PHYSIOLOGICAL INVESTIGATION OF CROWN ROT DISEASE DEVELOPMENT IN WHEAT (*TRITICUM AESTIVUM*)

A Thesis submitted by

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ABSTRACT

Crown rot (CR), caused by *Fusarium pseudograminearum (Fp)*, is a serious soil-borne disease of wheat and barley both internationally and in Australia, where it causes $79 and $18 million, respectively, in lost yield per annum to the Australian wheat industry. CR has been exacerbated in recent years by reduced tillage practices and its control is principally based on management practices such as crop rotation which over time decreases field inoculum. Soil factors, including moisture, temperature, nutrients and stubble borne inoculum form complex relationships which play major roles in determining the severity of disease. Although commercial wheat cultivars with a low level of CR resistance are available to growers in some environments, the most promising sources of resistance identified to date are quantitative in character and currently in non-agronomic backgrounds. Further, understanding of fundamental mechanisms of crop development of resistance to CR is essential to breed new cultivars having strong resistance. The aim of this project is to investigate mechanisms involved in CR disease development in wheat (*Triticum aestivum*) caused by *Fp*. We hypothesise that colonisation of xylem vessels reduces water flow through the plant. Simultaneously to this, we also hypothesise that disruption of water flow and subsequent phloem colonisation will alter the dynamics of sugar transport in the plant, carbon and nitrogen content in stem, leaf and grain. The physiological mechanism of development of CR disease, caused by *Fp* is not fully understood. Three seedling, three glasshouse and three field experiments were conducted to examine the CR impact on shoot length, biomass, carbon and nitrogen content, stem water pressure, and leaf gas exchange including rate of photosynthesis, stomatal conductance, internal CO_2 concentration and transpiration rate of the plant canopy. This research explored the host reaction of different bread wheat genotypes which varied in susceptibility to CR at seedling, flowering and maturity growth stages across nine experiments. In this investigation, *Fp* had a negative impact on the fundamental physiological parameters measured across all genotypes. Significant reduction in gas exchange parameters were observed in all field experiments; however, only internal CO_2 increased in seedling experiments. Inoculated genotypes displayed a reduction in the wheat biomass and required higher stem water pressure. The findings further our understanding of the relationship between experimental conditions, plant genotypes, and inoculation status. They reveal the intricate physiological responses of wheat to *Fp* inoculation at different growth stages and within various plant tissues. Collectively, our discoveries clarify

the effects of *Fp* infection on wheat during crucial stages of growth, potentially reshaping our comprehension of disease resistance. This information provides a foundation for developing new cultivars that have improved resistance to crown rot, which offers substantial benefits in terms of agricultural productivity. Employing advanced breeding techniques based on comprehensive physiological knowledge can greatly enhance the ability of crops to withstand CR, thus ensuring food security in countries heavily reliant on wheat.

CERTIFICATION OF THESIS

I Rian Rashid Abdulsada declare that the PhD Thesis entitled *Physiological investigation of crown rot disease development in wheat (Triticum aestivum)* is not more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references, and footnotes.

This Thesis is the work of Rian Rashid Abdulsada except where otherwise acknowledged, with the majority of the contribution to the papers presented as a Thesis by Publication undertaken by the student. The work is original and has not previously been submitted for any other award, except where acknowledged.

Date: 13/01/2024

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STATEMENT OF CONTRIBUTION

The agreed-upon contribution percentage for the candidate and co-authors of the publications presented in this thesis is as follows:

Paper 1 (Chapter 3)*:* *Fusarium pseudograminearum* infected wheat lines vary in disease severity and gas exchange response under different watering regimes.

Rian R Abdulsada, Michael Thompson, Lucas Peitton, Alison Kelly and Cassandra D Percy published in **Plant Pathology Journal.**

Rian R Abdulsada (RRA) wrote the first draft and contributed 60% towards the concept of the manuscript, design of the experiments, inoculum preparation and data collecting. Cassandra Percy (CP) 20% towards the concept of the manuscript and design the experiments, revision of the manuscript critically and final editorial input. Lucas Peitton (LP) 10% towards the data interpretation and analysis, Alison Kelly (AK) 5% towards the data interpretation and analysis, and Michael Thompson (MT) contributed 5% towards the concept and final editorial input.

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Paper 2 (Chapter 4): *Fusarium pseudograminearum* impact bread wheat (Triticum Aestivum) physiological parameters in the field trials.

Rian R Abdulsada, Michael Thompson, Clayton Forknall, Alison Kelly, and Cassandra D Percy to be submitted to **Agronomy Journal.**

RRA wrote the first draft and contributed 60% towards the concept of the manuscript, design of the experiments, inoculum preparation and data collecting of the field sample. CP 20% towards the concept of the manuscript and design the experiments, revision of the manuscript critically and final editorial input. Clayton Forknall (CF) 10% towards the data interpretation and analysis, AK 5% towards the data interpretation and analysis, and MT contributed 5% towards the concept and final editorial input.

Paper 3 (Chapter 5): Fundamental physiological responses of *Triticum aestivum* infected with *Fusarium pseudograminearum* in glasshouse and field trials.

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RRA wrote the first draft and contributed 60% towards the concept of the manuscript, design of the experiments, inoculum preparation and data collecting of the field and glasshouse sample. CP 20% towards the concept of the manuscript and designed the experiments, revision of the manuscript critically and final editorial input. (LP) 10% towards the data interpretation and analysis, AK 5% towards the data interpretation and analysis, and MT contributed 5% towards the concept and final editorial input.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A-photosynthesis net rate

Bar-metric unit of pressure

C_i-internal CO_2 concentration

CLA-carnation leaf agar

cm-centimetre

CR-Crown rot

E- transpiration rate

Fg-Fusarium graminearum

Fp-.Fusarium pseudograminearum

G_s-stomatal conductance

GRDC-Grains Research and Development Corporation

ha-hectare

mm-millimeter

PDA-potato dextrose agar

qPCR-quantitative real time polymerase chain reaction

QTL-quantitative trait loci

CHAPTER 1: INTRODUCTION

Crown rot (CR), caused by *Fusarium pseudograminearum (Fp)*, is a serious soil-borne disease of wheat and barley both internationally and in Australia, where it causes $79 and $18 million, respectively, in lost yield per annum to the Australian grains industry (Murray et al. 2009). The prevalence of CR has been exacerbated in recent years by reduced tillage practices with its control primarily relying on the management procedures such as crop rotation to decreases field inoculum (Katan 2017). The complicated interactions between soil parameters, including moisture, temperature, nutrients, and stubble-borne inoculum, are important in influencing the severity of disease.

Wheat is a vital and fundamental crop that is in high demand for both production and consumption. This demand has increased as a result of population growth. Nevertheless, diseases including CR, which is identified by the browning of the base and the appearance of white heads, can result in substantial reductions in wheat yield (Percy et al. 2012). The occurrence of CR has become more extensive as a result of the growing adoption of minimum tillage methods, and the disease is exacerbated by water scarcity during crucial growth phases. Although breeding for tolerance and resistance to CR disease would be the most efficient method of control, the existing elite breeding material does not possess genetic resistance (Kazan & Gardiner 2018). The most promising sources of resistance identified to date are quantitative in nature and presently in non-agronomic backgrounds, even though commercial wheat cultivars with a low level of CR resistance are available to growers in certain environments (Liu & Ogbonnaya 2015).

It is imperative to understand the fundamental mechanisms of crop development that confer resistance to CR in order to develop novel cultivars that exhibit robust resistance. The objective of the current investigation is to improve our understanding of the variations associated with the development of CR disease in wheat caused by *Fp*. We hypothesise that colonisation of xylem vessels reduces water flow through the plant. Simultaneously to this, we also hypothesise that disruption of water flow and subsequent phloem colonisation will alter the dynamics of sugar transport in the plant, carbon and nitrogen content in stem, leaf and grain. The physiological processes underlying the development of CR caused by *Fp* are not yet elucidated. To examine these mechanisms, a series of experiments were carried out, encompassing three seedling trials, three glasshouse trials, and three field trials. These investigations examined the impact of CR on shoot length, biomass, carbon and nitrogen

content, stem water pressure, and leaf gas exchange, including rate of photosynthesis, stomatal conductance, internal CO_2 concentration and transpiration rate of the plant canopy.

Fusarium CR is a significant disease that has significant effects on wheat farming in many global regions. Nevertheless, the precise pathways that contribute to the development of diseases remains not fully understood. This knowledge is vital for enhancing the resilience of existing wheat breeding lines. Abundant empirical data indicates that water scarcity is a major factor in the development of CR disease in wheat (Cook 1973; Burgess et al. 2001; Smiley 2009) Fusarium CR is also expected to impair the crop's physiological water stress, perhaps leading to increased toxin production, transportation to the grain and other organs, and the manifestation of the disease. If there is a high level of toxin synthesis and efficient transport to the grain, and this occurs at the same time as vigorous division and expansion of the grain endosperm cells, it can result in the abortion of flowers and a greater loss of yield (Wallwork et al., 2000). In more extreme instances, this could lead to the formation of the "dead heads" frequently seen in plants damaged by CR (Yang et al., 2021). The aim of this project is to investigate physiological mechanisms involved in CR disease development in wheat, caused by *Fp*. We hypothesize that *Fp* resistance is largely varied between cultivars and the disease development is influenced by water supply. Further, we hypothesise that genotypes will vary in structural and morphological characteristics. The outcome of this research will contribute to the protection of this important crop, contributing to global food security.

Through the analysis of essential physiological processes, including the interactions between water and nutrients, we can identify the crucial characteristics that are accountable for resistance to CR. The results of these experiment will yield significant economic advantages for the industry and enhance global food security. Gaining knowledge about the basic principles of *Fp* infection can also offer valuable understanding of the physiological mechanisms underlying other diseases of cereals that affect the roots and crowns, such as take-all, eye-spot, and common root rot. This thesis will explore physiological pathways involved in CR progression under *Fp* infection. Specifically, it will examine variations in structural and morphological traits among wheat genotypes with differing susceptibilities to *Fp*, investigate physiological changes associated with gas exchange, and analyse modifications in water transport and carbon and nitrogen allocations. Through a detailed analysis of these physiological interactions, we aim to identify key traits responsible for CR resistance,

potentially offering insights that could enhance the resilience of wheat cultivars and ensure sustainable yield improvements.

CHAPTER 2: LITERATURE REVIEW

2.1. Wheat

Wheat is one of the most significant commercial crops produced and utilized globally, providing 21% of human and animal feed intake (Ortiz et al. 2008). Wheat belongs to the genus *Triticum* as grass from *Poaceae* family. *Triticum* has many species, such as *T. durum Desf* (durum wheat), variant *T. aestivum var compactum* (club wheat), and T.*aestivum L.* (bread wheat). Wheat is the 2nd most consumed crop as food after rice (81 kg, 46%), and delivers 20% of total caloric intake of human population (Erenstein et al. 2022). This genus of wheat is more complicated than other domesticated species, with seven chromosomes and three different ploidy forms depending on species: diploid (2x=2n=14), tetraploid (2x=4n=28) for the *T. turgidum ssp.* and hexaploid (2x=6n=42) for *T. asetivum*. Similar to all crops, wheat goes through various development stages including seed germination, seedling growth, tillering, stem elongation, booting, ear emergence, flowering, milk development, dough development and ripening (Miller 1999).

Globally Australia produces three per cent, almost 25 million tons perannum, which is mostly grown for the grain which is used for human consumption and animal feed (Aegic 2022). In Australia wheat is grown largely within a zone named the "Australian wheat belt." This wheat belt runs from southern Queensland, extends south and west to South Australia and southern portions of West Australia (Stephens 2010). Wheat accounts for approximately 90% of the total value of Australian grain production, making it a vital crop in the country. In 2006, Australia contributed between 8-15% of global wheat production by exporting 70% of its production (Trewin 2006). Australia is set to become the 3rd exporter (28.5 million tons) in 2022/2023 and it's going to have sufficient supplies of great quality of wheat grain (USDA 2022). Wheat takes between three to eleven months to mature, depending on the growth conditions, and is classified into spring and winter wheat types. Spring wheat, which is sown in late autumn and matures in late spring, is the most commonly grown type in Australia.

2.1.2. Wheat production in Australia

Wheat was the first crop produced in Australia, dating back to 1788, and now is grown in all Australian states. Figure 1 indicates that wheat is grown between the Great Dividing Range

and the margin of the dry inland areas. In Australia, wheat is grown largely within a zone named the "Australian wheat belt." This wheat belt runs from southern Queensland, extends south and west to South Australia and southern portions of West Australia. Approximately 90% of the total value of Australian grain production is accounted for by wheat, making it one of the most important grain crops in Australia. In 2006, Australia contributed between 8-15% of global wheat production by exporting 70% of wheat production (Trewin 2006). Currently, Australia is one of the top ten countries that export wheat (Table 1). Recently Australian wheat export raise 1.0 million tons to 19.5 million due to a large area of a land under crop (USDA 2016).

Exports	Quantity tonnes	Value in thousands of USD
Russia	37,267,014	7,918,294
USA	26,131,626	6,318,111
Canada	26,110,509	6,317,889
France	19,792,509	4,528,591
Ukraine	18,055,673	3,594,217
Australia	10,400,418	2,698,498
Germany	9,259,493	2,105,865
Argentina	10,196,931	2,029,494
Kazakhstan	10,196,931	2,029,494
Poland	4,689,130	1,047,399

Table 1 World top 10 wheat export for 2020, (FAOSTAT 2020)

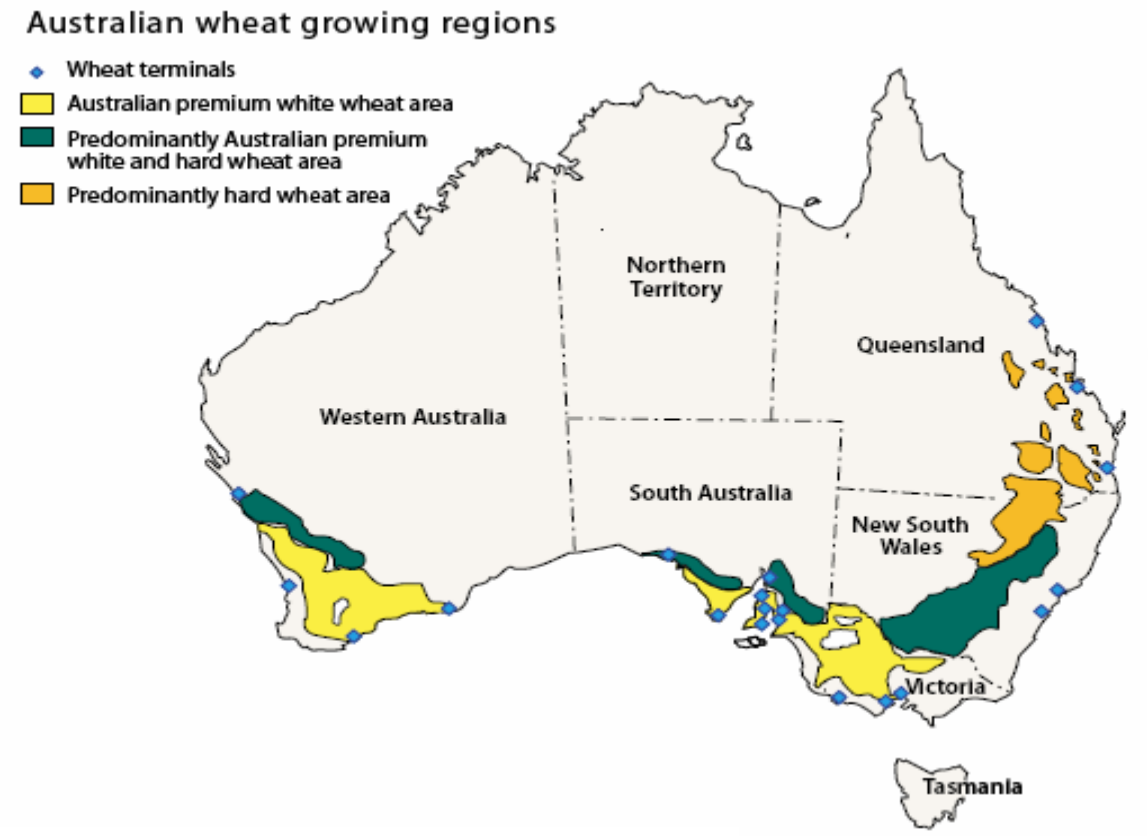

Figure 1. Australia wheat growing regions (ABARE, 2007)

2.2. Major factors limiting wheat production

The necessity of wheat for human and animal intake, along with tentative environmental conditions and disease, have generated significant pressure on wheat production globally. Water availability is considered to be one of the major challenges associated with wheat production. Insufficient water can result in yield losses and bad grain quality. The reduction of water availability is becoming more frequent due to climate change, and it is predicated to be drought affected by 30% by 2099 (Solomon 2007).

In addition, plant diseases also play a crucial role in limiting the yield potential of wheat. Fungi, nematodes, bacteria, and viruses have all been identified as biotic factors that contribute to disease. These pathogens directly or indirectly infect various organs of the plants, for example, leaves, stems, heads, and roots, causing an array of diseases that lead to severe yield losses and compromised grain quality. Among plant diseases, fungal diseases have been identified as

economically important, posing a significant challenge to Australian wheat production (Ophel-Keller et al. 2008). Plant fungal pathogens vary significantly between each other in their biology. Some pathogenic organisms complete their lifecycle within soil and are defined as soil borne plant pathogens. This kind of pathogen is confined to and dispersed in soil. Moreover this pathogen is mostly associated with diseases of root and stem bases of the plant. Hillocks and Waller (1997) claimed that soil borne pathogens cause great damage and huge yield losses. Wheat diseases cost average loss of $913 million annually to Australian wheat industry (Murray & Brennan 2009). Nationally the five most important diseases dominating losses in wheat include stripe rust ($10.62 per hectare), yellow spot ($17.82 per hectare), *Septoranodorum* blotch ($9.07), *Partylenchusneglectus* ($6.13 per hectare) and *Fusarium pseudograminearum* causing crown rot (CR) ($6.63 per hectare) (Murray & Brennan 2009).

Research investigation in plant and environmental interactions, particularly water availability, is fraught with challenges as it is controlled by complex genetics (Budak, Kantar & Yucebilgili Kurtoglu 2013). Plant under water stress can be predisposed to fungal diseases. Previous studies reported that CR can severely develop its symptoms under water stress (Beddis & Burgess 1992; Smiley & Patterson 1996). The interaction between lower water availability and CR resistance changes significantly based on the levels of moisture stress or rainfall at late in the growing season (Dodman & Wildermuth 1987). This means CR is exacerbated by frequent episodes of lower water taking place during the grain filling growth stage and resulting in yield losses and damage to the entire crop.

2.2.1. Soil-borne pathogens in Cropping Systems

Soil-borne pathogens comprised of several classes of microorganisms such as fungi, oomycetes, nematodes and viruses. Despite being highly diverse in their features, these pathogens unite under a group owing to their soilborne pathogenesis. The lifecycle of these pathogens involves soil, at least during one part of their lives. Soil's abiotic and biotic components highly influence the pathogens. Further, agricultural practices such as irrigation, tillage, manure application and fertilization also impact pathogenesis by the microorganisms (Katan 2017).

Soil borne pathogens can be divided into two groups soil inhabitants and soil invaders. Soil inhabitants are the ones who have a longer survival period as opposed to the soil invaders which survive for a shorter period of time. The soil-borne pathogens usually infect the plants through belowground organs but may also reach the upper parts of the plant. When pathogens encounter dead and decaying plant tissues, they grow at the sites as saprobes or saprophytes (Koike et al. 2003).

Symptoms associated with soil-borne pathogenesis include visible lesions, rots and wilt. If unattended such symptoms can cause plant mortality, hence are regarded as the major pathogens. On the other hand, minor pathogens are the ones that are present on root tips or root cortical cells. These minor pathogens mainly cause suppression of plant growth and stunting (Gamliel & Katan 1991.). However, such categorization of minor and major pathogens may not always hold true. In some instances a major pathogen may cause only partial stunting, whereas a minor pathogen may become highly destructive (Mazzola & Strauss 2014).

Most of the soil-borne pathogens cause root or crown rots and vascular wilts. Such infections result in seed decay and damping off of the seedlings. The multicellular fungi are the most frequent causative microorganism, responsible for the soil-borne diseases. Based on the morphological and biological characteristics the fungal pathogens are divided into five groups- *Plasmodiophoromycetes, Zygomycetes, Oomycetes, Ascomycetes* and *Basidiomycetes.* Resilient structures such as the melanized mycelium, chlamydospores, oospores and sclerotia allow longer survival of the soil borne pathogens. Formation of sclerotia in soil has been reported for several soil-borne plant pathogens of the ascomycetes and basidiomycetes group. Fungal sclerotia provide a rich source of nutrients for the fungi. Although the majority of the sclerotial parasites are necrotrophs, an initial biotrophic phase has been observed in some species (Koike et al. 2003).

Establishment of pathogenesis by the pathogen is highly determined by fungistasis and the production of root exudates. Fungistasis is a property of natural soils whereby germination of propagules is inhibited. The phenomenon is widespread in the soils with normal biological activity. It acts to prevent the germination of many fungi. Apart from fungi, the phenomenon of fungistasis is also prevalent for other soil microorganisms such as soil bacteria (soil microbiostasis). Revealing the life cycle of soilborne pathogens and their hosts' responses can provide potential tools for their management (Katan 2017).

2.3. Crown Rot

Crown Rot (CR) is predominantly caused by *Fusarium pseudograminearum (Fp)* in Australia. Common names used in the past and internationally for CR include dryland root rot, dryland foot rot, foot rot, Fusarium crown rot, and Fusarium root rot. CR was first observed and recorded in Australia in 1951 on the Darling Downs in Queensland (McKnight & Hart 1966). Since this time CR has been recorded in all wheat growing regions in Australia, including Victoria (Chambers 1972), New South Wales (Burgess et al. 1981), South Australia (Grewal, Graham & Rengel 1996) and Western Australia (Burgess et al. 2001). CR also has been reported in other parts of the world, including South Africa (Van Wyk et al. 1987), Pacific Northwest of North America (Smiley & Patterson 1996), Egypt and Syria (Burgess et al. 2001) and western Canada (Mishra et al. 2010). The widespread occurrence of this disease can lead to major yield losses in wheat worldwide (Wallwork 2000).

2.3.2. Fusarium

The genus *Fusarium* in association with plant disease is considered to be harmful to animals and humans. Crops can be infected with different species of *Fusarium* which can cause serious disease and yield losses such as CR and head blight on wheat (Desjardins 2006). These pathogens are not just capable of decreasing grain yield but can also produce a range of secondary metabolites known as mycotoxins, for instance, *fumonisins, zearalenones* and *trichothecene* (Desjardins 2006). These mycotoxins have been studied extensively due to the threat to plant, animals and humans, particularly in association with the internationally important disease Fusarium Head Blight (FHB). Nevertheless, the mycotoxin synthesis and transit within the plant, also the impact of water stress which is prevalent in Mediterranean climates, on these mycotoxins during CR infection, remains unexplored. . These understandings are essential to improve the crop resistance to CR.

2.3.3. Fusarium characteristics

Many studies have shown that there is a clear phenotypic and genotypic differentiation in *Fusarium*. Burgess, L., Wearing, A. and Toussoun, T. (1975) reported the existence of two groups which differed in morphology within the species *Fusariumgraminearum*. Group 1 was described as homothallic and unable to produce perithecia on potato dextrose agar (PDA) or

carnation leaf agar (CLA) media. However, it can produce macroconidia on (CLA) with pale orange sporodochia (figure 2). The mycelium produces a distinctive pink/red colour on PDA.

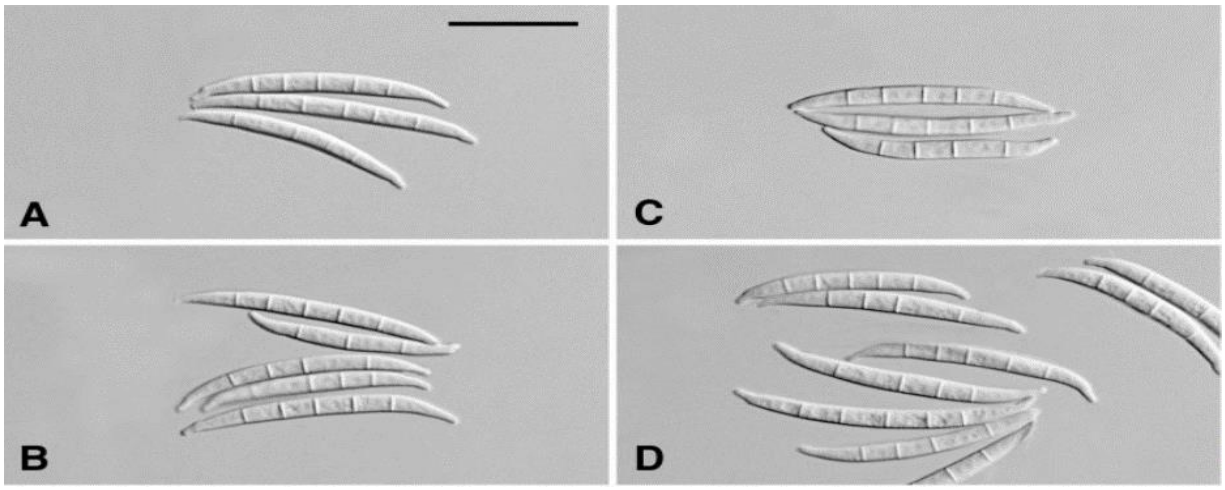

Figure 2. Macroconidia of *Fusarium pseudograminearum*; scale bar = 25 µm. (Leslie, Summerell & Bullock 2006).

Francis and Burgess (1977) also provided evidence of two groups of isolates which could be distinguished by their ability to form perithecia on PDA and CLA media and they were termed *Fg* Group 1 and Group 2. *Fg* Group 2 is the pathogen responsible for FHB in wheat and stalk rot in maize. Cultural and genetic characteristics have been provided later by Aoki and O'Donnell (1999) when they reclassified isolates of group 1 as *Fp* based upon phylogeny analysis of DNA sequences of ß-tubulin introns and exons from Group 1 and Group 2. Moreover, the morphology of macroconidia can be used to distinguish the two groups. Whereby, the conidium of *Fg* can be distinguished by the wide formation of the upper region, while *Fp* conidium (Figure 2) are widest at the mid-region of their length (Francis & Burgess 1977; Aoki & O'Donnell 1999). Many studies later have confirmed this result (Benyon, Burgess & Sharp 2000; Mui-Keng & Niessen 2003; Monds et al. 2005; Scott & Chakraborty 2006).

2.3.4. Life cycle of Fusarium pseudograminearum

Fp is a widely distributed soil borne pathogen which can be found in infested plant debris where it's able to survive for several years (Wallwork 2000; Leslie, Summerell & Bullock

2006). It is unknown how far the *Fp* can grow through the soil, however, it is postulated that direct physical contact between the debris and the host is required to start the infection (Burgess et al. 2001; Backhouse 2006). The lifecycle of *Fp* includes two stages. The first stage is the sexual stage, which doesn't play an important role in initiating the infection and is rarely found in the field (Chakraborty et al. 2006). The second stage is the asexual stage, which is important in causing damage to wheat (Stephens et al. 2008). Therefore, detail understanding of asexual growth of CR in the wheat shoot is essential to develop a comprehensive understanding of the mechanisms of disease development.

Fp can survive in the soil as chlamydospores. The hyphae germinate and contact the first emerging seedlings. Purss (1966) reported that when wheat is planted in soil containing infected debris, the sub-crown internode or leaf sheath bases will come into contact with the fungus when the wheat grows. The coleoptile is the first tissue to come into contact with *Fp* inoculum as it grows towards the soil surface. However, infection is not limited to this stage, but occurs from the seedling stage through to maturity (Purss 1966; Burgess et al. 1981). The fungus colonises the lower stem tissue as mycelium and remains in these host tissues at the end of the growing season, where the cycle begins again. Both *Fp* and *Fg* are also able to cause head blight, however, this is very rare with *Fp* (Burgess et al. 1987).

2.3.4. Crown Rot disease symptoms

Fp infection can cause pre- or post-emergent seedling blight. CR symptoms can be seen as a browning or discoloration of the coleoptile, sub-crown internode and leaf sheaths (Burgess et al. 2001; Percy, Wildermuth & Sutherland 2012). Diseased roots exhibit external discolouration of tissue and brown lesions can be observed on seminal and secondary roots. Purss (1966) and Chakraborty et al. (2006) reported that in severe CR infection, the root system can entirely collapse. Burgess et al. (2001) reported that infection of stem bases from roots is considered to be infrequent, however crown roots could be colonised from crown or stem tissue. As the infection progresses a honey brown discoloration (Figure 3a) can be observed on the internodes of each tiller (Purss 1966). This browning on the stem can be seen as high as the sixth internode in severe infections (Malligan 2009).

