# Exploring Synergistic Effects of Soil Microorganisms in the Wheat Rhizosphere

## Evaluating the Role of Native Soil Bacteria and Arbuscular Mycorrhizal Fungi for *Fusarium* Disease Suppression

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Degree project/Independent project • 30 credits
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Faculty/Department: Crop Production Ecology
Programme/Education: Plant Science for Sustainable Production
SLU Ultuna, 2024

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|---|---|
| **Credits:** | 30 credits |
| **Level:** | Advanced (A2E) |
| **Course title:** | Master Thesis in Biology |
| **Course code:** | EX0895 |
| **Programme/education:** | Plant Biology for Sustainable Production – Master's Programme |
| **Course coordinating dept:** | Department of Aquatic Science and Assessment |
| **Place of publication:** | Uppsala, Sweden |
| **Year of publication:** | 2024 |
| **Cover picture:** | Electra Lennartsson |

| **Keywords:** | *Fusarium Crown Root Rot, plant growth promoting bacteria, native bacterial strains, Fusarium spp., spring wheat* |

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# Abstract

This thesis aimed to investigate the underlying processes of potential synergistic effects of native soil bacteria exhibiting plant growth-promoting traits and arbuscular mycorrhizal fungi (AMF) in suppressing *Fusarium* Crown and Root Rot in spring wheat cultivars. Through greenhouse pot experiments, the efficacy of microbial interventions was evaluated. Despite encountering weaker results in terms of disease suppression than initially expected, this thesis still manages to show significant results with the introduction of AMF and a local microbial community provided by Hasta gård may have in terms of disease suppression. Specifically, observations in spring wheat cultivar Diskett align with existing literature as a promising cultivar in showcasing the positive effects AMF may have, and the synergy it could potentially establish below ground in terms of plant resilience challenged with fungal diseases. These results, however, should be approached with caution as more tests and replicates for statistical robustness are needed. They could potentially be used as a springboard for further studies in that direction in the pursuit of sustainable practices in the realm of beneficial soil bacteria and fungi acting as biocontrol agents.



*Keywords*: *Fusarium* Crown Root Rot, plant growth promoting bacteria, native bacterial strains, *Fusarium* spp., spring wheat cultivars.

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# Abbreviations

| | |
|---|---|
| AMF | Arbuscular Mycorrhizal Fungi |
| EM1 | Effective Microorganisms 1 |
| FCRR | *Fusarium* Crown and Root Rot |
| IPM | Integrated Pest Management |
| ISP2 | International Streptomyces 2 |
| LB | Luria-Bertani |
| PDA | Potato Dextrose Agar |
| PGPR | Plant Growth Promoting Rhizobacteria |
| RQ | Research Question |

# 1. Introduction

*'Bacterial communities inside of and around plant roots help sound the alarm and man the barricades when pathogens storm the botanical gates.'*
- The Hidden Half of Nature: The microbial roots of life and health, by Montgomery & Bikké

Amidst the tumultuous landscape of recent years, food security and access to safe, healthy nourishment have become increasingly precarious (FAO 2023). From the dawn of the global COVID-19 pandemic to the ongoing conflict resulting from Russia's invasion of Ukraine; both countries being major players in food production and the former in fertiliser trade, the stability of our agricultural systems have been severely tested (FAO 2023). These crises, which often occur in combination, are compounded by the relentless impacts of climate change, culminating in a sharp surge in food and energy prices in March 2022, an increase that has yet to abate (FAO 2023). In the face of such challenges, the importance of wheat crop health and productivity cannot be overstated (Willocquet et al. 2021), particularly as we sustainably strive for crop resilience.

Wheat (*Triticum aestivum*) is a staple food for billions of people worldwide. In fact, this translates into over 800 millionhectares of land accounting for wheat production by providing essential nutrients, and 15% calorie intake in diets across diverse cultures and regions (FAOSTAT 2022). However, the susceptibility of wheat crops to pests and diseases, as well as environmental stressors, such as climate change and globalisation, pose a significant threat to yield quantity and quality. These losses are estimated to escalate further in the coming years (Lobell et al. 2011). With climate change as a catalyser, these disruptions can have far-reaching consequences, exacerbating food insecurity, and inflation, and undermining social stability.

Plant pathogens pose a significant threat to staple crops. The resulting diseases have been associated with substantial yield losses globally, ranging from 10% to 28.1% (Savary et al. 2019). Fungal diseases, including those caused by *Fusarium* species, present persistent challenges to wheat cultivation (Bottalico & Perrone 2002). Conventional agricultural practices, including heavy reliance on pesticides, monocultures, limited crop rotations and vulnerability to invasive species, have heightened susceptibility to *Fusarium*-related diseases (Goswami & Kistler 2004) by disrupting soil microbiota and creating conditions that act as a substrate for disease proliferation. All these factors collectively may aggravate the buildup of *Fusarium* pathogens belowground and thus increase the risk of infections caused by *Fusarium* strains. Despite efforts in disease suppression, prevention, and management, the efficacy of conventional methods remains limited, often leading

to environmental degradation and long-term soil deterioration and depletion (Champeil et al. 2004). The prevalence of *Fusarium* pathogens in cereal crops, such as wheat, barley, and maize, only accentuates the urgency for sustainable disease management strategies (Leslie & Summerell, 2006). In particular, *Fusarium* crown and root rot (FCRR) represents strenuous challenges, impacting yield quantity and quality (Smiley et al. 2005).

In response to these challenges, a paradigm shift towards harnessing beneficial soil microorganisms to enhance plant health and resilience is underway (Bonfante & Genre 2010; Pieterse et al. 2014). This continuous exploration of soil microbiota arises from the recognition that these microorganisms are essential in shaping plant-microbe interactions, nutrient cycling, and overall ecosystem functioning (Yu et al. 2019; Hnini et al. 2024). More research is needed to investigate the interaction between microorganisms, to utilise the potential of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPRs) as sustainable biocontrol agents against *Fusarium*-related challenges in wheat (Berruti et al. 2015; Sharma et al. 2021). Although there is documented evidence that there is a potential to mitigate *Fusarium*-related diseases whilst simultaneously promoting plant growth (Nadeem et al. 2014; Liu et al. 2019) there are undoubtedly parts about the synergistic interactions between these microorganisms that have yet to be discovered to form the larger underground puzzle of what is occurring in the hidden half which is what this thesis will seek to explore.

## 1.1 *Fusarium*

### 1.1.1 *Fusarium* – Related Diseases

*Fusarium* Crown and Root Rot (FCRR) is a fungal disease caused primarily by soil borne pathogens such as *Fusarium culmorum* (Kazan & Gardiner 2018; Bozoğlu et al. 2022) as well as *Fusarium graminaerum* (Kulik et al. 2023) and *Fusarium pseudograminearum* (Beccari et al. 2011; Wang et al. 2015; Figueroa et al. 2018).

*Fusarium* fungi are notorious for causing diseases in various crops, including cereals, vegetables, fruits, and ornamental plants, the manifestation of which varies depending on the host plant and environmental conditions. *Fusarium* head blight (FHB) predominantly affects the reproductive structures of cereal crops, leading to yield losses and grain quality deterioration (Dean et al. 2012; Yu et al. 2021). Often underlooked is the significant threat posed by *Fusarium* in compromising the below-ground structures and impeding water and nutrient absorption in the FCRR disease (Dean et al. 2012).

In this thesis, our focus specifically centres on *Fusarium* diseases impacting the roots and root crown of wheat plants.

### 1.1.2 *Fusarium* Crown and Root Rot (FCRR) Symptoms on Wheat

Infection of *Fusarium* spp. is manifested as symptoms in both shoots and roots. Early signs of FCRR, such as wilting and leaf decolouration, appear to be similar to drought or nutrient deficiency (Wang et al. 2015). Depending on the growth stage of the plant when infected, symptoms of brown necrotic discolouration of the roots and coleoptiles, with early infection causing seedling blight and killing the emerging plant (Beccari et al. 2011; Scherm et al. 2013). This can expand throughout the crown and infect the base of the stem showing signs of honey-brown discolouration just above the soil line (Li et al. 2012; Bozoğlu et al. 2022). This initial phase is followed by tiller abortion, wherein whiteheads with shriveled white grains form, often alongside premature bleaching of the entire head. In advanced stages, FCRR ultimately leads to yellowing of leaves, and, in severe cases, plant death (Al-Tovi & Haleem 2018). Evidently, FCRR can result in grain yield loss and quality reduction (Moya-Elizondo 2013).

Understanding these symptoms is key for early detection and effective management strategies to mitigate the impact of FCRR on cereal crops. So far, efforts to manage FCRR conventionally, involve the application of fungicides. Nevertheless, this approach is fraught with ecological and economic drawbacks. Fungicide resistance in *Fusarium* spp. is well-documented (Lamichhane et al. 2016), necessitating constant innovation and increasing chemical input costs for farmers. Furthermore, the environmental implications of fungicide use, including soil and water contamination, further indicate the unsustainability of relying solely on chemical solutions (Köhl et al. 2019).

### 1.1.3 Global Impact of FCRR

The economic repercussions of FCRR are far from negligible; with yield losses extending up to 35% depending on environmental conditions and irrigation practices. In Australia, the recorded estimate of annual yield losses exceeded AUD 90 million (Petronaitis et al. 2022). Moreover, FCRR caused by *Fusarium pseudograminearum* is emerging as a major threat in various regions, including China, Africa, and the Pacific Midwest of the USA (Blum et al. 2019).

Beyond the immediate yield reduction, the quality of harvested grains is compromised, impacting both food and fodder utilisation. In *Fusarium* head blight primarily, it is documented that *Fusarium* produces mycotoxins, a collection of different classes of secondary metabolites which are toxic substances to human and animal health (Ji et al. 2019). Additionally, the persistence of *Fusarium* spp. in the soil further aggravates the problem, as the pathogen can survive for extended periods in crop residues, creating a continual threat to subsequent wheat crops (Beccari et al. 2011). Last but not least, the disease impairs the vulnerability of crops to other stress factors by impacting wheat plants' physiological processes, such as nutrient uptake and water utilisation (Goswami & Kistler 2004).

The global spread of FCRR highlights the need for effective disease management strategies to mitigate its impact on cereal crops. Despite extensive research on *Fusarium*-related diseases, several areas remain underexplored, indicating potential knowledge gaps in this field. There is a considerable lack of knowledge on how *Fusarium* species and strains interact with their environment. Understanding the complex relationships between *Fusarium* pathogens, soil conditions, and disease development is crucial for implementing targeted disease management practices.

Given the challenges posed by FCRR on wheat crop health, exploring alternative avenues, with an eco-friendly and cost-effective approach, becomes critical. In this context, documented beneficial microorganisms residing in the rhizosphere, such as arbuscular mycorrhizal fungi (AMF), plant growth promoting rhizobacteria (PGPR), and the patented effective microorganism (EM1), have emerged as potential candidates for disease mitigation. My thesis explores how these microbial allies can aid in FCRR disease suppression in wheat and, if so, what their potential roles and interactions are for practical application.

## 1.2 Beneficial Microbes

### 1.2.1 Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizal fungi (AMF), belonging to the Glomeromycota phylum, are recognised as ubiquitous symbiotic organisms in the rhizosphere, forming mutualistic relationships with plant roots. A wide range of plant species, estimated to be within the 70-90% range, engage in symbiosis with AMF (Brundrett & Tedersoo 2018). During this symbiotic association, AMFs form structures called arbuscules (hence their name) inside plant root cells to enable nutrient exchange, while their hyphae extend into the soil, exploring new resources. Operating within

the roots and rhizosphere, AMFs interact not only with the plant but also with its rich surrounding microbial community.

Numerous studies have demonstrated the positive impact of AMFs on plant growth, nutrient uptake, and stress tolerance (Smith & Read 2008; Rahimzadeh & Pirzad 2017; Yang et al. 2023). AMFs facilitate the uptake of essential nutrients such as phosphorus and nitrogen, thereby sustaining plant vigour and resilience against pathogen attacks. Moreover, AMFs can induce systemic resistance in plants, priming them to mount more effective defense responses against invading pathogens (Jung et al. 2012). Induced resistance refers to the heightened defensive state in plants triggered by biological or chemical stimuli, safeguarding untreated plant parts against various pathogens or herbivores (Pieterse et al. 2014). Induced resistance operates locally and systemically, leading to a broad-spectrum defense mechanism through the activation of latent defenses, orchestrated by interconnected signaling networks (Yan et al. 2002; Choudhary et al. 2007; Bekkar et al. 2018).

Beyond nutrient uptake, AMFs possess other properties that contribute to their biocontrol potential (Weng et al. 2022). These include the production of antifungal compounds, modulation of plant hormone levels, and enhancement of the plant's antioxidative defense system (Weng et al. 2022; Zhao et al. 2022; Wahab et al. 2023). The symbiotic association with AMFs may also contribute to improved water utilisation, making plants more resilient to environmental fluctuations (Bonfante & Genre 2010).

In addition to their role in promoting plant growth and nutrient uptake, studies have shown that AMF can enhance disease resistance against *Fusarium* pathogens (Hnini et al. 2024). While the precise mechanisms underlying AMF-mediated disease resistance against *Fusarium* pathogens are still to be fully unveiled, AMFs seem to have revealed potential avenues for enhancing plant resilience and combating fungal infections such as the ones caused by certain *Fusarium* strains. For instance, AMF was observed fortifying plants against *Fusarium* wilt (Pu et al. 2022). Similarly, the combination of *Trichoderma harzianum* with AMF was shown to curb and suppress *Fusarium* wilt in tomatoes (Mwangi et al. 2011), emphasising the value of leveraging diverse beneficial fungi to combat pathogen threats while fostering plant growth concurrently. AMF's role in priming plant defenses and enhancing resistance to *Fusarium* infections (Kapoor 2008) indicates an interconnection among these organisms.

Collectively, these studies and mechanisms accentuate the intricate interplay between nutrient acquisition and other physiological processes in plant defense mechanisms against *Fusarium* pathogens, contributing to our strive to harness AMF-*Fusarium* interactions for sustainable agriculture by promoting resource-use

efficiency and reducing the dependency on synthetic fertilisers. The literature, although providing success stories such as the case of tomato (Mwangi et al. 2011; Tahiri et al. 2022) and banana (Rodríguez-Romero et al. 2005), yet the ways of harnessing this kind of beneficial interaction is not as well studied with wheat in comparison; which is the main reason this knowledge gap was taken upon making wheat the crop of interest in my thesis.

### 1.2.2 Plant Growth Promoting Rhizobacteria (PGPR)

Plant Growth-Promoting Rhizobacteria (PGPR) are beneficial soil bacteria which colonise the plant roots, thereby offering various advantages to host plants from a large range of phyla. The benefits PGPRs exhibit are enhanced nutrient uptake (Rana et al. 2012) and solubilisation (Berendsen et al. 2012), increased stress tolerance such as drought stress (Khoury et al. 2020), and pathogen protection (Backer et al. 2018).

PGPRs can provide pathogen resistance driven by their multifaceted positive effects on plant health and disease suppression (Goswami & Kistler 2004). Firstly, several studies have highlighted the efficacy of specific PGPR strains in suppressing *Fusarium* and other fungal diseases in studies, through induced systemic resistance (ISR) (Pieterse et al. 2014; Backer et al. 2018; Meena et al. 2020). Examples of PGPR strains inducing systemic resistance against *Fusarium* spp., include *Pseudomonas fluorescens,* (Yan et al. 2002; Wang et al. 2015; Müller et al. 2018) and *Bacillus* spp.(Bekkar et al. 2018), and *Bacillus subtilis* (Lakshmanan et al. 2013; Yu et al. 2021).

Another way PGPRs can provide pathogen resistance is through direct antagonism of pathogenic fungi by PGPR. This includes the production of antifungal compounds (Gardiner et al. 2013; Chowdhury et al. 2021), nutrient and space competition (Lugtenberg & Kamilova 2009; Chen et al. 2021), and fungal cell wall degradation as a result of lytic enzymes like chitinase and β-1,3-glucanase that ultimately aid to the control of root rot in plants (Won et al. 2019). For example, siderophore production by certain PGPR strains limits the iron availability to fungal pathogens like *Fusarium* (Pieterse et al. 2014).

The examples above highlight the potential of PGPRs as complementary to chemical pesticides, to managing fungal root and crown diseases like FCRR; and by doing so, enhance crop health and productivity (Rana et al. 2012; Chandran et al. 2021), an approach aligning with the implementation of integrated pest management (IPM) practices (Lamichhane et al. 2016). Moreover, the synergistic interactions between AMF and PGPRs have been shown to confer enhanced disease

resistance and improved plant performance compared to individual treatments (Lakshmanan et al. 2013). The intricate crosstalk between AMF and PGPRs involves hormonal signaling, induced systemic resistance, and modulation of microbial communities in the rhizosphere (Nadeem et al. 2014).

In practical terms, the application of AMF and PGPRs as bioinoculants in agriculture has gained attention (Hnini et al. 2024) in terms of utilising their beneficial interactions with plants alongside understanding plant defense mechanisms. While AMF primarily focuses on improving nutrient uptake and disease resistance in plants, PGPR contributes to root development, growth promotion, and disease suppression.

In this thesis, we wanted to put these effects to the test. To do that, we wanted, apart from AMF, to test native PGPRs and their commercial bacterial inoculant counterparts. Since one of our experiments found native potential PGPRs strains we wanted to observe if there was a significant difference in impact between natively adapted strains in comparison to microbial communities readily available in the market. Below we describe the two products we selected for the scope of this thesis which were EM1 and Hasta gård.

