tools like CRISPR-Cas, is expected to enhance downstream utility and to provide tools to assemble and manipulate eukaryotic genomes.

Currently, over one-fifth of yeast species has been sequenced[56]. Yeast genome sizes can vary significantly. The model yeast *S. cerevisiae* has a genome of 12 Mb with approximately 6,000 protein-coding genes[57], while the smallest known genome belongs to *S. kudriavzevii*, with only 8.5 Mb and around 4,000 protein-coding genes. In contrast, *Aureobasidium* species, known for their metabolic versatility and biotechnological applications, have some of the largest yeast genomes, averaging 28.4 Mb with approximately 24,000 protein-coding genes (NCBI Genome database). Increasingly accessible genome-sequencing methods have provided valuable insights into yeast ecology and evolution, supported by large datasets[58]. The growing availability of yeast genomes has made comparative genomics a powerful tool to perform in-depth characterization of genetic variations among species and strains. This approach has been extensively applied to wine yeast strains to improve winemaking through targeted selection and the creation of new strains[52,59]. Different wine strains influence various aspects of winemaking, such as flavor profiles. By leveraging these genomic techniques, novel and improved strains can be developed to meet public demand[59]. To illustrate the yet untapped potential of phyllosphere yeasts, we performed an extensive literature search on the availability of yeast genomes[6]. This study once more illustrated the gap between biochemistry and biotechnology studies, and ecology-related subjects, which only cover 6.6% of all articles regarding yeasts.

Emerging applications of phyllosphere yeasts against mycotoxigenic plant pathogens

Interest in engineering the plant microbiome to improve plant growth and health is rapidly developing[1]. Beneficial plant-associated microbes, also referred to as ″plant probiotics″, can offer a wealth of benefits to their host plants[60]. While much attention has traditionally focused on plant-growth-promoting (rhizo)bacteria, there is growing interest in microbes for biotic stress tolerance. The use of microbial-based pesticides is anticipated to rise globally as part of efforts to adopt more sustainable agricultural practices[61]. Yeasts are widely employed in the food and feed industry and are generally regarded to be safe; they are not reported to produce toxic compounds such as mycotoxins, and they have low nutritional requirements, while bulk-growth for biotechnological applications can be accomplished with fermenters.

In the context of exploring yeasts for their potential to enhance biotic stress tolerance of crops, a recent study by Chai et al. (2022) examined the impact of fungal pathogens on global wheat production and assessed the associated risks. Their study revealed that 80% of the global wheat-growing area is threatened by four major fungal pathogens, namely *Fusarium graminearum* (causing Fusarium head blight, FHB), *Puccinia triticic*na (leaf rust), *Puccinia striiformis* f. sp. *tritici* (stripe rust), and *Puccinia graminis* (stem rust). The risk posed by FHB is especially alarming, with a global average risk infection of 86.8%, and as high as 98.9% in developing countries[62]. Annual wheat losses due to FHB are estimated to range from 2.3 – 3.9%, translating to economic annual losses of $2.9 – $4.9 billion US dollars. Severe FHB outbreaks used to occur every four to five years in regions prone to the disease. However, likely due to global warming, the frequency of FHB epidemics has increased, with outbreaks now occurring every one to two years in China[63]. FHB not only causes significant yield losses due to shivered and empty grains, but also contaminates cereals with various mycotoxins. Strikingly, more than 60-80% of all grains are contaminated by at least one of these toxins, surpassing EU legal food safety limits in 20% of cases[64]. These secondary molecules remain stable at high temperatures and low pH. The most prevalent, and economically important, mycotoxin is deoxynivalenol (DON), or its most relevant derivatives DON-3-glucoside, 3-acetyl-DON and 15-acetyl-DON, with an incidence rate of up to 59% in cereals[64–66]. DON binds to ribosomes, thereby inhibiting protein synthesis causing various acute or chronic health problems in humans, including anorexia and immune dysregulations[64]. Numerous strategies, including resistance breeding, chemical application and biological control, have been deployed to mitigate FHB and DON contamination. Breeding for resistant genes to FHB is multigenic and greatly influenced by environmental interactions. Plant resistance is further complicated due to the high genetic variability of *F. graminearum* species[67]. The application of chemical fungicides is moderately effective in reducing FHB incidence, the timing of application is crucial and the accumulation of these chemicals in the environment and the food chain has made these methods unwanted[68]. Consequently, the focus has shifted towards environmentally friendly alternatives based on biocontrol agents including yeasts[69,70].