Figure 3. Typical symptoms of crown rot- a) Stem browning. b) Whitehead under dry finish season. (Source: Percy, C 2016).

The infection of the head via the stem is rare (Burgess et al. 1981). Infection can result in the development of white heads (dead heads), which contain no grain (Figure 3b). Burgess et al. (1981) reported that the formation of white head results from a disruption to the translocation system, causing premature ripening of the plants. Further studies have reported that increases in protein content are associated with increasing CR, resulting in reduced grain quality (Smiley et al. 2005). In addition, glass house assays postulated that all tissue including the spikes contain mycotoxins (Mudge et al. 2006) which are harmful to humans and animals (Mudge et al. 2006).

2.3.5. Economic impact of CR

In Australia CR of wheat and barley is an economically threatening disease, particularly in southern Queensland and NSW. Wallwork (2000) reported that under favourable conditions this disease can reduce the crop yield potential up to 50%. In the growing season during 1995-1996, the losses of wheat yield were estimated at $21.3 million in the northern wheat belt in Australia (ABARE 1996). According to Ogbannaya (2014) the average yield losses of Australian wheat industry are reported at ~ $100 million annually due to the CR disease.

2.3.6. Management of crown rot

2.3.6.1. Chemical control

Complete chemical control against major soil pathogens of wheat including *Fp* have not been found yet (Paulitz, Okubara & Schroeder 2010). Early investigation in Australia of CR chemical control by using fungicide seed treatment such as Agrosan, Ceresan, copper carbonate and hexachlorobenzene (McKnight & Hart 1966), have claimed that the fungicide seed treatment can provide protection against CR at lower concentration. Other early work in vitro by Klein and Burgess (1987) have utilized seed dressing techniques including the active ingredient carboxin, fenarimol and triadimefon which were effective against the hyphal growth but were not effective against seed borne disease. Other seed treatment investigated by Moya-Elizondo and Jacobsen (2016) when they applied seed with Difenoconazole-mefenoxam have caused a decline the rate of CR infection up to 50% under controlled environment conditions. Akgül and Erkilic (2016) investigated different kind of fungicides as wheat seed treated with tebuconazole where it was effective for the control of *F. culomorum* under growth room conditions. In Australia currently the only product used for CR suppression is Rancona® Dimension. This product has worked against seedling blight when it is used as a standalone management strategy and shows that it has limited effectiveness in reducing yield loss due to CR diseases (Alahmad 2018). A project funded by the GRDC in Australia has applied fungicides with irrigation during seedling growth stage to reduce the CR disease severity and resulted in small improvements on yield (GRDC 2016). Moreover, a study by Zhang et al. (2022) have tested different fungicides to determine their effectiveness in managing wheat Fusarium crown rot (FCR), which is mainly caused by *Fp*. The researchers evaluated 12 fungicides using both laboratory and field experiments. During the seedling stage, Cruiser Plus and Celest proved to be the most effective treatments, with relative control efficacies of 58.40% and 57.60%, respectively. The application of fungicides resulted in a noticeable decrease in disease severity and a notable improvement in plant growth when compared to untreated controls. Most of the fungicides that were tested demonstrated some level of effectiveness against CR, although their performance varied depending on the assessment methods used (Zhang et. al, 2022). A recent study conducted by Yang et al. (2022) found that the application of hexaconazole and lentinan (LNT) as a seed dressing can successfully manage soilborne diseases in wheat, specifically wheat sharp eyespot. This combination treatment demonstrated exceptional long-term control and remarkable disease suppression in field tests, surpassing the effectiveness of using either agent individually. Significantly, the treatment showed

effectiveness even at lower doses, indicating a powerful collaboration between hexaconazole and LNT. This collaboration has the potential to decrease the reliance on chemical fungicides, leading to a reduced environmental impact and lower chances of resistance development (Yang et, al., 2022). Although chemical control of FCR is currently the most effective method, the repeated use of fungicides can lead to a decrease in sensitivity of Fusarium isolates to these chemicals. This, in turn, increases the likelihood of severe plant disease (Yang et, al., 2022). Despite the progress made in CR management strategies, there is still a considerable need to thoroughly investigate the physiological mechanisms responsible for CR in wheat. It is crucial to gather precise information on the complex physiological processes that drive the development of CR.

2.3.6.2. Biological control

Novel approaches have been utilized to manage CR infections. The absence of completely resistance cultivars raised the needs for biological control against CR, which have been applied on many occasions. Biological control of CR investigations has applied *Fusarium* species such as *Fusarium nygami* and *Fusarium equiseti* along with other biological control agent such as *Alternaria infectoria* and *Trichoderma harzianum* (Wong, Mead & Croff 2002; Luongo et al. 2005; Moya-Elizondo & Jacobsen 2016). These investigations indicated that higher levels of antagonism with *Fp* by the applied agent, but the effectiveness was constrained by humidity and temperature, were low temperature and humidity resulted in low in antagonism (Singh, Backhouse & Kristiansen 2009). In another study, Wong, Mead and Croff (2002) observed that a reduction of *Fp* survival in the straw along with high temperature and high moisture occurs after applying *Trichoderma viride* as biological agent. A further study conducted by Luongo et al. (2005) screened 135 saprophytic fungi to investigate their ability to lower the sporulation rate of *Fusarium* species on maize and wheat residues. It was found that several antagonists, including *Clonostachys rosea* and non-pathogenic *Fusarium* species were able to reduce the sporulation and improve displacement on crop residues. Another recent study indicated that *Bacillus halotolerans* QTH8 has been stated to effectively inhibit the growth of *Fp* and other plant pathogens, leading to a reduction in the severity of wheat CR and improved plant growth metrics. This discovery highlights the potential of *Bacillus halotolerans* QTH8 as a highly effective biocontrol agent (Li et al., 2022). Moreover, the antimicrobial compounds synthesised by QTH8 demonstrated strong resilience in different environmental circumstances,

highlighting the ecological and health benefits of employing biological rather than chemical fungicides (Li et al., 2022).

A glasshouse assay of Moya-Elizondo and Jacobsen (2016) when applying integrated novel approaches by mixing fungicides with biological agents (*Bacillus mycoides*), revealed increased protection against CR by induced systemic acquired resistance. Despite their effectiveness in reducing *Fp* infestation in glasshouse experiments, however, when applied in field, non-significant differences were reported between the treated seed and non-treated seed (Moya-Elizondo & Jacobsen 2016). The research conducted by Feng et al. (2023) demonstrated the efficacy of the *Chaetomium globosum* strain 12XP1-2-3 in mitigating Fusarium crown rot in wheat. This resulted in significant decrease of the disease and enhanced crop yields, while also positively impacting the microbial community in the rhizosphere. Nevertheless, there is a lack of comprehensive exploration of the physiological mechanisms that cause CR diseases in wheat, which reveals a significant gap in our current understanding. Although there have been advancements in biological management strategies, there is a lack of comprehensive data regarding the intricate physiological mechanisms that determine the progression of CR disease in wheat. It is essential to address this lack of knowledge in order to improve disease management strategies and strengthen the long-term viability of wheat production.

2.3.6.3. Cultural control

Crop rotation has been extensively studied as means of effectively controlling CR in agricultural conventional practice. This practice strategy, particularly when non-cereal crops are included in the rotation, has been demonstrated to significantly reduce the incidence and severity of CR (Burgess et al. 2001; Summerell et al. 2001; Lamprecht et al. 2006). For instance, incorporating a non-host crop such as sorghum into the rotation has been shown to alleviate crop losses due to CR infection (Burgess 2005; Simpfendorfer 2005).

Necrotrophic pathogens include *Fp,* as saprophytes can survive in infected stubble. Yet, there is no complete resistance available and cultural control measures rely on crop rotation and stubble management. In molecular biology research conducted in Eastern Australia, quantitative real-time polymerase chain reaction (qPCR) was used to measure the levels of *Fp* and *F. culmorum* DNA in collected soil samples. The final data revealed that the DNA of CR was significantly higher in samples collected after cereal crops, compared to non-cereal crop

samples. These findings suggest that cereal crops may be more susceptible to CR due to the higher levels of pathogen DNA in the soil (Evans et al. 2010).

Chickpea (*Cicer arietinum*) has been shown to significantly reduce the incidence of CR on wheat used as a break crop in the Northern Wheat Belt of New South Wales (Felton et al. 1998). Oilseeds, such as canola, have been successful of CR reduction on winter cereals in high rainfall cropping regions of Australia (Norton et al. 1999). In another investigation, Canola and mustard have been used as break crops in the northern region, and the results indicated a significant decrease in CR incidence (Oram et al. 1999). Norton et al. (1999) have exposed that the use of *Brassica spp*, as break crop in the Southern Wheat belt of Australia has significantly contributed to reduced CR and increased the next seasons of wheat yield. In Northern New South Wales, a study by Kirkegaard et al. (2004) investigated the impact of planted prior crops, particularly, oilseed, legumes, and cereals on the incidence of CR. The result finds that the most effective at increasing yield and decreasing the Fp inoculum, potentially due to their rapid residue decomposition, however, little evidence of biofumigation from *Barssica* tissue was observed.

Stubble management, including both burning and removal, can reduce the amount of fungal inoculum available for the next growing season (Summerell et al. 2001). However, stubble can also create favorable conditions for fungal infection of seedlings due to its impact on soil surface moisture (Liddell & Burgess 1988; Swan, Backhouse & Burgess 2000). Research has shown that there is a significant relationship between soil water levels and the ability of the fungus *Fp* to infect seedlings, with a range of 0.3-0.7 MPa (Liddell & Burgess 1988). Therefore, both stubble burning and integrating stubble into the soil can significantly reduce inoculum levels and disease incidence (Summerell & Burgess 1988; Burgess et al. 1996). Martin, McMillan and Cook (1988) study the management practice of the Northern Wheat Belt of New South Wales, indicated that stubble burning is typically conducted soon after harvest, which occurs in December or January. Moreover, stubble burning over a two year period can reduce diseases incidence by approximately 47%, with the greatest reduction occurring in the following year (Summerell & Burgess 1988). However, the removal of stubble technique can also have negative impacts, such as increasing soil erosion during summer and reducing the amount of stored water available to plants during early stages of growth, particularly in dry climate areas (Freebairn, Loch & Cogle 1993; Burgess et al. 2001). Stubble burning is not currently a common agricultural practice due to the concerns about its potential impacts. More

importantly, both cultural control, crop rotation and stubble management can reduce the disease incidence, but they do not eliminate the disease.

A recent field study by Petronaitis et al. (2022) have examined the effects of different harvest heights on the management of FCR in wheat. This research discovered that taller standing stubble facilitated the vertical spread of the *Fp* fungus within the stubble after harvest, while shorter stubble restricted its growth to the height of the cut stubble. It was noted that stripper fronts, which result in taller stubble, could potentially lead to an increase in disease-causing organisms present in the stubble, particularly in moist fallow conditions. The research conducted field experiments over three seasons and found that shorter stubble restricts saprotrophic colonization of FCR, but it does not have any negative impact on soil moisture levels. It is important to consider adjusting the harvest height in order to effectively manage FCR while still reaping the advantages of stubble retention practices (Petronaitis et al. 2022). Regardless of progress in traditional and cultural management strategies, there is still a strong need to better understand the physiological mechanisms that contribute to the progression of CR. It is of vital significance to address this issue in order to enhance disease management and secure the long-term sustainability of wheat production.

2.3.6.4. Genetic Resistance and tolerance

The development of cereal crop varieties with improved genetic resistance to CR disease is crucial, as agricultural practice aimed at reducing the incidence of these diseases may not always be economically feasible. To date there is no complete resistance to CR diseases commercially available, where resistance refers only to partial resistance as being measured by disease symptoms and/ or fungal biomass (Kazan & Gardiner 2018). It has been proposed that genetic resistance to CR can manifest in two forms. The first is host resistance to infection and diseases progression, and the secondis the suppression of inoculum accumulation in stubble or/and soil profile (Wildermuth et al. 1997; Fetch Jr, Steffenson & Nevo 2003; Chen et al. 2015). Moreover, resistance in wheat refers to the ability of the plants to limit CR disease by reducing disease symptoms or the level of fungal biomass (Liu et al. 2012; Davies 2016). Tolerance in wheat is defined as the ability of the plant to produce sufficient yield even when infected with CR, for example, although barley may express similar disease symptoms to wheat, it can be considered more tolerant due to its ability to maintain yield in the presence of the pathogen (Wildermuth & Purss 1971; Klein, Burgess & Ellison 1989). Resistance to CR

has been widely studied to understand plant responses to CR infection, while tolerance has often been overlooked (Davies 2016). However, other studies suggest that growing tolerant genotypes may be more beneficial for growers than partially resistant varieties that also exhibit tolerance (Kazan & Gardiner 2018).

Stem base browning has been used as benchmark for evaluating resistance to CR. A field investigation utilising qPCR found a positive correlation between fungal biomass and stem base browning in wheat and barley after two years of observation these association, however, were significantly more pronounced in certain internodes of plant diseases (Knight & Sutherland 2015). Other researchers have not found a direct correlation between stem base browning and host resistance, despite the observed correlation between browning symptoms and the level of fungal biomass in infected plants (Knight et al. 2012).

Two forms of host resistance to CR have been identified, seedling resistance (SR) and adult plant resistance (APR). These forms have been found to be associated with shallow and deep crown formation in the soil profile (Wildermuth, McNamara & Quick 2001). Liu and Ogbonnaya (2015) conducted a comprehensive review of studies on CR resistance and tolerant cultivars, with focus on identifying resistance-associated quantitative trait loci (QTL) in wheat and barley germplasm. While several resistant sources were identified in these studies, to date, few moderately resistant to resistant varieties have been registered (GRDC 2024). Since this time, many studies on wheat resistance to CR disease have been conducted in glasshouse and field trials to select the partially resistant lines (Wallwork et al. 2004). From these studies, QTL that control CR resistance have also been identified (Bovill et al. 2010). QTL are fragments of DNA associated with a quantitative trait such as resistance. QTL have been identified for CR resistance in various wheat populations (Collard et al. 2005; Tamburic-Ilincic et al. 2009; Bovill et al. 2010). Genetic studies have reported that there are three sources of partial resistance to CR; the commercial varieties Kukri, 2-49 and W21MMT20. Resistance of adult Kukri plants was tested in populations grown in open ended tubes placed in outdoor terraces. The QTL mapped in Kukri was located on chromosome 4B near the semi-dwarf gene *Rht1 (Wallwork et al. 2004)*. Based on seedling assay, Collard et al. (2005) found two QTL conferring CR resistance in the breeding line 2-49. One found on chromosome 1DL explained up to 21% of phenotypic variance. The second QTL, located on chromosome 1AL, explained up to 10% of the variance (Collard et al. 2005). Based on seedling assays of W21MMT20, Bovill et al. (2006) identified a QTL conferring CR resistance located on 5D, which explained

up to 28% of the phenotypic variance. Tamburic-Ilincic et al. (2009) identified the QTL on 5B conferring CR resistance in the Wuhan/Nybai. This would explain 13.4% of phenotypic variance. Another QTL located on 2D was only significant in one of the three trial assays, which explained 10.2% of the variance. A further investigation done by Bovill et al. (2010) with crosses of W21MMT20 and 2-49 identified QTL conferring resistance located on chromosome 3BL, which explained 49% of phenotypic variance and was designated as *Qcrs.cpi-3B.* QTL in this region has been confirmed in three different genotypes: Ernie, Macon and Otis (Li et al. 2010; Poole et al. 2012). These findings suggest that resistance to CR is very complex with a large number of minor effects QTLS associated with CR resistance. Therefore, understanding of broad physiological mechanism of wheat resistance to CR is essential to improve the resistance in wheat. Moreover, recent studies have identified partial levels of resistance in bread wheat lines and these have been incorporated into breeding programs to create new wheat cultivars with improved levels of resistance and tolerance to *Fp* infection (Forknall, Simpfendorfer & Kelly 2019; Kelly et al. 2021). In more recent years, Rahman et al. (2021) reported several marker-trait associations (MTAs) with resistance to Fusarium crown rot in wheat. They particularly pointed out that the A genome is the main host for substantial MTAs, with a total of 515 identified across different treatments and years. In addition, QTLs that are associated with important characteristics linked to crop yield, such as the weight of a thousand kernels (TKW) and overall grain were identified. These associations were observed in both scenarios wherein the plants encountered to a pathogen and when they were not (Rahman et al., 2021). By linking this extensive genetic analysis with the as-yet not investigated physiological mechanisms that drive the spread of CR in wheat, we could gain valuable knowledge that can be used to enhance disease resistance and improve the sustainability of wheat production. In summary, it is appearing that there are currently no completely resistant varieties to CR available, but there are partially resistant, quantitively inherited traits presented in most segregated populations that could be utilized for marker-assisted selection for CR resistance. Although there have been important breakthroughs in genetic research understanding the physiological processes of CR resistance and tolerance will further improve the ability of breeding companies to develop varieties with enhanced resistance and tolerance to CR.

2.3.7. Crown rot stages of infection and fungal gene expression

Few studies have been conducted which investigate host response and gene expression during CR colonisation in wheat. Stephens et al. (2008) investigated CR infection with real time quantitative chain reaction PCR (RT-qPCR) and histological examinations. This study reported that there are three stages of CR infection. The first stage is the first spore germination with superficial hyphal structure on mat at inoculation point, however this stage is associated with the increase of fungal biomass.

The second stage includes two formations. One is the formation of the adaxial epidermis of the outer leaf sheath, and the other formation is mycelia growth from the inoculation point at the crown, which is accompanied by a drop in fungal biomass. The last stage is the extensive fungal colonisation of the internal crown tissue which is associated with increasing rate of fungal biomass. Fungal gene expression was investigated throughout each of these stages. Similar patterns of expressions have been found for some genes among these different stages, but they were different for others. Moreover, significant similarities were also found between the infection processes of early stages of FHB and CR (Stephens et al. 2008).

From a selection of 26 wheat defence genes Desmond et al. (2006) observed eight genes reproducibly expressed in inoculated seedlings of two wheat cultivars: *B-glucanase, wheatwin, thaumatin-like protein, peroxide, PR1.1, PR10* and *TaGLP2a*. These genes were induced in the CR partially resistant cultivar Sunco and the susceptible cultivar Kennedy, however, some of these genes were induced faster in the partially resistant cultivar than the susceptible cultivar. The above mentioned defence genes were induced in both cultivars when pre-treated with methyl jasmonate (MJ) before inoculation with *Fp.* MJ as a volatile organic compound used in plant defence pathways has been proven in different studies to induce gene expression in wheat which resulted in delay in disease symptom development (Desmond et al. 2006; Motallebi et al. 2015). In addition, most of the genes stimulated by *Fp* were also induced by the application of the toxin deoxynivalenol (DON) of *Fp* during CR infection. These findings suggest that plant defense against *Fp* works in an auto catalytic manner.

2.4. Crop physiological change by fungal infection

The infection of crops with a pathogen involves changes of metabolic pathways such as photosynthesis, growth development and its nutrients (Jha & Mohamed 2022). The

biochemical, molecular and physiological research tools provide close observations to the details to cellular level, which was not possible three decades ago. Plants are attacked by a variety of pathogens, such as bacteria, fungi, oomycetes, and nematodes. Usually, the pathogens are of three types based on their mode of action or lifestyles: necrotrophs, biotrophs and hemibiotrophic. In case of the necrotrophs, the pathogens kill the plant tissues and thereby fulfill their requirement for nutrients from dead tissues. Biotrophs on the other hand procure nutrients from the living cells. However, an intermediate of the two named hemi-biotrophs have been identified (Laluka & Mengiste 2010). Hemibiotrophic pathogens derive nutrition from the host cell by initially adopting the biotrophic phase in the early stages of infection and subsequently transitioning to the necrotrophic mode in the later stages of their life cycle (Glazebrook 2005). Frequent necrotrophic fungi, such as *Fusarium, Ramularia, Botrytis, Cercospora, Helminthosporium, Rhynchosporium, Sclerotinia, Verticillium*. or *Alternaria,* species, produce disguised appressoria and homogenous infection hyphae within the host (Condon et al. 2013). In certain fungal infections, the ability to produce a phytotoxin is directly related to their pathogenicity. Fungal phytotoxins can be categorised into two groups: host-selective toxins (HSTs) and non-host-selective toxins (NHSTs). Necrotrophs and hemibiotrophs utilise distinct mechanisms to promote disease. Nevertheless, they both deploy comparable or even identical tools such as host-selective toxins (HSTs) and protein effectors (Ding et al. 2011). The hemibiotrophic *Fg*, which is responsible for causing Fusarium head blight in wheat, results in significant financial losses. The defence against Fusarium head blight is controlled in a specific order by salicylic acid (SA) and jasmonic acid (JA) during the initial and subsequent phases of the infection, respectively (Ameye et al. 2015). During the initial stage, a defence mechanism involving JA and ET is activated to combat fungal growth and sporulation. This mechanism triggers the transcription of a specific group of genes that encode antimicrobial peptides, PR proteins such as lipid transfer proteins, thionins, and defensins in the resistant lines. Additionally, proteases and mycotoxins were stimulated by an alternate route (Gottwald et al. 2012). In contrast, a transcriptome investigation using whole RNASeq in maize cultivars revealed that the activation of SA-related genes in both resistant and susceptible genotypes was insignificant three days after being infected with *F. verticillioides*. Furthermore, the activation of often responsive JA- and ET-responsive PR genes and transcription factors, including as LOXs, PR10, and ACC oxidase (which control ET levels via regulating 1-aminocyclopropane-1-carboxylic acid oxidase) and chitinases, was discovered to have a stronger induction in a resistant line (Lanubile et al. 2014). While the benefits can be made

through the progress in modern science, there is still much to discover regarding the physiological mechanisms behind the development of CRin wheat. In order to investigate this issue, the study conducted an intensive physiological analysis on a diverse set of wheat varieties. These varieties exhibited different levels of resistance to CR and were tested in controlled environments as well as in the field, at various stages of growth. These findings are crucial as they introduce the complex interactions and responses of various genotypes to CR, thus laying the foundation for the development of strong and sustainable wheat cultivars. In addition, understanding these physiological responses is vital not just for wheat, but also for bolstering resistance in other crops, ultimately aiding global food security and sustainable agriculture.

2.4.1. Gas exchange data in crops health

Photosynthesis is a ubiquitous process that takes place in a variety of green organs, such as leaves (Smith et al. 1997), young stems (Nilsen 1995), green fruits (Cipollini & Levey 1991), and ears before they mature (Kriedemann 1966), and it serves as a source of both the material basis and the energy supply for a number of physiological and metabolic processes. The plant pathogen substantially impacts the physiological processes of the plant. Investigations into the fundamentals of plant physiology have been carried out on crops that have been infected with fungal pathogens. These pathogens have been shown to have an effect on gas exchange processes, such as photosynthesis, stomatal conductance, transpiration rate, and intercellular CO_2 concentrations.

Pathogens can cause photosynthetic changes. For instance, Lorenzini et al. (1997) claimed that *Fusarium oxysporum f. sp. lycopersici* or *Verticillium albo-atrum* are able to influence the photosynthesis rate of susceptible tomato leaves. *Colletotrichum musae* and *Fusarium moniliforme* infections inhibit maize leaf photosynthesis and reduce chlorophyll content (Pinto et al. 2000).

Wheat has a longer growth period than other food crop species such as maize and rice, making it more susceptible to pathogens and diseases during its growth and development (Duveiller, Singh & Nicol 2007). Amongst these diseases, FHB caused primarily by hemibiotrophic *Fg* species, stripe rust caused by the biotrophic parasite *Puccinia striiformis f. sp. tritici* (*Pst*), and wheat powdery mildew caused by the obligate biotrophic fungus *Blumeria graminis f. sp. tritici*

(*Bgt*) are mostly destructive due to their ability to decrease yield and quality, resulting in significant economic losses in the production of wheat. Powdery mildew can appear across the wheat growth cycle, with the optimal temperature being 15-20 C (Yang 2016). *Blumeria graminis* pathogens primarily infect the leaves, but they can also infect green awns, glumes, and stalks (Gao 2018). These organs vary significantly in their position in the plant, but because of their strong photosynthetic capabilities, they are usually considered photosynthetic source organs at the developmental stage (Wang, Wei & Zheng 2001).

A comparison study found a decrease in *A* and has been reported in wheat during the early stages of infection by the blast pathogen *Pyricularia oryaze* before visible symptoms appear (Debona et al. 2014). *Pyricularia oryaze* differs from CR in that the main symptoms appear more on the spike and spikelet than the stem and leaves (Igarashi, 1986). Yang et al. (2016) discovered an alteration in the mitigation of *A* in FHB resistant genotypes after *Fg* inoculation, but no change in the *A* parameter in FHB susceptible genotypes. Another photosynthetic study by Hu et al. (2020) of wheat infected by biotrophic fungal *Bgt* which cause powdery mildew of leaves of two wheat lines (L658 susceptible) and (L958 resistance), demonstrated the differential expression of photosynthesis related genes most likely led to a lowering in *A* which is related to inhibition of peroxidase (POD) and catalase (CAT) to generate two stages of hydrogen proxied H_2O_2 due to infection of *Bgt*.

The gas exchange scientific research can offer crucial data about the mechanism of plant resistance to pathogen infections (Francesconi & Balestra 2020; Bharath, Gahir & Raghavendra 2021). Research demonstrates that pathogen infection mechanisms vary from host to host, which may reprogram plant metabolism to be more beneficial to infection (Rojas et al. 2014). Current studies by Aucique-Pérez et al. (2020) and Silva et al. (2022) suggest that lower concentrations of photosynthetic pigments cause physiological changes prompted by hemi-biotrophic and necrotrophic fungal pathogens due to hydrolytic enzyme action and non-selective toxin attack on chloroplasts and protein.

Other gas exchange parameters, including stomatal conductance and high intercellular and transpiration rate, are related to one another, also reported in crops investigations. A study by Tatagiba et al. (2016), claimed that the gas exchange and antioxidant metabolism of *Monographella albesscens*-infected rice leaves revealed a reduction in photosynthesis with low stomatal conductance and high intercellular CO_2. Other pathological systems investigations typically have interpreted this as an indication of biochemical, rather than diffusive,

photosynthesis limitations (Dallagnol et al. 2011; Resende et al. 2012). Lower transpiration rate due to infection have been observed by Debona et al. (2014) when he demonstrated a decrease transpiration rate in wheat infected with *Pyricularia oryaze* compared to the controls wherein the observed lower transpiration was ascribed to the control by stomatal conductance. A detailed investigation of gas exchange parameters in the current study provides valuable insights into the impact of CR on wheat physiology, laying the groundwork for improving disease management strategies. This study addresses a significant gap in physiological research by providing insights into the complex mechanisms involved in plant-fungal interactions. It offers a more comprehensive understanding of how CR impacts gas exchange processes and plant health, contributing to the existing knowledge in this field.

2.4.2. Disturbance of water transport system with infected pathogen

Infection can inhibit plant growth and development by disturbing the uptake of nutrient and water, or by producing toxins that interfere with normal plant function, where this disruption results in destroyed or stunted plant growth which can lead to death (Dong et al. 2012). Pathogen attack on plant water relations can have serious consequences for plant health and productivity. Plants with altered water relations may display symptoms such as wilting, yellowing leaves, and reduced growth. In extreme cases, altered water relations can kill plants.

It is critical to use proper cultural practices and control measures to mitigate the effects of pathogen attack on plant water relations. Maintaining proper soil moisture levels, using appropriate irrigation techniques, and applying appropriate pesticides to control and prevent pathogen infections are all examples of this (Ayres 1978). When pathogens spread within the xylem of their hosts, and as they expand there, the polysaccharides and pectolytic enzymes they secrete as well as the pathogen's growing biomass block the vessels. Gums, mucilages, and tyloses are produced in response by the host, which can drastically reduce water flow through such vessels by 95% (Walters 2015). In turn, this may lead to water deficits in the leaves, which may close the stomata and lower the rate of transpiration. For instance, infection with *Verticillium dahliae*, in field experiments on potato decreased stomatal conductance and transpiration, resulted in an increase in leaf temperature (Resende et al. 1996). These findings indicated that isolates of *V. dahliae* cause severe defoliation in cocoa, while others cause wilting and desiccation, but no defoliation.