### 1.2.3 Commercial Microbial Inoculants

*Effective Microorganism 1 (EM1)*

EM1 (Agriton Sverige AB, Kungsängen, Sweden), a commercial microbial inoculant, has been vastly utilised in various agricultural practices, profiting from their beneficial properties to enhance soil health by introducing a diverse microbial community, plant growth, nitrogen fixation, bioremediation processes, and disease suppression, etc. (Antoszewski et al. 2022). EM1 consists of more than 80 different microorganisms collected from nature and recreated in a laboratory environment. The group's actinobacteria, photosynthetic bacteria, and lactic acid bacteria have been documented to have a more imperative role in the mixture, living in symbiosis with each other, according to the Swedish provider (Agriton Sverige) where EM1 was purchased.

It all started in the early 1980s, when Dr. Teguo Higa, while studying microbiology in Okinawa, Japan, discovered a remarkable combination of naturally occurring bacteria and yeasts with extraordinary properties. This blend, now known as EM1, was patented and copyrighted by Dr. Higa (Kuwayama & Higashinakagawa 2019). Initially used as a spray on crops, EM1 demonstrated promising results, increasing yields and eliminating the need for chemical pesticides and fungicides (Köhl et al.

2019). More recently, researchers presented EM1's efficacy in promoting improved root architecture in wheat and enhancing phosphorus uptake in rice crops (Beura et al. 2020).

Whilst EM1 seems to yield promising positive effects in enhancing soil multifunctionality and plant growth, the implications of introducing foreign microbial inoculants do not come without its pitfalls. Shifts in soil microbial communities resulting from EM1 introduction may lead to the emergence of functionally redundant microbes, which could influence ecosystem dynamics and resilience (Bargaz et al. 2018), as well as the structure and functioning of soil microbial communities (Li et al. 2024).

To put this in the context of this thesis, questions have risen regarding the impact that commercial microbial inoculants have on natively originating soil microbial populations (Canfora et al. 2021), and Scandinavia is no different. In turn, that instigates discussions on whether globally utilised products such as the widely used EM1 are truly optimal for enhancing crop microbial communities or if there exist alternative approaches that are yet to be explored and thus systematised.

*Hasta Gård*

In search of other commercial products to be tested in this thesis, another, (unreleased) product was acquired, this time provided by Hasta gård, an organic farm on the outskirts of Arboga. Their mission is to focus on regenerative farming methods and the utilisation of natural resources to produce high-quality, organic food (Hasta Gård 2024). They have also developed their own EM1 equivalent from soil microorganisms, which is claimed to be contributing to plant health by using local microbial communities. It was thought that it would be interesting to look into this product as well in this thesis and see if there would be comparatively more benefits with the inoculation of the Hasta gård product in contrast to EM1 which is manufactured in 59 countries worldwide (EM1 2024).

EM1 and Hasta gård were thus put to the test to investigate how they compare to natively adapted rhizobacteria strains in the soil. While commercial inoculants offer the advantage of standardised formulations and known microbial compositions, native soil rhizobacteria may possess unique traits and adaptations tailored to local environmental conditions. By conducting side-by-side comparisons, the idea was to try and identify which microbial communities are most effective at promoting disease suppression and enhancing plant health under specific growing conditions. By focusing on natural symbiotic relationships within the rhizosphere, the research recognises that the health of agricultural systems is intricately linked to broader ecological dynamics.

## 1.3 Aim, Research Questions & Hypotheses

The primary aim of this thesis was to assess whether native soil bacteria possessing plant growth-promoting characteristics, in the presence or absence of AMF, differ in their efficacy in suppressing FCRR compared to commercially available microbial inoculants in wheat cultivars in Sweden. This was investigated through a series of greenhouse experiments aimed at comparing disease suppression outcomes between treatments utilising native soil bacteria versus commercial microbial inoculants, and their interaction with AMF. In pursuit of this objective, the study aimed to address the research questions (RQs) outlined below, along with their respective objectives and anticipated outcomes based on hypotheses aligning with peer-reviewed literature.

$RQ_1$: Do we find bacterial isolates with plant growth-promoting properties in Swedish soils?

RQ1 aims to detect the presence of native bacterial strains from Swedish soils that show potential for promoting plant growth in wheat cultivars, through Experiment 1. By having identified such strains, the hypothesis here is that, in terms of plant growth and health, it is likely to find native strains that would beneficially interact with the plant in terms of plant growth, whether above or below ground or both. My assumption was that this difference would be visible when comparing a variety of phenotypic traits depending on the mechanism of action they employ in this situation.

$RQ_2$: How do different spring wheat varieties respond to *Fusarium* crown and root rot (FCRR) susceptibility when inoculated with pathogenic *Fusarium* spp.?

Secondly, I wanted to assess how different wheat crop varieties respond to various *Fusarium* spp. that were inoculated with distinctly different infection methods. Specifically, $RQ_2$ seeks to understand whether certain crop varieties respond with greater susceptibility to *Fusarium* infection compared to other genotypes, through Experiment 2. I hypothesised that different wheat crop varieties will exhibit varying tolerance levels to FCRR which, in turn, manifested in their phenotypic traits and nutrient acquisition levels assessed within the scope of this study.

$RQ_3$: How do native PGPRs affect FCRR disease suppression in comparison to their commercial equivalents in wheat?

With $RQ_3$ I wanted to focus on the effects PGPR would exhibit on disease suppression in wheat (with cv. Diskett). Disease occurrence and its respective progress, severity, and plant growth parameters were quantified in Experiment 3 to evaluate the efficacy of microbial treatments in phenotypically expressing plant

resilience against FCRR. My hypothesis was that native PGPRs would exhibit greater adaptation to Swedish wheat compared to commercial equivalents (EM1 and Hasta gård) of beneficial soil microbes as their traits may have coevolved alongside the respective wheat varieties. It was anticipated that this adaptation would be evident in the statistical analysis of phenotypic traits and nutrient data acquired during the study.

$RQ_4$: How do the beneficial bacterial inoculants interact with arbuscular mycorrhizal fungi (AMF) in the suppression of FCRR and overall plant health?

Lastly, $RQ_4$ digs into the separate but also synergistic effects of the added variable effect of AMF separately but also in combination with beneficial soil bacteria on disease suppression in wheat cultivars. Treatments in Experiment 3 involve the application of AMF, native and commercial PGPR microbial communities, and every subsequent combination thereof, which were administered to wheat cultivars, which are additionally challenged with *Fusarium* sp. pathogenic agents. Disease incidence and severity, along with plant growth variables, were quantified to assess the efficacy of different treatment combinations in enhancing plant resilience against FCRR. I hypothesised that the synergy of AMF and bacterial inoculants would be more beneficial in terms of plant growth and health. This anticipated outcome was hypothesised to surpass the efficacy observed when solely relying on either fungi or bacteria for disease suppression.

# 2. Materials & Methods

## 2.1 Biological Material

### 2.1.1 Plant Cultivars

The plant material utilised in the experiments comprised seeds from selected crop varieties sourced from Lantmännen Lantbruk (Svalöv, Sweden), a crop breeding company. The spring wheat (*Triticum sativum*) cultivars used were Happy, Diskett, and Bjarne, commercial cultivars grown in Sweden.

Before planting, the seeds were germinated to ensure uniformity and consistency across all experimental treatments. The seeds were placed on fitted filter paper in petri dishes, that were sprayed with water for sufficient humidity to facilitate germination. Petri dishes containing 10-15 seeds were stored and wrapped in aluminum foil at room temperature conditions in the laboratory. Germination progress was regularly monitored and within 2-3 days (depending on the cultivar) the emerging seedlings were ready to be transplanted. Each seedling was grown in individual 3L pots, filled with low-nutrient S-jord (**Supplementary Table 1**) in the greenhouse.

Since all of the cultivars were spring cultivars, and we had no abiotic stress treatment, greenhouse conditions were set to mimic natural Swedish growing conditions conducive to plant growth and development that these cultivars were adapted to. In the greenhouse, the day-night cycle was maintained at 19h:5H (light and darkness), with temperatures set to 18°C during the day and 12°C at night. Artificial lights were used to achieve a light intensity of up to 300 µmol to supplement natural light. Aphid and whitefly sticky traps were set up in each block. Watering occurred three times a week and was done on the pot plates instead of directly on the soil to avoid the inoculants being washed out or diluted to the point of result distortion. No supplementary soil nutrients or fertilisers were added.

### 2.1.2 Bacterial Isolates

Bacterial strains were initially isolated from experimental soil sites near Bjertorp (Västergötland) post-winter wheat harvest.

The soil samples underwent homogenisation and suspension in PBS (phosphate buffer solution), then applied to Luria-Bertani (LB) or International Streptomyces 2 (ISP2) (**Supplementary Table 2; Supplementary Table 3**). The resulting colonies were individually analysed, including Gram staining and assessment of colony colour, morphology, and cell characteristics. Out of the initial 90 isolates, 14 were chosen based on their varied characteristics to ensure they would also be distinct species or strains.

The 14 selected strains underwent further cultivation and preparation to serve as inoculants for wheat inoculation in Experiments 1 and 3. When not used, we kept their respective glycerol stocks in the freezer until further use.

Glycerol stocks for the selected strains were prepared by combining 500 µL overnight cultures with 500 µL 50% glycerol in a 2 mL tube. Freeze at -80°C. To retrieve bacteria, the frozen cultures were scraped with a sterile tool without allowing thawing. During transfer procedures in the laboratory, cultures were maintained with a liquid-nitrogen cooled container to ensure their preservation.

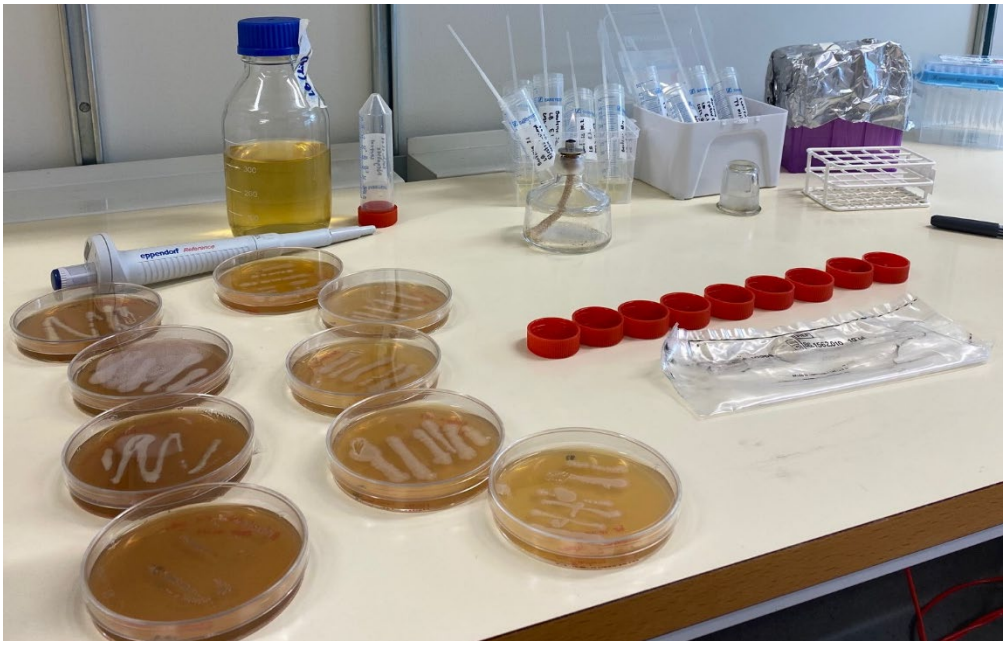



*Figure 1 - Bacterial cultures transfered from LB and ISP2 medium petri dishes to liquid medium (Methods in Experiment 1 and 3).*

Inoculum preparation involved transferring bacterial strains from the -80°C stock freezer onto Petri dishes with appropriate medium using an inoculation loop under sterile conditions within a laminar flow hood. Subsequently, bacterial cultures were streaked onto Petri dishes with LB or ISP2 medium using a flame-sterilised stick-loop technique. Cultures were then incubated at 28°C for 2-3 days to facilitate optimal growth. Upon readiness for transfer to liquid medium, LB and ISP2 were autoclaved. Using a sterile pipette, a colony was selected from the agar plate and dropped into the liquid bacterial medium, then swirled. This was loosely covered and incubated at 28°C for 12-24 hours in a shaking incubator. After incubation, growth was assessed; a hazy appearance indicated successful incubation. A negative control of only liquid medium was always included to check for contamination. After one day of growth, a second transfer to 50 mL liquid medium was performed and returned to the orbital shaker in the incubator, no more than 24 hours before inoculating the plants.

Following the above steps, the seedlings were prepared for inoculation with 2 mL of liquid bacterial medium in each pot. Each sample was then centrifuged at 4000 rpm to concentrate the spores into pellets. After the removal of the supernatant was removed, the pellets were resuspended in sterile water. This step was repeated twice. This solution was then inoculated by adding the specified amount with a pipette onto where each seedling was potted.

### 2.1.3 Fungal Isolates

Experiments 2 and 3 involved fungal isolates, for which the following procedures were conducted for preparation, cultivation, and inoculation.

The fungal isolates were chosen based on their relevance to the research objectives as they are typically known to cause cause root or crown diseases in wheat; namely *Fusarium graminearum* and *Fusarium culmorum.* Four of them were acquired by the Crop Production Ecology department (labelled as VPEx), one by the Associate Professor Mukesh Dubey of the Department of Forest Mycology and Plant Pathology (labelled as PH-1, also known as NRRL 31084 (Cuomo et al. 2007)), and one from the company Lantmännen (labelled as LM) (see **Supplementary Table 4** for *Fusarium* overview). The colony of *Fusarium graminearum* characters on PDA is off-white to pale yellow or rose/orange towards becoming brown after more days followed by the culture initiation with dense mycelium on the surface and darker brown colour on the bottom. Similarly, the colony of *Fusarium culmorum* on PDA seemed to be a darker shade of red/brown with a darker brown colour on the bottom.

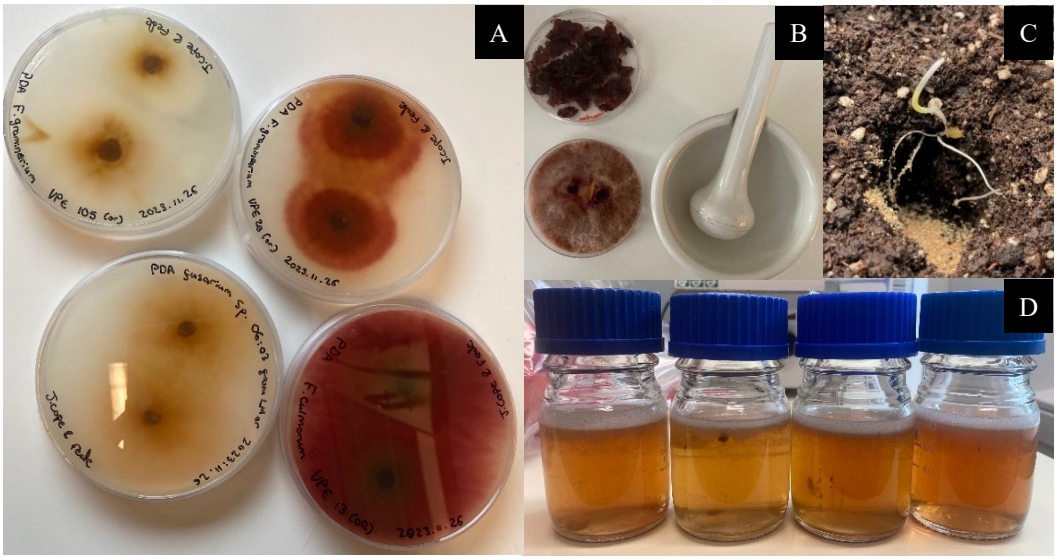



*Figure 2 - Fungal isolates and the three infection methods employed in Experiment 2. (A) Fungal cultures in PDA medium. (B) Crushed PDA medium (also used in Experiment 3). (C) Wheat powder in soil with seedling. (D) Mung bean liquid broth.*

Fungi grown in conventional Petri dishes containing PDA were subjected to ambient room temperature for growth and placed on a windowsill to incubate (**Figure 2A**). At two-week intervals, agar plugs 8 mm in diameter with growing mycelium of the selected fungal indicator strains, were aseptically cut out using a sterile cork borer and transferred onto the centre surface of a new agar plate with PDA medium. This process was reiterated consistently throughout the experiment. Once the fungal cultures had sporulated adequately on agar plates, the fungal inoculum was prepared for subsequent plant inoculation procedures. This process entailed the careful harvest of fungal spores from the agar plates.

The following cultures were used in Experiment 2 when testing different infection methods.

Spores derived from the *Fusarium* strains were collected and subsequently blended with wheat meal (Kungsörnen Vetekli from Lantmännen). This composite mixture was then cultured in sealed plastic bags under dark conditions for two weeks. Subsequent observation revealed visibly discernible hyphae formation (**Figure 2C**).

Similar to the aforementioned method, *Fusarium* strains initially cultured on PDA plates were subsequently transferred to bottles filled with boiled mung bean to make liquid cultures in mung bean broth (**Figure 2D, Appendix**). These bottles were then transferred to the orbital shaker operating at 100 rpm, facilitating incubation at room temperature.

## 2.2 Experimental Design

To address the above-formulated research questions, a series of systematic experiments were developed to build upon the foundation of the experimental design in a preliminary pilot study done in the summer of 2023 (Carter 2023). In the greenhouse, we undertook three distinct experiments to investigate microbial interactions within the wheat rhizosphere. A graphical illustration of the experimental setup is presented in **Figure 3**.