Biological control is defined as the use of microbial antagonists or their properties to prevent or suppress pathogen infections. This can be adopted at different developmental stages of the host plant. Namely, pre-harvest, in-field application to limit contamination, and post-harvest application of micro-organisms or enzymes for detoxification. Several microbes or microbial consortia have demonstrated the ability to inhibit the production or degrade DON via two mechanisms, namely absorption to the cell wall or biodegradation[71]. Studies suggest that the composition of the cell wall plays a pivotal role in binding DON, where the content of β-(1,3)-D-glucans plays a major role in absorption efficiency[72]. For instance, freeze-dried *S. cerevisiae* cells were used to decontaminate wort (e.g. ground grains used for fermentation), successfully removing 10 – 17% of DON and 30 – 70% of another mycotoxin zearalenone (ZEN). *S. cerevisiae* has also been reported for its ability to inhibit *F. graminearum* and degrade DON[73,74]. Application of *Debaryomyces hansenii* to grains of winter wheat resulted in almost complete removal of DON, compared to the control group which had concentrations of 141.36 µg/kg[75], while field experiments with *Cryptococcus flavescens* decreased FHB disease severity by 42% and reduced DON levels by 41%, although not statistically significant[76,77].

A promising alternative to synthetic pesticides is the use of microbial VOCs, which suppress pathogens from a distance without requiring direct contact between the beneficial microbial strain(s) and the pathogen. Additionally, the high volatility also allows for broad and uniform distribution of the active VOCs, a key challenge in open-field agriculture[78]. Yeasts have been widely demonstrated to suppress fungal pathogens through the production of VOCs. *A. pullulans*, for example, has been shown to inhibit *Alternaria alternata* and *Botrytis cinerea*, likely through the production of ethanol and 2-phenylethanol, which were proposed to disrupt membrane composition, induce electrolyte loss and cause oxidative stress[48,49]. The production of 2-phenylethanol also inhibited the growth of apple fruit pathogens such as *Penicillium expansum*, *P. digitatum*, and *P. italicum*[50]. Additionally, *Wickerhamomyces anomalus* produces ethyl acetate, which inhibited postharvest decay on strawberry caused by *B. cinerea*[79]. *Metschnikowia pulcherrima* has also been found to inhibit *Alternaria alternata*, the causal agent of grey spot rot on loquat fruits, partly through the production of 3-methyl-1-butanol, 2-phenylethanol and 1-hexanol[80]. However, despite their strong antagonistic properties and significance as eukaryotic model organism, only a limited number of yeast-based biocontrol products have reached the market[21]. Regulatory hurdles for biopesticides are often stringent and time-consuming, requiring extensive efficacy and safety testing. Additionally, variability in field performance further limit their availability[21].

Outstanding questions

What is the diversity and spatiotemporal distribution of yeasts in the wheat phyllosphere?

Which adaptive traits enable yeasts to effectively colonize the phyllosphere?

How widespread is the antagonistic activity of phyllosphere yeasts against fungal pathogens?

How diverse is the volatilome of phyllosphere yeasts and what is their role in the interactions with fungal pathogens?

What molecular mechanisms govern yeast-pathogen interactions?

Can phyllosphere yeasts degrade mycotoxins produced by fungal pathogens and/or inhibit their biosynthesis?

Can phyllosphere yeasts prime the innate immune system of the host plant to suppress pathogen infection?

How can phyllosphere yeasts be leveraged for sustainable agricultural practices?

Thesis outline

The overall aim of my PhD thesis is to study the taxonomic and functional diversity of yeasts in the wheat phyllosphere and to elucidate their interactions with the mycotoxigenic fungal wheat pathogen *Fusarium graminearum*. To disentangle the different modes of action, I used an integrated approach involving *in vitro*, *in vivo*, and *in planta* bioassays, genomics, transcriptomics, and metabolomics (Figure 2).

Current knowledge on yeasts stems primarily from industrial and medical research focusing on *Saccharomyces cerevisiae*, known for its role in fermentation (e.g. beer, wine and bread), and the human pathogenic yeast *Candida albicans*. **Chapter 2** highlights the natural roles that environmental yeasts can play in the plant phyllosphere environment. We explored the diversity, dynamics, interactions and genomics of plant-associated yeasts and identify the knowledge gaps between phyllosphere and medical/industrial yeasts, exemplifying that so far, we have only scratched the surface of their full functional potential.

The phyllosphere is known for its diverse biotic (e.g. pathogen invasion) and abiotic (e.g. limited nutrient availability and oscillating temperatures) stresses, creating a hostile environment for its microbial inhabitants. Therefore, successful colonization of this dynamic environment requires specific adaptive traits. To investigate the ability of phyllosphere yeasts to withstand different stresses, yeasts were isolated from the wheat flag leaf. In **Chapter 3**, we characterize a diverse selection of yeasts for various adaptive traits, including flexible carbon utilization, tolerance to a range of temperatures, biofilm formation and antagonism towards the cereal wheat pathogen *Fusarium graminearum*. We highlight the ability of several yeast genera, especially *Aureobasidium*, *Metschnikowia* and *Papiliotrema* to inhibit *Fusarium* through the production of diffusible and volatile compounds.

In **Chapter 4**, we perform an extensive genomic analysis to profile the functional potential of a selection of 96 phyllosphere yeasts, representing 14 different genera. We further profile various enzyme groups with CAZymes that may be beneficial for phyllosphere yeasts, including the production of lactamases, secondary and volatile compounds, and their ability to degrade various complex polysaccharides. Additionally, we also perform comparative genomics to identify traits that might be beneficial for leaf-associated yeasts.