Wheat stem pathogen fungal infections can have a significant impact on wheat plant water relations, resulting in negative effects on plant growth and development. The disruption of the plant's vascular system is one of the primary mechanisms by which fungal infections impact plant water relations. Fungal colonization of the stem and the production of toxins harm the plant's xylem and phloem, preventing water and nutrients from flowing from the roots to the rest of the plant. This can result in decreased water uptake, increased water loss, impaired nutrient transport, and decreased plant growth and development (Singh et al. 2011). Histopathological study of the colonization investigation by Knight and Sutherland (2013) found that mycelium of *Fp* was able to colonise xylem and phloem of infected wheat seedlings. The development and growth rate of mycelium was slower in susceptible genotypes. In this study, water transport of wheat infected by *Fp* have been tested in glasshouse experiments. In addition, some vascular wilt pathogens produce specific toxins that result in altered water relations of infected hosts. Observations have been noted for banana plants infected with *Fusarium oxysporum* f.sp. *cubense*. Results revealed reduction in stomatal conductance and transpiration rate. The loss of water was higher in infected plants compared to the controls. Fusaric acid, produced by *F. oxysporum* f.sp. *cubense*, was identified as the causative agent that resulted in the water loss (Dong et al. 2012). The observed results were confirmed when similar changes in water relations were noted on treatment of banana plants with fusaric acid produced due to the pathogen infection. Moreover, this is the first investigation to utilise stem water pressure measurement of wheat stems infected with *Fp*. We hypothesis that colonization of xylem vessels reduces water flow through the plant. This study's comprehensive examination of stem water transport parameters improves our comprehension of water dynamics in CR infected wheat, offering essential insights for plant pathology research. The discovery of these results is crucial for the progress of physiological research and the enhancement of disease control methods aimed at mitigating the effects of fungal infections on the water dynamics of plants.

2.5. Carbon and Nitrogen content of crops

The most important essential elements in plants, animals and microorganisms are Carbon (C) and nitrogen (N). Giavalisco et al. (2011) informed that C and N behave as limiting aspects for plant growth and crop yield by directly contributing to the fundamental of plant metabolism. The transfer of C and N between shoots and roots via the xylem and phloem is critical. N is

necessary for C metabolism because it is involved in protein synthesis. Carbon compounds are also required for N absorption, nitrate reduction, N_2 fixation, and amino acid metabolism in order to produce C skeletons, metabolic energy, and reductants. An increase in carbohydrate content has been linked to the suppression of genes involved in photosynthesis and N metabolism, as a significant amount of fixed C is required to provide the C skeletons that act as acceptors for incorporating N into amino acids to form proteins and other nitrogenous compounds (Kurusu et al. 2014). A more recent study by Buster et al. (2023) amid to examine the main cause-and-effect relationship and additional agricultural impacts of N availability over time on the severity of CR and has discovered that lower concentrations of urea (0.03 M and 0.15 M) had a significant positive effect on the in vitro growth of *Fp*. However, higher concentrations of urea (0.3 M) and all concentrations of ammonium nitrate, except the lowest (0.03 M), had a detrimental effect on fungal growth compared to the control group.

C and N not only contribute to plant nutrition, but they can also act as signalling molecules to regulate nutrient absorption, assimilation and photosynthesis, as well as plant growth and crop yield, by regulating gene expression, enzymatic processes and signalling pathways (Goel et al. 2016). Three genes, nitrate transporters (LIN1/NRT2.1), glutamate receptor (GLR1.1), and oversensitive to sugar 1 (OSU1), have been identified as being involved in the regulation of C and N signalling in Arabidopsis plants (Gent & Forde 2017). Zhang et al. (2018) investigated the balance of C and N metabolism in cyanobacteria as a model for photosynthetic organisms. The researchers discovered that 2-phosphoglycolate (2-PG), produced by Rubisco's oxygenase activity, and 2-OG, produced by the tricarboxylic acid (TCA) cycle, serve as C and N starvation signals, respectively. The researchers determined that the signalling role that 2-OG and 2-PG perform in regulating the balance between C and N is likely to be conserved in other photosynthetic organisms. Specifically, Zhang et al. (2018) noted that this role is likely to be conserved in plants. *Escherichia coli* bacteria have also been shown to exhibit the role of 2-OG as a critical regulatory metabolite in maintaining a healthy equilibrium between the C and N metabolisms (Huergo & Dixon 2015).

Pathogens can have a significant impact on the plant’s function including in C and N metabolism. Microorganisms, as classified into biotrophic to necrotrophic, also other pathogens, have neutral or mutualistic interactions such as mycorrhiza and plant growth-promoting rhizobacteria in the root region, or endophytes and epiphytes in the vegetative

region, those can include competition, commensalism, mutualism, and parasitism, all of which affect the metabolism of C and N (Baslam et al. 2020).

Pathogens such as fungi, bacteria and viruses can colonize either sink or source organs and disrupt the source-sink balance due to the heterotrophic colonizing agent's requirement for sugar supply from host plants (Baslam et al. 2020). This interaction constructively can benefit both the host and the invader (Bago, Pfeffer & Shachar-Hill 2000; Adeniji & Babalola 2020). Moreover, these pathogens can hijack host cells plants to suppress host immunity and take advantage of their nutrients, primarily sugars, for survival and reproduction without returning any benefits to the host. Plant-biotrophic fungi interactions such as rust and powdery mildew, are being used more frequently as models for studying pathogen-related changes in C and N metabolism and partitioning.

Research led by Sutton, Henry and Hall (1999) on the uptake of C-derived metabolites and the competition between sugars in the wheat powdery mildew association exposed that sucrose is hydrolyzed prior to uptake in that system. Another study noticed efficient and increased C and N metabolism, phytoalexin accumulation, and lignification, as well as increased accumulation of proteins related to pathogenesis, in *Fusarium oxysporum*-infected chickpea roots (Kumar et al. 2016). Likewise, Desalegn et al. (2016), proposed the existence of an adjustment of C- and N-derived metabolites (secondary metabolism, amino acids, and TCA) and proteomes (proteins related to pisatin biosynthesis) in reaction to *Didymella pinodes* infection in pea plant metabolism. The current literature highlights a significant lack of understanding of the physiological mechanisms that drive this pathogenesis, specifically in clarifying the changes in C and N levels in affected wheat. Comprehending the complex changes in C and N levels caused by CR infection is critical for understanding how wheat plants respond metabolically to fungal diseases. This knowledge provides valuable insights for improving disease resistance and maximizing the health and productivity of crops.

CHAPTER 3 - PAPER 1: *FUSARIUM PSEUDOGRAMINEARUM* INFECTED WHEAT LINES VARY IN DISEASE SEVERITY AND GAS EXCHANGE RESPONSE UNDER DIFFERENT WATERING REGIMES.

This chapter investigates the associations between water availability, plant physiology and the infection of wheat by *Fusarium pseudograminearum*. Importantly this study sets out to address the long-held supposition about the role that water deficit plays in the exacerbation of crown rot in wheat. A selection of wheat genotypes with varying susceptibility to crown rot have been evaluated for the impact of disease under both field capacity and reduced water conditions. Additionally, physiological parameters associated with water and gas exchange were measured in the same trials.

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Fusarium pseudograminearum infected wheat lines vary in disease severity and gas exchange response under different watering regimes

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Abstract

Crown rot (CR; *Fusarium pseudograminearum*) is a serious disease in winter cereals. Soil type, temperature, nutrients, water availability and stubble-borne inoculum levels play major roles in determining disease severity. This paper reports the impact of two different watering regimes on the disease severity and gas exchange of *F. pseudograminearum* infected bread wheat for the first time. *Fusarium pseudograminearum* inoculated and noninoculated genotypes with different susceptibility to CR were watered to either field capacity or a reduced watering regime in three controlled environment experiments. Rate of photosynthesis, stomatal conductance, internal CO_2 concentration and transpiration rate were measured using a portable photosynthesis system, together with disease severity of leaf sheaths at 28 days after planting. Significant differences in disease severity were reported between watering treatments with reduction in CR symptoms in the partially resistant genotypes in the reduced water treatment. Photosynthesis, stomatal conductance and transpiration rate were significantly decreased across most genotypes when inoculated with *F. pseudograminearum*. Differences in gas exchange between inoculum treatments were more evident in plants watered to field capacity. Water availability has been reported to be one of the crucial factors for initiating *F. pseudograminearum* infection and subsequent development of CR disease. This research demonstrates significant variation in genotype-related responses to the complex interactions of *F. pseudograminearum* infection and water treatment, with a negative impact of both limited soil water availability and CR disease severity on plant gas exchange in bread wheat.

KEYWORDS
bread wheat, crown rot, *Fusarium pseudograminearum*, photosynthesis, transpiration, water availability

1 | INTRODUCTION

Crown rot (CR) is a serious stubble-borne disease of wheat and barley, important in Australia and internationally (Kazan & Gardiner, 2018). CR was estimated to cause annual yield losses in Australia valued at AU$79 million and $18 million per annum, in wheat and barley, respectively (Murray & Brennan, 2009, 2010). CR is caused by a number of *Fusarium* species. Within Australia, these *Fusarium* species include *Fusarium culmorum*, *F. graminearum* and predominantly *F. pseudograminearum* (Fp) (Obanor & Chakraborty, 2014). In seedlings, the most characteristic symptoms are necrotic lesions on the coleoptile, which develop to a brown discolouration on the subcrown internode and leaf sheaths (Kazan & Gardiner, 2018; Percy et al., 2012). The infection cycle of Fp consists of three stages. In the first stage, Fp infects the plant and proliferates around the site of infection. In the second stage, termed the lag stage, a small increase in fungal biomass and symptom development is observed. The last stage is necrotrophic where the pathogen invades the internal stem crown tissue and causes the development of lesions and stem browning in the tillers of more mature plants (Beccari et al., 2011; Stephens et al., 2008). Both *F. graminearum* and Fp have been shown to behave as hemibiotrophic in the lag phase (Kazan et al., 2012).

Agricultural practices in Australia, such as minimum tillage used to improve soil moisture, have led to significant increases in the occurrence of CR (Kazan & Gardiner, 2018). Since the adoption of minimum or no till systems, control of CR is principally based on crop rotation to a nonhost or the use of partially resistant varieties, which over time decreases field inoculum (Wildermuth et al., 1997). To date, only partial levels of resistance to CR are available in cereal varieties (Kazan & Gardiner, 2018). Genetics studies have focused on screening wheat and barley populations to identify quantitative trait loci (QTLs) closely linked to resistance genes, leading to identification of 13 QTLs in the partially resistant hexaploid lines 2-49, Sunco, W21MMT70 and IRN479, which are each used in this study, and CPI 133814 (Bovill et al., 2006, 2010; Collard et al., 2005; Martin et al., 2015). QTLs on chromosome 1DL of 2-49 and IRN497 were identified at the seedling stage. However, QTLs on chromosomes 1AS, 1BS and 4BS in 2-49 bread wheat genotypes and on chromosome 2BS contributed by Sunco were observed at both the seedling stage and maturity in field trials. Bovill et al. (2006) identified three QTLs on chromosomes 2B, 2D and 5D in W21MMT70 at the seedling stage.

Soil type, temperature, water availability, nutrients and stubble-borne inoculum levels form complex relationships that play major roles in determining the severity of CR disease (Felton et al., 1998). Additionally, water availability is one of the most crucial factors for initiating CR infection and subsequent development of disease symptoms. Liddell and Burgess (1985) found that initial CR infection of wheat seedlings is favoured in moist soil when the water potential is between −0.3 and −0.7 MPa and that low infection occurs when the water potential is less than −1.5 MPa. In contrast, Beddis (1992) examined the relationship between soil water potential and seedling infection and observed that the incidence of infection was increased in both a tolerant and non-tolerant wheat variety grown under moisture stress or low water potential, which assists the pathogen to colonize seedlings. Further investigation is required to comprehensively understand the relationship between Fp infection, water availability and the subsequent development of disease symptoms in wheat varieties.

Plant–water relations can be directly altered and damaged by pathogen infection. The symptoms of infected plants can be similar to symptoms of water deficiency, for example, wilting. Previous physiological studies have indicated that *Fusarium* spp. are able to cause occlusions in the host xylem vessels (Walters, 2015). Only limited studies have been conducted on the infection process and host response of wheat to infection with Fp. Knight and Sutherland (2016) investigated Fp colonization in bread wheat, durum wheat and barley stems harvested at 10, 16 and 22 weeks after inoculation. During the infection process, all cell types, including vascular tissues, were colonized in stems of the cereals examined. Those authors suggested that blockage of vascular tissue and the subsequent restriction of water and nutrient translocation within the plant contributes to the reduction in grain yield and the establishment of white heads in susceptible genotypes. However, further information on the relationship between CR development and water stress across wheat varieties is required.

Both water deficiency and pathogen infection can induce a defensive response in the plant in order to maximize survival. The changes in water status may trigger hydrological signals via pressure volume changes in sensing cells or cause temporary cavitation in leaf veins, leading to increased stomatal conductance and water loss through transpiration (Christmann et al., 2013). Moreover, reports on water stressed wheat indicate direct relationships between water deficit and decreases in gas exchange parameters including photosynthesis (*A*), stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (*E*; Sharifi & Mohammadkhani, 2016; Zhao et al., 2020). Other reports have also indicated a reduction in gas exchange parameters due to fungal infection. Gas exchange parameters can vary depending on the growth development stage. In adult crops, leaves are the primary source and the roots, stems and grain are the sink, whereas in the seedling phase leaves can act as both the source and the sink (Yang & Luo, 2021). In the current research, we examine the disease severity and gas exchange response of wheat seedlings infected with Fp under different water regimes.

Understanding the physiological response of wheat genotypes with different levels of resistance to Fp is important for the future development of resistant and tolerant germplasm, enabling better management of CR disease. To the best of our knowledge, this is the first report of the effect of Fp infection on the gas exchange of bread wheat genotypes. We hypothesized that CR disease development is influenced by water availability. In addition, it was hypothesized that the wheat seedlings may vary in gas exchange response to both pathogen infection and watering regime.

2 | MATERIALS AND METHODS

2.1 | Plant material and experimental setup

To test the effect of water stress on the disease severity of six wheat genotypes inoculated with Fp, a series of seedling experiments were conducted in a controlled environment growth room at the Leslie Research Facility, Department of Agriculture and Fisheries, Toowoomba, Qld, Australia. Three replicated experiments were conducted in 2016 and in 2017 with six wheat genotypes that were selected according to their previously established susceptibility to CR (Table 1). Four treatments were applied to the six bread wheat genotypes consisting of a Fp-inoculated and a noninoculated pot each watered to either field capacity or reduced water (67% of field capacity). Each treatment by genotype combination was replicated three times forming a total of 72 pots as experimental units in each experiment; treatments were randomized to pots and arranged in the experiment following a randomized complete block design.

Soil used in this study was a self-mulching black Vertosol of the Irving clay soil association (Thompson & Beckmann, 1959), obtained from the Darling Downs, Qld, Australia. Soil was mixed with river sand (50% sand:50% soil) and heated to 80°C with a steam sterilizer for 40 min and air-dried for 7 days. No fertilizer was added to the mix. Plastic pots (7.5–9 cm diameter × 10 cm height = 500 cm^3) were filled with 295 g of dry soil and moistened to field capacity. The soil was levelled, and eight seeds of each genotype were lightly pushed into the surface. Seeds were covered by 160 g of sieved dry soil and the surface levelled. Then, 0.45 g of ground Fp inoculum was sprinkled over the soil surface of the inoculated pots and another 40 g of dry fine soil added to each pot. Fp inoculum consisted of sterilized wheat grain inoculated with a mixture of five aggressive isolates used in routine CR disease screening at the University of Southern Queensland (Percy et al., 2012). All pots were placed in the growth room set to 25°C/21°C in a 12 h day/night schedule and a light energy of 600 $\mu mol\, m^{-2}\, s^{-1}$. The inoculum was activated after 7 days by watering each pot to approximate field capacity or reduced water on a digital scale. Weights of 600 and 565 g were calculated to represent field capacity and reduced water (67% of field capacity), respectively. At this point, plants were thinned to five plants per pot. Pots were watered every 24 h to either field capacity or reduced water for the remainder of the experiment.

2.2 | Gas exchange and disease severity measurements

At 28 days after inoculum activation, gas exchange measurements were taken from plants in the controlled environment growth chamber using a portable photosynthesis system (LI-6400; LICOR). The measurements were taken from the first expanded leaf on two plants per pot. The temperature in the 6-cm^2 chamber was set at 25°C and the leaf level temperature was maintained at 1700 $\mu mol\, m^{-2}\, s^{-1}$ using an in-built LED lamp (red/blue). Vapour pressure deficit was maintained between 1.9 and 2.1 kPa within this chamber during measurements. Each leaf was allowed 10–15 min to reach a steady state before measurements were taken. Rate of photosynthesis (A), stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E) were recorded between 10:00 and 12:00 hours. Following gas exchange measurements, the plants were harvested, and excess soil was washed from roots and the lower crown region. After washing, plants were assessed for disease severity. Disease severity was measured on a 0%–100% rating scale of visual discolouration on the first four leaf sheaths of each of the five plants per pot. Rating of each tissue occurred in 5% increments where 0% is no discolouration and 100% is complete tissue discolouration.

2.3 | Data analysis

An across experiment analysis of data from the gas exchange parameters (photosynthesis, stomatal conductance, internal CO_2 concentration and transpiration rate) and disease severity assessments was conducted using a linear mixed model. A separate analysis was conducted for each trait, with the two groups of traits (gas exchange and disease severity) requiring different modelling approaches according to the structure of the experimental material measured. Additionally, as the assumptions of normality and homoscedasticity of the residuals were not fulfilled for disease severity, this trait was transformed using a square root transformation.

TABLE 1 Crown rot susceptibility of each wheat line and cultivar as defined in the published literature and 2014 Queensland wheat variety guide.

Genotype	Crown rot response	Reference
2-49	Partial resistance	Collard et al. (2005)
W21MMT70	Partial resistance	Bovill et al. (2006)
IRN497	Partial resistance	Wildermuth et al. (2001)
Sunco	Moderately susceptible	GRDC and DAFF (2014)
EGA Gregory	Susceptible	GRDC and DAFF (2014)
Livingston	Susceptible–very susceptible	GRDC and DAFF (2014)

Abbreviations: GRDC, Grains Research and Development Corporation; DAFF, Queensland Department of Agriculture, Fisheries and Forestry.

A single pot in each experiment formed the experimental unit, and five plants per pot formed the observational units. While gas exchange traits were measured on the first expanded leaf of an individual plant, namely one value per observational unit, disease severity was measured from the first four leaf sheaths of each plant, thus four values from the same observational unit. The models fitted to each trait included experiment, genotype, inoculum and water treatment, along with their respective interactions, as fixed effects. Additionally, for the disease severity trait, a fixed effect of leaf was also included, along with all resulting interactions with the previously described terms. Terms describing the structure of the experimental material (replicate blocks, pots, plants) were included as random effects in both models, with additional terms for leaf sheaths included in the disease severity model. Heterogeneity of residual variance was modelled between experiments and, where significant, the complexity of the residual variance structure was extended to allow for heterogeneous residual variance between inoculum treatment groups within and between experiments. Furthermore, for disease severity, an unstructured covariance model was considered to account for residual correlation between leaves of the same plant.

Models were fitted using the ASReml-R package (Butler et al., 2017) in the R statistical computing environment (R Development Core Team, 2020) with variance components estimated using residual maximum likelihood (Patterson & Thompson, 1971). The significance of fixed effects was tested using a Wald conditional test, while the significance of increasingly complex residual variance models was tested using a log-likelihood ratio test. All significance testing was performed at the 5% level.

3 | RESULTS

3.1 | Effect of water stress on disease severity of wheat genotypes infected with *F. pseudograminearum*

Disease severity was assessed for each plant based on the visual appearance of brown/black lesions from infection on the first four leaf sheaths of each wheat genotype seedling. During disease rating of IRN479, a dark purple colour was observed, which was difficult to distinguish from typical CR visual discolouration. For this reason, it is expected the disease severity measurements in IRN497 may be overestimated.

There was a significant Water×Inoculum×Genotype water interaction for disease severity ($p=0.003$) in the leaf sheaths measured at 28 days after planting (Table 2). Average visual discolouration across the leaf sheaths was up to 60% in the Fp-inoculated treatments watered to field capacity, with W21MMT70 and 2-49 significantly lower than the other genotypes (Figure 1a). The average visual discolouration of the leaf sheaths in Sunco, IRN497 and 2-49 was significantly lower in the reduced water treatment compared to the field capacity treatment, while differences in disease severity between watering regimes were not significant in Livingston, EGA Gregory and W21MMT70 (Figure 1a). Visual discolouration of the leaf sheaths was low in the noninoculated treatments under both field capacity and reduced water treatments (<1.5%) of all genotypes (Figure 1b).

TABLE 2 Significant effects and interactions for disease severity and rate of photosynthesis (A), stomatal conductance to water vapour (g_s), internal CO_2 concentration (C_i), and transpiration rate (E) of inoculated and noninoculated bread wheat seedlings watered to field capacity or reduced watering.

Variable	Source	p
Disease severity	Water×Inoculum×Genotype	0.003
	Experiment×Inoculum×Genotype	0.002
A	Genotype×Inoculum	<0.0001
	Inoculum×Water	<0.0001
g_s	Genotype×Inoculum×Water	0.024
C_i	Genotype×Inoculum×Water	0.020
E	Experiment×Inoculum	0.006
	Genotype×Inoculum×Water	0.032

A significant Experiment×Inoculum×Genotype interaction for disease severity ($p=0.002$) was also detected (Table 2), which indicated the variation between experiments in the average visual discolouration observed across genotypes. In Experiment 2, lower levels of disease were recorded in the inoculated treatments of several genotypes than were recorded in Experiments 1 and 3 (Figure 2a). The ranking of genotypes also varied between experiments. For example, the highest disease severity recorded in Experiments 1 and 2 was for EGA Gregory, while this cultivar was ranked in the middle range of disease severities in Experiment 3. The average visual discolouration of the leaf sheaths was low in the noninoculated treatments with a maximum of 4% disease severity recorded in EGA Gregory in Experiment 1 (Figure 2b).

3.2 | Leaf gas exchange parameters

Significant Genotype × Inoculum ($p<0.0001$) and Inoculum × Water ($p<0.0001$) interactions were reported for the average of rate of photosynthesis (A) (Table 2). The value of A in the noninoculated treatments was higher in the partially resistant genotypes (IRN497, W21MMT70 and 2-49) compared to the susceptible genotypes (EGA Gregory and Livingston) and the moderately susceptible genotype Sunco. Conversely, only slight differences were observed in A between genotypes inoculated with Fp, with the average A significantly lower in the Fp-inoculated treatments compared to the noninoculated treatments across all genotypes (Figure 3a). Overall, the average A value in the reduced water treatments was significantly lower than the treatments watered to field capacity in both the Fp-inoculated and noninoculated treatments, with the lowest A reported in the inoculated reduced water treatments (Figure 3b).

A significant Genotype×Water×Inoculum interaction was recorded for the values of stomatal conductance (g_s) ($p=0.024$), C_i ($p=0.020$) and E ($p=0.032$) (Table 2). In the noninoculated treatments,

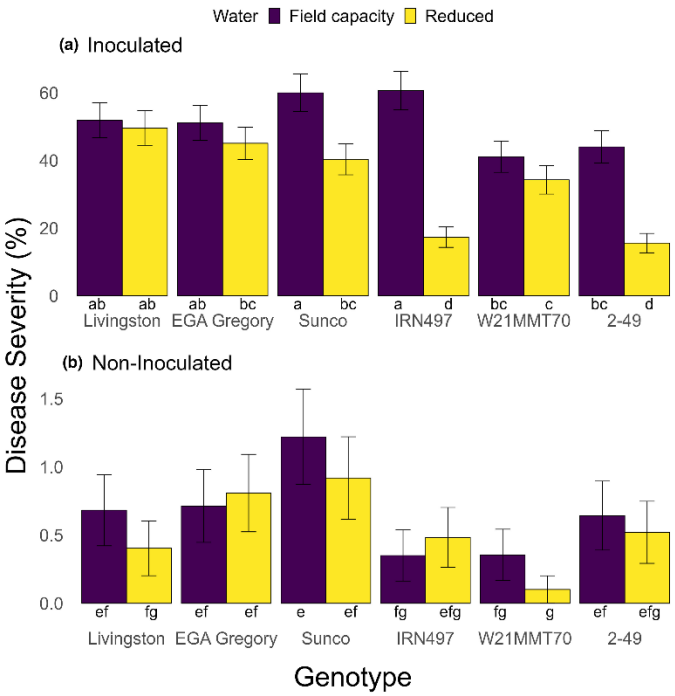

FIGURE 1 Disease severity of (a) *Fusarium pseudograminearum* inoculated and (b) noninoculated bread wheat seedling leaf sheaths, watered to either field capacity or reduced watering (67% of field capacity). Plants were assessed 28 days after planting and results averaged across three experiments. Data = mean ± *SE*; $n = 45$. Different letters represent values that are significantly different between genotypes, inoculum and water treatments ($p = 0.0015$).

all genotypes watered to field capacity recorded higher values of g_s compared to the reduced water treatment, with the exception of cv. Livingston, which had low g_s in both inoculated and noninoculated treatments under both watering regimes (Figure 4a). Inoculation with Fp significantly reduced g_s in all genotypes watered to field capacity (except Livingston) and only in EGA Gregory and Sunco in the reduced water treatments. EGA Gregory watered to field capacity without inoculation recorded the highest g_s value (0.32 mol^{-2} s^{-1}) followed by Sunco with the same treatments (0.28 mol^{-2} s^{-1}) (Figure 4a). The lowest g_s value (of 0.13 mol^{-2} s^{-1}) was recorded in EGA Gregory inoculated with Fp in the reduced water treatment.

The value of internal CO_2 concentration (C_i) was significantly higher in treatments watered to field capacity compared to the reduced water regime in both Fp-inoculated and noninoculated genotypes, with the exception of Livingston and Sunco in the noninoculated treatments (Figure 4b). Significantly higher C_i values were recorded in the inoculated genotypes compared to the noninoculated genotypes, with the exception of Sunco and EGA Gregory in the reduced water treatment and 2-49 under both water treatments (Figure 4b).

The value of transpiration rate (E) was significantly lower in plants with the reduced water treatment compared to those watered to field capacity in all genotypes in both the inoculated and noninoculated treatments, with the exception of Fp-inoculated Livingston. Inoculation with Fp also reduced the value of E irrespective of water regime (Figure 4c). Transpiration rate was highest for W21MMT70 (4.6 mmol H_2O m^{-2} s^{-1}) followed by 2-49 (4.1 mmol H_2O m^{-2} s^{-1}) and EGA Gregory (3.6 mmol H_2O m^{-2} s^{-1}) when watered to field capacity without inoculation. The lowest E value was recorded in EGA Gregory (1.1 mmol H_2O m^{-2} s^{-1}) grown under reduced water and Fp inoculation. A significant interaction between experiment and inoculum ($p = 0.006$) was also observed for E where the value of E was highest in Experiment 3 and lowest in Experiment 1 for noninoculated genotypes and highest in Experiment 1 and lowest in Experiment 3 for the Fp-inoculated genotypes (data not shown).

4 | DISCUSSION

In the last 20 years, CR research in Australia has concentrated on identifying and integrating genetic resistance into commercial cultivars, disease evaluation methodologies, and production loss in infected wheat and barley (Forknall et al., 2019; Hollaway et al., 2013; Kelly et al., 2021; Percy et al., 2012). Limited information is available on how CR disease develops and how the host responds to infection in relation to soil water availability. In this study, we demonstrate the negative impact of limited soil water availability and CR infection on plant gas exchange in seedlings of six bread wheat genotypes with varied susceptibility to Fp in an environmentally controlled growth chamber.

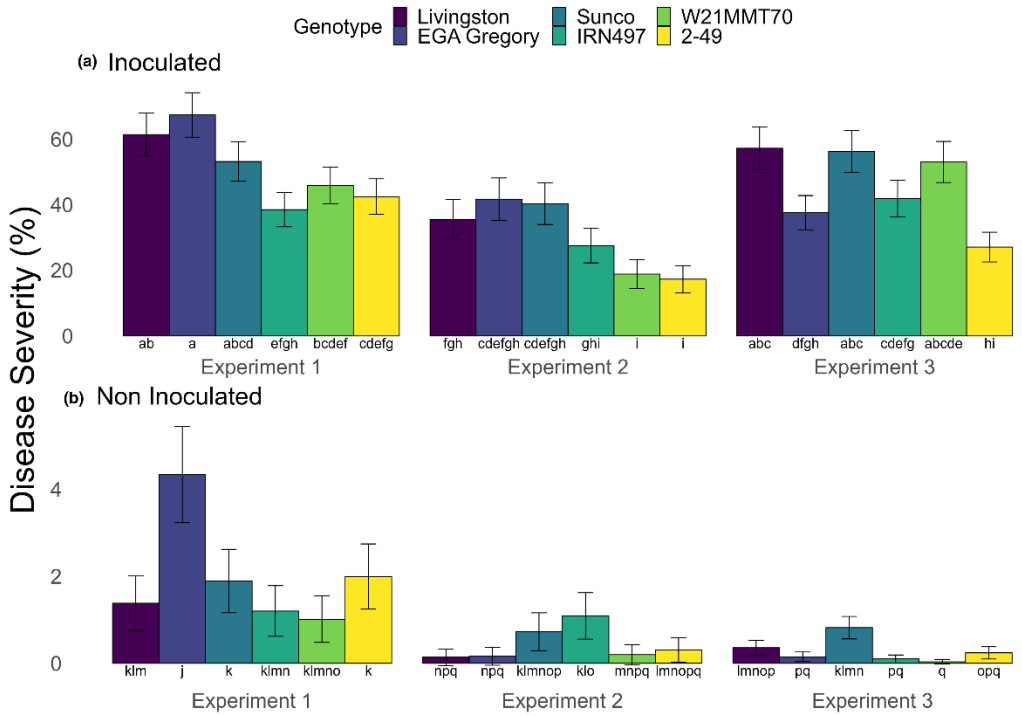

FIGURE 2 Disease severity of (a) *Fusarium pseudograminearum* inoculated and (b) noninoculated bread wheat seedling leaf sheaths, assessed 28 days after planting for each of the three experiments averaged across the water treatments. Data = mean ± *SE*; $n = 30$. Different letters represent values that are significantly different between genotypes, experiments and inoculum treatments ($p = 0.0015$).