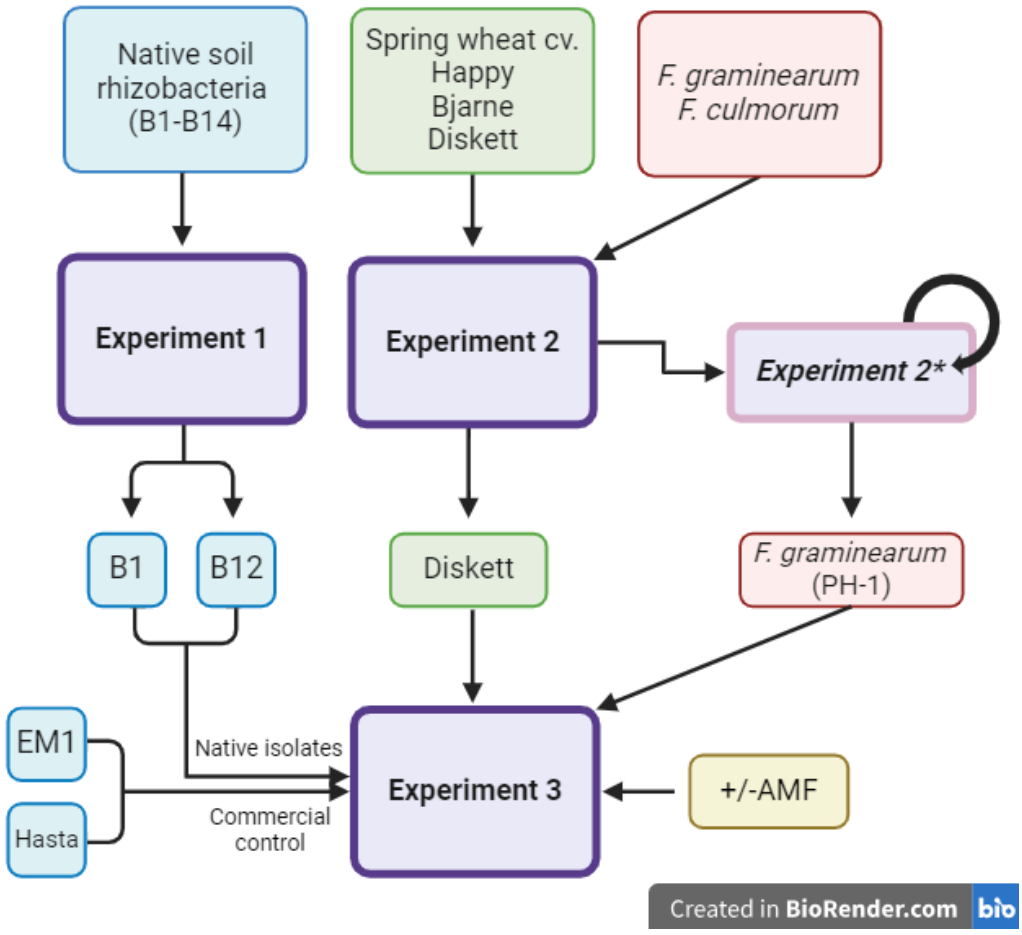



*Figure 3 - Experimental design as a flow chart (Biorender 2024). Experiments 1 and 2 (and a partial repetition of the latter) determined the design of Experiment 3 where AMF and commercial bacterial inoculants were added on top of that.*

Experiment 1 tested 14 soil rhizobacteria strains on spring wheat cultivar Diskett with the primary objective of selecting two strains exhibiting growth-promoting attributes.

Experiment 2 generated an assessment of FCRR disease expression in three spring wheat cultivars, induced by various pathogenic *Fusarium* spp. for which three distinct infection methods were tested.

Experiment 3 was based on the outcomes of Experiment 1 and Experiment 2 respectively. Spring wheat cultivar Diskett was subjected to different treatments and inoculations; *Fusarium graminearum*, beneficial microbial inoculants; namely commercial (EM1 and Hasta gård) and the selected native soil bacterial strains, and AMF.

### 2.2.1 Experiment 1

In this experiment, I assessed the plant growth-promoting abilities of 14 bacterial strains isolated from Swedish soil. These strains were evaluated for their efficacy in promoting growth in the spring wheat cultivar Diskett. The evaluation included both non-destructive and destructive measurements (as described below) to comprehensively analyse the impact of bacterial inoculation on plant growth.

Experiment 1 was conducted in the greenhouse using 3L pots filled with low-nutrient S-soil (**Appendix**). Diskett seedlings were prepared for germination as described before. The experiment comprised three replicates of the spring wheat cultivar Diskett with 15 bacterial treatments (1 control and 14 native bacterial strains). The experimental design adopted was randomisation within each replication block. The following measurements were taken to acquire insight into the overall growth response of cv. Diskett to bacterial inoculation.

Non-destructive measurements involved equipment such as the SPAD (Soil Plant Analysis Development) meter, which is commonly used to measure leaf chlorophyll content, and Spectra Crop analysis, which has been deemed able to assess overall plant health.

The SPAD (Soil Plant Analysis Development) meter is a portable and straightforward diagnostic tool that can aid in advanced interpretations of the photochemical processes in plants (Xu et al. 2019). The SPAD value derived from the meter serves as an indicator of relative chlorophyll concentration within the leaves, capturing a snapshot of the prevailing conditions at a specific moment. To acquire a more representative overview, multiple measurements were conducted from the emergence of the first true leaf (excluding the coleoptile leaf) until the day of plant harvest, allowing for the evaluation of SPAD values over time.

The Spectra Crop generates values represented as Fv/Fm (ratio of variable fluorescence to maximal fluorescence) (Carstensen et al. 2019). The range of 0.75

to 0.85 is indicative of minimal stress and efficient utilisation of light in the photosynthetic process. If the value drops below 0.75 it suggests suboptimal light utilisation, potentially impeding plant growth and development (SpectraCrop 2019).

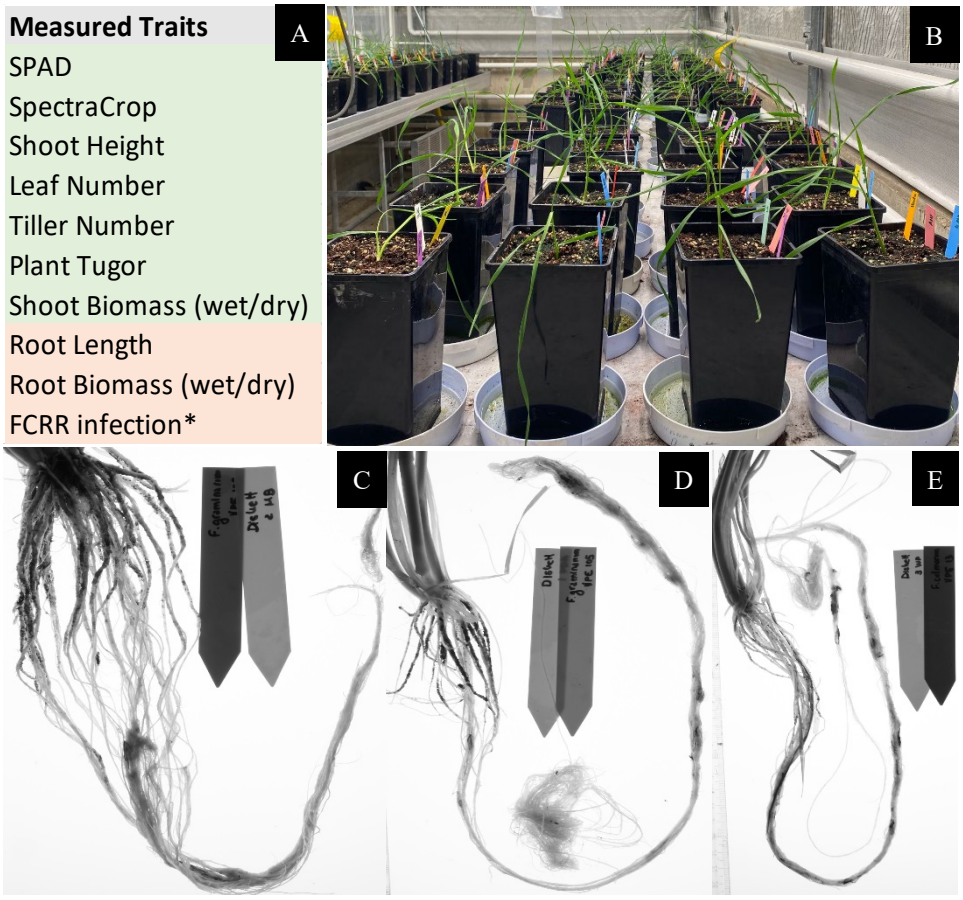



*Figure 4 – Overview of trait measurements (all 3 experiments) and FCRR evaluation on wheat cultivars (Experiment 2 and 3). (A) Measured traits . (B) Greenhouse where the experiment was conducted. (C) (D) (E) cv. Diskett infected with Fusarium strains by MB, PDA, and WP respectively. *signifies that it was also used in repetition of Experiment 2 with the Fusarium graminearum (PH-1) strain.*

SPAD and SpectraCrop values were taken every 2-3 days to track plant development over time. Aside from that measurement, I also gathered data for shoot height, leaf number, and tiller number, and evaluated plant tigor (**Figure 4**).

Harvest days involved taking the pots with their respective plants to be processed so as to acquire the destructive alongside the non-destructive measurements described above. During this process I preserved as much of the root system as possible, avoiding breakage during the extraction process by removing excess soil and soaking the roots before washing.

Destructive measurements were conducted at the end of the experiment which in this experiment was 28 days after sowing (DAS). These measurements included assessing root length to evaluate root system development and determining dry and wet shoot and root weights to quantify biomass accumulation. I also counted leaves and tillers.

Post-cleaning, these roots were carefully placed on dampened paper to prevent them from drying up during the interim period. Photos were taken using a light box and a DSLR camera (Canon EOS 2000D 18-55mm DC). Each plant was photographed with a label and a ruler to adjust the scale when analysing the photos with ImageJ Fiji software. After all the measurements were taken, the leaves and the roots were packaged in paper bags and were oven-dried at 70°C for at least 24 hours.

### 2.2.2 Experiment 2

In Experiment 2, I examined how wheat cultivars respond to *Fusarium* inoculation treatments. To do so, I selected three distinct spring wheat cultivars: Diskett, Happy, and Bjarne, and four diverse *Fusarium* strains. These strains were subsequently introduced to the wheat plants using three different infection methods that I evaluated. To assess the phenotypic traits of the wheat cultivars, I collected data both through non-destructive and destructive measurements. This comprehensive approach allowed me to analyse the effects of pathogenic *Fusarium* strains on plant growth.

The pot experimental design consisted of 3 replicates for each of the distinct infection methods (3), strains (4+1), and cultivars (3), resulting in a total of 135 pots (**Table 1**).

*Table 1 - Overview of Experiment 2*

| Cultivar | *Fusarium* strain | Infection method | Replicates | Total number of pots |
|---|---|---|---|---|
| Happy | *F. graminaerum* VPE 20 | wheat powder | | |
| Diskett | *F. graminaerum* VPE 105 | agar plug (PDA) | | |
| Bjarne | *F. culmorum* VPE 13 | liquid spore dimension | | |
| | *Fusarium* sp 06:03 | | | |
| | Negative control | | | |
| 3 | 5 | 3 | 3 | 135 |

The three distinct infection methods that were employed, ensured the seedlings' root system was in contact with the inoculated fungal strain (**Figure 2**).

PDA was crushed with some Milli-Q water a mortel and a pestle until it reached a jam-like consistency (**Figure 2B**). Upon infection, 1 teaspoon was applied to the soil adjacent to the seedlings. Wheat powder of which 2 teaspoons were applied to the soil adjacent to the germinated seedling (**Figure 2C**). Liquid spore suspension (mung bean broth): Seedlings of wheat cultivars were root dipped in a liquid spore suspension for a couple of seconds, made from a 2-week-old culture on PDA (**Figure 2D**).

The extent of pathogenicity was identified through root phenotypic observations, as in Experiment 1, with the additional evaluation of the root system for the presence and/or effect of the *Fusarium* inoculum using a published guide by Al-Tovi and Haleem (2018). This data paired with observations from the shoot (such as leaf shape, colour, and turgor), aided in obtaining an overview of the plant's health. Plants were also photographed and dried as in Experiment 1.

Note: After completing Experiment 2 and before starting Experiment 3, I obtained another *Fusarium graminearum* strain (PH-1). To assess its pathogenicity in the three wheat cultivars, Diskett, Happy, and Bjarne, I partially repeated the experiment testing PH-1 against VPE105. The PH-1 strain was then selected to be used in the following experiment.

### 2.2.3 Experiment 3

Building upon the outcomes of experiments 1 and 2, I sought to investigate the disease resistance against *Fusarium* in spring wheat. Due to the lack of space in the greenhouse and a restricted timeline accounting for the time needed to acquire and process all the data, I chose the cultivar Diskett (also used in Experiment 1).

On cv. Diskett, I applied several different treatment combinations that I wanted to test. The larger variance within treatments was the bacterial communities. We had 5 different ones within that treatment; namely the 2 bacterial strains (B1, from LB medium; and B12 from ISP2 medium) that displayed the starkest significance amongst the initial 14 strains in Experiment 1 and are native to Swedish soil. We chose to test these against two commercial products; namely, the international microbial inoculants EM1 (Agriton Group, Sweden), a proprietary local inoculum from Hasta gård (Hasta, Västmanland), and a control treatment.

Furthermore, combinations of rhizobacterial strains, both with and without commercially available AMF (Ugro Rhiza1200, Simply Organic S.L., Barcelona, Spain) were tested to assess their efficacy in suppressing *Fusarium* disease and attempt to explore potential synergistic effects with the bacterial strains, on disease

mitigation. This involved testing combinations of bacterial strains with and without AMF to determine if AMF enhances the effectiveness of bacterial PGPRs in suppressing *Fusarium* disease. For all the different combinations of treatments, there were four replicates, resulting in a total of 80 pots prepared with S-jord soil (low nutrient; **Table 2**).

*Table 2 - Overview of Experiment 3*

| Cultivar | Infection Method | *Fusarium* strain | Bacterial Treatment | AMF | Replicates | Total |
|---|---|---|---|---|---|---|
| Diskett | agar plug (PDA) | *F. graminaerum* | EM1 | +AMF | | |
| | | Negative control | Hasta gård | - AMF | | |
| | | | LB strain | | | |
| | | | ISP2 strain | | | |
| | | | Negative control | | | |
| 1 | 1 | 2 | 5 | 2 | 4 | 80 |

The evaluation involved measuring the expression of the disease and assessing the potential enhancement in disease resistance conferred by rhizobacteria, selected from the results of experiment 1 as well as the commercial products available in the market. Plants were photographed and dried as in Experiment 1.

## 2.3 Statistical Analysis & Data Visualisation

Statistical analysis was conducted for all traits measured, as well as root fractions of total plant biomass (in both wet and dry biomass).

In Experiment 1 the analysis utilised the Kruskal-Wallis test, a non-parametric equivalent of ANOVA with a further post-hoc Dunn test, as well as ANOVA and post-hoc Tukey test. In experiments 2 and 3 an ANOVA was employed, followed by Tukey tests when the statistical results were significant and deemed scientifically interesting. The post-hoc test was utilised to pinpoint the specific traits or factors responsible for the observed differences in terms of pairs of means as it compares all possible pair groups. The aforementioned statistical procedures were performed using the statistical software "R" version 4.0.4 (R Core Team, 2024), with *Fusarium* treatment, bacterial inoculants, and cultivar genotype serving as the independent variables, where appropriate. R also facilitated the generation of statistically robust results by considering correlations between the different traits

and variables taken into account. Statistical significance was judged at the level $P \leq 0.05$. This data analysis approach allowed for insights into the relationships and variations present in our data, enhancing the quality and reliability of our findings.

Statistical analyses were performed using the statistical programming language R (R Core Team 2024). Normality tests were conducted for all experiments using the Shapiro-Wilk test. The packages dplyr (Wickham et al. 2023) and tidyr (Wickham et al. 2024b) were used for data manipulation, and ggplot2 (Wickham et al. 2024a) was used for data visualisation with colour palette wesanderson (Ram et al. 2023).

# 3. Results

## 3.1 Experiment 1

From the data collected for this experiment, I was able to look into how different bacterial treatments (B1-B14) respond in terms of phenotypic traits and chlorophyll analysis in Diskett. One trait that particularly stood out as statistically significant was the dry root biomass fraction (*ANOVA*, p=0.04765*) (**Figure 5**), employed to visualise disparities regarding, in this case, the wheat cultivar's resource allocation patterns in terms of bacterial treatment. More specifically, a variance of effect can be observed; with B1 and B12 being in contrasting extremes in terms of root dry weight : total dry weight ratio. B1 showed an increased allocation to root biomass, whereas B12 showed an increased allocation to the shoot biomass. Regarding the other 12 strains, we see that B11 showcases a similar effect as the control, while the rest (with the exception of B4 and B10), appear to manifest lower dry root comparative biomass.