Chapters 5 and **6** dive deeper into the volatile-mediated interactions initially described in **Chapter 3**. We profile the volatilome of a selection of 40 yeasts via GC-MSD analysis (**Chapter 5**) and highlight differences between yeast phyla and genera. We investigate the reciprocal influence of yeast (e.g. *Metschnikowia*) volatiles on *F. graminearum*. Additionally, VOCs were identified that play a role in the volatile-mediated interactions between specific yeasts and *F. graminearum*. **Chapter 6** addresses the transcriptional changes induced in yeasts and *Fusarium* via volatiles. This chapter further aims at investigating if/how yeasts modulate the production of toxic compounds produced by *F. graminearum*, in particular the mycotoxins DON and ZEN.

Besides being a hostile environment for microbes, the phyllosphere is also known for its complex interactions among micro-organisms. In **Chapter 7**, we investigate the ability of a selection of phyllosphere yeasts to inhibit mycotoxigenic *F. graminearum* in a more natural habitat, i.e. wheat leaves and heads. By combining various experimental approaches, we investigate the complex interaction dynamics between two phyllosphere yeasts and *F. graminearum*. We further focus on the ability of yeasts to modulate the production of mycotoxins through the degradation of precursors, and the effect of these mycotoxins on yeast growth.

Chapter 8 integrates these findings and discusses the ability of phyllosphere yeasts to adapt to various external stresses. We also give a future perspective on the use of yeasts as biocontrol agents and highlight the knowledge gaps needed to be addressed before this microbial technology can be successfully implemented as a novel sustainable practice in modern-day agriculture.

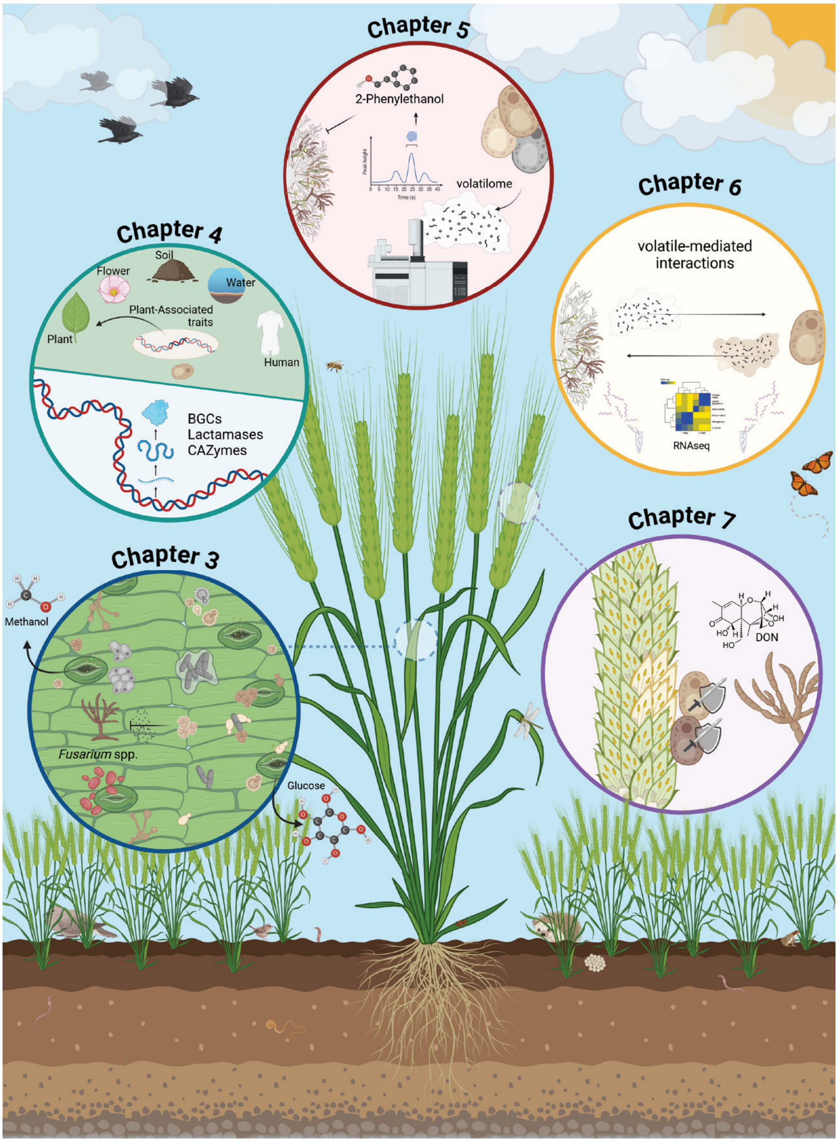

Figure 2. Schematic overview of the experimental chapters presented in this thesis

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