This study provides evidence that Fp-inoculated wheat seedlings watered to field capacity produced higher visual discolouration on the leaf sheaths than when grown under a reduced watering regime. This is not consistent with previous reports demonstrating that water stress can enhance the CR proliferation in seedlings in controlled environment conditions (Beddis, 1992; Li et al., 2008). The water stress achieved in those two studies is likely to be more severe than the reduced watering treatment imposed in our experiments, where differences in disease severity between the water treatments were only significant in the partially resistant lines 2-49 and IRN497 and the moderately susceptible cv. Sunco. Our result indicates that partial resistance in seedlings is more pronounced when water is limiting. Ma, Du, et al. (2015) identified the QTL *Qheb.mda-3B* on chromosome 3B of wheat, which can control the content of malondialdehyde, a product of lipid peroxidation used as a parameter to assess the cellular damage of plants attributed to water stress. It was argued that such QTLs can form a strong association with drought tolerance located in the same region as *Qcrs.cpi-3B*, which was previously identified to control the resistance to *Fusarium* CR infection in wheat. A recent study by Buster et al. (2022) reported yield reductions in bread and durum wheat varieties in both field and controlled environment conditions, even when water was not limited, suggesting that the negative effects of Fp infection on yield are not just restricted to situations where water is limiting.

Photosynthesis is ultimately a substantial factor in plant development, and it is very sensitive to water deficits (Sharma et al., 2020). In our study, gas exchange measurements have been collected at the seedling stage, where plants can act as both the source and sink for photosynthesis, due to the accumulation of photosynthates that are required for growth and development (Yang & Luo, 2021).

Water stress was shown to strongly impact the gas exchange parameters in this study, significantly reducing the photosynthetic capacity of all genotypes. This result is in agreement with previous studies on wheat genotypes that demonstrated a reduction in *A* during water stress treatments (Ashraf & Harris, 2013; Chaves & Oliveira, 2004; Liu et al., 2016; Ma, Duan, et al., 2015; Sharifi & Mohammadkhani, 2016; Wu & Bao, 2011; Zhao et al., 2020). Other studies on plant physiology have also shown that drought stress can cause changes in the photosynthesis process, with Sun et al. (2013) identifying it as one of the first processes to be impacted. When

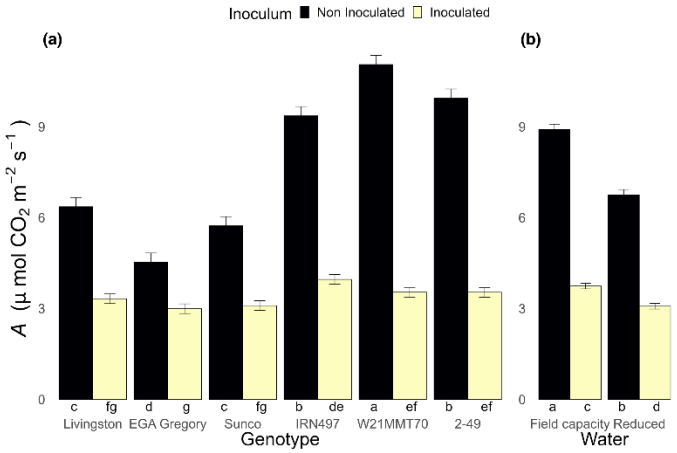

FIGURE 3 Rate of photosynthesis (a) in *Fusarium pseudograminearum* inoculated and noninoculated bread wheat seedlings, assessed 28 days after planting for (a) six genotypes with varying crown rot resistance averaged across experiments and water treatments, and (b) plants watered to either field capacity or reduced watering (67% of field capacity) averaged across genotypes and the three experiments. (a) Data = mean ± *SE*; $n = 36$. Different letters represent values that are significantly different between genotypes and inoculum treatments ($p = 0.0001$). (b) Data = mean ± *SE*; $n = 90$. Different letters represent values that are significantly different between water and inoculum treatments ($p = 0.0001$).

plants are exposed to drought stress, one of the first responses is to reduce transpiration by closing the stomata. Stomata regulate the exchange of CO_2 and water in plants, and their closure helps to limit water loss. However, this also leads to a decrease in CO_2 absorption and the transportation of nonstructural carbon (NSC), which is crucial for photosynthesis, and can lead to carbon starvation that affects various other plant processes (McDowell & Sevanto, 2010; Sevanto, 2014). Carbon starvation can cause stunted growth and negatively impact respiration under mild to moderate water deficit conditions (Pinheiro & Chaves, 2011).

In this study, in the noninoculated genotypes, the plants watered to field capacity had higher g_s, C_i and E than the plants in the reduced water treatments. These findings are in line with previous studies by Ma, Duan, et al. (2015), Saeidi and Abdoli (2015) and Zhao et al. (2020), where water deficit of wheat was reported to cause stomatal closure leading to a decreased availability of CO_2. Water stress decreases turgor pressure within the cell, and stomata react by partial closing to limit the transpiration to prevent excessive water losses, which in turn leads to a decrease in A, C_i and E (Thapa et al., 2018).

This is the first report of changes in gas exchange parameters of wheat seedlings varying in their response to CR infection, grown under reduced and field capacity water treatments. Our results demonstrate a strong negative impact on A across all wheat genotypes infected with Fp, reducing A in inoculated genotypes by approximately 60% and 70% for plants watered to field capacity and in the reduced water treatment, respectively. Thus, the lowest level of A was observed in the inoculated genotypes under the reduced water treatment. Yang et al. (2016) reported a reduction in A in Fusarium head blight (FHB)-resistant genotypes after *F. graminearum* inoculation but insignificant changes in the A parameters in FHB-susceptible genotypes. However, in our study, all genotypes (from susceptible to partially resistant) demonstrated significant reductions in A when inoculated with Fp, although the differences in A between the inoculated and noninoculated treatments were more pronounced in the partially resistant genotypes IRN497, W21MMT70 and 2-49. Hu et al. (2020) demonstrated the downregulation of photosynthesis-related genes in two wheat lines L658 (susceptible) and L958 (resistant) infected by the biotrophic powdery mildew fungus *Blumeria graminis* f. sp. *tritici*. This decrease in A was reported to be most likely related to inhibition of peroxidase (POD) and catalase (CAT), which regulate hydrogen peroxide (H_2O_2).

Fp generally infects the lower crown region, including the subcrown internodes, leaf sheaths and stem tissues. Although photosynthetic measurements were taken on the leaf blade tissue without disease symptoms, the A activity in the infected plants was found to be lower than in the noninoculated controls. Bastiaans (1991) and Debona et al. (2014) also reported a reduction in the A activity in the noninfected area in wheat during early infection by the blast pathogen *Pyricularia oryaze*. This reduction in A resulted from a toxin produced by the fungus that had diffused into the surrounding tissue, causing tissue disintegration, which compromised water uptake as well as phototranslocation in leaves far from the infection site. The relationship between photosynthesis and mycotoxin production in Fp CR infections requires further investigation.

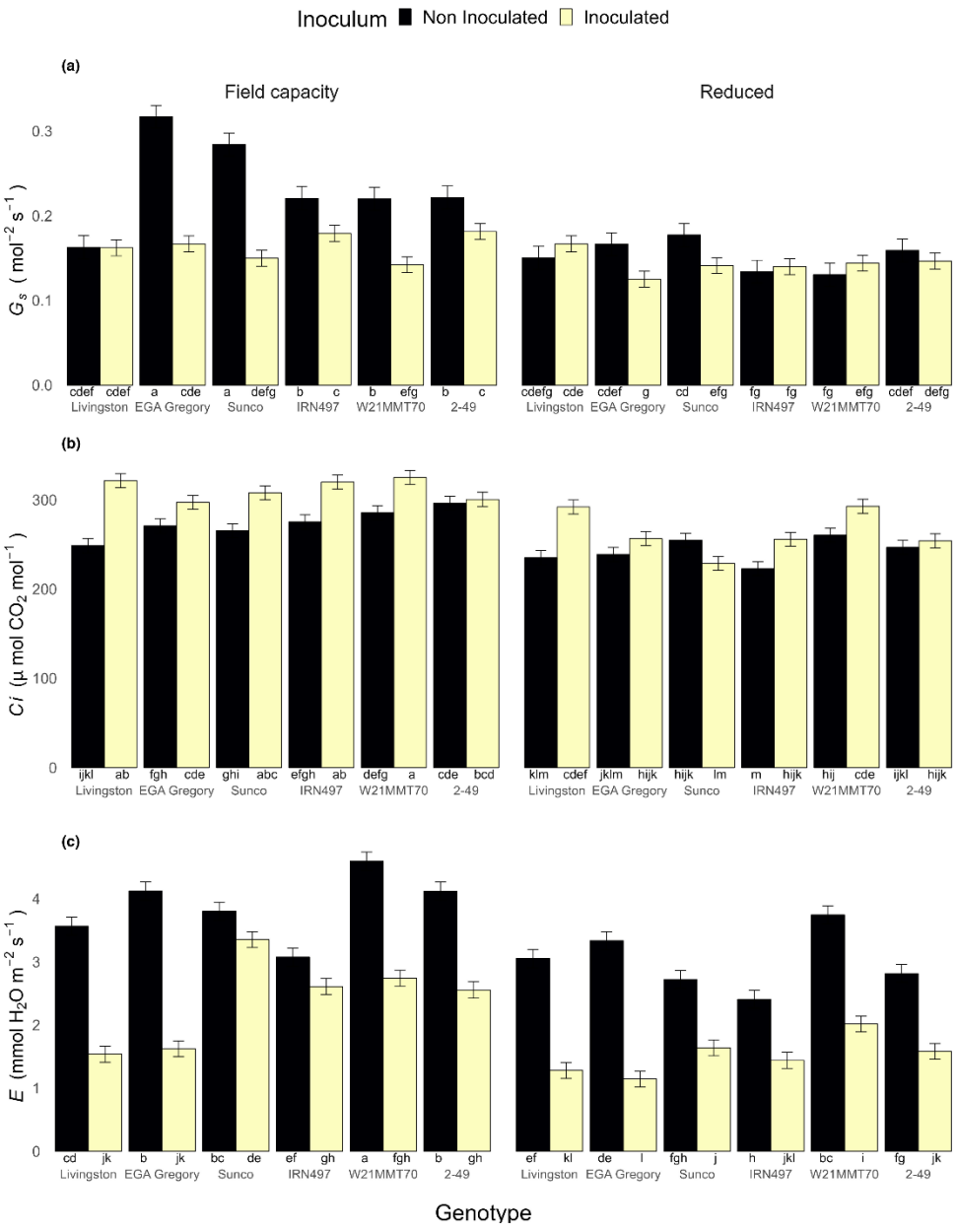

FIGURE 4 (a) Stomatal conductance (G_s), (b) internal CO_2 concentration (C_i), and (c) transpiration rate (E) of *Fusarium pseudograminearum* inoculated and noninoculated bread wheat seedlings, watered to either field capacity or reduced watering (67% of field capacity). Plants were assessed 28 days after planting and results averaged across three experiments. Data = mean ± *SE*; $n = 18$. Different letters represent values that are significantly different between genotypes, inoculum and water treatments ($p = 0.024$, 0.020 and 0.006 for a, b and c, respectively).

All genotypes inoculated with Fp recorded a significantly lower value of g_s, compared to the noninoculated genotypes, when watered to field capacity, with the exception of the susceptible cv. Livingston. Genotypes that were inoculated also exhibited higher C_i than the noninoculated plants in both the watering treatments with the exception of cv. Sunco. These data are in line with Tatagiba et al. (2016), who demonstrated a reduction in *A* with decreased g_s and increased C_i of rice leaves infected with *Monographella albescens*. Similar results have also been reported in other pathosystem investigations, and these findings have usually been interpreted as an indication of biochemical, rather than diffusive, limitations to photosynthesis (Dallagnol et al., 2011; Resende et al., 2012). Furthermore, high C_i values at advanced stages of fungal infection suggest that there are biochemical limitations that restrict CO_2 influx into the carboxylation sites on chloroplasts, due to a reduction in RuBisCO activity (Aucique-Pérez et al., 2017).

Wheat infected with fungal species have been reported to display a reduction in *A* activity due to poor RuBisCO performance, low inflow of CO_2 from the atmosphere to carboxylation sites in the leaf tissue and an increase in *E* (Debona et al., 2014; Rios et al., 2017). In addition, reduced *A* was observed in several biotrophic fungal infections including wheat infected with *Pyricularia* (Perez et al., 2014), and wheat infected by *Bipolaris sorokiniana* (Rios et al., 2017). The reduction in *A* during these infections was claimed to be linked to the non-stomatal factors such as decreased mesophyll conductance. Additionally, these fungal infections have been observed to alter the dissipation of light energy in wheat plants, with decreases in photochemical processes and increases in non-photochemical mechanisms. This leads to a decrease in photosynthetic pigments, such as β-carotene, xanthophylls, total chlorophylls and lutein, on the leaves of infected wheat plants.

The average *E* of the wheat genotypes in this study was lower in the Fp-infected plants than in the noninoculated controls in both watering treatments. Debona et al. (2014) observed lower *E* in wheat infected with *P. oryaze* compared to the controls. The observed lower *E* was ascribed to the control by g_s. Our current findings agree well with the work of Bermúdez-Cardona et al. (2015) where lower *E* was argued to be associated with the stomatal closure of leaf maize infected with *Stenocarpella macrospora* in order to avoid the excessive water loss. Silveira et al. (2019) also suggested that maize infected with *Exserohilum turcicum* can produce a reduced *E* compared to the noninoculated plants due to wilting leaves at the early stage of fungal infection. While in our study *E* was significantly reduced in all inoculated genotypes grown under both watering treatments, variation in *E* was observed. The highest level of *E* in the inoculated treatments was recorded in the moderately susceptible cv. Sunco, followed by the partially resistant lines IRN497, 2-49 and W21MMT70, when watered to field capacity. Additionally, the damage induced by Fp hyphae colonizing the vascular tissue may result in a reduction in the level of *E* in infected genotypes. Knight and Sutherland (2016) reported significant Fp hyphal colonization of vascular bundles that was expected to cause the vascular system in wheat plants to become dysfunctional. Additionally, the blocking of vascular tissue in susceptible genotypes may disrupt hydraulic conductivity to a greater extent than in partially resistant genotypes.

Gas exchange parameters are associated with each other and, consistent with our observation on this host pathogen interaction, a lower rate of *A* may be linked with a reduction in g_s and increase in C_i (Bermúdez-Cardona et al., 2015; Rios et al., 2017). In this case, the *A* reduction might have resulted from limited CO_2 fixation at the biochemical level or reduction in CO_2 influx due to stomatal closure (Debona et al., 2014). The observed gas exchange parameters among the six wheat genotypes included in this study indicated significant variation in genotype-related responses to the complex interactions of Fp infection and water treatment. It is possible that Fp may have been able to interfere with the wheat primary metabolism including amino acid, hormonal signals and organic acid for support and extension of fungal infection, and certain changes were observed among cultivars regardless of their level of resistance to CR. Further research on the biochemical and physiological response of wheat genotypes infected with Fp will improve our understanding of the mechanisms of resistance and tolerance to CR and therefore enable better selection of improved CR-resistant and -tolerant wheat varieties for integration into the Australian and international wheat breeding programmes.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing interests to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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3.2 Link and Implications

This study aimed at understanding the relationships between water deficit, plant physiology and *Fp* infection in wheat. Importantly this investigation sets out to empirically address a long held supposition about the role that water deficit plays in the exacerbation of CR in wheat. A small panel of wheat varieties that differ in their response to CR disease under well-watered and water deficit condition were examined. In the same experiments, measures of physiological parameters related to water and gas exchange were also taken. The entire study was essentially undertaken in three repeated runs under controlled growth conditions. The next investigation delves into the differences of wheat genotype response to CR under field conditions. Three field trails were sampled at two developmental stages (flowering and maturity). The incidence of infected tillers, the severity of the disease visual symptoms on the stem, and the sub-crown internode were reported. Physiological parameters including assessments of leaf, stem and head dry weight, head counts, tillers numbers and gas exchange parameters, are defined in plus and minus inoculated field experiments.

CHAPTER 4 – PAPER 2: *FUSARIUM PSEUDOGRAMINEARUM* IMPACTS BREAD WHEAT (TRITICUM AESTIVUM) PHYSIOLOGICAL PARAMETERS IN THE FIELD

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In this experimental chapter, the effects of *Fusarium pseudograminearum* (*Fp*) invasion on wheat in field environment was examined. The outcome of this vast field investigation has an important insight into the intricate interactions between wheat and *Fp*, also the diverse responses exhibited by different wheat cultivars with different levels of resistance to crown rot (CR). This study seeks to consolidate these findings and investigate the physiological mechanisms that control the relationship between wheat and *Fp*, through the analysis of the effects of *Fp* infection on crucial factors like gas exchange and biomass production. By incorporating the provided results into the current body of knowledge, this chapter on field experiments will contribute to the main goal of this thesis, which is to improve the comprehension and management of crown rot, a significant challenge for the wheat industry worldwide.

4.1. Abstract

Crown rot (CR) caused by *Fusarium pseudograminearum* (*Fp*) is a significant wheat disease in Australia and internationally. Complete resistance to crown rot has not been identified in wheat. Partial levels of resistance are available in breeding lines, however these partially resistant lines are non-agronomic and typically low yielding. Physiological differences between the susceptible and partially resistant wheat genotypes are not known. In this study the physiology of twelve bread wheat genotypes differing in susceptibility to crown rot were investigated in plus and minus inoculated field experiments conducted under different environmental conditions across three years. CR plant height, plant biomass and disease severity rate were measured at flowering and at maturity, whilst photosynthesis net rate, stomatal conductance and transpiration rate were measured at flowering only. *Fusarium pseudograminearum* infection caused a reduction in susceptible and very susceptible cultivars in shoot length, tiller numbers, biomass and head number across the three field experiments. The reduction in photosynthesis net rate was accompanied with reduced stomatal conductance and transpiration rates in the three experiments. The physiological investigations presented in this research provide useful information for understanding the disease development in the field and its importance for breeders and farmers.

4.2. Introduction

Crown rot (CR) disease caused predominantly by the fungus *Fusarium pseudograminearum* (*Fp*) is one of the most devastating soil borne diseases of wheat across most producing countries (Backhouse et al. 2002; Smiley 2009; Alahmad et al. 2020; Simpfendorfer et al. 2020; Petronaitis et al. 2021). Although all major winter cereals can be colonized by *Fp,* the pathogen's main impact is on bread wheat (*Triticum aestivum L.*), durum wheat (*Triticum turgidum L*. spp. durum (Dest.)) and barley (*Hordeum vulgare L*). While oats (*Avena sativa L.*) can be infected by the pathogen and may exhibit little to no disease symptoms (Percy et al. 2012; Kazan et al. 2018). Under natural inoculum levels, yield losses can reach 10 to 35 percent (Klein et al. 1991; Smiley et al. 2005; Murray et al. 2010). Yield losses in wheat in Australia were estimated at $79 million dollars, annually (Murray et al. 2009).

The most distinct visual symptoms of *Fp* infection are a brown discoloration of stem bases and the formation of whiteheads, observed at flowering time, when the plant undergo moisture stress (Forknall et al. 2019). However, infection can occur at any stage of the plants lifecycle

and lesions will be observed on crown tissues from the seedling stage. Histopathological studies by Knight et al. (2016) report that infection occurs most widely via stomatal apertures and continues to evolve into the parenchymatous hypoderm. Hyphae spread vertically through the tissues from the culm base, first through the hypoderm and pith cavity in culm tissues (Knight et al. 2016). Fungus colonization can be observed in susceptible and partially resistant cultivars; however, the partially resistant genotypes have significantly less colonization than susceptible genotypes (Percy et al. 2012).

The most common agronomic practice for managing CR involves stubble management and soil fertility (Cook 2001; Lamprecht et al. 2006), crop rotation (Kirkegaard et al. 2004), inter-row sowing (Verrell et al. 2017), and growing wheat varieties with improved resistance or tolerance to the disease (Forknall et al. 2019). In addition, applying fungicides to seeds or in-furrow at planting can reduce early-season pathogen growth (Akgül et al. 2016; Moya-Elizondo et al. 2016). Agricultural practices in Australia have changed dramatically in the last years, with crop rotation and no-tillage being widely used to conserve moisture (Chakraborty et al. 2006). These practices, as well as as genetic diversity and structure have the potential to influence pathogen dynamics (McDonald et al. 2002). The *Fp* has the potential to produce hazardous mycotoxins like deoxynivalenol (DON) and its acetylated forms, 3-acetyl deoxynivalenol (3-ADON) and 15-acetyl deoxynivalenol (15-ADON) (Obanor et al. 2014). The grain's quality can be reduced by these mycotoxins which also have the potential to contaminate food and feed sources (De Boevre et al. 2012). Monds et al. (2005) reported in a study in New Zealand there is another type of type B trichothecene mycotoxin that can be produced by *Fp* is known as nivalenol (NIV). Yet there have been no reports of *Fp* infected wheat in Australia producing 15-ADON.

Plant biomass studies conducted by (Saad et al. 2022) demonstrated that five winter cereals including barley (cv. Grimmett), bread wheat (cv. Livingston), durum wheat (cv. Hyperno), oat (cv. Genie), and triticale (cv. Endeavour), that were inoculated with *Fp* and *F. culmorum* strains demonstrated a greater plant dry weight from tillering to maturity, also differences between inoculated and non-inoculated. This result might be attributed to the increased stem numbers observed at flowering and maturity in the treatments inoculated with *Fp* and *F. culmorum*, compared to the non-inoculated control. In this investigation, plant height and weight have been measured for twelve genotypes under field conditions.

Fungal pathogen and plant interactions can be linked to changes in pathways such as photosynthesis (Hill-Ambroz et al. 2006). Photosynthetic source organs are plant organs, such

as leaves, that can perform photosynthesis. Photosynthetic sink organs, which include stems, roots and fruits, are the organs that store the organic matter produced by photosynthesis. The photosynthetic source and sink can be modified in accordance with the development stage, for example, at the seedling stage, leaves can act as both the source and sink for photosynthesis during growth and development. Gas exchange can be disrupted by abiotic stress (Takahashi et al. 2011; Hou et al. 2016) and biotic stress such as pathogen infection (Lu et al. 2018). Further, the plant photosynthetic and source-sink relationship can be impacted when infected by necrotrophic, biotrophic or hemi biotrophic fungi (Biemelt et al. 2006). The relationship between photosynthetic sources and non-photosynthetic sinks is maintained in dynamic balance (Luo et al. 2009; Luo et al. 2013). Disturbance in this balance can occur when plant pathogen attack is likely to manifest as changes in gas exchange parameters because of the regulation of cellular signaling homeostasis (Rodriguez-Brljevich et al. 2010; Wituszyńska et al. 2013).

Photosynthetic studies of three major diseases of wheat have presented gas exchange changes and source and sink relationship due to infections (Yang et al. 2021). Wheat powdery mildew caused by *Bulmeria graminis* f. sp. *tritici* (*Bgt*) and wheat stripe rust caused by the biotrophic fungi *Puccina striiformis* f. sp. *tritici* which both predominantly infect the leaf tissue are considered source diseases (Chen et al. 2014; Yang, M. et al. 2016). Fusarium head blight (FHB) caused by *Fusarium graminearum* (*Fg*) infects the flowering parts of the plants and is considered a sink disease. Gas exchange parameters in ears and leaves infected by *Fg* were found to be significantly different (Li et al. 2018). After inoculation, a decrease in gas exchange parameters was observed in the sources and sinks of the abovementioned diseases. These results suggest that modifications in the photosynthesis parameters observed during the initial stages of wheat infection by stripe rust and powdery mildew contribute to the plant's resistance development. However, there is currently no study indicating an association between *Fp*-infected wheat and gas exchange parameters. Despite differences in the site of infection on the crop, such as leaves, head or stem, yet it may cause distinct physiological and biochemical changes in the host plant, resulting in varying effects on crop yield (Yang et al. 2021). Yang, et al. (2016) demonstrated lower gas exchange parameters in FHB susceptible cultivars than in the resistant genotypes. Various wheat leaf diseases can cause reduction in the amount of green leaf tissue, leading to decrease in the chlorophyl content of the infected plant part. This decrease in chlorophyll content can impact the plant’s ability to perform photosynthesis, which can ultimately reduce crop yield (Rosyara et al. 2010).

Lower CO_2 assimilation may cause oxidative stress in chloroplasts due to increased generation of reactive oxygen species (ROS) by response inhibition of photosynthetic electron transport (Takahashi et al. 2008; Sonoike 2011). The carboxylation efficiency and photochemical capacity of Photosystem II (PSII) has been demonstrated to decrease under *Fusarium* o*xysporum* infection on other crops such as tomato and banana, negatively affecting Rubisco and the PSII reaction center (Pshibytko et al. 2006; Dong et al. 2016). Previous research reported an association between gas exchange and fungal infection of wheat by *Pyriculria oryzae* (wheat blast). *Pyriculria oryzae* caused a decrease in the photosynthetic capacity of infected wheat leaves, reducing photosynthesis by more than 50% and stomatal conductance and transpiration rate more than 30% (Debona et al. 2014; Aucique-Pérez et al. 2019). It has been theorized by Rojas et al. (2014) that these decreases in photosynthesis may be the result of physiological modifications to the photosynthetic process brought on by the wheat blast infection. Moreover, certain pathogens have been discovered to use sophisticated mechanisms to alter the metabolism of their host plants during infection, with the asymptomatic phase playing a critical role in their success. These mechanisms allow pathogens to infect their hosts even when the infection is not immediately apparent. To date, wheat genotypes with complete resistance to *Fp* have not been observed and only a few varieties with partial resistance are available (Kazan et al. 2018). As a result, wheat and its fungal pathogens can provide an ideal pathogen system for investigating the role of photosynthesis in the development of wheat resistance (Yang et al. 2021). The physiological response of *Fp* inoculation in wheat genotypes possessing different resistance statuses to crown rot has not been reported. This information is important to better understand the impact of crown rot on the host and to improve the future development of resistant genotypes.

In the current study, the physiological response of bread wheat genotypes with a known varying CR resistance response to *Fp* infection have been measured under field conditions. Disease severity, including assessments of lesion development, wheat leaf, stem and head dry weight, head count, tillers numbers and gas exchange parameters have been defined in plus and minus inoculated field experiments Differences between wheat genotypes with varying levels of resistance to CR, as well as the impact of infection on these physiological traits in wheat have been determined. Wheat cultivars with partial resistance to CR will exhibit better physiological performance when infected with *Fp* compared to susceptible and very susceptible genotype. Specifically, we hypothesize that CR partial resistant genotypes will: Maintain higher biomass production, reveal less severe declines in gas exchange parameters, demonstrate reduced

disease severity, and suffer less pronounced reductions in tiller numbers and head counts when infected with *Fp* compared to susceptible and very susceptible genotypes. Finding the specific physiological mechanisms underlying the variance reactions of resistant susceptible and very susceptible wheat cultivars to *Fp* infection will provide breeders with a broader range of tools to select wheat genotypes with improved resistance to CR.

4.3. Methodology

4.3.1. Field site location

Three field experiments were carried out at the Wellcamp field station, Department of Agriculture and Fisheries, QLD, Australia (27°33'54" S 151°51'15" E) in 2016, 2017 and 2018. The soil type at this site is a self-mulching black Vertisol of the Irving clay soil association (Australian black earth) (Thompson et al. 1959). In each of the experiments, urea (100 kg N/ha) was applied three weeks before sowing at a depth of 50 mm. Rainfall were collected across the three years from the Moyola weather station, 8.1 km from the site of the experiments (Table 2). The three field experiments were planted in early July each year and flowering stages were harvested between mid October and beginning of November with mature plants harvested between beginning and middle of December of each year.