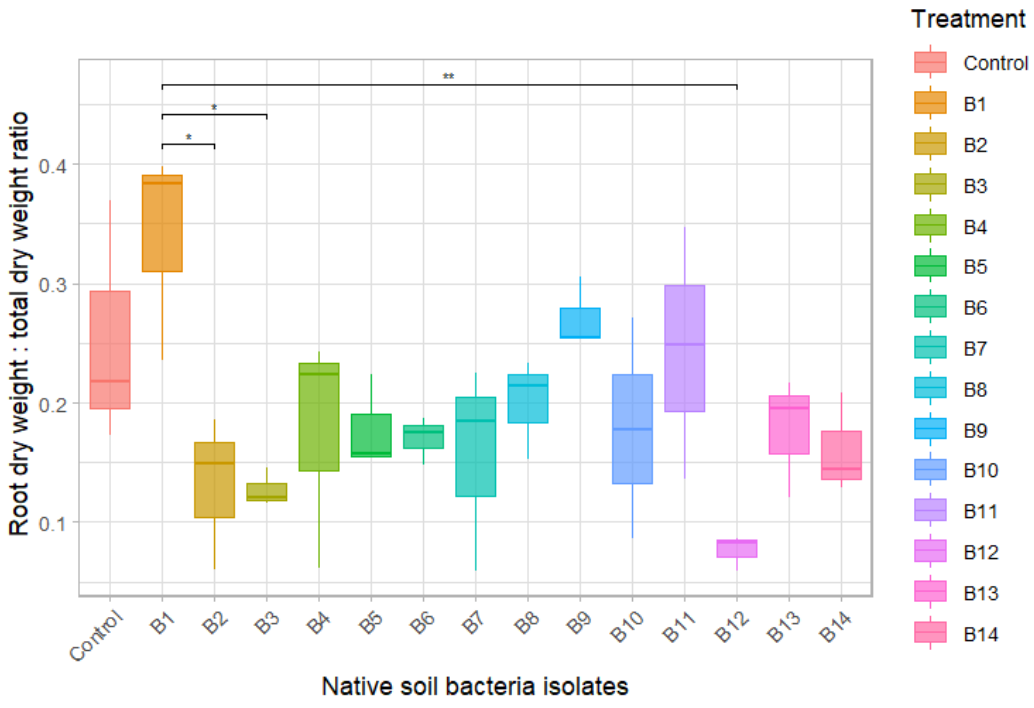



*Figure 5 - Root dry weight : Total plant dry weight ratio per bacterial treatment. Control indicates the absence of bacterial treatments. Boxplots are calculated based on three replicates. ANOVA was performed, followed by Tukey post-hoc analysis which showed that B1 and B12 were promising potential candidates with plant growth-promoting traits (Experiment 1).*

In further exploration of the effects various bacterial treatments (B1-B14) exhibit on spring wheat, we observed some interesting results albeit not statistically significant but perhaps worth our attention.

**Supplementary Figure 1** shows a wide range of height spanning from as low as 33 cm to as long as 50 cm; values that range from either direction in comparison to the control. Lastly, the Fv/Fm values from the SpectraCrop remained within the optimal health range (0.75-0.85) (according to the manufacturer (SpectraCrop 2019) in all of the bacterial treatments (**Supplementary Figure 2**). Considering Fv/Fm as one of several plant health metrics one can utilise, this observation serves as supportive evidence that the bacteria did not exert a negative effect on the overall health of the plant.

## 3.2 Experiment 2

Overall, below-ground traits showed more significant variance in response to the *Fusarium* treatment in comparison to above-ground traits (**Figure 6**). The ANOVA results for the various traits provide insights into the influence of several factors that were tested in Experiment 2. Cultivar type appears to be a significant factor across multiple metrics, the most prominent ones being root length (*ANOVA*, p < 2e-16***) and root dry weight (*ANOVA*, p = 0.0235**). Interestingly, the interaction between genotype and infection method was significant in both root and shoot length, signifying a potential combined effect of these two factors. The *Fusarium* variable seems to act similarly with root length (*ANOVA*, p = 5.61e-07***), and shoot length (*ANOVA*, p = 1.83e-06***), though its influence on other variables is less marked. On the contrary, the infection method does not seem to exhibit a significant effect across most metrics although it must be noted that the wheat meal method was disregarded quite early on in the harvest period due to a lack of results regarding the *Fusarium* treatment which is why it is not included in the graphs.

Note: The occurrence of missing values in the Bjarne*PDA treatment group is attributed to plant mortality before harvest, i.e. 28DAS, and will be the case for the graphs that follow within Experiment 2.

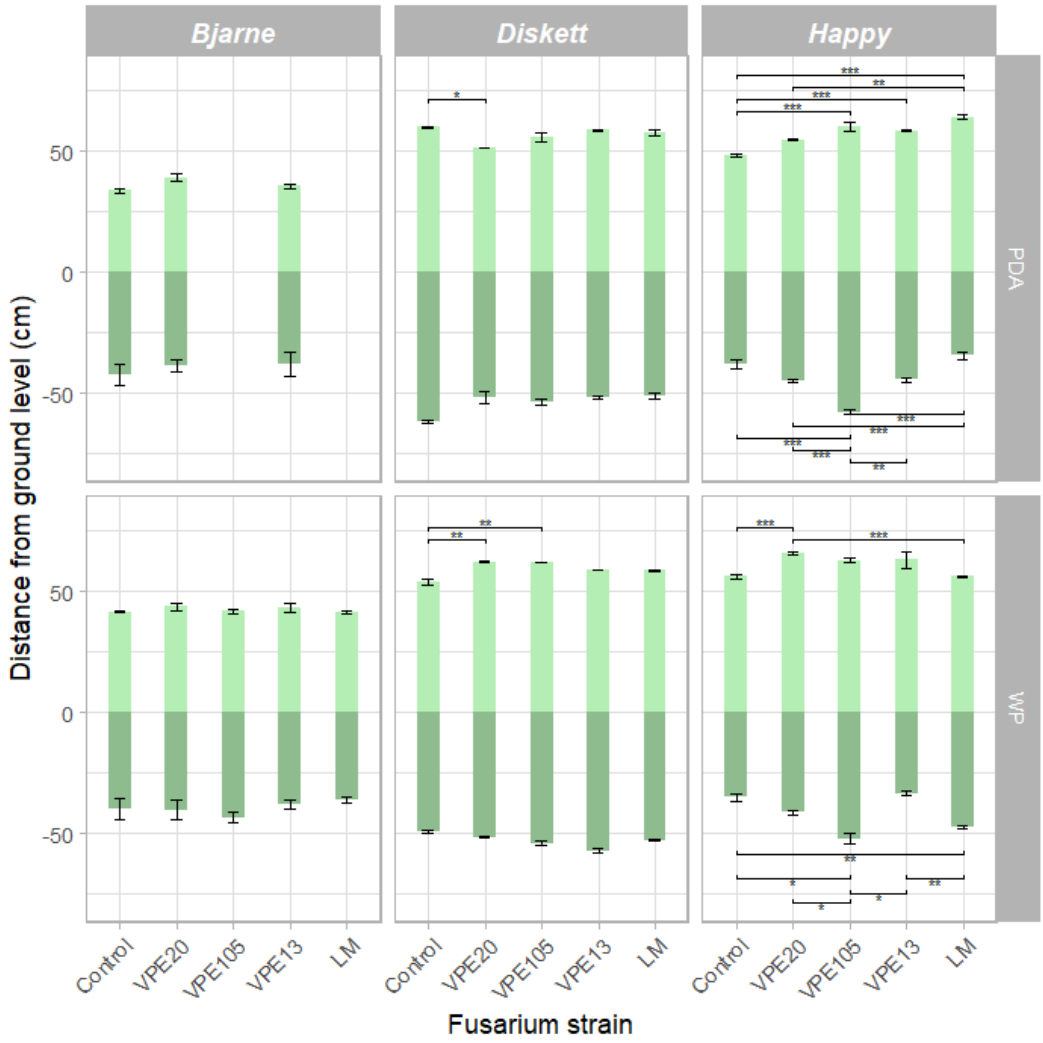



*Figure 6 – This mirrored bar chart presents an overview of the experimental variables tested in Experiment 2. It indicates the above ground (shoot height, light green) along with the below ground (root length, dark green). The various treatments the plants underwent are shown by the Fusarium strain, the infection method of said strains, as well as the genotypic variability using different spring wheat cultivars. ANOVA (root length, p = 5.61e-07***), and (shoot length, p = 1.83e-06***) and post-hoc Tukey was performed, the latter showed as lines with asterisks on the graph where statistically significant.*

An observation of the data reveals a pronounced phenotypic variance, indicating a higher level of variance particularly below ground compared to above-ground height assessments and that is made clearer when looking at this mirrored bar chart assembling all the conditions and combinations the cultivars were exposed to under this experiment. Moreover, the statistical analysis highlighted significant between the cultivars Happy and Diskett across *Fusarium* treatments differences in root dry weight (*ANOVA*, p= 0.00870**) (**Supplementary Figure 3**).

### 3.2.1 Experiment 2*

Experiment 2 was partially repeated when a new strain of *Fusarium graminearum* was presented to us. This experiment tested the *Fusarium* treatment with *F. graminearum* from the VPE department (VPE105), the *F. graminearum* provided by Associate Professor Mukesh Dubey (PH-1), and the negative control. I used the three original cultivars to test for cultivar variability as well. From this, the PH-1 isotype was used in Experiment 3. Dry root biomass to total dry biomass (*ANOVA*, p= 0.0411*) exhibited the lowest values across cultivars and *Fusarium* treatment in cv. Diskett with the new *F. graminearum* strain (PH-1) as shown in **Figure 7**, a result which, although not statistically significant, was taken into consideration when deciding the cultivar and *Fusarium* strain for Experiment 3.

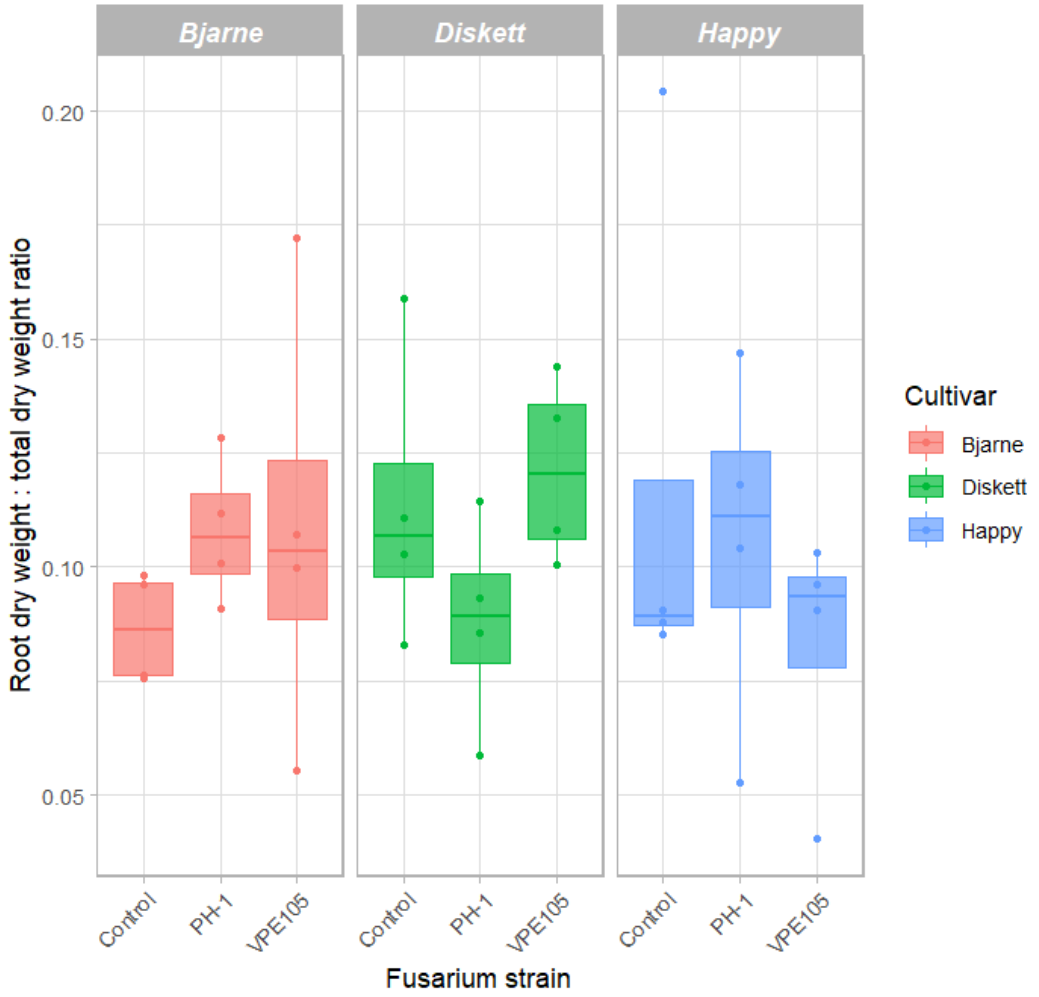



*Figure 7 – Root dry weight : total dry weight ratio in terms of Fusarium treatment where the cultivars are accounted for in the facet grid. In Diskett, the PH-1 treatment is the one that prompted us to consider when designing Experiment 3 where we used the new F. graminearum strain we acquired by Associate Professor Mukesh Dubey. However, no statistical differences were observed amongst cultivars and Fusarium treatment.*

Moreover, **Supplementary Figure 4** provides a temporal depiction, in the form of a time series, illustrating the variation in SPAD values recorded at multiple intervals throughout the duration of the experiment. A discernible trend emerges from the depicted data, wherein the SPAD values associated with the Bjarne cultivar exhibit a comparatively homogeneous trajectory over time. In contrast, the SPAD values corresponding to the Diskett and Happy cultivars manifest a more pronounced pattern characterised by fluctuations between highs and lows in terms of *Fusarium* treatment. Additional data which further supported the decision to select the new strain was indicated in **Supplementary Figure 6** where a significant difference is observed in terms of shoot length with the *Fusarium*-treated plants exhibiting lower values compared to the control treatment.

## 3.3 Experiment 3

In Experiment 3 we have an additional variable; that of AMF. We observe a statistically significant difference between the presence and absence of AMF in the plant root (*ANOVA*, p=0.0325*). More specifically, the root length (**Figure 8a**) seems to be decreasing whilst simultaneously, root dry weight seems to be increasing in the presence of AMF (**Figure 8b)**.

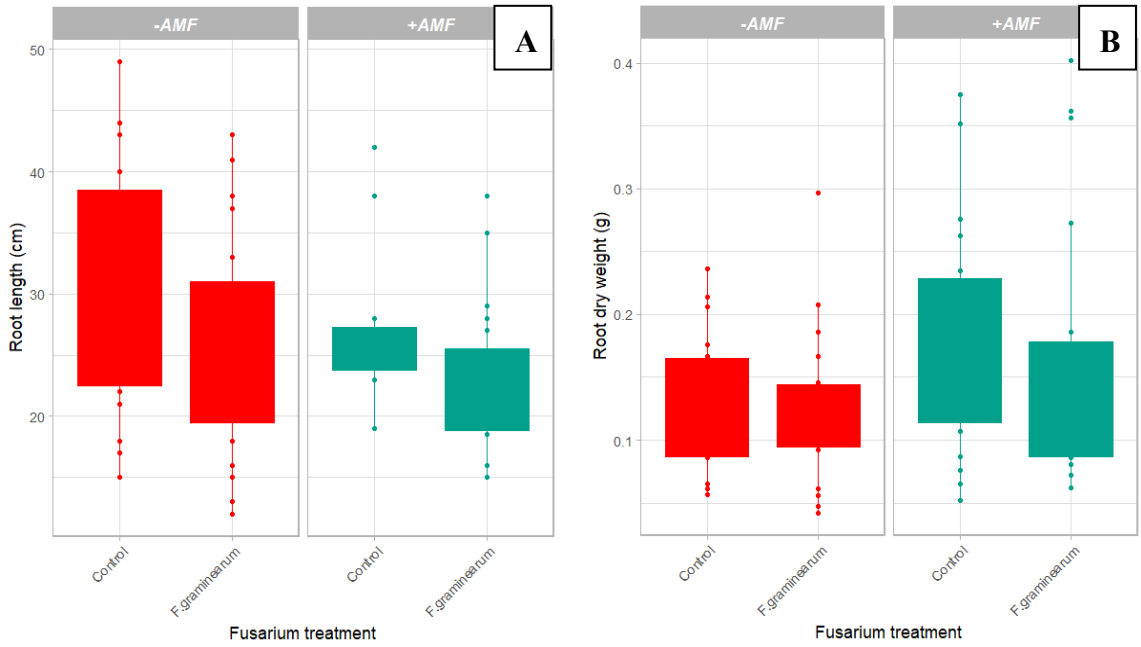



*Figure 8 - Root Dry Weight (g; A) and Root Length (cm; B) per Fusarium treatment with new F.graminearum strain accounting for AMF treatment. A) Root length decreases in the presence of AMF whilst B) root dry weight simultaneously increases. ANOVA was performed that showed that the AMF treatment was statistically significant (p=0.0325*). (Experiment 2*).*

When accounting for bacterial treatments , we also see a somewhat similar pattern. With AMF the difference in increase in root dry weight is most apparent in the plants treated with both Hasta and AMF (**Supplementary Figure 5**). Conversely, when looking at the AMF effect in terms of the root length, the decrease in values is more prominent in the native bacterial isolates (**Supplementary Figure 6**).