4.3.2. Experimental design

Twelve wheat genotypes with a known susceptibility to crown rot (Table 1) were sown into plus and minus *Fp* inoculated plots in the 2016 and 2017 experiments. Each experiment was arranged as a strip plot design, where the inoculum was confined to a row and the genotypes randomized as paired (+/- inoculum) plots within each pair of rows. Each paired plot was replicated six times with measurements taken on three replicates at flowering time and on three replicates at maturity. 20 seeds of each genotype were planted at 5cm depth into each plot using a cassette delivery system on a Glen E Lee planter (Kingaroy engineering, Australia). Inoculum was delivered into the furrow above, the seed at planting with a Microband distributor at a rate of 2g/m row. Inoculum consisted of *Fp* colonized millet (mixture of five aggressive isolates) supplied by the soilborne disease team at UniSQ. Ten plants from each of three replicates of each treatment (Table 1) were hand harvested at each of the two harvest times: flowering (Zadoks growth stage GS60 – 69) and maturity (Zadoks growth stage GS90 – 99) in each field experiment. A third field experiment was conducted in 2018 as described above however only

six genotypes (Table 1) were included, as extra measurements were taken on these plots (not reported in this paper).

4.3.3. Disease severity rating

At flowering and maturity, the number of diseased tillers per plant were counted for each of the ten plants per plot and the disease severity was then measured by recording the percentage of brown discoloration on the lower 15 cm of each stem by using a 0-100% rating scale where 0 = no discoloration and 100% = completely discolored tissue. Disease severity was measured on the main tiller and two primary tillers from each of the ten plants. Discoloration was identified as a honey brown to dark brown color.

4.3.4. Plant height, tiller number and weight

Plant height of each plant was taken by measuring from the base of the tiller to the tip of the longest leaf at flowering and from the base of the stem to the tip of the longest head at maturity. Tiller number was counted. Heads, leaves and stems were separated for each of the ten plants per plot and placed in paper bags. Samples were dried in a dehydrator (Wessberg Martin Engineering Pty Ltd, dryer/oven, Germany) at 60°C for 72 hours. Afterwards the dried samples were weighed.

4.3.5. Gas exchange measurements

Gas exchange measurements were taken on the 10 plants from each inoculated and non-inoculated plot prior to harvest at the flowering growth stage. Photosynthesis (*A*), stomatal conductance (*Gs*), internal CO_2 concentration (C_i) and transpiration rate (*E*) were recorded by using a portable photosynthesis system (LI-6400, LICOR, USA). Leaf level temperature was maintained at 1700 µmol quanta m^{-2} s^{-1} using an in-built LED lamp (red/blue). Gas exchange measurements were conducted on the flag leaf of each plant. Each leaf allowed 5 to 7 minutes to reach a steady state before measurements were taken as described by (Seneweera et al. 2002). Measurements were taken between 10:00 am and 2:00 pm.

4.3.6. Data Analysis

Traits related to plant height, tiller number and weight, along with disease severity and incidence, were analysed separately using a linear mixed model framework. For each trait,

terms to account for the four-way factorial structure of experiment, genotype, inoculum treatment and harvest time, along with the associated interactions between terms, were fitted as fixed effects. Terms to account for the experimental design structure at each experiment were fitted as random effects. Visual diagnostics revealed substantial heterogeneity of residual variance between experiments, inoculum treatments and harvest times. The need to model this heterogeneity was confirmed through formal model comparison tests and subsequently included in the model.

A similar framework was used for the analysis of the gas exchange parameters, however given these traits were only measured at flowering, terms corresponding to harvest time were omitted from the model. Furthermore, for these traits, heterogeneous residual variance was modelled between combinations of experiment and inoculum treatment, the need for which was indicated by visual diagnostics and confirmed by formal model comparison tests.

Predictions of the fixed effects were provided as empirical best linear unbiased estimates (e-BLUEs). Models were fitted using the ASReml-R package (Butler et al. 2017) in R (R Core Team, 2019), whereby variance components were estimated using residual maximum (Patterson et, al. 1971).

4.4. Results

4.4.1. Disease severity

There were three significant interactions for the average disease severity in stems, including a genotype x inoculum x harvest ($p<0.0001$), and experiment x inoculum x harvest ($p<0.0001$), and experiment x genotype x inoculum ($p<0.0001$) (Supplementary Table 1).

Non-inoculated genotypes of the three experiments displayed low disease ratings (<10%) compared to inoculated genotypes (Figures 1, Supplementary Figure 1and 2). In the genotype x inoculum x harvest ($p<0.0001$) interaction, inoculated Livingston and Mace demonstrated higher disease severity > 50%, at flowering, followed by Gregory and Suntop >30% (Figure 1). At maturity the disease severity of only Livingston was >50%, followed by Mace >40%, Suntop, Syn110, Gregory and Sunco >30%, compared to the partially resistant genotypes.

At the flowering growth stage, the disease severity in the inoculated genotypes was higher in the 2018 experiment compared to the 2016 and 2017 experiments, whereas at maturity the

disease severity in the inoculated genotypes was highest in the 2017 experiment, followed by the 2018 experiment and the 2016 experiment (Supplementary Figure 1).

In the experiment x genotype x inoculum interaction (*p=0.0001)*, genotypes Mace was > 40%, followed by Gregory, Livingston and Syn110 > 30% in 2016. In 2017 the disease severity of Livingston and Mace was higher > 55% compared to other genotypes. In 2018 the disease severity of Livingston was higher > 50% (Supplementary Figure 2).

4.4.2. Tiller number

There was a significant interaction between experiment x genotype x inoculum x harvest *(p= 0.001)* for the tiller number (Supplementary Table 1). Inoculated plants that demonstrated partial resistance to the disease had a tiller number ranging from 6.27 to 10.53 during flowering, and from 6.79 to 11.67 during maturity (left side of Figure 2). Inoculated plants that were susceptible to the disease had a tiller number ranging from 5.93 to 12.40 during flowering, and from 4.72 to 13.07 during maturity (right side of Figure 2). Inoculated plants that were very susceptible to the disease had a tiller number ranging from 5.84 to 10.67 during flowering, and from 6.23 to 12.93 during maturity. Inoculated plants that are moderately resistant or tolerant to the disease had a tiller number ranging from 5.75 to 10.13 during flowering, and from 6.88 to 12.40 during maturity.

In the 2016 experiment a reduction in tiller number was observed in the inoculated genotypes in AUS29529, Mace and Suntop at flowering and in Syn110, Sunguard and AUS29529 at maturity (Figure 2). In 2017, a reduction in tiller numbers was observed in the inoculated genotypes in Livingston, Mace, Gregory, Suntop, Syn 110 and Sunco at maturity. In the 2018 experiment only Gregory and 2-49 had a reduced tiller number in the inoculated gentypes at maturity (Figure 2).

4.4.3. Head number

A significant experiment x genotypes x inoculum x harvest interaction for head number was reported (*p=0.010*) (Supplementary Table 1). Overall, the number of wheat heads were higher in 2016 compared to 2017 and 2018. In the 2016, 2017 and 2018, it was observed that the non-inoculated genotypes exhibited a higher number of flowering and mature heads in comparison to the inoculated genotypes. The head number of the genotypes in the 2016 field experiment at flowering were reduced when inoculated compared to non-inoculated genotypes, however, the differences were non-significant, with the exception of Mace, Suntop, Syn110, Sunco and AUS29529 where head number was lower under inoculation (Figure 3). At maturity, Sunco

indicated an increase in head number when inoculated, on the other hand, the inoculated genotypes of Livingston, Sunguard, and AUS29529 had a reduced head number compared to the non-inoculated treatments. In the 2017 field experiment, it was observed that only the 2-49 genotype exhibited a significant difference between the inoculated and non-inoculated treatments at flowering. Furthermore, the inoculated Syn110 and Mace had the greatest reduction in head number compared to the non-inoculated treatments at maturity. In the 2018 field experiment the head number in the inoculated genotypes was only reduced in the cultivar Gregory at maturity (Figure 3).

4.4.4. Shoot length

Significant experiment x genotype x inoculum and experiment x genotype x harvest interactions were detected for shoot length ($p<0.0001$) (Supplementary Table 1). The average shoot length of genotypes varied among the three field experiments, with a higher shoot length in the 2016 and 2018 experiment compared to the 2017 experiment. Also, in the 2017 experiment IRN497, W21MMT70 and 2-49 were significantly taller than the other genotypes at maturity (Supplementary Figure 3). In all three experiments, inoculated genotypes exhibited a reduced shoot length in comparison to non-inoculated genotypes with the exception of W21MMT70 in the 2016 experiment, Sunco in the 2017 experiment and Sunguard in the 2018 experiment (Figure 4).

4.4.5. Stem dry weight

There was a significant experiment x genotype x inoculum x harvest interaction for stem dry weight ($p= 0.004$) (Supplementary Table 1). The average total stem weights in the 2016 experiment were higher than those recorded in the 2017 and 2018 experiment (Figure 5). In the 2016 experiment the stem weights were reduced in the inoculated Syn110, Sunco AUS29529 and GW95-703*C15 at flowering and in Livingston, Sunco and IRN497 at maturity. In the 2017 experiment the stem weights were reduced in all of the inoculated genotypes at flowering with the exception of Gregory, Syn110, Sunco and IRN497. At maturity the stem weights were reduced in the inoculated Mace, Gregory and Suntop in the 2017 experiment. In 2018, differences between inoculated and non-inoculated genotypes were mostly non-significant at flowering and maturity (Figure 5).

4.4.6. Head dry weight

A significant experiment × genotype x inoculum x harvest interaction was reported for the head dry weight (*p=0.04*) (Supplementary Table 1). Differences between inoculated and non-inoculated genotypes were mostly non-significant at flowering, with the exception of Syn110, AUS29529 and IRN497 in the 2016 field experiment. All head weights were reduced in the inoculated genotypes of the 2016 field experiment at maturity with the exception of Syn110, IRN497, W21MMT70 and 2-49 (Figure 6). In the 2017 field experiment, at flowering, the differences between inoculated and non-inoculated genotypes were not significant. At maturity the head weight of the inoculated genotypes was reduced in Livingston, Mace, Gregory, Suntop, AUS29529 and IRN497 (Figure 6). The findings from the 2018 field experiment at flowering growth revealed that there were no significant differences between inoculated and non-inoculated genotypes. The head weights of Livingston, Gregory, and 2-49 were significantly lower in the inoculated genotypes at maturity (Figure 6).

4.4.7. Leaf dry weight

There was a significant experiment x genotypes x harvest (*p< 0.001*) interaction for leaf dry weight (Supplementary Table 1). Overall, a higher leaf dry weight was recorded in the 2016 field experiment compared to the 2017 and 2018 experiments at both flowering and maturity (Supplementary Figure 4).

4.4.8. Gas exchange

Gas exchange parameters including photosynthesis (*A*), stomatal conductance (*Gs*) and transpiration rate (*E*) were taken from the flag leaf of each plant at the flowering harvest only for 2016, 2017 and 2018 experiments. We point out that the highest *A* was recorded in the non-inoculated genotypes in the 2016 trial, followed by the 2018 trial.

A significant interaction between harvest x genotype x inoculum was observed for *A*, for 2016 *(P<0.007),* 2017 (*p<0.001*) and 2018 (*P=0.014*) (Supplementary Table 2). The *A* in the 2016 and 2018 experiments was higher than that observed in the 2017 experiment (Figure 8). A significant reduction in *A* was recorded in all inoculated genotypes compared to non-inoculated genotypes across all three field experiments (Figure 7). The least reduction in photosynthesis was observed in Sunco in the 2017 experiment.

A significant harvest x genotype x inoculum interaction was reported for *Gs,* for 2016 (*P<0.000*), 2017 (*p<0.001*), and 2018 (*P<0.000*) (Supplementary Table 2). Stomatal conductance was significantly reduced in the inoculated genotypes in all three experiments with the exception of Livingston, IRN497 and 2-49 in the 2016 experiment (Figure 8).

A significant harvest x genotype x inoculum interaction for 2016 (*P=0.008*), 2017 (P<0.001) and 2018 (P=0.070), was observed in the field experiment for *E* (Supplementary Table 2). Transpiration was significantly reduced in the inoculated genotypes in all three experiments with the exception of Livingston, Syn110, Sunco and GW95-703*C15 in the 2016 experiment (Figure 10). The highest *E* in the non-inoculated genotypes was reported in 2-49 (3.66 H_2O m^{-2} s^{-1}) in 2016, AUS29529 (3.58 H_2O m^{-2} s^{-1}) in 2017 and Wylie (3.38 H_2O m^{-2} s^{-1}) and 2-49 (3.24 H_2O m^{-2} s^{-1}) in 2018. The highest *E* in the inoculated genotypes was reported in Livingston (3.21 H_2O m^{-2} s^{-1}) in 2016, Sunguard (3.04 H_2O m^{-2} s^{-1}) in 2017 and Livingston (3.09 H_2O m^{-2} s^{-1}) and Sunguard (2.89 H_2O m^{-2} s^{-1}) in 2018 (Figure 9).

4.5. Discussion

The current research aimed to investigate physiological differences in twelve wheat genotypes which vary in their susceptibility to crown rot caused by *Fusarium pseudograminearum.* Measurements were taken at the flowering and maturity growth stages across three field experiment seasons. In this study, significant differences in the development of disease symptoms on tillers, plant biomass including tiller and head numbers, stem, leaf, and head weight, shoot length and gas exchange parameters were observed between inoculated and non-inoculated genotypes across the field experiments of 2016, 2017 and 2018.

This research follows on from previous controlled environment experiments (Abdulsada et, al. 2023), where the fundamental physiological parameters of wheat genotypes are examined under field conditions, with the impact of climate considered on the disease severity and the host reaction during the three field seasons. The variation of disease severity caused by *Fp* infection among the three field experiments was observed at both flowering and maturity growth stages. The level of disease severity was more pronounced in 2018 at flowering and in 2017 at maturity compared to the 2016 seasons. Fluctuations in rainfall were seen during the three seasons. In 2016 and 2018 there was abundant rainfall during the beginning of the season and early spring, followed by a period of dryness towards the end of the season when the crops reached maturity. Conversely, in 2017, there were dry conditions at the start and end of the

season, with a significant amount of rainfall in October after flowering of the season during the flowering period. The severity of crown rot was significantly higher in all inoculated genotypes compared to non-inoculated genotypes. While rainfall and temperature varied across seasons, the partially resistant genotypes demonstrated lower disease severity in all experiments.

The susceptible genotype Mace was observed to have the greatest disease severity in the 2016 and 2017 experiments, followed by the susceptible genotype Livingston across all experiments when inoculated with *Fp.* Recent field experiments conducted by Saad et al. (2022) also demonstrated the cultivar Livingston to express high disease severity due to *Fp* inoculation. The finding of this study is also consistent with Abdulsada et al. (2023) where the disease severity was highest in susceptible and very susceptible such Gregory and Livingston in glasshouse experiments, also lower disease severity was observed in partial and moderate resistance genotypes such as 2-94, IRN497, W21MMT70, and Sunco. The lower disease severity was observed in the partially resistant genotypes IRN497 and Wylie. Moreover, the partially resistant and moderate resistant and tolerant genotypes was not as low as IRN497 and Wylie Given that maybe the distinct genes are accountable for CR resistance during the initial growth phases of wheat (Yang et al., 2010).

The shoot length of most genotypes was reduced under inoculation across all of the experiments, indicating the negative impact of *Fp* infection on plant development. Saad et al. (2021) reported a reduction in shoot length of a single variety of several cereals infected with *Fp.* In this investigation an important finding emerged regarding the variation in shoot length among different genotypes. A significant difference was observed between the susceptible, very susceptible and tolerant genotypes. However, it is worth mentioning that the partially resistant genotypes did not exhibit substantial differences in shoot length when compared to the other genotypes. Genetic research by Liu et al. (2010) and Yan et al. (2011) has demonstrated that CR infection in wheat is associated with a reduced height (*Rht*) loci located on different chromosomes and reported that short canopy wheat varieties have better resistance to CR. Another data of a real time PCR and histological analysis of barley infected with *Fp* showed that the cell density of dwarf isolines was higher compared to tall isolines and may act as a physical barrier to spread of FCR in cereal crops (Bai et al. 2015). However, the majority of the partially resistant genotypes in our study are tall lines and this area of research requires further investigation. This observation adds to our understanding of the varying responses of different genotypes to the experimental conditions.

In general, there was also a decrease in stem weight in the inoculated genotypes compared to the non-inoculated genotypes. Additionally, head dry weight was also reduced across most genotypes, particularly significant at maturity in the 2016 and 2017 experiments. The differences in head dry weight between the inoculated and non-inoculated genotypes were more pronounced in the susceptible genotypes compared to the partially resistant genotypes. This can be partly explained by the significant reduction in head number, particularly in the drier 2017 season. On occasion an increase in head number in the inoculated genotypes was observed, and this was associated with an increase in tiller number. Other glasshouse investigations on Livingston inoculated with *Fp* have indicated a significant decrease in shoot dry weight. The findings of this study align with the research conducted by Saad et al. (2022), who also conducted a two-year field investigation. The results of both studies indicate that there is a decrease in the total plant dry weight of Livingston when infected with *Fp*. In the present research, the total head number did not demonstrate a significant difference between the flowering and maturity growth. This finding can be attributed to the fact that *Fp*, the pathogen under investigation, primarily infects the lower stem.

The findings of this study reveal a significant reduction in photosynthesis (*A*), stomatal conductance (*Gs*), and transpiration (*E*) in all the genotypes when inoculated, as observed in the three field experiments conducted. The findings of this study demonstrate that CR displayed a significant impact on gas exchange parameters in all genotypes examined. However, it is important to indicate that the magnitude of this response varied among the different genotypes. This variability can be attributed to the varying field conditions experienced during the three-year duration of the experiment. This supports the findings of the investigation on wheat seedlings infected with *Fp* under controlled conditions, where gas exchange parameters of all genotypes (from susceptible to partially resistant to CR) were significantly reduced (Abdulsada et, al. 2023).

Gas exchange measurements are crucial for understanding the physiological alterations taking place in wheat stems infected by *Fp* under field conditions. Prior work has mostly concentrated on the effects of fungal infections on wheat ears and leaves, documenting decreases in *A* (Yang et al., 2021). However, there has been little investigation into the gas exchange processes occurring in soil and stubble-borne disease where symptoms occur on wheat stems. Gas exchange experiments have been conducted on various fungal diseases, including wheat powdery mildew caused by the biotrophic fungus *Blumeria graminis f. sp. Tritici* (*Bgt*), FHB

caused by *Fg* species, and stripe rust induced by the biotrophic parasite *Puccinia striiformis* (*Pst*), at different growth stages (Yang et al., 2021). The results of these studies have provided insight into the disturbances in *A* and gas exchange parameters caused by these diseases. Debona et al. (2014) investigated the *A* processes of wheat plants infected with the blast pathogen *Pyricularia oryzae*. The study found that *A* was reduced even before obvious symptoms developed, particularly during the early stages of growth. *Pyricularia oryzae* infection primarily affects spikes and spikelets, while CR mainly affects stems and crown regions. Another study by Rojas et al. (2014) have suggested that pathogens can extensively alter plant metabolism to promote infection during their interaction with host plants. Recent studies have revealed that the reduction in *A* pigment observed in infected wheat stems can be ascribed to physiological alterations caused by hemibiotrophic and necrotrophic fungal diseases. These alterations are linked to the activity of hydrolytic enzymes and the broad-spectrum toxins that specifically affect chloroplasts and proteins (Aucique-Pérez et al., 2020; Silva et al., 2022). In addition, Hu et al. (2020) investigated wheat plants that were infected with the biotrophic fungus *Blumeria graminis f. sp. Tritici*, which is known to cause powdery mildew in leaves. The researchers detected a decrease in the activity of genes related to *A*. Rios et al. (2017) have presented comparable declines in *A* in their study on wheat that was infected with *Bipolaris sorokiniana*.

The stomatal conductance of genotypes in this field study appears to be negatively influenced by *Fp* infection. The observed decrease in *Gs*, was found to be consistent across all inoculated genotypes compared to non-inoculated genotypes. Prior research has indicated a correlation between the decrease in photosynthetic rate *A* and a decrease in *Gs* as a result of the interaction between the host and pathogen (Lopes et al., 2001; Bermúdez-Cardona et al., 2015; Tatagiba et al., 2016; Yang, S. et al., 2016). Our findings align with the study conducted by Yan et al. (2016), which demonstrated a decrease in *Gs* in wheat plants infected with FHB while exhibiting resistance to the disease. Furthermore, Bender et al. (1999) conducted a comprehensive investigation on the stomatal activities of tomato plants infected by *Pseudomonas syringae* (*Pst*). Their findings revealed that *Pst* has the capability to synthesize a polyketide toxin known as coronatine, which subsequently induces modifications in stomatal performance. Stomata, being integral to the regulation of gas exchange, have a profound physiological connection to the intricate mechanisms of photosynthesis and respiration. Additionally, they contribute to the canopy's ability to fend off pathogen invasion, as highlighted by Prats et al. (2007).

In this investigation, all inoculated genotypes had a consistent decrease in the transpiration rate (*E*) in all three field experiments. The findings are consistent with the study conducted by Debona et al. (2014), which examined wheat leaves infected with *Pyricularia oryzae*. The researchers observed a decrease in *E* compared to the control samples, indicating that the reduction in *E* may be controlled by *Gs*. Furthermore, our results align with the research conducted by Bermúdez-Cardona et al. (2015), which demonstrated that decreased levels of *E* in maize leaves infected with *Stenocarpella macrospora* were linked to the closing of stomata. This closure serves as a mechanism to reduce excessive water loss. Multiple investigations support our findings, suggesting that infections caused by fungal pathogens might result in a decrease in *E* (Silveira et al. 2019; McGrath et al. 1990; Bassanezi et al. 2002; Alves et al. 2011; Dallagnol et al. 2011). The observed variation in gas exchange characteristics across the 12 wheat genotypes included in this experiment highlight the substantial diversity in genotype-specific reactions to the complex interaction between *Fp* infection under field conditions.

It is very important to notice that *Fp* as a hemibiotrophic/biotrophic fungal pathogen has the capability to strategically manipulate the tested fundamental physiological parameters of the infected wheat under field conditions. Previous microbiological literatures on hemibiotrophic fungal have provided an explanation. In order for plant pathogens to interact in a compatible manner, they must first overcome the intricate multilayered defence system (Abera Gebrie, 2016). Fungal chitin scavengers and shields prevent chitinases from damaging the fungal cell wall and chitin fragments, for instance, the effector of *Cladosporium fulvum* can hold functional chitin-binding domain (van Esse et al., 2007). To defend the cell walls of fungi, plants release beta-1,3-glucanases; however, many pathogens generate an inhibitor protein known as glucanase. Other effectors, including detoxifying enzymes called phytoalexins and proteinase inhibitors, may also contribute to the pathogen's success (Abera Gebrie, 2016). Attachment, host identification, penetration and proliferation are all steps in pathogenesis that biotrophic fungi must accomplish by constructing an infection structure. Complex regulatory processes and controlled gene expression limit structure creation (Kahmann, 2001).

The following characteristics are present in biotrophic fungi that contribute to their valuable virulence activity: elaborate structures for infection; secretory activity that is limited, particularly in terms of lytic enzymes; interfacial layers that are rich in carbohydrates and protein and separate the fungal and plant plasma membranes; the ability to suppress the host defence for an extended period; and haustoria that are utilised for the absorption and

metabolism of nutrients. In order to protect their effectors from plant receptor molecules, biotrophic fungi have various defence mechanisms. The resistance of the plant will not be effective once the pathogen passes defences. Subsequently, the plant decreases the release of salicylic acid as a signalling molecule (Mendgen and Hahn, 2002). Hence, *Fp* possible influence on wheat's main metabolism, including amino acid composition, hormone signalling, and organic acid synthesis, could potentially facilitate fungal infection.

The previous finding of metabolic changes which in turn have resulted in difference in wheat physiological parameters were present in different types of wheat cultivars, regardless of their resistance to crown rot. This emphasizes the complex nature of the interactions between plants and pathogens, and the resulting physiological adaptations. In conclusion, this research has identified the physiological response of 12 bread wheat genotypes with varying levels of resistance to CR when they were inoculated with *Fp* in a field setting. The results signified that *Fp* caused a significant reduction in height and biomass in very susceptible and susceptible wheat, as well as a reduction in photosynthesis and transpiration rates and stomatal conductance in all genotypes, with the greatest reduction observed in very susceptible and susceptible wheat. This study provides a pathological and physiological important information to improve our understanding of the development of CR across different wheat genotypes with varying levels of susceptibility. Therefore, further research is needed to fully understand the plant signaling pathways of genotypes that differ in their susceptibility to CR. Additionally, histological, fluorescence imaging of chlorophyll, biochemical, and molecular genetics investigations may help to establish a clearer relationship between commercial wheat and CR. In the future, it would be useful to replicate this study with a larger sample size and a longer observation period to further understand the physiological response of different genotypes to *Fp* infection.

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Table 4.1: Wheat genotypes included in the 2016, 2017 and 2018 field experiments and their response to Crown Rot diseases.

Genotype	**Reaction to CR**	**Year of experiments**	**Reference**
2-49	Partial resistance	2016, 2017 and 2018	Collard et al. (2005)
W21MMT70	Partial resistance	2016 and 2017	Bovill et al. (2006)
IRN497	Partial resistance	2016 and 2017	Wildermuth et al. (2001)
Sunco	Moderate resistance/tolerance	2016 and 2017	GRDC and DAFF (2014)
Syn110	Moderate resistance/tolerance	2016 and 2017	The University of Sydney (2014)
AUS29529	Partial resistance	2016 and 2017	Nicol et al. (2012)
Sunguard	Moderate resistant	2016, 2017 and 2018	GRDC and DAFF (2014)
Suntop	Susceptible	2016, 2017 and 2018	GRDC and DAFF (2014)
Gregory	Susceptible	2016, 2017 and 2018	GRDC and DAFF (2014)
Mace	Very susceptible	2016 and 2017	GRDC and DAFF (2014)
Livingston	Very susceptible	2016, 2017 and 2018	GRDC and DAFF (2014)
GW95-703*C15	Partial resistance	2016 and 2017	Bovill et al (2006)
Wylie	Moderate resistance/tolerance	2018	Zheng et al. (2014)

Table 4.2: Monthly rainfall (mm) for 2016, 2017 and 2018 collected at the Moyola station (5.9km from Wellcamp). Station Number: 041369; State: QLD; Opened: 1972; Latitude: 27.52°S; Longitude: 151.88°E; 559m. http://www.bom.gov.au/climate/data

2016	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Monthly total	75.0	72.8	41.8	5.4	6.0	95.3	42.1	45.4	91.0	31.0	73.5	39.2	618.5
2017	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Monthly total	79.6	23.0	185.5	11.6	17.3	18.8	37.8	2.8	0	160.5	25.4	90.6	652.9
2018	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Monthly total	19.2	149.4	59.0	8.0	5.2	14.4	10.6	7.8	17.6	108.6	39.4	44.4	483.6
Total	173.8	255.2	286.3	25	28.5	128.5	90.5	56	108.6	300.1	138.3	174.2	

Figure 4.1

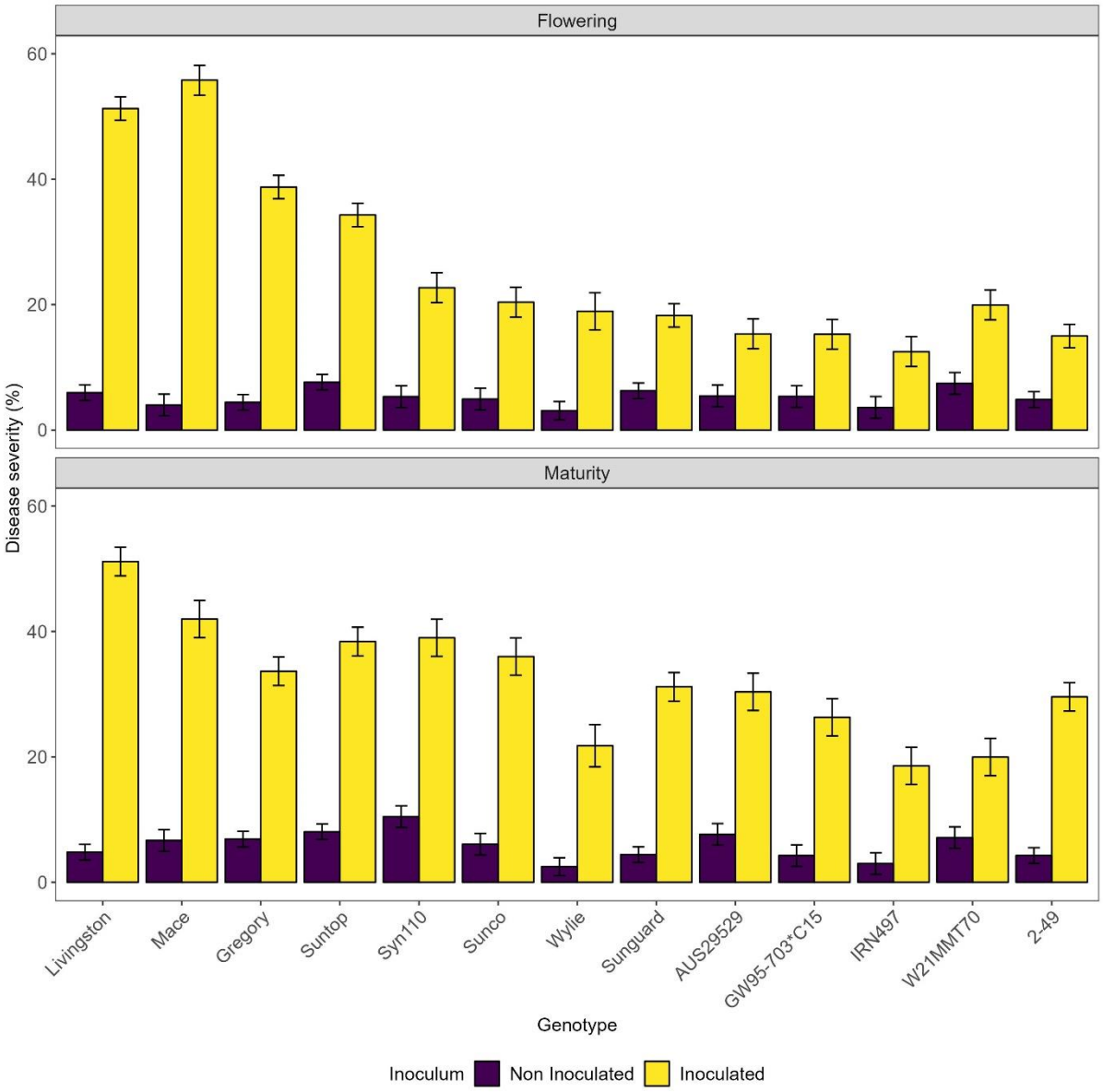

Flowering
Maturity
Disease severity (%)
60
40
20
0
Livingston
Mace
Gregory
Suntop
Syn110
Sunco
Wylie
Sunguard
AUS29529
GW95-703*C15
IRN497
W21MMT70
2-49
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 4.2

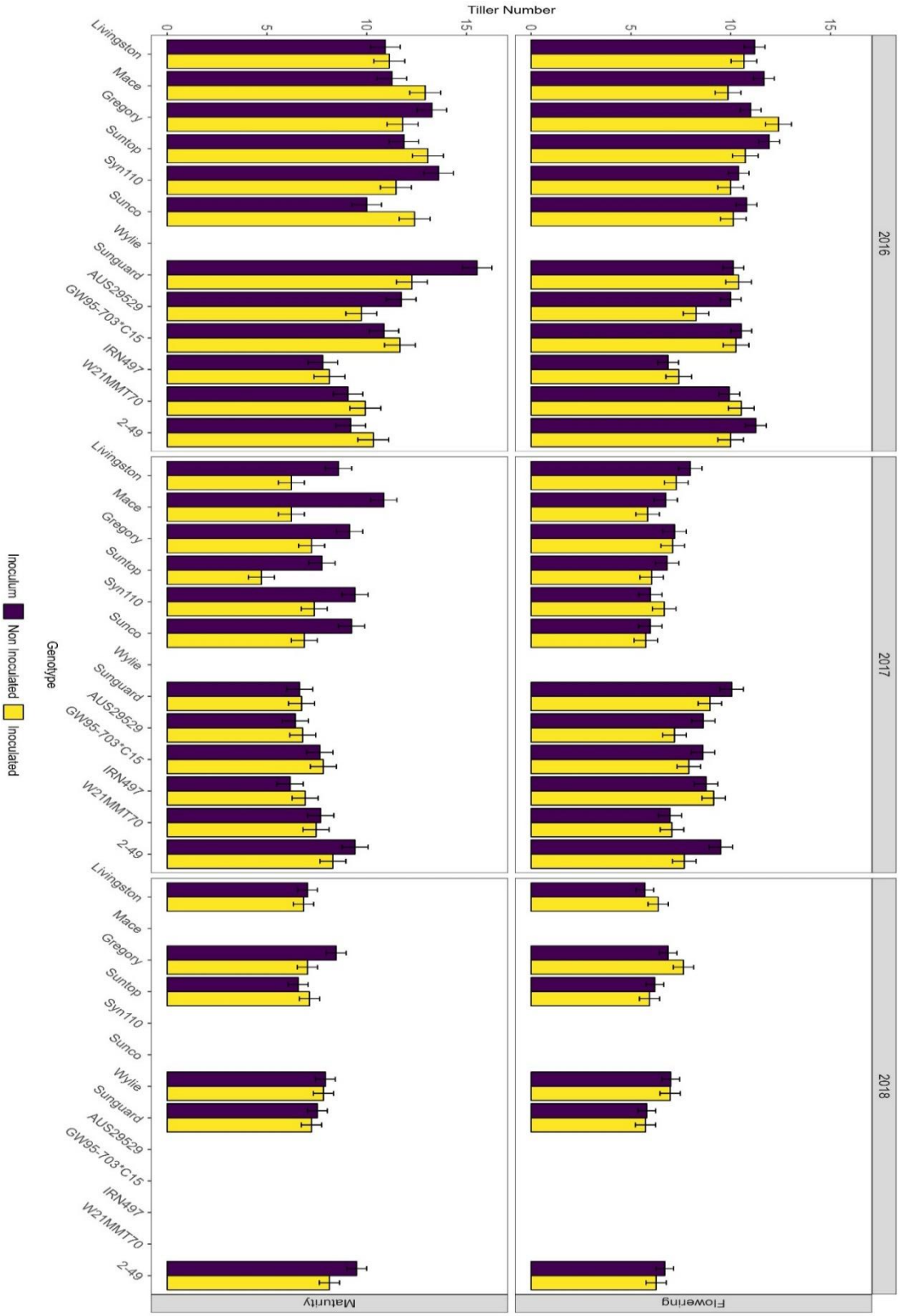

Tiller Number
0
5
10
15
2016
2017
2018
Flowering
Maturity
Genotype
Livingston
Mace
Gregory
Suntop
Syn110
Sunco
Wylie
Sunguard
AUS29529
GW95-703*C15
IRN497
W21MMT70
2-49
Inoculum
Non Inoculated
Inoculated

Figure 4.3

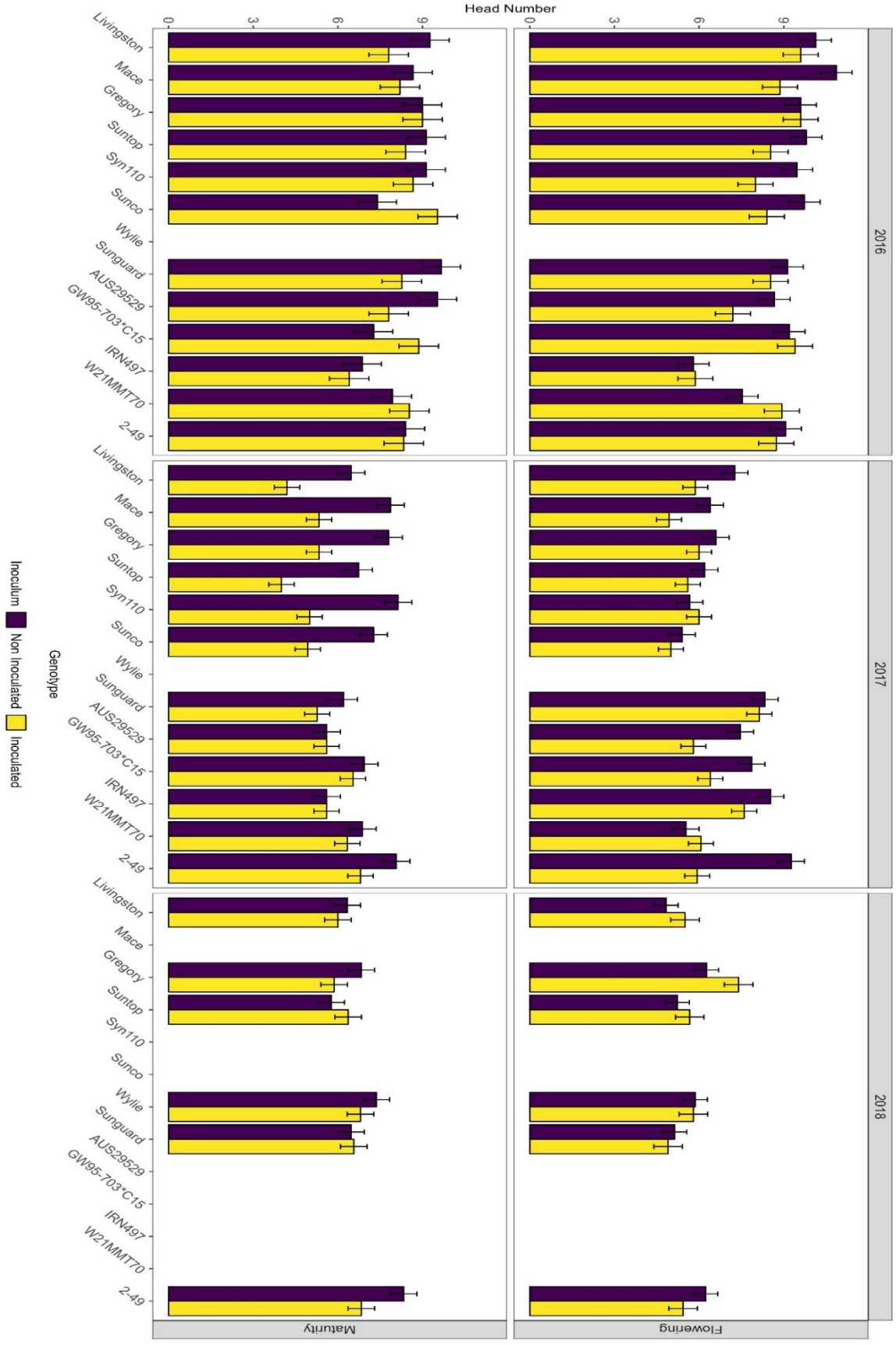

Head Number
0
3
6
9
Livingston
Mace
Gregory
Suntop
Syn110
Sunco
Wylie
Sunguard
AUS29529
GW95-703*C15
IRN497
W21MMT70
2-49
2016
2017
2018
Flowering
Maturity
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 4.4

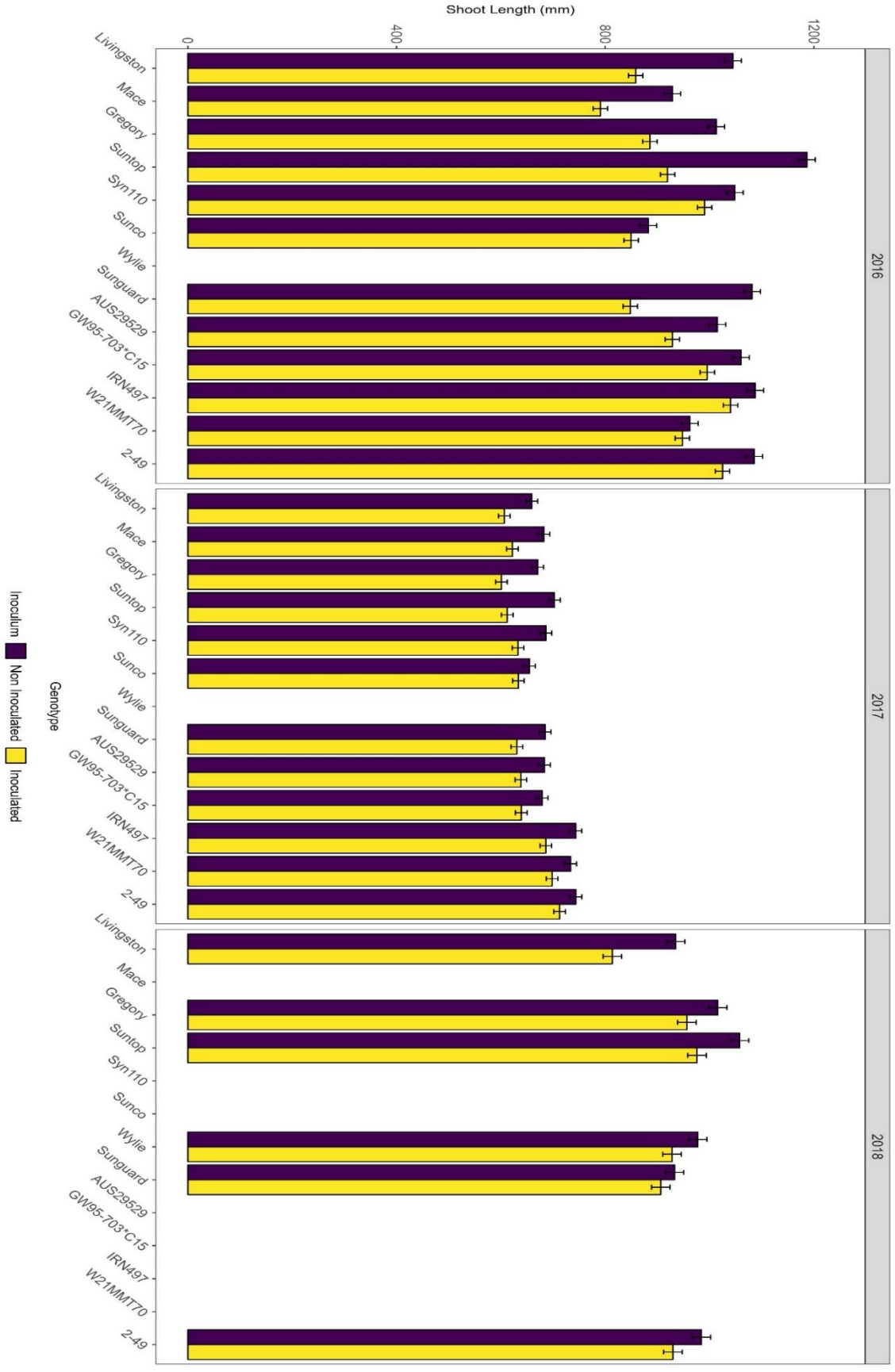

Shoot Length (mm)
0
400
800
1200
2016
2017
2018
Livingston
Mace
Gregory
Suntop
Syn110
Sunco
Wylie
Sunguard
AUS29529
GW95-703*C15
IRN497
W21MMT70
2-49
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 4.5

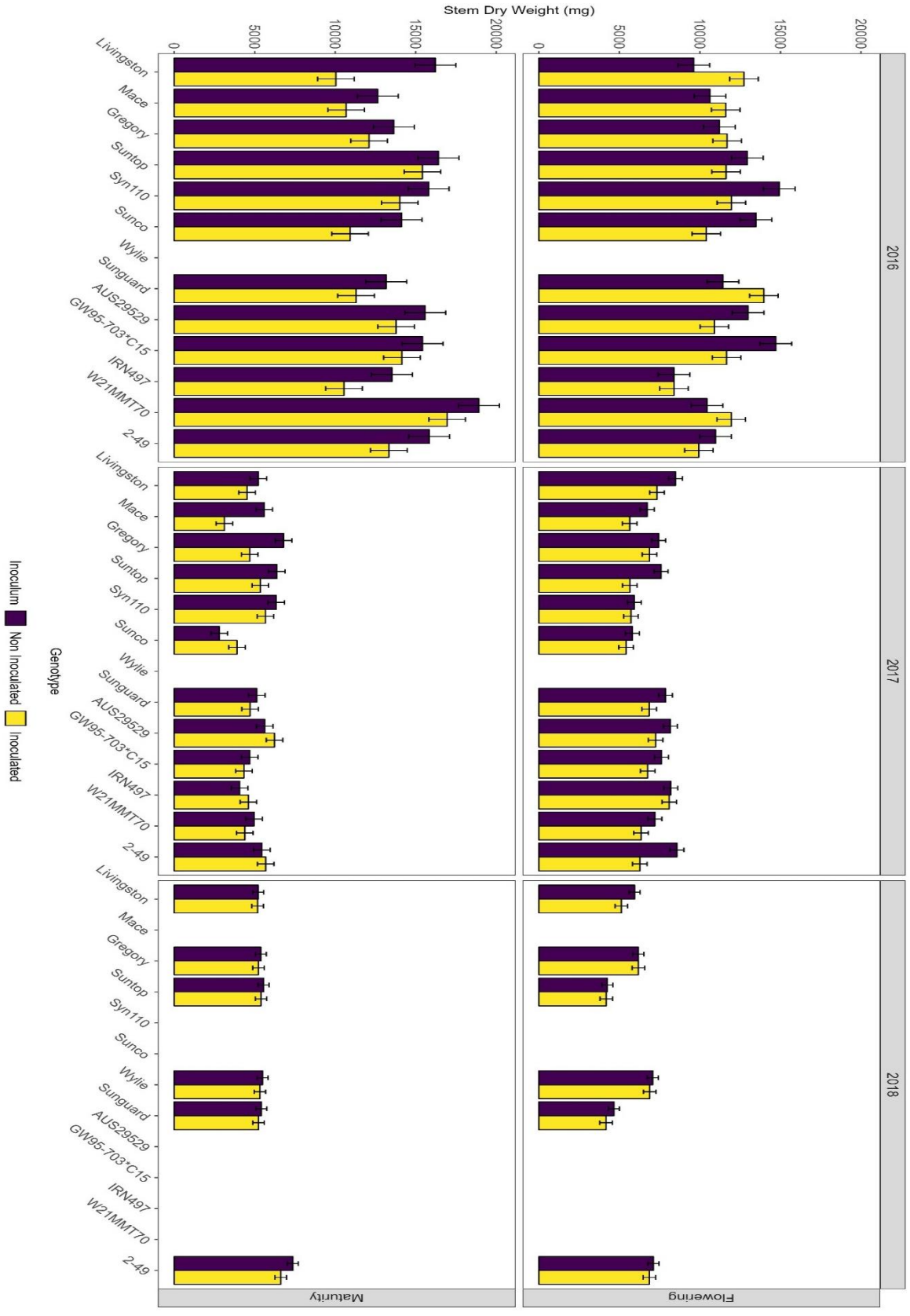

Stem Dry Weight (mg)
0
5000
10000
15000
20000
Livingston
Mace
Gregory
Suntop
Syn110
Sunco
Wylie
Sunguard
AUS29529
GW95-703*C15
IRN497
W21MMT70
2-49
2016
2017
2018
Flowering
Maturity
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 4.6

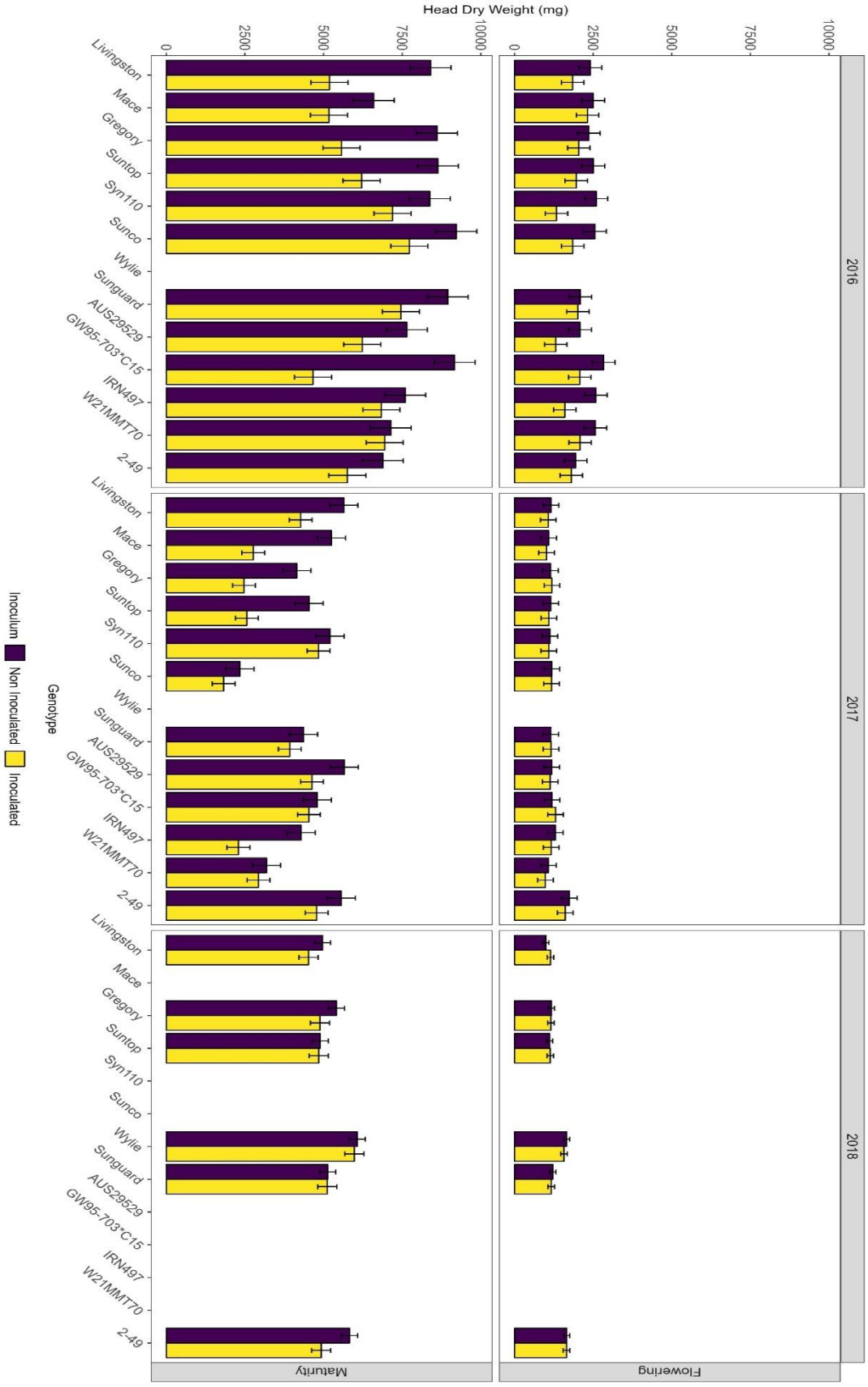

Head Dry Weight (mg)
0
2500
5000
7500
10000
Livingston
Mace
Gregory
Suntop
Syn110
Sunco
Wylie
Sunguard
AUS29529
GW95-703*C15
IRN497
W21MMT70
2-49
2016
2017
2018
Maturity
Flowering
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 4.7

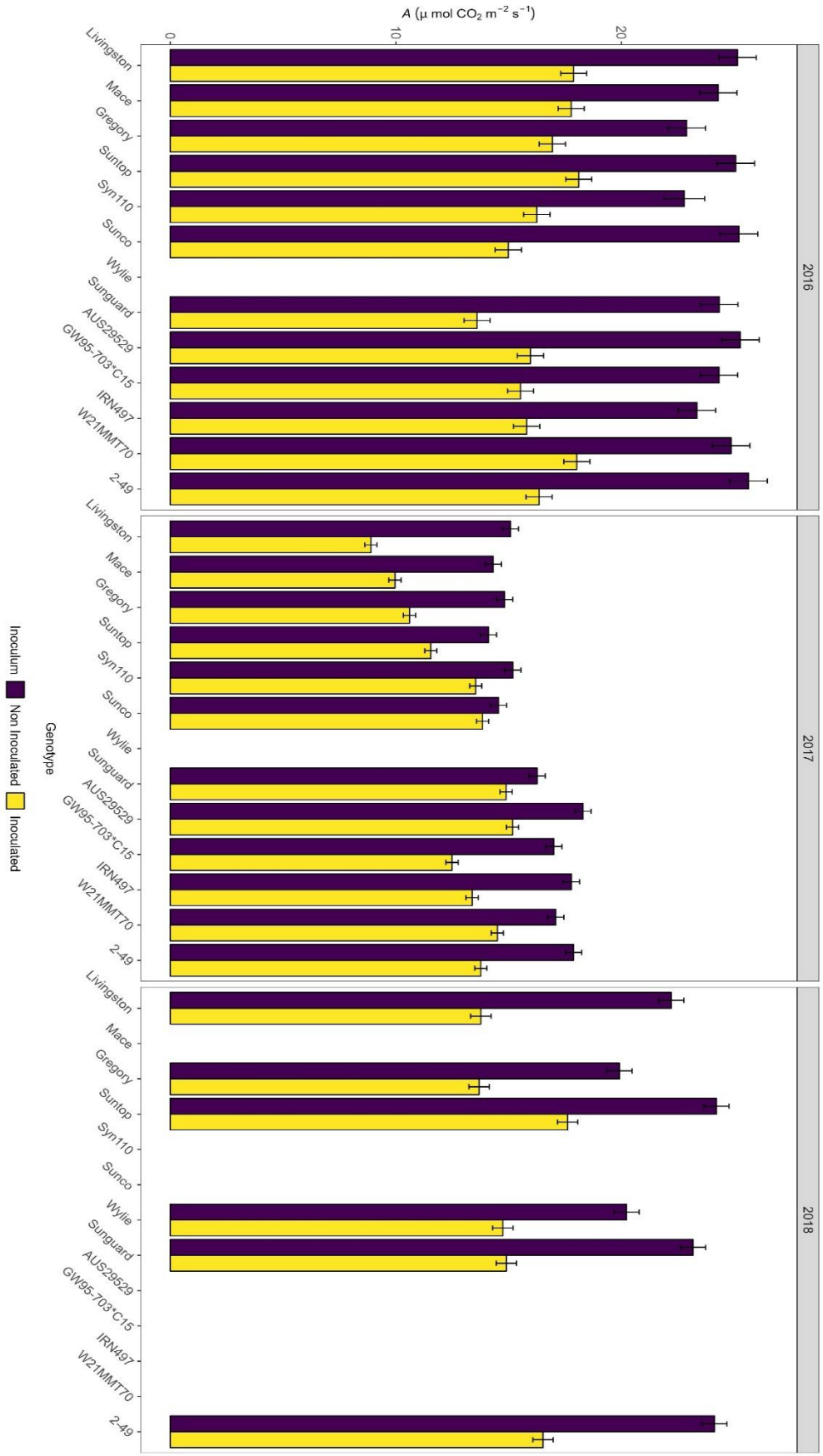

A (μ mol CO2 m−2 s−1)
0
10
20
Livingston
Mace
Gregory
Suntop
Syn110
Sunco
Wylie
Sunguard
AUS29529
GW95-703*C15
IRN497
W21MMT70
2-49
2016
2017
2018
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 4.8