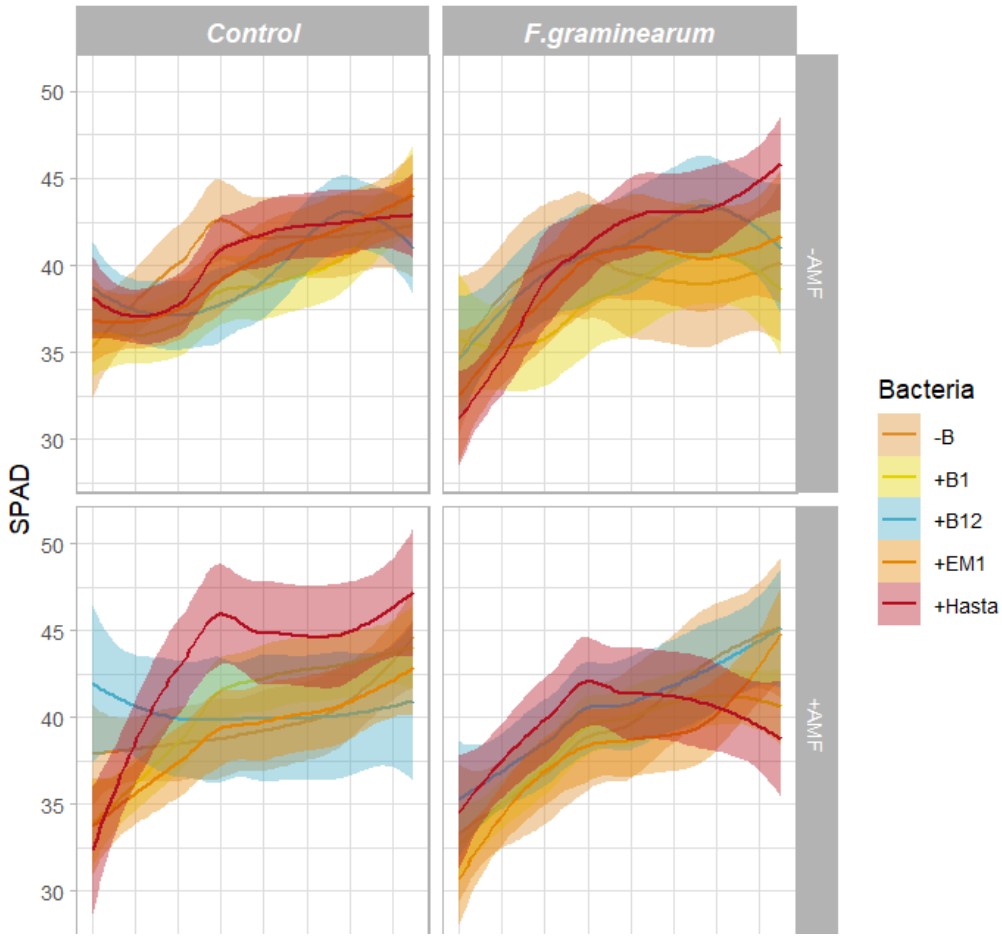



*Figure 9 - SPAD timerseries in Experiment 3 accounting for bacterial and AMF treatment. Every 2-3 days SPAD values were acquired to acquire an overview in the duration of the experiment. Hasta seems to be exhibiting a different trend in comparison to the rest, especially in the control with absence of AMF as well as in the presence of both AMF and F.graminearum.*

Lastly, statistically significant results were generated in terms of shoot length and *Fusarium* treatment (p=0.00203**) as well as the threefold combination of factors *Fusarium**AMF*Bacteria (p= 0.04078*) shown in **Supplementary Figure 7.** This is also shown through some notable trends in the SPAD time series (**Figure 9**). More specifically, attention is once again drawn to the Hasta treatment that seems to be exhibiting starker differences in comparison to its counterparts seemingly exposing a positive synergy between AMF and Hasta bacterial community.

# 4. Discussion

## 4.1 Interpretation of Results

In the discussion section, I will address the research questions formulated during the design phase of this thesis. To do so, I will analyse and interpret the results in light of the initial hypotheses, drawing on existing peer-reviewed literature in the field. I will subsequently summarise the results and reflections in terms of the challenges and limitations encountered within the scope of the 20-week work plan.

At the project's inception, the overarching idea of this project was to collect data that would provide evidence in answering the question of whether the native rhizobacteria can be deemed more suitable and capable of both exhibiting plant growth-promoting properties and disease suppression. How those interactions would, assuming they were positive for the plant's health, be affected when AMF is added; there has been documentation of a promising synergy between them and PGPRs in the face of biotic and abiotic stress factors (Hnini et al. 2024). As shown in this flow chart visualising an overview of what my work entailed (**Figure 3**), the first step to even begin answering any of these two larger scale questions was to source PGPR strains to work with (**Experiment 1**), then test the effect inoculated pathogenic *Fusarium* strains have on different spring wheat cultivars (**Experiment 2**) and draw these results together with the added variable of the effect of the AMF presence/absence (**Experiment 3**) – all conducted with the intention of testing a series of hypotheses based on published data.

### 4.1.1 PGPRs: Native vs Commercial

By testing an array of native soil rhizobacteria in terms of their potential plant growth-promoting properties, two strains with different morphological characteristics when cultured and incubated in their respective media also exhibited variance in terms of dry root biomass (**Figure 5**) (which could be indicative of a potential role in the plant's resource (re)allocation, were selected as potential natively originated PGPR candidates to be used in the final Experiment 3. That led us to describe them as such; answering the $RQ_1$ with a positive tone; albeit with some reservation. Further tests, such as looking into their mechanism of action (Chandran et al. 2021), will need to be conducted for higher robustness if the project intended to make use of them is larger in numbers and ambition.

At this stage, it was decided to put them to test in the final experiment's bacterial treatment against two commercial microbial communities that would act as the commercial control vs the native isolates. EM1 and a product from Hasta gård were utilised as the commercial aspect of the bacterial treatment albeit they have quite stark differences in how they are recommended to be applied. EM1 is a product utilised in a wide range of countries, crops, and environmental conditions (Li et al. 2024); whereas the one from Hasta gård claims to be re-applying microbes that were originally isolated from local Swedish soils and returned to the source as their mission statement emphasises (Hasta Gård 2024). Hasta gård appears closer to the native isolates B1 and B12 in comparison to EM1 although this is merely speculative based on the approach the manufacturers have; the former being a patented product and the latter with contents that are being vaguely described which makes it harder to conclude without making more detailed content analyses ourselves.

Regardless, the commercial products do represent a mix of microbes making up a microbial community inoculant whereas the isolates are the sole strains of the native rhizobacteria. Taking this contrasting nature among the bacterial treatments employed in Experiment 3 into account, it is important to note that microbial communities are generally viewed favourably due to their potential for adaptability, with different species thriving under various conditions by altering their numbers and composition levels according to the presented needs (Trabelsi & Mhamdi 2013). This versatility contrasts with bacterial isolates, which can be riskier yet more efficient when their application mechanisms are well-understood. Additionally, the presence of resident microbial communities in the soil and crop cannot be overlooked (Nogrado et al. 2021). These established dynamics and interactions underground play a significant role in the success or failure of introducing new microbial communities (Trabelsi & Mhamdi 2013; Canfora et al. 2021) or isolates (Gołębiewski et al. 2023). The bacterial isolates are more likely to be unstable and more unpredictable short term in the ways they could potentially skew the ecology of established rhizobiome (Nogrado et al. 2021); a probability compounded by the added factors of plant species and genotype and specific traits present in the inoculated bacteria (Zhong et al. 2019).

In my thesis, we draw inconclusive results making it difficult to take a stance for the conditions set for our purpose in testing their effect in terms of effectiveness in *Fusarium* disease suppression in wheat. The original hypothesis was that native isolates would probably perform better in terms of plant growth in contrast to their more broad-spectrum commercial counterparts. However, after acquiring information from the Hasta gård contact with whom we had meetings about their product, maybe theirs is a more tailored inoculant for Swedish soils as they seem

to advocate for the recirculation of local products making their way back to the soil. There is some interesting variation in the inoculation of the Hasta gård community in comparison to the isolates and EM1 and its effect also seems to stand out especially when combined with AMF (**Supplementary Figure 5)**, implying a potentially interesting combination that could be further investigated with more experiments and replicates. Even if optimal strain selection ensures the compatibility and efficacy of microbial interventions, maximising their potential for disease suppression (Kirby et al. 2017) that type of research is a whole project on its own and was not included in the initial experimental design.

Upon reflection of $RQ_3$, considering the comparison between our isolates and the commercial products, further investigation is warranted to determine their efficacy as PGPRs. Circling back to the initial hypothesis, however, there is some indication aligned with the assumption that native isolates perform better in terms of plant growth and disease suppression.

### 4.1.2 FCRR Disease (?)

One of the objectives of this thesis was to test how wheat responds to *Fusarium* inoculation in terms of inoculation method and physiological responses of the respective cultivars. Such information could have potentially been indicative of variations in their susceptibility to *Fusarium* infection and more specifically FCRR disease expression. Essentially it was expected that from Experiment 2 we would have gained insight into which *Fusarium* strain was the most virulent in the greenhouse experiment and also, which cultivars were the most resilient or susceptible to the disease exposure. After contemplating the method intended to apply regarding the evaluation of the disease and the ways I intended to quantify it based on the diagnostic guide by Al-Tovi & Haleem 2018 on four distinct disease progression levels, the outcome was disappointingly no visible disease was detected, neither in the roots nor root crown nor any expected leaf discolouration or loss of turgor signs attributed to FCRR expression. That is important information to retain as this was the case for Experiments 2 (and repetition) and Experiment 3. Therefore, all the indirect effects in terms of phenotypicing and chlorophyll analysis should be considered with caution.

In the quest of trying to answer $RQ_2$, it is important to acknowledge that although we didn't observe visual symptoms of the disease during the experiment, certain below-ground phenotypic traits showed variance, some more affected than others compared to the respective controls. These traits, which could have indicated the presence of the pathogen, were scrutinised further to make assumptions about the potentially non-visually apparent FCRR disease.

Firstly, the a higher variance in root length, compared to the shoot height.of interaction response to the treatments in Experiment 2 in comparison to a more homogenous variability in above-ground traits. Root dry weight variations across different cultivars proved statistically significant in relation to *Fusarium* strains, showcasing some variance amongst the three spring wheat cultivars.

The decision-making process regarding the selection of a cultivar for experimentation involves careful consideration of various factors to ensure the reliability and validity of research outcomes. While conducting observations on the root length variance in Happy was intriguing, our ultimate choice gravitated towards the utilisation of Diskett. Primarily, our prior testing of bacterial isolates was conducted specifically on the Diskett cultivar. The idea was to maintain experimental consistency and avoid the introduction of extraneous variables in this decision-making process. Additionally, insights from previous field trials and pilot studies revealed suboptimal performance of Happy under experimental conditions. Consequently, the decision to shift towards Diskett was to potentially maximise the likelihood of obtaining discernible manifestations of FCRR as supported by the partial repetition of Experiment 2 with the new *Fusarium* graminearum strain (**Figure 7)**. Last but not least, Diskett, characterised by intermediate root traits, showed to be promising in utilising AM symbiosis as a supplementary strategy for nutrient uptake, suggesting a complementary relationship between roots and AM fungi (Torppa et al. 2023). Thus, the selection of Diskett was not only based on experimental considerations but also on its potential to effectively interact with soil microbes. In summary, the selection of Diskett as the cultivar for experimentation was grounded in an evaluation of experimental considerations, including prior testing protocols, experiential observations, and the overarching objective of maintaining the interpretability of research outcomes.

### 4.1.3 The AMF Effect

In the context of $RQ_4$, which delves into the interaction between beneficial bacterial inoculants and AMF in FCRR suppression and overall plant health, our findings shed light on the separate yet synergistic effects of AMF and bacterial inoculants on disease suppression in wheat cultivars. My hypothesis suggested that the synergy between AMF and bacterial inoculants would yield greater benefits in terms of plant growth and health compared to relying solely on either fungi or bacteria for disease suppression.

Throughout our study, several key observations were made regarding plant morphological traits and the significance of AMF colonisation. Height and root

length exhibited statistical significance in relation to *Fusarium* presence or absence, acting as an indicator of stress potentially caused by the pathogen presence on plant morphology. Existing literature does highlight the complex nature of mycorrhizal function and its synergistic use with *Fusarium* strains in agricultural contexts, further supporting the relevance of our findings (Hoeksema et al., 2010). The AMF treatment, in this thesis, showed to decrease in root length, and yet its significance in root dry weight where it showcased an increase in AMF presence, suggested potential AMF root colonisation (**Figure 8)**, which unfortunately could not be confirmed through root sampling and microscopy due to time constraints.

## 4.2 Reflections

It is well established that the rhizosphere serves as a hotspot for diverse microbial communities, whose intricate interplay influences plant health and disease resistance (Kirby et al. 2017). However, deciphering and manipulating these interactions for effective disease management is accompanied by significant challenges, highlighting the ever-prevalent focus on understanding rhizosphere ecology. After all, plants have evolved mechanisms to attract and maintain microbial populations based on their functional capabilities, suggesting a co-evolutionary relationship between plants and associated microbes (Theis et al. 2016).

### 4.2.1 Limitations and Challenges

Throughout my thesis, I encountered various challenges and limitations that influenced the experimental design and results. These challenges, rooted in the complexities of plant-microbe interactions and experimental constraints, shaped the course of my research. Below, I will describe the main problems and limitations that I faced.

The primary challenge was the non-visual confirmation of the FCRR symptoms in the wheat plants. At first, that occurrence made us question the strains we used in Experiment 2 in terms of their pathogenicity, as they could have lost their ability to display their pathogenicity in wheat due to their prolonged isolation from host plants. This assumption was partially rejected due to two reasons. Firstly, the acquisition of a new *F. graminearum* strain that had recently been in contact with a host with which it did show pathogenicity towards it, when inoculated in the conditions of my experiment it still did not provide visual evidence in terms of FCRR expression. Secondly (albeit too late in the thesis timeline to be able to repeat experiments), I looked into other factors that could have contributed to this odd

result. Another diagnostic guide put together by Hagerty et al. (2021) presents that drought stress and *Fusarium* pathogenicity seem to be positively correlated. However, while reducing watering frequency could potentially mitigate *Fusarium* pathogenicity, I was reluctant to introduce yet another variable in Experiment 3 that could potentially further confound the results, particularly in short-term studies like mine. It is something to consider, however, especially in greenhouse experiments when such issues occur.

Another condition that could have impacted the progress of the FCRR disease was the selection of the type of soil used. Research also highlighted the importance of soil composition in *Fusarium*-related diseases. For instance, *Fusarium* wilt severity in watermelon was found to vary depending on the soil media used for cultivation (Meru & McGregor 2020). Additionally, *Fusarium* has been associated with root rot in sweet potatoes, with studies showing that *Fusarium*-cultured soil mixed with sterile sand and soil can lead to root rot incidence (Kim et al. 2022). The impact of *Fusarium* on plant health extends to various crops, as seen in studies on bean production, where *Fusarium*-infested soils influenced the growth and yield of white beans differently based on soil types (Naseri 2014). With that in mind and considering that I had high organic matter (peat) which inhibits *Fusarium*, together with the discussion that occurred during my master's thesis defense about the avoidance of using soil with organic matter opting for sand instead or some degree of sand mix with low nutrient soil might have been more appropriate.

Moreover, another possible explanation for what occurred in this thesis could be that the longer duration for the plants to grow could have aided if, according to, (Deshmukh & Kogel 2007), the delay of root rot disease occurrence and its connection to reduced root symptoms as found. On the same token, we are unsure, regarding what type of relationship occurs between disease progression and fungal DNA levels.

Lastly, logistical constraints, such as restricted space and limited replicates, posed challenges in generating statistically significant results, particularly given the substantial phenotypic variance observed across different treatments and wheat cultivars.

### 4.2.2 Future Avenues

If this type of experimental design is further investigated there are some alterations and complementary experiments, I would suggest considering depending on the objective.

In terms of altering experimental methodologies, the prospect of conducting dual cultures of *Fusarium* and bacteria of interest emerged as a potential avenue for investigating microbial interactions (Balouiri et al. 2016). The challenge here could be to find an appropriate medium where both *Fusarium* strain and bacterial cultures could grow into potentially generating data about their interactions. The infection of plants would then occur at a later stage. Considerations regarding the timing of inoculation also surfaced, that inoculating before the seedling stage could enhance disease resistance. This question prompts reflection on the developmental stage at which microbial interventions are most effective, a topic worthy of further exploration with the caveat that if seeds are so affected by the fungal inoculation that they do not reach the next growth stage then that creates another form of challenge to deal with.

By integrating insights from strain selection, microbial-mediated disease suppression, gene regulation, and root exudate dynamics, a holistic understanding of rhizosphere ecology is essential for developing effective and sustainable disease management strategies. It would be interesting to see what can be translated from the lab to the greenhouse and ultimately to the field if useful results are generated.

# 5. Conclusion

The generated findings from my thesis are characterised by the intricacy of the mechanisms of the complex interactions occurring below in terms of soil beneficial microbes and plant health, particularly in the context of disease suppression and overall crop resilience. Undeniably, there is a versatility driven by the different roles the diversity of microbial interactions takes based on the different stress combinations the plant and its second genome are challenged with. That being said, I did observe some potential in the AMF synergy exhibited with the Hasta gård microbial community though more experiments need to be conducted for result robustness.

Addressing the root of this research gap necessitates a holistic approach. This is further aggregated if management practices such as precision agriculture and unraveling at least partially of what is happening below ground are accompanied by interdisciplinary research and a collective effort for sustainability by making use of the immense potential of soil microorganisms. The future of plant breeding and agriculture is using beneficial microbes as it is promising in solidifying resilient food production systems which aligns with the Sustainable Development Goals set forth by the United Nations.