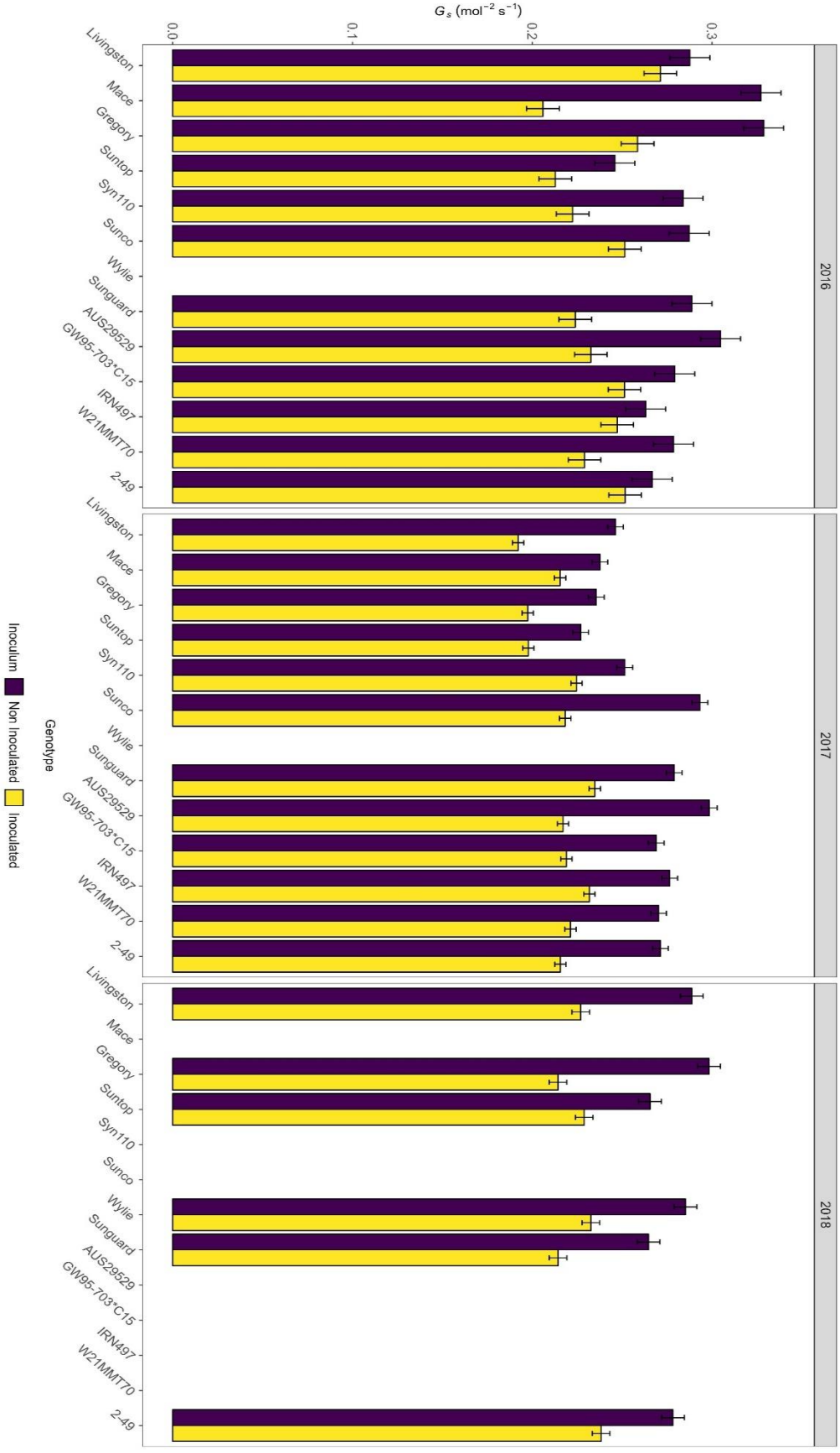
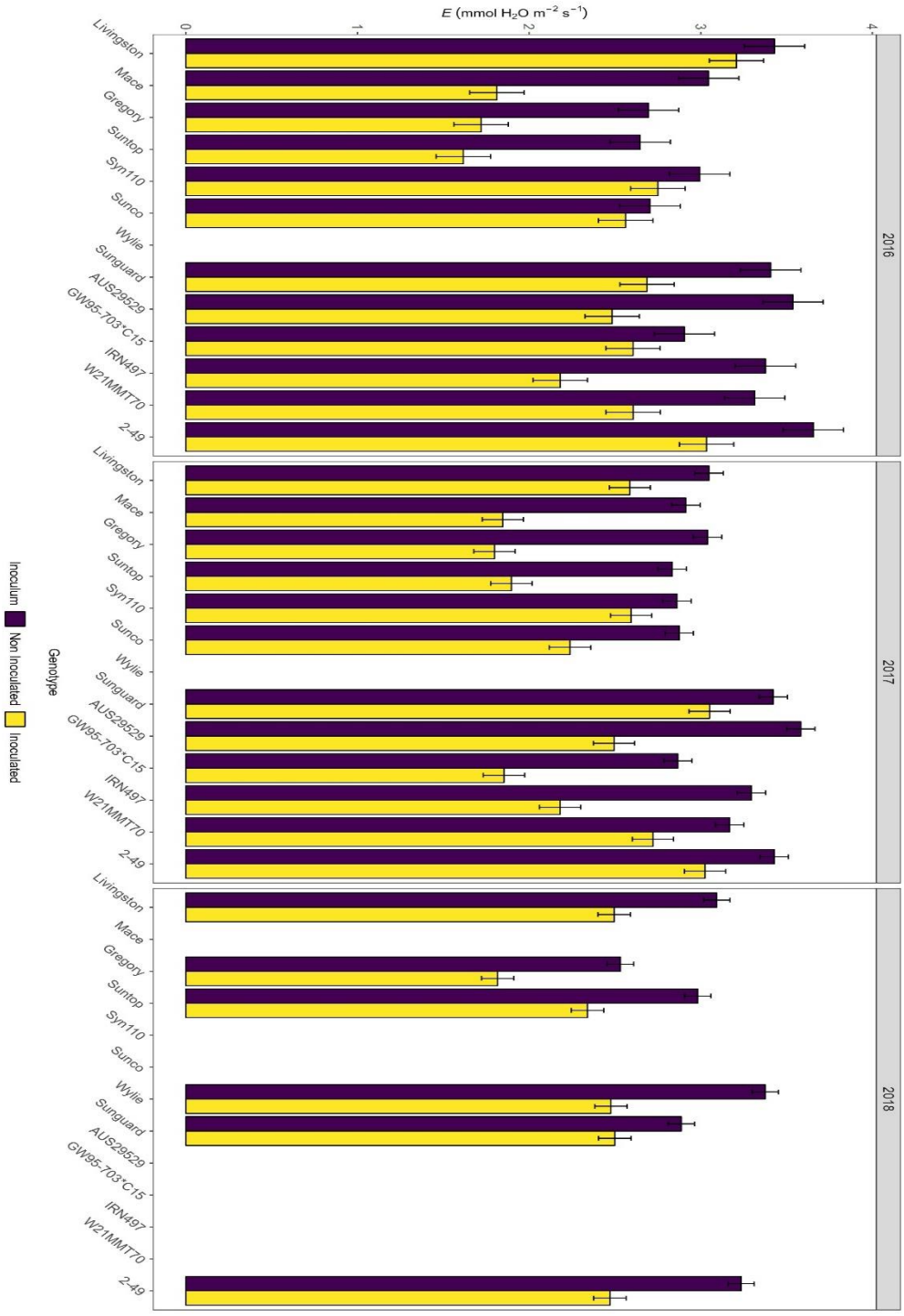

G_s (mol^{-2} s^{-1})
0.0
0.1
0.2
0.3
Livingston
Mace
Gregory
Suntop
Syn110
Sunco
Wylie
Sunguard
AUS29529
GW95-703*C15
IRN497
W21MMT70
2-49
2016
2017
2018
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 4.9


E (mmol H2O m−2 s−1)
0
1
2
3
4
Livingston
Mace
Gregory
Suntop
Syn110
Sunco
Wylie
Sunguard
AUS29529
GW95-703*C15
IRN497
W21MMT70
2-49
2016
2017
2018
Genotype
Inoculum
Non Inoculated
Inoculated

Supplementary Tables and Figures

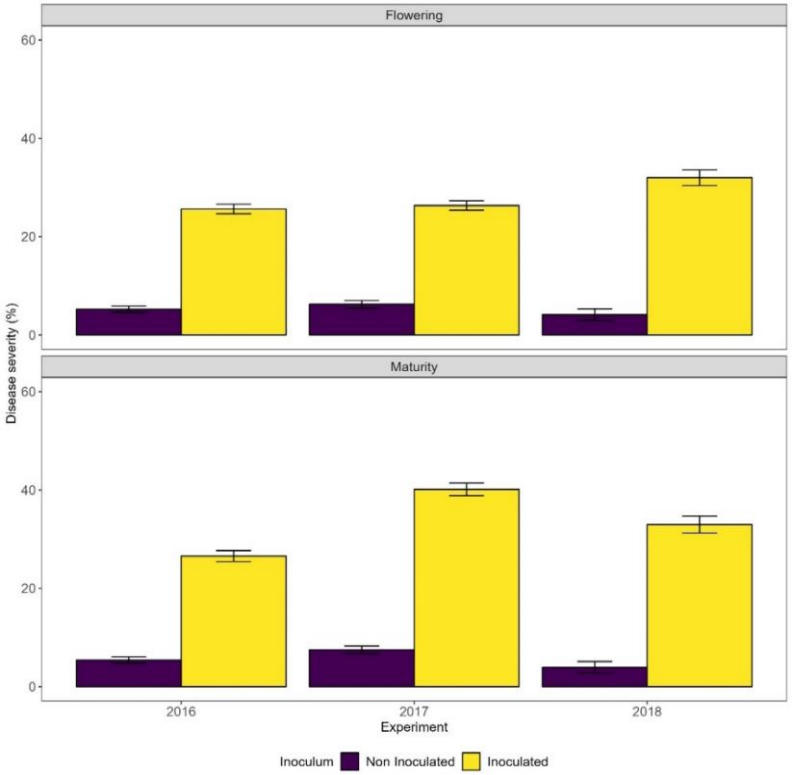
Supplementary Table 1: Analysis output of average disease severity, tiller number, shoot length, head number, stem dry weight, head dry weight and leaf dry weight of wheat genotypes inoculated and non-inoculated with *Fp* across three field experiments.

	Source	denDF	F.inc	F.con	Pr
Average disease severity of stems	Exp:Genotype:Inoculum	195.2	3.63	3.53	P< 0.0001
	Exp:Inoculum:Harvest	241.4	13.05	13.22	P< 0.0001
	Genotype:Inoculum:Harvest	324	5.91	5.91	P< 0.0001
Tiller number	Exp:Genotype:Inoculum:Harvest	200.8	2.691	2.691	P< 0.0001
Shoot length	Exp:Genotype:Inoculum	132.3	6.179	6.304	P< 0.0001
	Exp:Genotype:Harvest	126.6	6.161	6.047	P< 0.0001
Head number	Exp:Genotype:Inoculum:Harvest	209.9	2.1	2.1	P= 0.010
Stem dry weight	Exp:Genotype:Inoculum:Harvest	104.1	2.466	2.466	P= 0.003
Head dry weight	Exp:Genotype:Inoculum:Harvest	265	1.736	1.736	P= 0.044
Leaf dry weight	Exp:Inoculum:Harvest	195.3	10.43	7.392	P< 0.0001

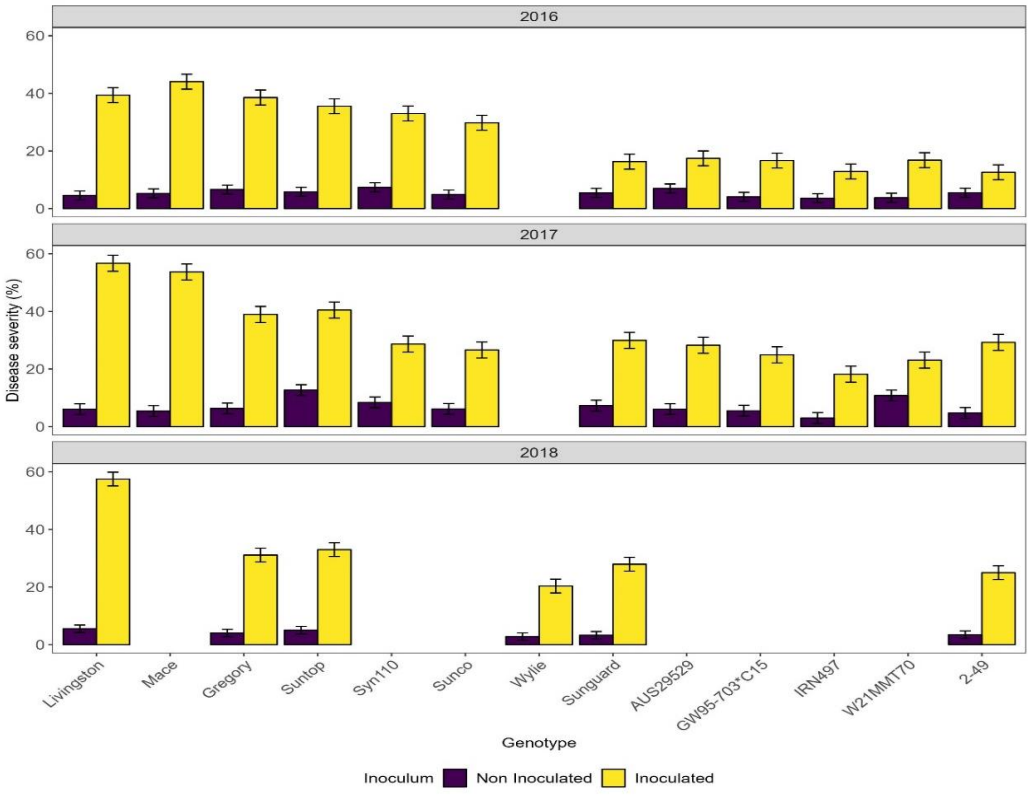
Supplementary Table 2: Analysis output for gas exchange parameters at flowering time of wheat genotypes inoculated and non-inoculated with *Fp* across three field experiments.

Gas exchange parameters	Year	Source	denDF	F.inc	F.con	Pr
Photosynthesis rate	2016	Genotype:Inoculum:Harvest	41	2.859	2.859	P< 0.007
	2017	Genotype:Inoculum:Harvest	107.4	12.41	12.41	P< 0.0001
	2018	Genotype:Inoculum:Harvest	10	5.044	5.044	P= 0.014
Stomatal conductance	2016	Genotype:Inoculum:Harvest	48	3.9	3.9	P< 0.000
	2017	Genotype:Inoculum:Harvest	48	9.016	9.016	P< 0.0001
	2018	Genotype:Inoculum:Harvest	348	4.646	4.646	P< 0.000
Transpiration rate	2016	Genotype:Inoculum:Harvest	22	3.319	3.319	P= 0.008
	2017	Genotype:Inoculum:Harvest	20.6	9.082	9.082	P< 0.0001
	2018	Genotype:Inoculum:Harvest	348	2.063	2.063	P= 0.070

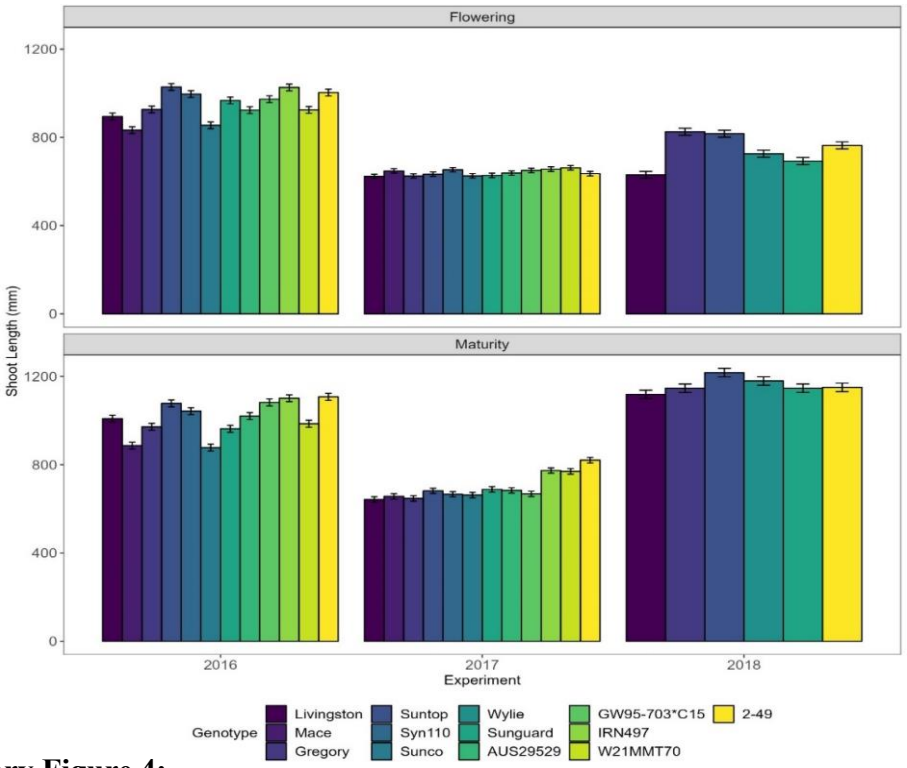
Supplementary Figure 1:



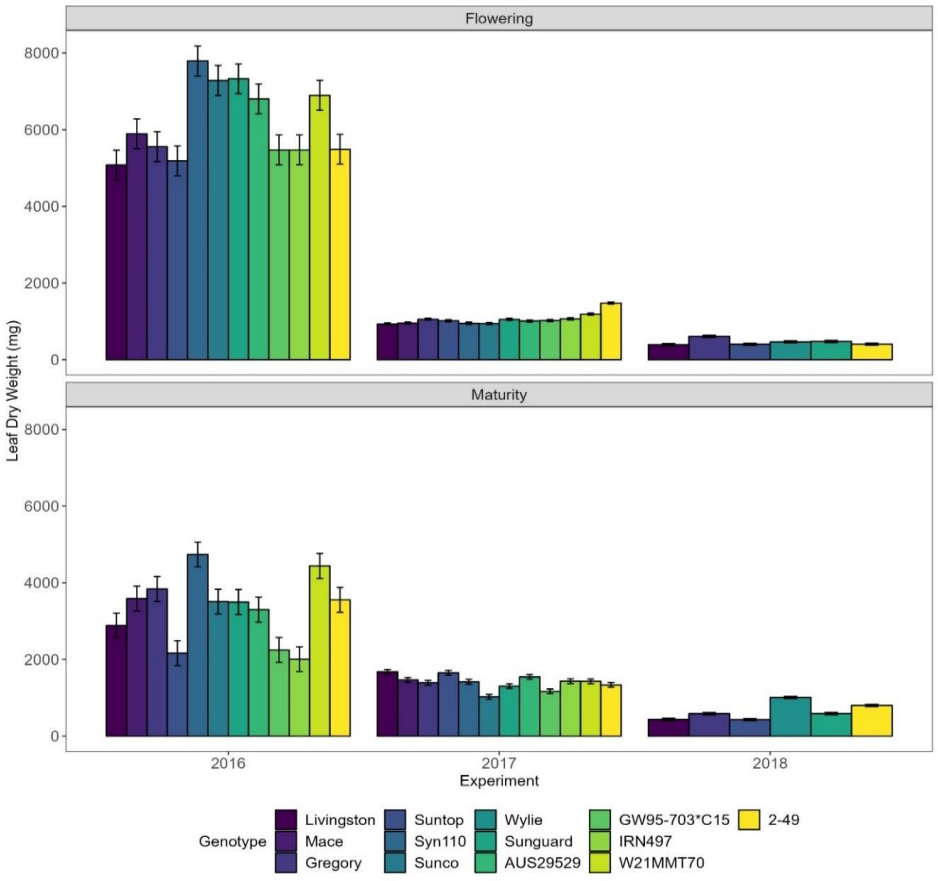
Supplementary Figure 2:



Supplementary Figure 3:



Supplementary Figure 4:



4.6 Link and Implications

This study aimed to improve our understanding of the physiology of *Fp* infection in wheat under field conditions. Importantly this investigation has examined large sets of wheat genotypes that differ in their response to CR at two developmental stages (flowering and maturity). Through the analysis of the effects of *Fp* infection on crucial factors of gas exchange and biomass production defined in plus and minus inoculated field experiments. The next chapter includes three field and glasshouse experiments, with plus and minus *Fp* inoculated plants, sampled at two developmental stages (flowering and maturity). In this next chapter the physiological measurements have been expanded to include stem water pressure, leaf area and carbon and nitrogen analysis in different plant parts.

CHAPTER 5 – PAPER 3: FUNDAMENTAL PHYSIOLOGICAL RESPONSES OF WHEAT (*TRITICUM AESTIVUM*) INFECTED WITH *FUSARIUM PSEUDOGRAMINEARUM* IN GLASSHOUSE AND FIELD TRIALS.

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2 Plant Protection Department, University of Misan, Amarah Governorate, 62001, Iraq.

3 Grains Research and Development Corporation (GRDC), Toowoomba, QLD 4350, Australia.

4 Department of Agriculture and Fisheries, Queensland, Australia.

Crown rot (CR) disease is a significant barrier to the development of wheat and barley globally. It is caused by fungus *Fusarium pseudograminearum* (*Fp)*. The objective of this study is to improve our comprehension of CR advancement in wheat by conducting a thorough analysis of essential physiological responses. This inquiry focuses on evaluating many characteristics, such as stem water pressure, biomass dynamics, leaf area, and carbon-nitrogen content analysis, as well as conducting further biomass measurements building on the datasets that have been presented in the previous chapter. This study again examines a range of genotypes which differ in susceptibility to CR in plus and minus *Fp* inoculated glasshouse and field trials. The data from this methodical investigation further develops our understanding of the CR disease and its effects on the wheat industry.

5.1. Abstract

Crown rot (CR) caused by *Fusarium pseudograminearm* (*Fp*) is a major disease of wheat and barley around the world. To date, wheat genotypes with complete resistance to *Fusarium pseudograminearm* have not been observed. Understanding the relationship between CR development, biomass, stem water pressure and carbon and nitrogen content is crucial. Inoculated and non-inoculated bread wheat genotypes with different CR susceptibility were tested to determine the difference between the inoculated and non-inoculated in both glasshouse and field trials. All genotypes consistently exhibited symptoms of CR upon inoculation. However, significant variations were reported in measurements across the glasshouse and field trials. Disease severity differed among genotypes at flowering and maturity. At flowering, in all trials, susceptible genotypes decreased in some biomass measurements such as head number, head weight, stem weight and leaf area compared to partially resistant genotypes. Head and dry weight, leaf area, and stem dry weight decreased in most genotypes when inoculated in glasshouse and field trials. Stem water pressure of inoculated stems of the six wheat genotypes increased. The flag leaf area displayed differences between inoculated and non-inoculated in total leaf area. Also, carbon and nitrogen content differed among inoculated plant parts. This research demonstrates the negative impact of CR disease severity on plant fundamental physiological measurement across genotypes which varied in susceptibility to *Fp* infection.

5.2. Introduction

Crown Rot (CR) presents a persistent impediment for wheat cultivation across diverse geographical areas, including Australia (Alahmad et al., 2018). *Fusarium pseudograminearum* (*Fp*) is the predominant fungal pathogen responsible for CR in Australia, occurring more frequently than *Fusarium culmorum* and *Fusarium graminearum* (Bockus et al. 2001; Akinsanmi et al. 2004; Nicol et al. 2004; Smiley et al. 2005) This disease has a substantial impact on both the quantity and quality of crops. In Australia, CR has been reported to cause a reduction in wheat yields by approximately 10%, leading to an estimated annual economic loss of AUD88 million (Murray & Brennan 2010). If not effectively managed, losses have the potential to increase to AUD434 million (Murray & Brennan 2009). Hollaway et al. (2013) reported on the impact of CR on yield in bread wheat and durum wheat within Australia, observing reductions ranging from 8% to 36% and 24% to 52%, respectively.

In addition to restricting crop productivity, *Fp* produces trichothecene mycotoxins, which are secondary metabolites that have the potential to negatively affect the quality of grains by contaminating food and feed products. The trichothecenes also demonstrate acute phytotoxic properties and have been identified as virulence factors in the infection of vulnerable host plants (De Boevre et al. 2012; Kazan & Gardiner 2018). The *Fp* variations reveal one of the three B type trichothecene chemotypes, namely deoxynivalenol (DON) + 3-acetyl-deoxynivalenol (3ADON), deoxynivalenol (DON) + 15-acetyl-deoxynivalenol (15ADON), or nivalenol (NIV) (Kirkegaard et al. 2004; Monds et al. 2005). Ward et al. (2002) proposed that the diversity in toxicity and bioactivity of trichothecene compounds is potentially regulated by a mechanism known as balancing selection. This mechanism is believed to have an impact on the pathogen's fitness and population dynamics.

Fusarium pseudograminearum is capable of surviving and overwintering on crop residues, such as stubble, which serves as the main reservoir for inoculum (Kazan & Gardiner 2018). During this period, the fungus exists in the form of mycelium or chlamydospores (Bennett 1930). These structures have the ability to germinate and develop, giving rise to sporodochia that produce asexual macroconidia (Leslie & Summerell 2008). These macroconidia are responsible for infecting the host plant and initiating disease symptoms when artificially introduced (Leslie & Summerell 2008). The spreading of the pathogen can be effectively achieved through the use of infected seeds (Klein & Burgess 1987; Marasas & Knox-Davies 1988). Yet the main source of inoculum in the field remains uncertain (Kazan & Gardiner 2018). The mycelium that arises from germinated macroconidia primarily invades the host plant by entering through stomata, thereby initiating infection in the coleoptile (Knight & Sutherland 2013). The organism progresses towards the subcrown internode, leaf sheaths, and subsequently infiltrates the epidermal tissues of the stem (Knight & Sutherland 2013). The described series of events leads to the invasion of the hypodermis, characterised by the unique occurrence of stem browning which in turn, the pathogen infiltrates the vascular tissues, as elucidated by Knight and Sutherland (2016). Additionally, previous research has demonstrated that *Fp* has the ability to spread from the base of the stem to the inflorescence through the pith parenchyma (Mudge et al. 2006). The colonisation of the pathogen is not constrained to a particular segment but rather has the ability to infest a minimum of three lower internodes within the host plant. The obstruction of water and nutrient transport in plants has been

attributed to the presence of mycelium and spores within the vascular tissues, resulting in the development of the distinctive white heads (Knight et al. 2012).

Strategies that have been recommended to manage CR include, inter-row sowing (Verrell, Simpfendorfer & Moore 2017), crop rotation (Kirkegaard et al. 2004), tillage and stubble management, and maintaining appropriate soil fertility (Cook 2001; Lamprecht et al. 2006). Tillage can reduce inoculum levels, however, this practice can reduce the crop yield by removing moisture that can be utilised during the season (Burgess et al. 2001). Combining biocontrol agents with fungicides has also shown an additional level of protection against CR. However, the applicability of these effects under field conditions for CR caused by *Fp* remains unclear (Moya-Elizondo & Jacobsen 2016). Currently, most of the bread wheat and all of the durum wheat cultivars are classed as very susceptible to moderately susceptible to CR (Percy, Wildermuth & Sutherland 2012; Lush et al. 2018), with no genotypes reported with complete resistance to *Fp*. Developing commercial cultivars with higher levels of CR resistant and tolerance will provide growers with an additional option for controlling CR. Partial levels of resistance occur in bread wheat lines and these have been incorporated into breeding programs to create new wheat cultivars with improved levels of resistance and tolerance to *Fp* infection (Forknall, Simpfendorfer & Kelly 2019; Kelly et al. 2021). Currently, most of the bread wheat and all of the durum wheat cultivars are classed as very susceptible to moderately susceptible to CR (Percy, Wildermuth & Sutherland 2012; Lush et al. 2018). However, there are no genotypes with complete resistance to *Fp*. Therefore, developing CR resistant genotypes can provide growers with an additional option.

Plant-pathogen interactions directly impact vegetative growth and development of crops, although the responsible mechanism of biomass reduction varies due to the habit of the pathogen (Walters 2015). Infection of host plants with a pathogenic fungus can cause major changes in the plant's structure and function (Mur, 2017). Biomass studies of wheat infected with *Fp* presented a reduction in shoot length and weight in one cultivar (Saad et al. 2021, 2022). The current study presents evidence of the detrimental effects of CR disease severity on fundamental physiological measurements across seven bread wheat varieties which range in susceptibility to *Fp* infection. Moreover, this is the first investigation to utilise stem water pressure measurement of wheat stems infected with *Fp*. We hypothesis that colonization of xylem vessels reduces water flow through the plant. The interaction between plants and

pathogen is complex, however, it can be influenced by many factors. Among these factors are Carbon (C) and Nitrogen (N) content.

Nitrogen is a fundamental and a necessary inorganic nutrient for plant development and a major constituent of nucleic acids, protein and secondary metabolites (Scheible et al. 2004). N availability can influence plant resistance to abiotic and biotic stresses. For instance, a previous investigation reported that plant resilience to abiotic stress can be altered by N supply due to the effect on plant development patterns and N-mediated singling transduction (Xuan, Beeckman & Xu 2017). Prior investigations have investigated various aspects of C and N metabolism, such as the regulation and interaction of these processes, plant-microorganism interactions, and how environmental stress affects C and N metabolism (Baslam et al. 2020). Furthermore, studies on the genomic, transcriptomic, proteomic and metabolic engineering aspects of C and N metabolism have revealed new insights into the molecular mechanisms underlying these processes (Zhang et al. 2010; Amiour et al. 2012; Huang et al. 2016). Despite this extensive research, no previous study has specifically examined the C and N content of wheat infected with *Fp*. As a result, this research makes a significant contribution to the field.

The primary objective of this study is to improve our comprehension of CR progression in wheat through the examination of fundamental physiological reactions. This encompasses the evaluation of various parameters such as stem water pressure, biomass, leaf area and C and N content in relation to the severity of the disease... This investigation hypothesis that wheat cultivars with different susceptibility to *Fp* infection will show a distinct physiological reaction when inoculated. Wheat cultivars with partial resistance to CR will possess a higher stem water pressure, biomass measurements, leaf area, and carbon-nitrogen content compared to more susceptible and very susceptible genotypes when inoculated with *Fp*. Moreover, the colonisation of *Fp* in the xylem vessels of the inoculated susceptible and very susceptible wheat genotypes will distract water flow via the plant, result in negative physiological effects compared to partial resistant genotypes. The research consists of both inoculated and not inoculated genotypes that range in susceptibility to *Fp* infection. The evaluation of these genotypes was conducted in both controlled glasshouse conditions and natural field conditions. Significantly, despite the existence of numerous previous studies on the metabolism of C and N, there has been a lack of specific investigation into the content of C and N in wheat infected with *Fp*. It highlights the importance of comprehending the plant physiological mechanisms

that regulate these processes, particularly in relation to wheat infected with *Fp*. This highlights the originality and unique contribution of this research to the scientific domain.