# References


Al-Tovi, B. & Haleem, R. (2018). Occurrence and isolation of some *Fusarium* species associated with crown and root rot disease in some wheat fields of Duhok, IKR, Iraq. *Journal of Zankoy Sulaimani - Part A*, 2ndInt.Conf.AGR, 331–342. https://doi.org/10.17656/jzs.10679

Antoszewski, M., Mierek-Adamska, A. & Dąbrowska, G.B. (2022). The Importance of Microorganisms for Sustainable Agriculture—A Review. *Metabolites*, 12 (11), 1100. https://doi.org/10.3390/metabo12111100

Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S. & Smith, D.L. (2018). Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. *Frontiers in Plant Science*, 9. https://www.frontiersin.org/articles/10.3389/fpls.2018.01473 [2023-11-24]

Balouiri, M., Sadiki, M. & Ibnsouda, S.K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6 (2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Bargaz, A., Lyamlouli, K., Chtouki, M., Zeroual, Y. & Dhiba, D. (2018). Soil Microbial Resources for Improving Fertilizers Efficiency in an Integrated Plant Nutrient Management System. *Frontiers in Microbiology*, 9. https://doi.org/10.3389/fmicb.2018.01606

Beccari, G., Covarelli, L. & Nicholson, P. (2011). Infection processes and soft wheat response to root rot and crown rot caused by *Fusarium* culmorum. *Plant Pathology*, 60 (4), 671–684. https://doi.org/10.1111/j.1365-3059.2011.02425.x

Bekkar, A.A., Zaim, S. & Belabid, L. (2018). Induction of systemic resistance in chickpea against *Fusarium* wilt by *Bacillus* strains. *Archives of Phytopathology and Plant Protection*, 51 (1–2), 70–80. https://doi.org/10.1080/03235408.2018.1438819

Berendsen, R.L., Pieterse, C.M.J. & Bakker, P.A.H.M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science*, 17 (8), 478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Berruti, A., Lumini, E., Balestrini, R. & Bianciotto, V. (2015). Arbuscular Mycorrhizal Fungi as Natural Biofertilizers: Let's Benefit from Past Successes. *Frontiers in Microbiology*, 6, 1559. https://doi.org/10.3389/fmicb.2015.01559

Beura, K., Pradhan, A.K., Ghosh, G.K., Kohli, A. & Singh, M. (2020). Root Architecture, Yield and Phosphorus Uptake by Rice: Response to Rock Phosphate Enriched Compost and Microbial Inoculants. *International Research Journal of Pure and Applied Chemistry*, 33–39. https://doi.org/10.9734/irjpac/2020/v21i1930275

Biorender (2024). *BioRender*. https://app.biorender.com/illustrations/6606f0508357c96496ab91de [2024-04-02]

Blum, A., Benfield, A.H., Sørensen, J.L., Nielsen, M.R., Bachleitner, S., Studt, L., Beccari, G., Covarelli, L., Batley, J. & Gardiner, D.M. (2019). Regulation of a novel *Fusarium* cytokinin in *Fusarium pseudograminearum*. *Fungal Biology*, 123 (3), 255–266. https://doi.org/10.1016/j.funbio.2018.12.009

Bonfante, P. & Genre, A. (2010). Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. *Nature Communications*, 1 (1), 48. https://doi.org/10.1038/ncomms1046

Bottalico, A. & Perrone, G. (2002). Toxigenic *Fusarium* species and Mycotoxins Associated with Head Blight in Small-Grain Cereals in Europe. *European Journal of Plant Pathology*, 108 (7), 611–624. https://doi.org/10.1023/A:1020635214971

Bozoğlu, T., Derviş, S., Imren, M., Amer, M., Özdemir, F., Paulitz, T.C., Morgounov, A., Dababat, A.A. & Özer, G. (2022). Fungal Pathogens Associated with Crown and Root Rot of Wheat in Central, Eastern, and Southeastern Kazakhstan. *Journal of Fungi*, 8 (5), 417. https://doi.org/10.3390/jof8050417

Brundrett, M.C. & Tedersoo, L. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist*, 220 (4), 1108–1115. https://doi.org/10.1111/nph.14976

Canfora, L., Costa, C., Pallottino, F. & Mocali, S. (2021). Trends in Soil Microbial Inoculants Research: A Science Mapping Approach to Unravel Strengths and Weaknesses of Their Application. *Agriculture*, 11 (2), 158. https://doi.org/10.3390/agriculture11020158

Carstensen, A., Szameitat, A.E., Frydenvang, J. & Husted, S. (2019). Chlorophyll a fluorescence analysis can detect phosphorus deficiency under field conditions and is an effective tool to prevent grain yield reductions in spring barley (Hordeum vulgare L.). *Plant and Soil*, 434 (1–2), 79–91. https://doi.org/10.1007/s11104-018-3783-6

Carter, J. (2023). *Testing Novel Biocontrol Combinations for Fusarium sp. infection in Wheat*. https://medcraveonline.com/APAR/*Fusarium*-head-blight-and-crown-rot-on-wheat-amp-barley-losses-and-health-risks.html [2023-12-04]

Champeil, A., Doré, T. & Fourbet, J.F. (2004). *Fusarium* head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains. *Plant Science*, 166 (6), 1389–1415. https://doi.org/10.1016/j.plantsci.2004.02.004

Chandran, H., Meena, M. & Swapnil, P. (2021). Plant Growth-Promoting Rhizobacteria as a Green Alternative for Sustainable Agriculture. *Sustainability*, 13 (19), 10986. https://doi.org/10.3390/su131910986

Chen, L., Liu, H. & Ma, Q. (2021). The early transcriptional responses in rose induced by Bacillus velezensis CLA178 and Agrobacterium tumefaciens C58. *Journal of Phytopathology*, 169 (2), 73–82. https://doi.org/10.1111/jph.12960

Choudhary, D.K., Prakash, A. & Johri, B.N. (2007). Induced systemic resistance (ISR) in plants: mechanism of action. *Indian Journal of Microbiology*, 47 (4), 289–297. https://doi.org/10.1007/s12088-007-0054-2

Chowdhury, F.T., Zaman, N.R., Islam, M.R. & Khan, H. (2021). Anti-fungal secondary metabolites and hydrolytic enzymes from rhizospheric bacteria in crop protection: a review. *Journal of Bangladesh Academy of Sciences*, 44 (2), 69–84. https://doi.org/10.3329/jbas.v44i2.51452

Cuomo, C.A., Güldener, U., Xu, J.-R., Trail, F., Turgeon, B.G., Di Pietro, A., Walton, J.D., Ma, L.-J., Baker, S.E., Rep, M., Adam, G., Antoniw, J., Baldwin, T., Calvo, S., Chang, Y.-L., DeCaprio, D., Gale, L.R., Gnerre, S., Goswami, R.S., Hammond-Kosack, K., Harris, L.J., Hilburn, K., Kennell, J.C., Kroken, S., Magnuson, J.K., Mannhaupt, G., Mauceli, E., Mewes, H.-

W., Mitterbauer, R., Muehlbauer, G., Münsterkötter, M., Nelson, D., O'Donnell, K., Ouellet, T., Qi, W., Quesneville, H., Roncero, M.I.G., Seong, K.-Y., Tetko, I.V., Urban, M., Waalwijk, C., Ward, T.J., Yao, J., Birren, B.W. & Kistler, H.C. (2007). The *Fusarium graminearum* Genome Reveals a Link Between Localized Polymorphism and Pathogen Specialization. *Science*, 317 (5843), 1400–1402. https://doi.org/10.1126/science.1143708

Dean, R., VAN KAN, J.A.L., PRETORIUS, Z.A., HAMMOND-KOSACK, K.E., DI PIETRO, A., SPANU, P.D., RUDD, J.J., DICKMAN, M., KAHMANN, R., ELLIS, J. & FOSTER, G.D. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, 13 (4), 414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Deshmukh, S.D. & Kogel, K.-H. (2007). Piriformospora indica protects barley from root rot caused by *Fusarium* graminearum / Piriformospora indica schützt Gerste vor der von *Fusarium* graminearum verursachten Wurzelfäule. *Journal of Plant Diseases and Protection*, 114 (6), 263–268

EM1 (2024). *Learn how to live sustainably using EM microbial technology on agriculture and environment*. *EM Research Organization*. https://www.emrojapan.com/what-we-do/ [2024-04-02]

FAO (2023). *The State of Food Security and Nutrition in the World 2023*. FAO; IFAD; UNICEF; WFP; WHO; https://doi.org/10.4060/cc3017en

FAOSTAT (2022). *FAOSTAT*. https://www.fao.org/faostat/en/#data [2024-03-28]

Figueroa, M., Hammond-Kosack, K.E. & Solomon, P.S. (2018). A review of wheat diseases—a field perspective. *Molecular Plant Pathology*, 19 (6), 1523–1536. https://doi.org/10.1111/mpp.12618

Gardiner, D.M., Stephens, A.E., Munn, A.L. & Manners, J.M. (2013). An ABC pleiotropic drug resistance transporter of *Fusarium graminearum* with a role in crown and root diseases of wheat. *FEMS Microbiology Letters*, 348 (1), 36–45. https://doi.org/10.1111/1574-6968.12240

Gołębiewski, M., Sikora, M., Mazur, J., Szymańska, S., Tyburski, J., Hrynkiewicz, K. & Ulrich, W. (2023). Colonization of axenic beet by rhizosphere bacteria takes place in discreet phases regardless of bioinculation with next generation bioinoculant. https://doi.org/10.1101/2023.02.10.527839

Goswami, R.S. & Kistler, H.C. (2004). Heading for disaster: *Fusarium* graminearum on cereal crops. *Molecular Plant Pathology*, 5 (6), 515–525. https://doi.org/10.1111/j.1364-3703.2004.00252.x

Hagerty, C.H., Irvine, T., Rivedal, H.M., Yin, C. & Kroese, D.R. (2021). Diagnostic Guide: *Fusarium* Crown Rot of Winter Wheat. *Plant Health Progress*, 22 (2), 176–181. https://doi.org/10.1094/PHP-10-20-0091-DG

Hasta Gård (2024). https://hastagard.eco/om-hasta-gard/ [2024-04-02]

Hnini, M., Rabeh, K. & Oubohssaine, M. (2024). Interactions between beneficial soil microorganisms (PGPR and AMF) and host plants for environmental restoration: A systematic review. *Plant Stress*, 100391. https://doi.org/10.1016/j.stress.2024.100391

Ji, F., He, D., Olaniran, A.O., Mokoena, M.P., Xu, J. & Shi, J. (2019). Occurrence, toxicity, production and detection of *Fusarium* mycotoxin: a review. *Food Production, Processing and Nutrition*, 1 (1), 6. https://doi.org/10.1186/s43014-019-0007-2

Jung, S.C., Martinez-Medina, A., Lopez-Raez, J.A. & Pozo, M.J. (2012). Mycorrhiza-Induced Resistance and Priming of Plant Defenses. *Journal of Chemical Ecology*, 38 (6), 651–664. https://doi.org/10.1007/s10886-012-0134-6

Kapoor, R. (2008). Induced Resistance in Mycorrhizal Tomato is correlated to Concentration of Jasmonic Acid. *OnLine Journal of Biological Sciences*, 8 (3), 49–56. https://doi.org/10.3844/ojbsci.2008.49.56

Kazan, K. & Gardiner, D.M. (2018). *Fusarium* crown rot caused by *Fusarium* pseudograminearum in cereal crops: recent progress and future prospects. *Molecular Plant Pathology*, 19 (7), 1547–1562. https://doi.org/10.1111/mpp.12639

Khoury, C.K., Carver, D., Kates, H.R., Achicanoy, H.A., Zonneveld, M., Thomas, E., Heinitz, C., Jarret, R., Labate, J.A., Reitsma, K., Nabhan, G.P. & Greene, S.L. (2020). Distributions, conservation status, and abiotic stress tolerance potential of wild cucurbits ( *Cucurbita* L.). *PLANTS, PEOPLE, PLANET*, 2 (3), 269–283. https://doi.org/10.1002/ppp3.10085

Kim, S., Kim, T.H., Chung, M.-N., Lee, Y., Lee, I.B., Lee, H. & Park, W. (2022). Incidence Rates of Root Rot in Sweetpotato Caused by Cultivation Soil and Soil Microorganisms During Storage Periods. *Frontiers in Plant Science*, 13, 897590. https://doi.org/10.3389/fpls.2022.897590

Kirby, E., Murray, T.D., Schroeder, K.L. & Chen, X. (2017). Disease Management for Wheat and Barley

Kulik, T., Molcan, T., Bilska, K., Beyer, M., Pasquali, M., Diepeningen, A.V. & Myszczynski, K. (2023). Two distinct *Fusarium* graminearum populations colonized European wheat in the past two decades. Ruan, Y. (red.) (Ruan, Y., red.) *PLOS ONE*, 18 (12), e0296302. https://doi.org/10.1371/journal.pone.0296302

Kuwayama, H. & Higashinakagawa, T. (2019). The Life Cycle of *Dictyostelium discoideum* Is Accelerated via MAP Kinase Cascade by a Culture Extract Produced by a Synthetic Microbial Consortium. *Microbial Physiology*, 29 (1–6), 35–42. https://doi.org/10.1159/000504442

Köhl, J., Kolnaar, R. & Ravensberg, W.J. (2019). Mode of Action of Microbial Biological Control Agents Against Plant Diseases: Relevance Beyond Efficacy. *Frontiers in Plant Science*, 10, 845. https://doi.org/10.3389/fpls.2019.00845

Lakshmanan, V., Castaneda, R., Rudrappa, T. & Bais, H.P. (2013). Root transcriptome analysis of Arabidopsis thaliana exposed to beneficial Bacillus subtilis FB17 rhizobacteria revealed genes for bacterial recruitment and plant defense independent of malate efflux. *Planta*, 238 (4), 657–668. https://doi.org/10.1007/s00425-013-1920-2

Lamichhane, J.R., Dachbrodt-Saaydeh, S., Kudsk, P. & Messéan, A. (2016). Toward a Reduced Reliance on Conventional Pesticides in European Agriculture. *Plant Disease*, 100 (1), 10–24. https://doi.org/10.1094/PDIS-05-15-0574-FE

Li, H.L., Yuan, H.X., Fu, B., Xing, X.P., Sun, B.J. & Tang, W.H. (2012). First Report of *Fusarium* pseudograminearum Causing Crown Rot of Wheat in Henan, China. *Plant Disease*, 96 (7), 1065–1065. https://doi.org/10.1094/PDIS-01-12-0007-PDN

Li, J., Wei, J., Shao, X., Yan, X. & Liu, K. (2024). Effective microorganisms input efficiently improves the vegetation and microbial community of degraded alpine grassland. *Frontiers in Microbiology*, 14. https://doi.org/10.3389/fmicb.2023.1330149

Liu, Y.-X., Qin, Y. & Bai, Y. (2019). Reductionist synthetic community approaches in root microbiome research. *Current Opinion in Microbiology*, 49, 97–102. https://doi.org/10.1016/j.mib.2019.10.010

Lobell, D.B., Schlenker, W. & Costa-Roberts, J. (2011). Climate Trends and Global Crop Production Since 1980. *Science*, 333 (6042), 616–620. https://doi.org/10.1126/science.1204531

Lugtenberg, B. & Kamilova, F. (2009). Plant-Growth-Promoting Rhizobacteria. *Annual Review of Microbiology*, 63 (1), 541–556. https://doi.org/10.1146/annurev.micro.62.081307.162918

Meena, M., Swapnil, P., Divyanshu, K., Kumar, S., Harish, Tripathi, Y.N., Zehra, A., Marwal, A. & Upadhyay, R.S. (2020). PGPR-mediated induction of systemic resistance and physiochemical alterations in plants against the pathogens: Current perspectives. *Journal of Basic Microbiology*, 60 (10), 828–861. https://doi.org/10.1002/jobm.202000370

Meru, G. & McGregor, C. (2020). Effect of Various Soil Media on Disease Severity of *Fusarium* Wilt in Watermelon. *American Journal of Plant Sciences*, 11 (12), 1890–1898. https://doi.org/10.4236/ajps.2020.1112135

Moya-Elizondo, E.A. (2013). *Fusarium* crown rot disease: biology, interactions, management and function as a possible sensor of global climate change. *Ciencia e investigación agraria*, 40 (2), 235–252. https://doi.org/10.4067/S0718-16202013000200001

Mwangi, M.W., Monda, E.O., Okoth, S.A. & Jefwa, J.M. (2011). Inoculation of tomato seedlings with Trichoderma Harzianum and Arbuscular Mycorrhizal Fungi and their effect on growth and control of wilt in tomato seedlings. *Brazilian Journal of Microbiology*, 42 (2), 508–513. https://doi.org/10.1590/S1517-83822011000200015

Müller, T., Ruppel, S., Behrendt, U., Lentzsch, P. & Müller, M.E.H. (2018). Antagonistic Potential of Fluorescent Pseudomonads Colonizing Wheat Heads Against Mycotoxin Producing Alternaria and Fusaria. *Frontiers in Microbiology*, 9. https://www.frontiersin.org/articles/10.3389/fmicb.2018.02124 [2024-02-02]

Nadeem, S.M., Ahmad, M., Zahir, Z.A., Javaid, A. & Ashraf, M. (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnology Advances*, 32 (2), 429–448. https://doi.org/10.1016/j.biotechadv.2013.12.005

Naseri, B. (2014). Bean production and *Fusarium* root rot in diverse soil environments in Iran. *Journal of soil science and plant nutrition*, (ahead), 0–0. https://doi.org/10.4067/S0718-95162014005000014

Nogrado, K., Ha, G.-S., Yang, H.-J. & Lee, J.-H. (2021). Effect of Non-indigenous Bacterial Introductions on Rhizosphere Microbial Community. *Korean Journal of Environmental Agriculture*, 40 (3), 194–202. https://doi.org/10.5338/KJEA.2021.40.3.23

Petronaitis, T., Forknall, C., Brodie, G., Simpfendorfer, S. & Backhouse, D. (2022). "Killer Joules": spores of Bipolaris sorokiniana and *Fusarium* species are susceptible to microwave radiation. *Australasian Plant Pathology*, 51 (4), 433–440. https://doi.org/10.1007/s13313-022-00872-x

Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C.M. & Bakker, P.A.H.M. (2014). Induced Systemic Resistance by Beneficial Microbes. *Annual Review of Phytopathology*, 52 (1), 347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Pu, C., Ge, Y., Yang, G., Zheng, H., Guan, W., Chao, Z., Shen, Y., Liu, S., Chen, M. & Huang, L. (2022). Arbuscular mycorrhizal fungi enhance disease resistance of Salvia miltiorrhiza to *Fusarium* wilt. *Frontiers in Plant Science*, 13, 975558. https://doi.org/10.3389/fpls.2022.975558

R Core Team (2024). *R: The R Project for Statistical Computing*. https://www.r-project.org/ [2024-04-02]

Rahimzadeh, S. & Pirzad, A. (2017). Arbuscular mycorrhizal fungi and Pseudomonas in reduce drought stress damage in flax (Linum usitatissimum L.): a field study. *Mycorrhiza*, 27 (6), 537–552. https://doi.org/10.1007/s00572-017-0775-y

Ram, K., Wickham, H., Richards, C. & Baggett, A. (2023). *wesanderson: A Wes Anderson Palette Generator* (0.3.7). https://cran.r-project.org/web/packages/wesanderson/index.html [2024-04-02]
Rana, A., Saharan, B., Nain, L., Prasanna, R. & Shivay, Y.S. (2012). Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia. *Soil Science and Plant Nutrition*, 58 (5), 573–582. https://doi.org/10.1080/00380768.2012.716750
Rodríguez-Romero, A.S., Guerra, M.S.P. & Jaizme-Vega, M.D.C. (2005). Effect of arbuscular mycorrhizal fungi and rhizobacteria on banana growth and nutrition. *Agronomy for Sustainable Development*, 25 (3), 395–399. https://doi.org/10.1051/agro:2005039
Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N. & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, 3 (3), 430–439. https://doi.org/10.1038/s41559-018-0793-y
Scherm, B., Balmas, V., Spanu, F., Pani, G., Delogu, G., Pasquali, M. & Migheli, Q. (2013). *Fusarium* culmorum: causal agent of foot and root rot and head blight on wheat. *Molecular Plant Pathology*, 14 (4), 323–341. https://doi.org/10.1111/mpp.12011
Sharma, K., Gupta, S., Thokchom, S.D., Jangir, P. & Kapoor, R. (2021). Arbuscular Mycorrhiza-Mediated Regulation of Polyamines and Aquaporins During Abiotic Stress: Deep Insights on the Recondite Players. *Frontiers in Plant Science*, 12. https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2021.642101 [2024-03-02]
Smiley, R.W., Gourlie, J.A., Easley, S.A., Patterson, L.-M. & Whittaker, R.G. (2005). Crop Damage Estimates for Crown Rot of Wheat and Barley in the Pacific Northwest. *Plant Disease*, 89 (6), 595–604. https://doi.org/10.1094/PD-89-0595
Smith, S.E. & Read, D. (2008). Growth and carbon economy of arbuscular mycorrhizal symbionts. I: *Mycorrhizal Symbiosis*. Elsevier. 117–144. https://doi.org/10.1016/B978-012370526-6.50006-4
SpectraCrop (2019). plant_vitality_and_ptester.pdf
Tahiri, A., Meddich, A., Raklami, A., Alahmad, A., Bechtaoui, N., Anli, M., Göttfert, M., Heulin, T., Achouak, W. & Oufdou, K. (2022). Assessing the Potential Role of Compost, PGPR, and AMF in Improving Tomato Plant Growth, Yield, Fruit Quality, and Water Stress Tolerance. *Journal of Soil Science and Plant Nutrition*, 22 (1), 743–764. https://doi.org/10.1007/s42729-021-00684-w
Theis, K.R., Dheilly, N.M., Klassen, J.L., Brucker, R.M., Baines, J.F., Bosch, T.C.G., Cryan, J.F., Gilbert, S.F., Goodnight, C.J., Lloyd, E.A., Sapp, J., Vandenkoornhuyse, P., Zilber-Rosenberg, I., Rosenberg, E. & Bordenstein, S.R. (2016). Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes. *mSystems*, 1 (2), e00028-16. https://doi.org/10.1128/mSystems.00028-16
Torppa, K.A., Forkman, J., Maaroufi, N.I., Taylor, A.R., Vahter, T., Vasar, M., Weih, M., Öpik, M. & Viketoft, M. (2023). Soil compaction effects on arbuscular mycorrhizal symbiosis in wheat depend on host plant variety. *Plant and Soil*,. https://doi.org/10.1007/s11104-023-06250-w
Trabelsi, D. & Mhamdi, R. (2013). Microbial Inoculants and Their Impact on Soil Microbial Communities: A Review. *BioMed Research International*, 2013, 1–11. https://doi.org/10.1155/2013/863240
Wahab, A., Muhammad, M., Munir, A., Abdi, G., Zaman, W., Ayaz, A., Khizar, C. & Reddy, S.P.P. (2023). Role of Arbuscular Mycorrhizal Fungi in Regulating Growth, Enhancing Productivity, and Potentially Influencing

Ecosystems under Abiotic and Biotic Stresses. *Plants*, 12 (17), 3102. https://doi.org/10.3390/plants12173102
Wang, L.-Y., Xie, Y.-S., Cui, Y.-Y., Xu, J., He, W., Chen, H.-G. & Guo, J.-H. (2015). Conjunctively screening of biocontrol agents (BCAs) against *Fusarium* root rot and *Fusarium* head blight caused by *Fusarium* graminearum. *Microbiological Research*, 177, 34–42. https://doi.org/10.1016/j.micres.2015.05.005
Weng, W., Yan, J., Zhou, M., Yao, X., Gao, A., Ma, C., Cheng, J. & Ruan, J. (2022). Roles of Arbuscular mycorrhizal Fungi as a Biocontrol Agent in the Control of Plant Diseases. *Microorganisms*, 10 (7), 1266. https://doi.org/10.3390/microorganisms10071266
Wickham, H., Chang, W., Henry, L., Pedersen, T.L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., Dunnington, D., Brand, T. van den, Posit & PBC (2024a). *ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics* (3.5.0). https://cran.r-project.org/web/packages/ggplot2/index.html [2024-04-02]
Wickham, H., François, R., Henry, L., Müller, K., Vaughan, D., Software, P. & PBC (2023). *dplyr: A Grammar of Data Manipulation* (1.1.4). https://cran.r-project.org/web/packages/dplyr/index.html [2024-04-02]
Wickham, H., Vaughan, D., Girlich, M., Ushey, K., Software, P. & PBC (2024b). *tidyr: Tidy Messy Data* (1.3.1). https://cran.r-project.org/web/packages/tidyr/index.html [2024-04-02]
Willocquet, L., Meza, W.R., Dumont, B., Klocke, B., Feike, T., Kersebaum, K.C., Meriggi, P., Rossi, V., Ficke, A., Djurle, A. & Savary, S. (2021). An outlook on wheat health in Europe from a network of field experiments. *Crop Protection*, 139, 105335. https://doi.org/10.1016/j.cropro.2020.105335
Won, S.-J., Kwon, J.-H., Kim, D.-H. & Ahn, Y.-S. (2019). The Effect of Bacillus licheniformis MH48 on Control of Foliar Fungal Diseases and Growth Promotion of Camellia oleifera Seedlings in the Coastal Reclaimed Land of Korea. *Pathogens*, 8 (1), 6. https://doi.org/10.3390/pathogens8010006
Xu, Z., Luo, T., Rao, N., Yang, L., Liu, J., Zhang, C. & Hu, L. (2019). High Yield Achieved by Early-Maturing Rapeseed with High Light Energy and Temperature Production Efficiencies under Ideal Planting Density. *Crop Science*, 59 (1), 351–362. https://doi.org/10.2135/cropsci2018.04.0255
Yan, Z., Reddy, M.S., Ryu, C.-M., McInroy, J.A., Wilson, M. & Kloepper, J.W. (2002). Induced Systemic Protection Against Tomato Late Blight Elicited by Plant Growth-Promoting Rhizobacteria. *Phytopathology*®, 92 (12), 1329–1333. https://doi.org/10.1094/PHYTO.2002.92.12.1329
Yang, H., Berckx, F., Fransson, P. & Weih, M. (2023). Harnessing plant–microbe interactions to promote nitrogen use efficiency in cereal crops. *Plant and Soil*, 494 (1–2), 75–83. https://doi.org/10.1007/s11104-023-06278-y
Yu, C., Liu, X., Zhang, X., Zhang, M., Gu, Y., Ali, Q., Mohamed, M.S.R., Xu, J., Shi, J., Gao, X., Wu, H. & Gu, Q. (2021). Mycosubtilin Produced by Bacillus subtilis ATCC6633 Inhibits Growth and Mycotoxin Biosynthesis of *Fusarium* graminearum and *Fusarium* verticillioides. *Toxins*, 13 (11), 791. https://doi.org/10.3390/toxins13110791
Yu, K., Pieterse, C.M.J., Bakker, P.A.H.M. & Berendsen, R.L. (2019). Beneficial microbes going underground of root immunity. *Plant, Cell & Environment*, 42 (10), 2860–2870. https://doi.org/10.1111/pce.13632
Zhao, Y., Cartabia, A., Lalaymia, I. & Declerck, S. (2022). Arbuscular mycorrhizal fungi and production of secondary metabolites in medicinal plants. *Mycorrhiza*, 32 (3–4), 221–256. https://doi.org/10.1007/s00572-022-01079-0
Zhong, Y., Yang, Y., Liu, P., Xu, R., Rensing, C., Fu, X. & Liao, H. (2019). Genotype and rhizobium inoculation modulate the assembly of soybean

rhizobacterial communities. *Plant, Cell & Environment*, 42 (6), 2028–2044. https://doi.org/10.1111/pce.13519

# Popular science summary

**The hidden half: Can Soil Microbes be the Key to Sustainable Agricultural Practices? - Electra Lennartsson**

The European Union's farm-to-fork strategy has set ambitious goals, including a 50% reduction in pesticide use by 2023. This drive highlights the urgent need for alternative methods of crop protection and enhancement. One promising avenue lies beneath the soil, in the intricate relationships between plants and soil microbes. My recent research dives into this hidden world, revealing the potential of these tiny allies to transform farming practices.

Integrated Pest Management (IPM) serves as a guiding compass in minimizing reliance on chemical pesticides by harnessing natural ecological processes. It is becoming increasingly more recognised that the importance of soil-beneficial microorganisms plays an interconnected role in plant growth and development already established as ‘the plant’s second genome’. By understanding and leveraging these microbial interactions, we can reduce the need for synthetic chemicals while increasing yield productivity.

Two groups of soil microbes take centre stage for their beneficial properties: arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR). These microscopic allies form symbiotic relationships with plant roots, offering a range of benefits from improved nutrient uptake to disease suppression. Through rigorous experimentation, compelling evidence has emerged that wheat inoculated with AMF and PGPR shows promising results in terms of resilience when faced with pathogenic *Fusarium* strains.

Furthermore, there's growing interest among plant breeders in traits beneath the soil surface. Exploring root-related traits presents challenges, but it's increasingly clear how vital it is not to overlook this hidden half of below-ground traits and their interactions with the plant's genetic makeup.

Excitingly, there's potential synergy between these beneficial microorganisms. When used together, AMF and PGPR enhance each other's benefits, providing a

holistic approach to plant health. Can these natural allies help us reduce our reliance on synthetic inputs and transition to a more sustainable and resilient agricultural system?

Attaining sustainability requires more than just scientific inquiry; it demands a fundamental shift in mindset and agricultural practices. The actors needed to be involved in this strive for change is threefold. Farmers must be given economic and practical incentives to adopt practices that promote biodiversity and ecosystem resilience which can be facilitated by governments and policymakers by offering support through incentives and regulations that prioritize sustainable farming practices.

Solutions could lie just beneath our feet. Now is the time to dig deep and harness the power of nature's allies in our quest for a more sustainable food system. Interdisciplinary research and the pillars of sustainability must be prioritised to achieve this in the near future so that we can slowly shift away from the dependence on fertilisers affecting the soil’s health which is further aggravated by monocultures. One step at a time.

# Acknowledgments

I am deeply grateful for the invaluable support and assistance I received from the team at Ekologicentrum in SLU, Ultuna, without whom my thesis would not have been possible. I extend my heartfelt thanks to Martin Weih's group for generously allowing me to conduct my practical research training and master's thesis within their team. Special thanks go to Fede Berckx for all of her patience and guidance, Jada Carter for her work and support during the summer of 2023, Jonathan Edward Cope for his emotional support and body doubling me during long working hours, my beautiful daughter Maja Nepheli Momo Lennartsson with her hugs and smile that could dissipate my stress and anxiety, Ann Hanna Christina Hallberg for being an extremely good friend going above and beyond when I was in aid of a few hours of writing and provided food and taking care of Maja, and Bennet Arnaud Finn Wildenauer for coming over with food deliveries with all sorts of fruit and nuts, as well as reminding me to go home and rest. Last but certainly not least, work afternoon fikas and Ekopubs were the highlight of most of my social life and provided excellent escapism. I hope to meet you again both as friends and inspiring colleagues.

# 6. Appendix

Taxonomy for fungal material

*Fusarium culmorum* (W.G. Smith) Sacc. Kingdom Fungi; Phylum Ascomycota; Subphylum Pezizomycotina; Class Sordariomycetes; Subclass Hypocreomycetidae; Order Hypocreales; Family Nectriaceae; Genus *Fusarium*.

*Fusarium graminearum* (teleomorph stage: *Gibberella zeae*)
Kingdom Fungi; Phylum Ascomycota; Subphylum Pezizomycotina; Class Sordariomycetes; Subclass Hypocreomycetidae; Order Hypocreales; Family Nectriaceae; Genus *Fusarium*.

Mung bean broth protocol

- 40 gr of mung beans
- 1 litre of boiling water

Heat until the first peals loosen. Filtrate the beans through cheesecloth, aliquot the filtrate into smaller potions, and autoclave for 15 min at 121ºC. The medium should turn clear red.

## 6.1 Supplementary Tables

*Supplementary Table 1 - Hasselfors S-soil composition*

| Hasselfors S-jord soil composition |
|---|
| **pH value:** 5.5 – 6.5 |
| **Electrical conductivity** (± 25%): 20 mS/m |
| **Composition:** Light and dark peat, perlite, sand/rock milk, lime, mineral fertilizer, Root power |
| **Added nutrition per m³:** NPK 14-7-15 micro 0.9 kg |
| **Added amount in g/m³:** Nitrogen readily soluble (N) 125 |
| **Boron (B)** 0.3 |
| **Phosphorus (P)** 65 |
| **Copper (Cu)** 1.1 |
| **Potassium (K)** 140 |
| **Iron (Fe)** 1.0 |
| **Magnesium (Mg)** 220 |
| **Manganese (Mn)** 1.5 |
| **Calcium (Ca)** 1800 |
| **Molybdenum (Mo)** 0.5 |
| **Sulfur (S)** 70 |
| **Zinc (Zn)** 0.4 |

*Supplementary Table 2 - Luria-Bertani Medium by DMSZ*

| LB Medium | | |
|---|---|---|
| Tryptone | 10 | g |
| Yeast Extract | 5 | g |
| NaCl | 10 | g |
| Agar | 20 | g |
| Distilled Agar | 1000 | ml |
| pH | 7 | |

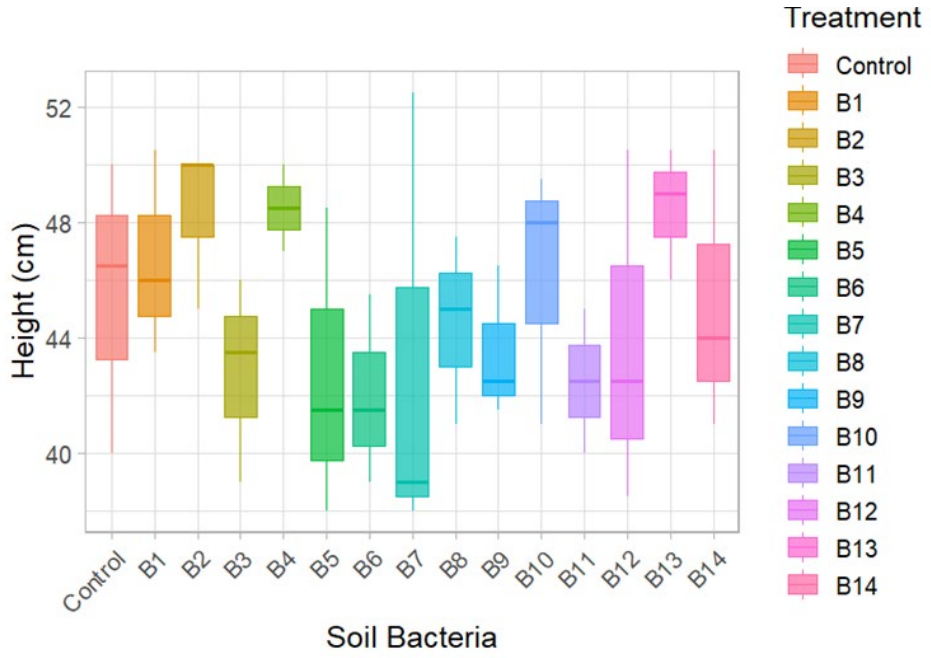
*Supplementary Table 3 - International Streptomyces 2 by DMSZ*

| ISP2 Medium | | |
|---|---|---|
| Yeast Extract (Difco) | 4 | g |
| Malt Extract (Difco) | 10 | g |
| Dextrose (Difco) | 4 | g |
| Agar | 20 | g |
| Distilled Water | 1000 | ml |
| pH | 7.2 | |

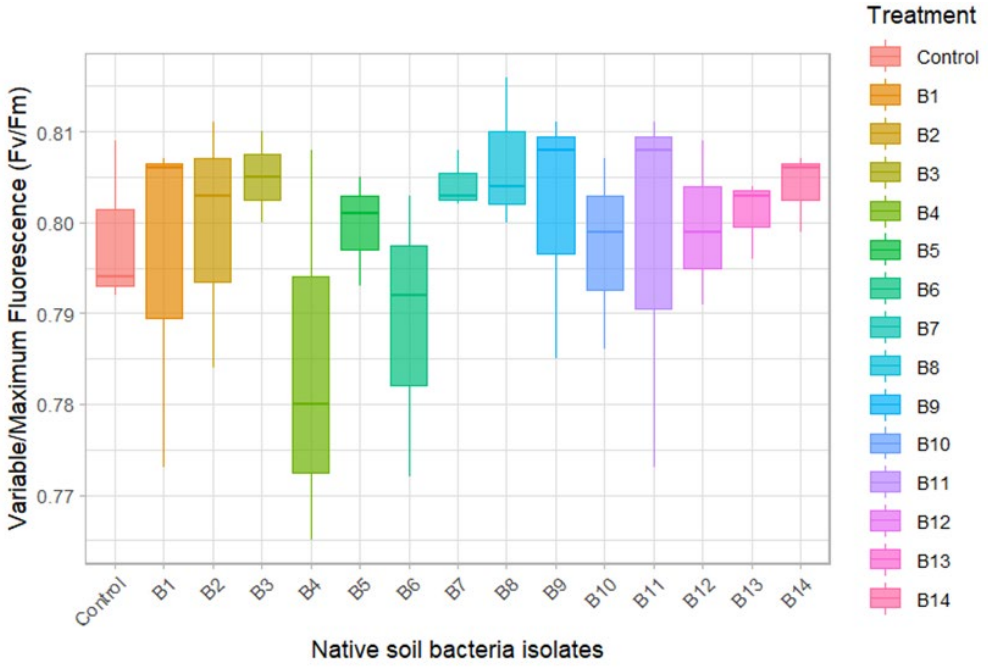
*Supplementary Table 4 - Fusarium strains used ( * signifies repetition).*

| ID Name | *Fusarium* strain | Experiment |
|---|---|---|
| VPE13 | *Fusarium culmorum* | 2 |
| VPE20 | *Fusarium graminearum* | 2 |
| VPE105 | *Fusarium graminearum* | 2* |
| LM | *Fusarium* sp. | 2 |
| PH-1 | *Fusarium graminearum* | 2* and 3 |

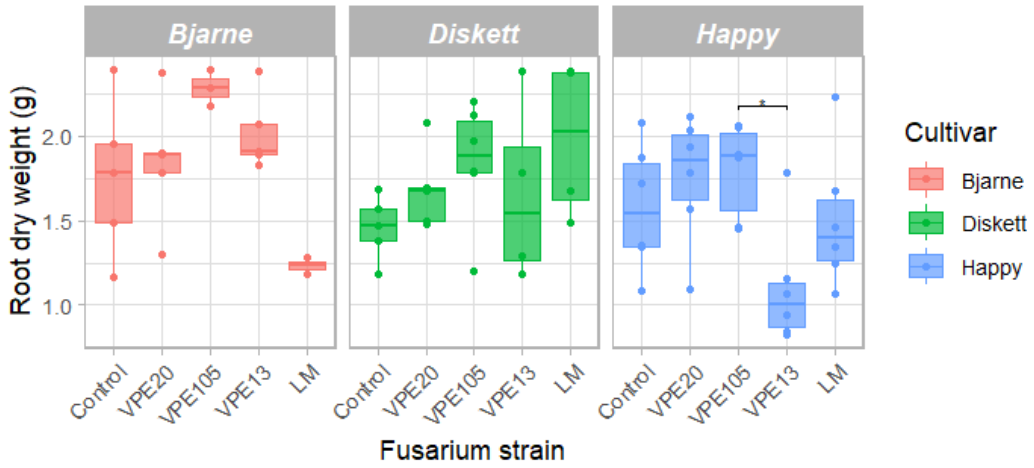
## 6.2 Supplementary Figures



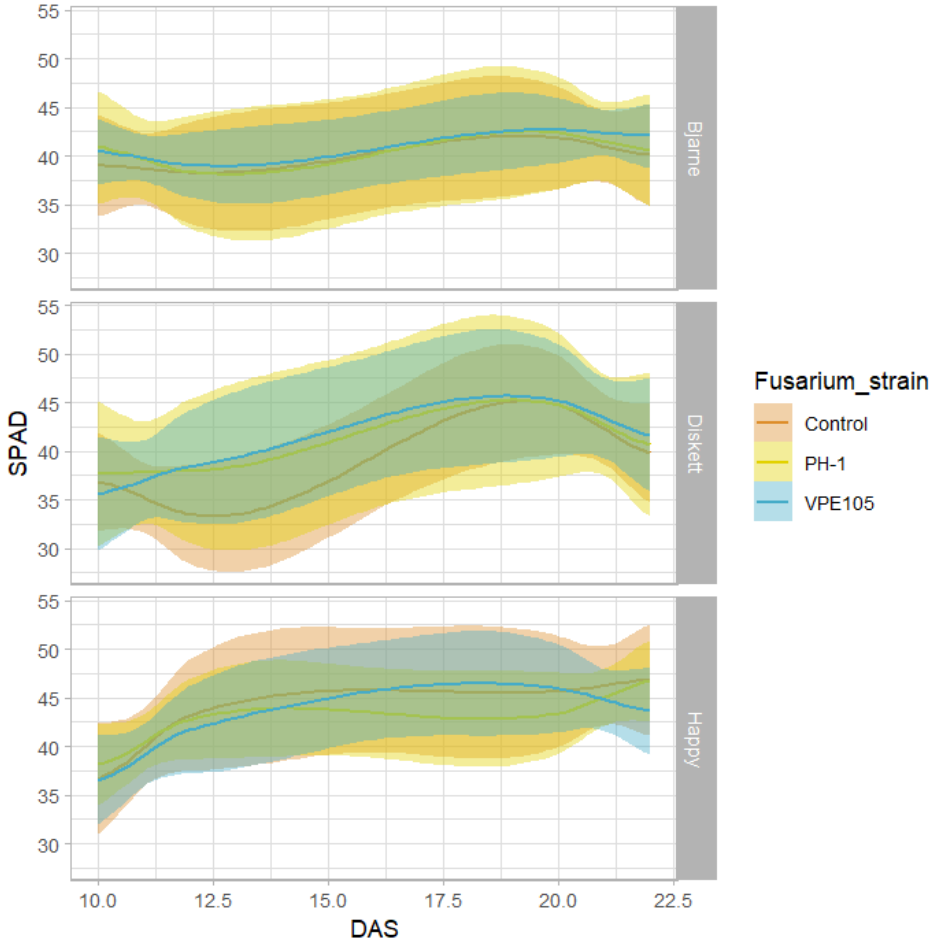

*Supplementary Figure 1 - Height per bacterial treatment. Boxplots are calculated based on three replicates. ANOVA showed that there is no statistical significance in this graph yet we do observe a large range in terms of shoot length. (Experiment 1).*



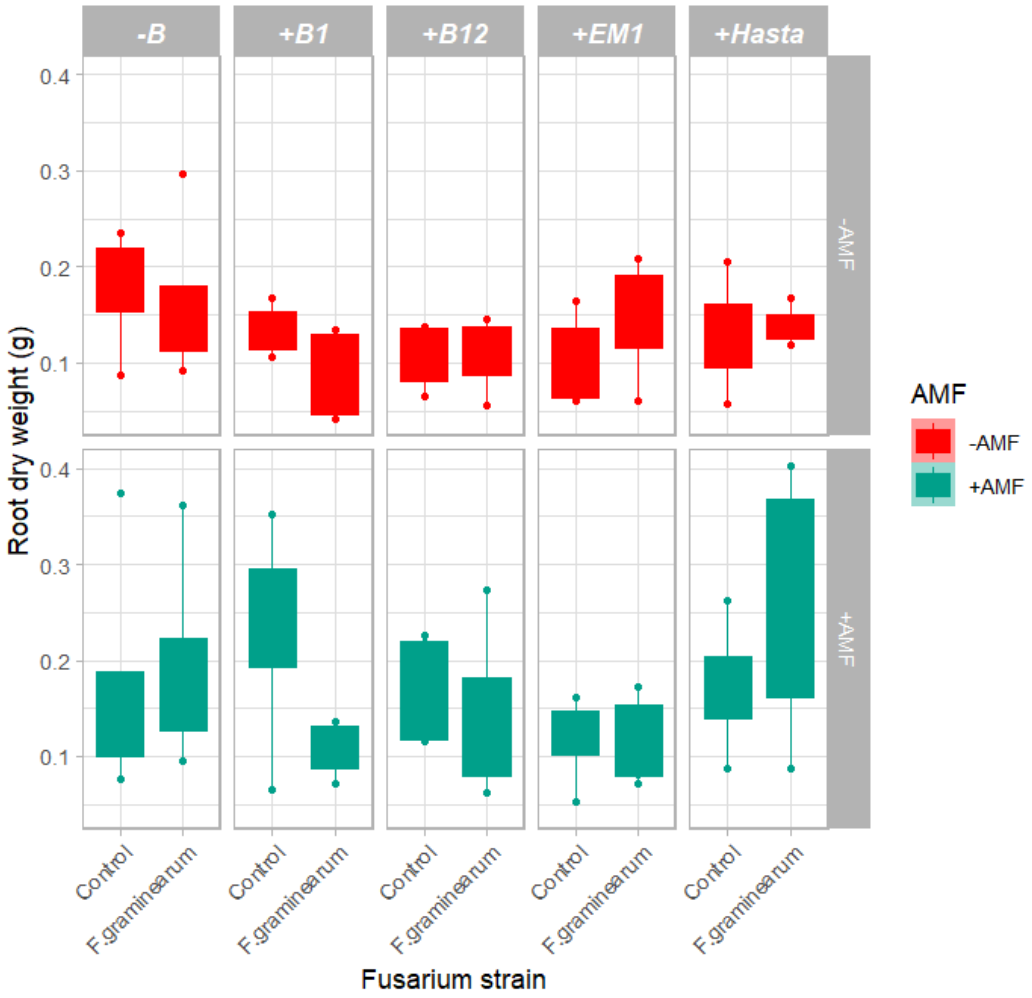

*Supplementary Figure 2 - Fv/Fm (Variable fluorescence/maximal Fluorescence ratio) values per bacterial treatment. Boxplots are calculated based on three replicates. ANOVA showed that there is no statistical significance in this graph. The height of the wheat plant does however exhibit a wide range of measurements so it could be the reason we see no statistical significance that we had a low number of replicates. This is only speculative, however, as more tests would be needed to test the effect of the native soil bacteria isolates in terms of wheat plant height (Experiment 1).*



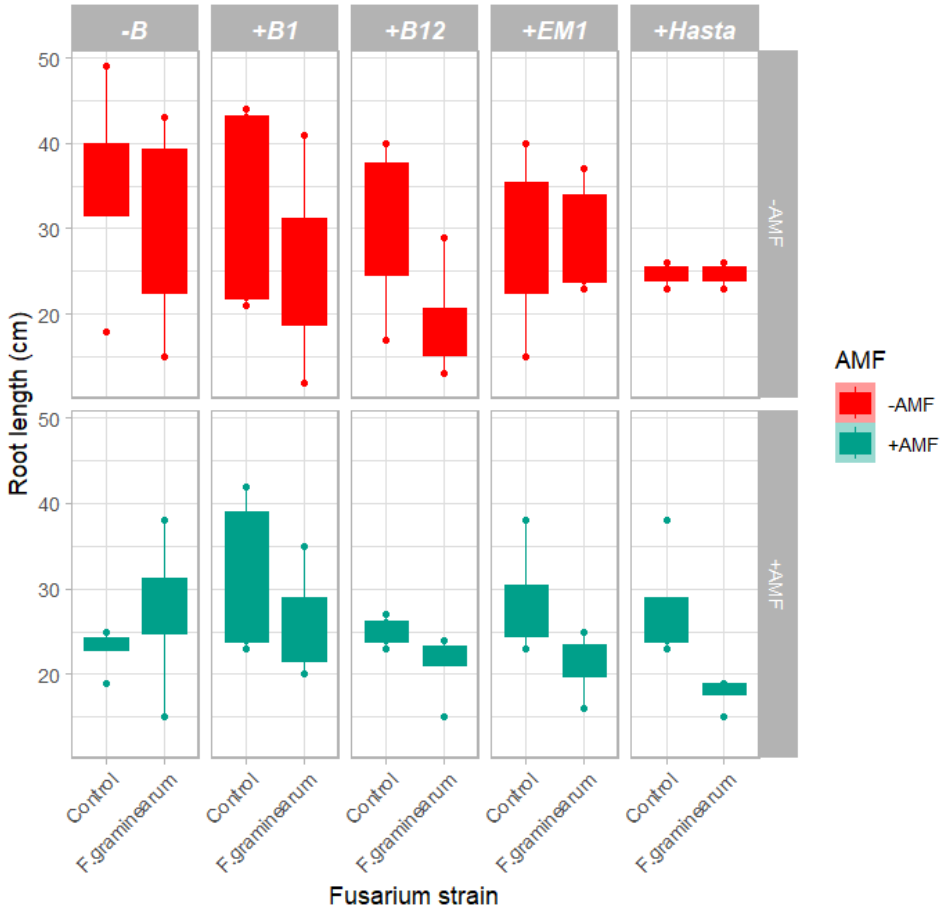

*Supplementary Figure 3 - Root dry weight per Fusarium treatment with cultivar type. (Experiment 2). Note: The WP method is missing as that method was disregarded. Root Dry Weight (g) per Fusarium strain*Cultivar. Here, I present an analysis of the impact of the spring wheat cultivar type when subjected to various strains of the pathogen, as discerned through the examination of root dry weight. Boxplots are calculated based on three replicates. ANOVA variance test was performed and we got p= 0.00870** for the root dry weight and cultivar*Fusarium treatment, followed by Tukey post-hoc analysis with p=0.0000160 cultivar Happy when comparing strain VPE105 and VPE13 as shown in the graph.*



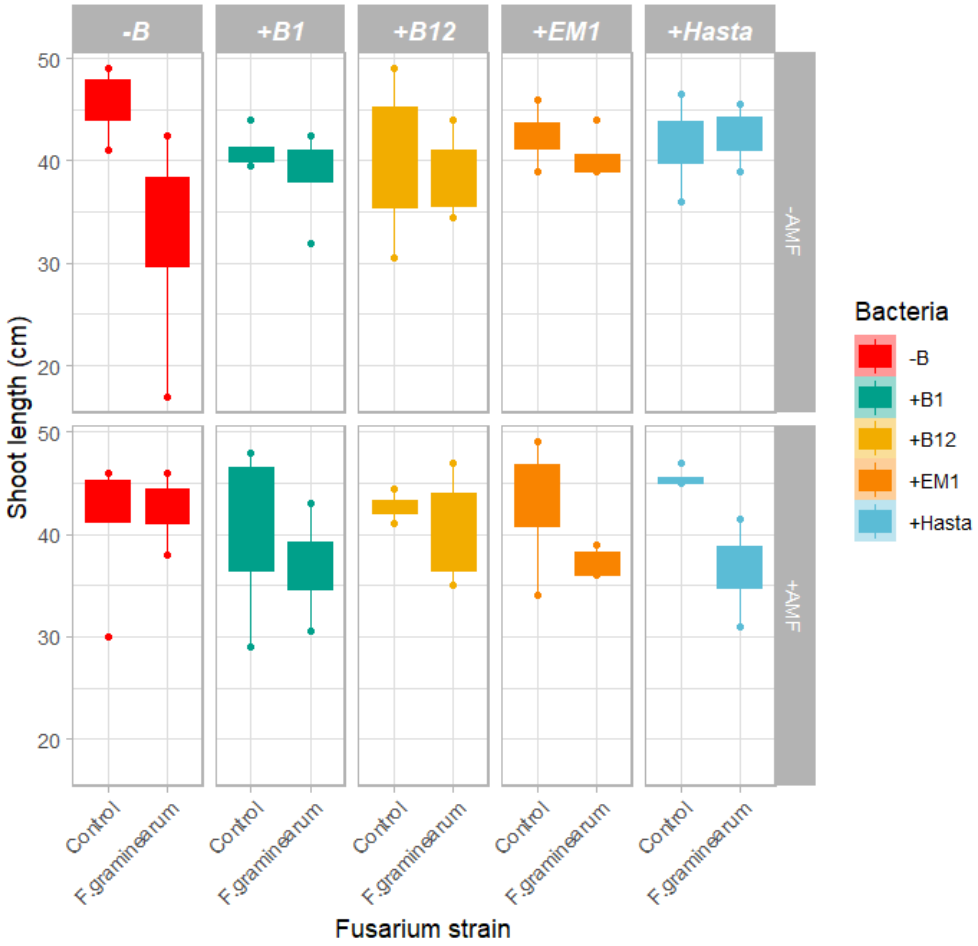

*Supplementary Figure 4 - SPAD time-series, SPAD values per DAS (days after sowing). Each grid is attributed to one wheat cultivar whose names are displayed in the right-hand column. The blue line indicates the presence of F. graminearum (PH-1) provided by Mukesh Dubey as well as the other F. graminearum strain (VPE105) from the VPE department. PH-1 seems to be exhibiting starker differences in SPAD values which could be indicative of the Fusarium treatment being more prominent in this comparison. (Experiment 2*).*




*Supplementary Figure 5 - Root dry weight in terms of Fusarium treatment also accounts for the bacterial and AMF treatment vertically and horizontally respectively. We can observe an ascending trend that occurs in dry root biomass when AMF is present. ANOVA showed statistical significance in terms of Root dry weight and AMF (p= 0.0325*) but we acquired no statistical significance with Tukey tests. (Experiment 3).*




*Supplementary Figure 6 - Root length in terms of Fusarium treatment also accounts for the bacterial and AMF treatment vertically and horizontally respectively. B1 seems to be working well with the presence of AMF in comparison to the other strains and communities. ANOVA tests were shown (p=0.0185*) in terms of Fusarium treatment and nonsignificant (but interesting) with AMF p= (0.0996 .) (Experiment 3).*




*Supplementary Figure 7. Shoot length(cm) in terms of Fusarium treatment. The facet grid showcases the different bacterial treatments of native soil rhizobacteria isolates together with commercial products. The AMF treatment is shown horizontally. From ANOVA we observed that Shoot length in terms of Fusarium*AMF*bacteria showed p=0.04078*(Experiment 3).*

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