5.3. Materials and Method

5.3.1. Glasshouse Experiments

Three glasshouse trials were conducted in 2018 and 2019. Glasshouse trial 1 was conducted at the Leslie Research Facility, Department of Agriculture and Fisheries (LRF-DAF-Qld), in Toowoomba, Australia. Glasshouse trials 2 and 3 were conducted at the Agricultural Science and Engineering Precinct, University of Southern Queensland, Toowoomba, Australia. Twelve replicates of plus and minus inoculated pots of each of five wheat genotypes (Table 1) were included in each glasshouse experiment. Three seeds were planted in each medium-size pot (Diameter 135 mm, Height 140 mm and 1.3 L volume) and thinned to one plant after emergence. The plant growth medium consisted of a black vertisol obtained from Wellcamp, Queensland, mixed with river sand (50% soil to 50% sand by volume). Soil was pasteurised at 70°C for 30 minutes and air dried for 2 weeks prior to storage in a sterile dry container until required. Plants were inoculated one week after planting by placing one *Fp* colonised wheat grain at the base of each plant so that it was touching, and a further one cm of sand/soil mixture was added to the pot to cover the inoculum. *Fusarium pseudograminearum* inoculum consisted of sterilised durum wheat grain inoculated with an aggressive *Fp* isolate used in routine crown rot disease screening at the University of Southern Queensland (Percy, Wildermuth & Sutherland 2012). Plants were maintained at a 25°C/18°C day/night schedule in a temperature controlled glasshouse. The first trial used sterilised water, while glasshouse trial 2 and glasshouse trial 3 trials used filtered rainwater, with the plant watered to approximate field capacity as needed.

5.3.2. Field Experiment

A field experiment was planted on the 26th of June 2018 at the Department of Agriculture and Fisheries Wellcamp Research Field Station, Wellcamp, Queensland, Australia (27°33’53” S 151°51’52” E). The soil at the site was a self-mulching black Vertisol of the Irving clay soil association (Australian black earth) (Thompson and Beckmann 1959). Six replicates of plus and minus *Fp* inoculated paired plots of each of six genotypes (Table 1) were planted into 3m plots at a depth of 6cm using a Glen E Lee planter (Kingaroy Engineering Works, Australia).

Plots were arranged in a strip plot design, where two replicates of each paired genotype were randomly allocated across three replicate blocks. Inoculum was randomly allocated to each strip and applied at a rate of 2.2 g/m row above the seed at planting. Inoculum consisted of sterilised millet grain colonised with a mixture of 5 aggressive *Fp* isolates used in routine crown rot disease screening at the University of Southern Queensland (Percy, Wildermuth & Sutherland 2012). Urea (100 kg N/ha) was applied three weeks before sowing at a depth of 50 mm and starter fertiliser (60kg/ha) was applied into the furrow at planting. The experiment was irrigated with 50mm on the 7/8/2018, and weed management was achieved through manual chipping throughout the season. Rainfall were collected across the three years from the Moyola weather station, 8.1 km from the site of the experiments (Table 3).

One set of plus and minus inoculated plots of each genotype from each of the three replicate blocks were sampled at flowering on the 18th of September 2018 (Zadoks growth stage GS60-69) and the second set of replicate plots were sampled at physiological maturity (Zadoks growth stage GS90-99) in November 2018.

5.3.4. Disease severity measurements

Disease severity ratings were conducted on plants sampled in both field and glasshouse trials at flowering (GS60-69) and maturity (GS90-99). Ten plants from each of 3 replicate plots (field experiment) and one plant of the 6 replicate pots (glass house experiments) of each of plus and minus inoculated genotypes were assessed. The number of diseased tillers per plant were counted and the disease severity was then measured by recording the percentage of brown discoloration on the lower 15 cm of each stem by using a 0-100% rating scale where 0 = no discoloration and 100% = completely discolored tissue. Disease severity was measured on the main tiller and two primary tillers from each plant. Discoloration was identified as a honey brown to dark brown color.

5.3.5. Stem water pressure

Following on from disease rating, the ten (field) and six (glasshouse) main stems from each treatment sampled at flowering time were tested for stem water potential. Testing of water stem pressure was conducted in the daytime (10:00 am- 01:00 pm). Firstly, the tiller was cut to fit the chamber ranging from 7 to 12 cm start from the lower stem (Figure 1 A and B) and measured

with a pressure bomb (PMS Instrument Co, model 1000, USA). Later the cut stem was covered by commercial straw, then the chamber was gradually pressurized with compressor gas until bubbles of sap appeared at the cut (the pressure of N_2 gas can direct the gas from the bottom of the stem to the top of the cut section) (Figure 1 C).

5.3.6. Flag leaf area

The ten (field) and six (glasshouse) main stems from each treatment sampled at flowering time were tested for the flag leaf area by using a leaf area meter (LI-3100C Area Meter; LI-COR, Lincoln, NE).

5.3.7. Biomass

The head, leaf and stem of each plant sampled for glasshouse and field experiments were separated and transferred to a dehydrator (Wessberg Martin Engineering Pty Ltd, dryer/oven Germany) and dried at 60°C for three days. After drying, the separated samples were individually weighed.

5.3.8. Carbon and Nitrogen content analysis

Following the plant drying and weighing, separated samples (grain stem, leaf) were ground by using a Cullatti (Cullati MFC grinder, CZ 13) micro-whisk mill with a sieve size 1mm. All leaf material, grain, and lower stem (0-7 cm) were collected to determine C and N content with the LECO CN628 elemental analyser (LECO, St. Joseph, MI, USA).

5.3.9. Data Analysis

An across experiment analysis of the stem water pressure, biomass, leaf area, C/N concentration and disease severity measurements was conducted using a linear mixed model. A separate analysis was conducted for each trait. Disease severity required a different modelling approach based on the structure of the experimental material measured. Additionally, to fulfil model assumptions, the disease severity trait was transformed using a square root transformation. Traits that were only measured in the field were analysed using a single trial analysis approach.

In each glasshouse experiment the pot formed the experimental unit and one plant per pot formed the observation unit. In the field experiment, the plot formed the experimental unit and ten plants per plot formed the observational units. While stem water pressure, biomass, leaf area and C and N content contained one value per observational unit, disease severity was measured from the main, second and third tillers of each plant, thus three values from the same observational unit. The models fitted to each trait included experiment, genotype, inoculum and harvest time, along with their respective interactions, as fixed effects. Additionally, for the disease severity trait, a fixed effect of tiller was also included, along with all resulting interactions with the previously described terms. Terms describing the structure of the experimental material - bench, replicate blocks and pots for glasshouse experiments and replicate and other blocking terms for the field experiment - were included as random effects in both models, with additional terms for tillers included in the disease severity model. Heterogeneity of residual variance was modelled between experiments and, where significant, the complexity of the residual variance structure was extended to allow for heterogeneous residual variance between inoculum and/or harvest treatment groups within and between experiments. Furthermore, for disease severity, an unstructured covariance model was considered to account for residual correlation between tillers of the same plant.

Models were fitted using the ASReml-R package (Butler et al. 2018) in the R statistical computing environment (R Core Team 2020), with variance components estimated using residual maximum likelihood (REML; Patterson & Thompson 1971). The significance of fixed effects was tested using a Wald conditional test, while the significance of increasingly complex residual variance models was tested using a log-likelihood ratio test. All significance testing was performed at the 5% level.

5.4. Result

5.4.1. Disease severity measurements in glasshouse and field trials.

Disease severity was assessed for each plant based on the visual appearance of brown/black lesions at the base of wheat tillers. Three tillers; the main (M), a secondary (S), and a tertiary (T), were collected at flowering and crop maturity to determine disease severity for glasshouse and field trials. A significant experiment x tiller x harvest interaction ($P = 0.0007$) was observed for disease severity (Table 2). In general, the main stem had the highest disease severity, followed by the secondary tiller and the tertiary tiller. No significant differences were reported

between tillers in the field trial at maturity (Figure 2).

A significant interaction was also observed between experiment x genotype x inoculum x harvest for disease severity (*P = 0.0002*) (Table 2). Low levels of disease were observed in non-inoculated genotypes across all experiments at both flowering and maturity (Figure 3). In the inoculated plants cvs. Livingston and Gregory generally had higher levels of disease than the partially resistant genotypes Sunguard, Sunco, 2-49 and Wylie, however, only Gregory was significantly higher than these genotypes in glasshouse trial 2, sampled at maturity. Disease severity in Suntop was higher than Sunguard, 2-49 and Wylie and lower than Livingston at maturity in the field trial. The highest levels of disease severity were recorded in Livingston in the field at flowering (62%) and glasshouse trial 1 at maturity (44%) (Figure 3 A and B).

5.4.2. Leaf area

A significant experiment x genotype x inoculum interaction ($P = 0.0034$) was reported for the Flag leaf area (Table 2). There were no significant effects or interactions for the field experiment (Data not presented). A significant reduction in leaf area was recorded in Livingston in glasshouse trial 3, in Sunco in glasshouse trials 1 and 2 and in 2-49 in glasshouse trials 1 and 3 (Figure 4). The average leaf area was not consistent across genotypes. Inoculated Gregory and Sunguard had greater leaf area compared to 2-49, Sunco, and Livingston in glasshouse trial 3 (Figure 4).

5.4.3. Head number

A significant experiment x inoculum x genotype interaction was observed for the number of heads ($p = 0.0031$) (Table 2). Generally, the inoculated plants had a lower head number then the non-inoculated plants. In glasshouse trial 1, this difference was significant in Livingston and Sunguard at flowering and Livingston at maturity (Figure 5A). In glasshouse trial 2 this difference was significant in Sunco at flowering and Livingston at maturity. In glasshouse trial 3, this difference was significant in Livingston, Gregory, Sunco and Sunguard at flowering, but only significant in Sunco and Sunguard at maturity. In the field trial the genotype 2-49, had a significantly lower number of heads in the inoculated plants compared to the non-inoculated plants at maturity. Head numbers were usually reduced by 1 or 2 heads/per plant (Figure 5B).

5.3.4. Head weight

A significant interaction between experiment x inoculum x genotype was observed for head weight (P = 0.0004). The average head dry weight at flowering was lower in inoculated Livingston in all three glasshouse trials and in Sunguard in glasshouse trials 1 and 2 (Figure 6A). No significant differences in head weight at flowering were reported in the field trial. The head dry weigh at maturity was significantly reduced in inoculated Gregory in glasshouse trial 1, Livingston and Sunco in glasshouse trial 2, Gregory, Sunco and Sunguard in glasshouse trial 3 and in Gregory and 2-49 in the field trial (Figure 6B).

5.3.5. Stem weight

A significant inoculum x experiment x harvest interaction (*P=0.0121*) was reported for stem weight (Table 2), averaged across genotypes. The stem dry weight was consistently higher in the non-inoculated treatment compared to the inoculated treatment across all trials, with the exception of glasshouse trial 2 at maturity (Figure 7). The greatest reduction in stem weight between inoculated and non-inoculated treatments was observed in glasshouse trial 1 at flowering (Figure 7A).

5.3.6. Stem water pressure

A significant experiment x inoculum interaction (*P = 0.0006*) was observed for stem water pressure (Table 2). Generally, the stem water pressure was highest in inoculated treatments compared to non-inoculated treatments across all experiments, however the difference between these treatments was greatest in the field trial (data not presented). There was also a significant genotype x inoculum interaction for stem water pressure (*P = 0.0135*) (Table 2). The stem water pressure was significantly higher in the inoculated genotypes compared to the non-inoculated genotypes with the exception of Sunco, whereby differences were not statistically significant (Figure 8). The highest stem water pressures were reported in the two inoculated susceptible genotypes, Livingston (14.3 bar) and Gregory (12.3 bar), compared to the other genotypes. The lowest stem water pressure when inoculated (9.1 bar) was observed in the tolerant genotype, Sunco (Figure 8).

5.3.7. Carbon content of head, stem and leaf

In terms of head C content, the interaction between the experiment x genotype x harvest was significant ($P < 0.0001$). Partially resistant genotype 2-49 displayed increased C content in the glasshouse 1 flowering. Wylie, a partially resistant genotype, exhibited the highest head C content during the field trial. During maturity, susceptible genotypes Livingston exhibited elevated head C content in the glasshouse trial 1 and glasshouse trial 2, and 2-49 was the highest in field trial (Figure 9).

The interaction between genotype x inoculum was statistically significant regarding stem C content ($P = 0.0004$). Generally, genotypes at maturity exhibited higher C content in the stem compared to a flowering. The average of C content inoculated genotypes was higher than the non-inoculated Inoculated genotypes. A very susceptible Livingston have observed a difference between inoculated and non-inoculated at flowering and, maturity inoculated and non-inoculated compared to other genotypes. Also, a moderate resistance Sunguard presented a difference between inoculated and non-inoculated (Figure 10). Moreover, other interaction between experiment, genotype, and harvest ($P = 0.0358$) (data not presented).

The analysis revealed a significant three-way interaction between the experiment x genotype x inoculum in relation to C content in the leaves ($P = 0.0004$). Few genotypes have presented a significant difference between inoculated and non-inoculated. Susceptible Gregory displayed a decrease when non-inoculated and increase when inoculated at glasshouse trial 1. However, at glasshouse trial 2, the very susceptible Livingston, showed increase when non-inoculated and decrease when inoculated. At glasshouse trial 3, Livingston reacted in contracts where increased when inoculated and decreased when non-inoculated. At field trial, only Sunguard showed difference compared to other genotypes where it is decreased when non-inoculated and increased when inoculated (Figure 11).

5.3.8. Nitrogen content of head, stem, and leaf

A significant experiment x genotype interaction for N content in the head was observed ($P < 0.0001$) (Table 2). The average of N content did not show any difference between inoculated and non-inoculated. However, the N content at maturity was higher compared to flowering growth. (Figure 12 A&B). For stem N content, a significant experiment and genotype interaction was observed ($P < 0.0001$) (Figure 13 A&B).

A significant interaction was observed for experiment x genotype x inoculum (*P = 0.0281)* for flowering and mature leaf N content. Significant variations were found between genotypes. In the glasshouse trial 1, highly susceptible Livingston reported a rise in N content when not inoculated, but a decrease when inoculated. In contrast, susceptible genotypes such as Gregory established a decrease in N content after inoculation but an increase in it when non-inoculated. After inoculation, moderately resistant Sunguard showed an increase in N content. However, in glasshouse trial 3, no significant differences were found. In the field trial, Suntop exhibited an increase in N content when inoculated but a decrease when not, whereas susceptible Gregory and partially resistant 2-49 showed an increase when inoculated but a decrease when not (Figure 14A). At maturity, only trials 2 and 3 in the glasshouse displayed differences. When inoculated, both the moderately resistant Sunco and the very susceptible Livingston in glasshouse trial 2 increased N content of leaves. Only partially resistant 2-49 showed reduced N content after inoculation in glasshouse trial 3 (Figure 14B).

5.4. Discussion

The research conducted on crown rot in the past two decades in Australia has primarily focused on identifying genetic resistance and integrating it into cultivars that are commercially viable. Additionally, researchers have also developed methodologies for evaluating the disease and quantifying the production losses experienced in wheat and barley crops affected by CR (Percy, Wildermuth ; Knight & Sutherland 2012; Hollaway et al. 2013; Forknall, Simpfendorfer & Kelly 2019; Kelly et al. 2021)**.** The focus of research on CR disease has been significant, but there is a noticeable lack of knowledge when it comes to understanding the complex developmental aspects of this disease, especially in terms of how the host plant reacts to infection. The lack of understanding regarding the overall influence of disease severity on basic physiological measurements and the varying vulnerability of different genotypes to *Fp* infection is a significant issue. In this investigation, fundamental physiological processes were investigated with significant differences reported in disease severity, head number and head dry weight, stem weight, plant stem water pressure, C and N content in head, stem and leaf tissues, sampled at both flowering and maturity, in response to *Fp* inoculation.

Initially, the brown discoloration that is associated with CR appears either as a small necrotic lesion on the coleoptile tissue followed by brown discoloration on the first leaf sheath and develops on the base of the first internode of stem tissue (Burgess et al. 2001; Kazan & Gardiner

2018). In our research, across each harvest time, the brown discoloration was recorded mostly at the stem base and developed into higher internodes in susceptible genotypes. Previous research reported the processes of fungal infection of internodes was mainly via stomata, with penetration hyphae growing across from the infected subtending leaf sheaths in close contact with the stem (Knight and Sutherland (2017).

In this study's experiments, the level of symptoms of CR in the glasshouse trials differed from CR in the field; however, they consistently revealed significant disease severity in the inoculated genotypes. Notably disease severity of the main tiller was significantly higher compared to second and third tillers at flowering across all glasshouse experiments. However, no significant differences between tillers were observed at maturity in the field trial. In our investigation, *Fp* caused great disease severity on Livingston and Gregory (44% - 62% severity) as very susceptible and susceptible respectively at flowering and maturity growth. The findings of this research are aligned with (Saad et al. 2022) where the *Fp* inoculated wheat genotype Livingston had the greatest disease severity on stem and sub-crown internode tissues in field trials. While crown rot symptoms might differ in the same field on the same host (Burgess et al. 2001), the findings of this study are also mostly consistence with chapter two (Abdulsada et al, 2023) and chapter three where the disease severity was higher in these susceptible and very susceptible genotypes. The disease severity of Suntop ranged from 35% to 38%, with Sunco exhibiting a moderate disease severity ranging from 10% to 25% in glasshouse experiments only. Lower disease severity was observed in the partially resistant genotypes, with 2–49 ranging from 5% to 25% in field and glasshouse experiments and Wylie ranging from 18% to 19% in the field experiment. These ranges are generally in line with the published literature (Percy, Wildermuth & Sutherland 2012; GRDC 2022; Saad et al. 2022).
Significant differences between *Fp* inoculated and non-inoculated genotypes were observed in the flag leaf area sampled at flowering, particularly in the glasshouse 3 experiment. However, the variations observed in leaf areas among different genotypes were not consistent in the glasshouse trials and non-significant in the field trial. The non-inoculated 2-49 and Livingston varieties exhibited larger leaf areas in comparison to Gregory, Sunguard, and Sunco, while Gregory and Sunguard exhibited larger leaf areas under inoculation. Sadras et al. (2000) conducted a study that provided evidence for the detrimental effects of *Verticillium dahilae* infection on sunflower plants. Specifically, their findings revealed a significant decrease in shoot biomass and leaf area, which serves as a clear indication of the wide-ranging influence of pathogen-induced stress on the overall morphology of plants. In addition, the research

conducted by Paul and Ayres (1987) sheds light on the impact of infections caused by necrotrophic pathogens on photosynthesis. For instance, common grass (*Senecio vulgaris*) inoculated with rust (*Puccinia legenophore*) in the leaf area resulted in reduced leaf areas in different plant species. The studies presented here collectively emphasise the intricate dynamics of interactions between hosts and pathogens, and how they can have various effects on leaf area responses. The inconsistent results obtained in our studies does not allow significant conclusions to be reached on the impact of *Fp* infection on the flag leaf area of bread wheat.

Our investigation reveals a new condition: a significant reduction in the quantity and dry weight of wheat heads due to infection by *Fp*. Our detailed investigation definitively shows that *Fp* infection has a significant impact on the reproductive structures of wheat, contrary to what was expected. A significant decline in the number of heads, with more pronounced reductions found in very susceptible, susceptible and tolerant genotypes. It is crucial to acknowledge that the environmental circumstances in both the controlled glasshouse and open field experiments played a significant role in the development of this fascinating set of results. The dry weight of stem tissue at flowering decreased when inoculated with *Fp* across genotypes in the glasshouse experiments. A reduction in dry weight was observed during the flowering stage when the plants were inoculated with *Fp*, in both the glasshouse trial 1 and glasshouse trial 3. Upon maturity, the reduction was observed in the glasshouse trial 3. Conversely, the remaining sample times exhibited no significant differences between inoculated and non-inoculated treatments. Saad et al. (2022) documented a notable increase in plant dry weight during the stages of flowering and maturity after inoculation with *Fp* in one field trial, characterised by a wet season. Moreover, the seedling investigations conducted by Saad et al. (2021) reported a consistent decline in plant dry weight across a range of winter cereals upon infection with *Fp.* These studies highlight the intricate and multifaceted dynamics inherent in the plant dry weight response to *Fp* inoculation, genetics and environmental conditions. Our findings highlight the variability observed across multiple experimental trials and growth stages, further emphasizing the complex nature of this plant pathogen interaction. This underscores the significance of accounting for a multitude of factors that impact plant biomass within the framework of fungal pathogen interactions.

Water stem pressure of inoculated stems of six of the seven wheat genotypes tested increased compared with non-inoculated plants. Differences between inoculated and non-inoculated

treatments was not significant in the tolerant cv. Sunco. All other inoculated genotypes required higher pressure, which means less water flow compared to the non-inoculated plants. Knight and Sutherland (2016) reported colonization of both xylem and phloem by *Fp* mycelium, leading to restricted water transfer. The development of fungal hyphae at infection sites can physically affect water transport, thus altering stem water status through pressure volume alternation in sensor cells and activating hydraulic signals (Hubbard et al. 2001; Christmann, Grill & Huang 2013). This is the first examination of stem water pressure for wheat infected by *Fp* that has been reported.

This is the first report on the impact of *Fp* infection on the C and N content in heads, leaves and stems. Tested genotypes have reported differences between inoculated and non-inoculated, however most differences were observed in leaves, most pronounced in the glasshouse and field trial including 2-49. Wylie, Livingston, Gregory and Sungaurd. Interestingly, genotypes that are prone to being affected by *Fp* revealed higher levels of C content when not exposed to the pathogen, which could indicate a fundamental variation in their metabolism that could impact their vulnerability to CR.

The leaf N content varied across genotypes and a range of responses to inoculation were recorded across the experiments, with significant variations at both the flowering and maturity growth stages. The range of responses among the genotypes following inoculation may indicate variations in metabolic adjustment to stress and could be crucial for comprehending the impact of N dynamics on disease resistance and development. Leaf N content at flowering was often higher in inoculated plants than non-inoculated ones in both glasshouse and field trials. Leaves have important functions in N metabolic processes, such as assimilation, nitrate reduction and amino acid transport, and provide support to other organs (Baslam et al. 2020). The content of the N head is achieved by assimilating N from the roots and through the recombination of previously assimilated N from the vegetative organs to the developing grain (Francesconi & Balestra 2020).

C and N may act as signals to regulate fundamental physiological activities such as photosynthesis, assimilation and absorption of nutrient in the interest of plant development through the signal transduction network (Palenchar et al. 2004; Goel et al. 2016). The study's findings emphasize the complex correlation between the C and N levels in wheat and its reaction to C restriction. The individual reactions of different genotypes to *Fp* infection, especially in relation to their C and N, serve as a basis for future studies aimed at understanding

the metabolic pathways and genetic determinants that determine resistance or tolerance to CR. Acquiring this understanding is essential for the advancement of improved wheat cultivars that possess enhanced resistance against CR.

The results of our study indicate that *Fp* effectively uses C and N resources from various parts of the plant, such as the head, stem and leaves. This use of nutrients leads to long-term advantages for the fungus. Controlling the proliferation of pathogens in the field of plant diseases can have a substantial impact on both the multiplication of pathogens and the defense mechanisms of plants. The complex relationship between *Fp* and wheat highlights the need for understanding how *Fp* utilizes C and N resources to develop while also affecting essential physiological indicators. According to the data presented by Neumann et al. (2004), the impact of N on diseases is more likely to be mediated by the host tissue's N content than by modifications to the canopy microclimate brought on by a larger canopy. N treatments given after canopy formation was complete, and hence after any meaningful influence on microclimate through canopy size, had a significant impact on epidemics.

Other opinion could support our findings that pathogens not only inhibit photosynthesis, which leads to the decrease of C reserves, but they also directly exhaust C reserves, speed up C consumption and raise the costs of restoration (Oliva, Stenlid & Martinez-Vilalta 2014). The majority of fungi have the capacity to metabolise accessible N sources by an array of mechanisms of regulation. Numerous studies have been carried out on N metabolism and regulation using the fungal model organisms Saccharomyces cerevisiae, *Aspergillus nidulans*, and *Neurospora crassa* (Marzluf 1997). These investigations have demonstrated that N supplies that can be easily absorbed, such as ammonium, glutamine, and glutamate, are preferable. However, when these primary sources are not readily available secondary sources such as nitrate, amino acids, and proteins can be utilised (Bolton & Thomma 2008). This suggests the presence of a physiological reaction that redistributes extra C and N to the leaves as a compensatory strategy during periods of stress, potentially revealing a continuous underlying pattern. Moreover, In the study conducted by Vary et al. (2015) the impact of acclimation to ambient and elevated levels of carbon dioxide (CO_2) on the development of *Septoria tritici blotch* disease symptoms on the moderately disease-susceptible wheat cultivar Remus., the findings indicate that prolonged exposure to CO_2 can have a favourable impact on the aggressiveness of the pathogen *F. graminearum* towards both susceptible and resistant germplasm.

In conclusion, this research has shown the significant variation of disease severity caused by *Fp* infection on seven wheat genotypes, which vary in susceptibility to CR at both flowering and maturity growth stages. Livingston and Gregory have shown the highest disease severity compared to other genotypes. The dry weight of stem tissue was reduced at flowering more than maturity harvest. Plant physiological investigation of *Fp* revealed that plant growth can be decreased due to infection. Water stem pressure has indicated that the colonization of *Fp* on infection site of the lower stem have a cause a restriction in water movement of inoculated wheat genotypes. Further physiological investigation is required to identify the disruption of water flow and subsequent phloem colonisation which could alter the dynamics of sugar transport in the plant, particularly the carbohydrate supply to the grain and roots. Variation of C and N was significantly observed in infected stem and leaf more than head, due to the infection site of *Fp.* This indicates that future studies are needed to understand the biochemical investigations such as mass spectrometry, mitochondrial proteome, and subcellular proteomics for gas exchange, C and N content of wheat during the development of CR infection of wheat in order to refine the knowledge of the cellular process network in susceptible and partially resistance genotypes to CR which in turn can contribute to better understanding of the metabolic process. The physiological analysis and data presented in this study provide valuable insights into the impact of infection on various aspects of wheat plants. This information can be utilized to develop effective disease management strategies and identify wheat breeds with high resistance to CR.

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Table 5.1: Wheat genotypes used in glasshouse and field trials and their response to crown rot as reported in the literature.

Genotype	**Crown Rot Response**	**Experiments**	**Reference**
2-49	partial resistance	Glasshouse and field trials	(Collard et al. 2006)
Sunguard	moderate resistant	Glasshouse and field trials	(Bovill et al. 2006)
Suntop	Susceptible	Field trial	(Wildermuth, McNamara & Quick 2001)
Gregory	Susceptible	Glasshouse and field trials	(Xie et al. 2006)
Livingston	Very susceptible	Glasshouse and field trials	(Burgess et al. 1996)
Wylie	Partial resistance	Field trial	(Zheng et al. 2014)
Sunco	Moderate resistance/tolerance	Glasshouse trial	(Wildermuth, McNamara & Quick 2001)

Table 5.2: Residual Maximum Likelihood (REML) Analysis output for disease severity, head number, head weight, stem weight, C and N of head, stem and leaf, stem water pressure, leaf water pressure, and leaf area of wheat genotypes inoculated and non-inoculated with Fp across three glasshouse trials and one field trial.

Trait	Significant Interaction	P-Value
Disease severity	Experiment x Genotype x Inoculum x Harvest	0.0002
	Experiment x Tiller x Harvest	0.0007
Head number	Experiment x Genotype x Inoculum x Harvest	0.0031
Stem weight	Experiment x Genotype x Harvest	<0.0001
	Experiment x Inoculum x Harvest	0.0121
Head weight	Experiment x Genotype x Inoculum x Harvest	0.0004
Carbon head	Experiment x Genotype x Harvest	<0.0001
Carbon stem	Experiment x Genotype x Harvest	0.0358
	Genotype x Inoculum x Harvest	0.0004
Carbon leaf	Experiment x Genotype x Inoculum x Harvest	0.0046
Nitrogen head	Experiment x Genotype x Harvest	<0.0001
Nitrogen stem	Experiment x Genotype x Harvest	<0.0001
Nitrogen leaf	Experiment x Genotype x Inoculum x Harvest	0.0281
Stem water pressure	Experiment x Inoculum	0.0006
	Genotype x Inoculum	0.0135
Flag leaf area	Experiment x Genotype x Inoculum	0.0034

Table 5.3: Monthly rainfall (mm) for 2018 at Moyola station http://www.bom.gov.au/climate/data Station Number: 041369; State: QLD; Opened: 1972; Latitude: 27.52°S; Longitude: 151.88°E; This station is 5.9km from Wellcamp.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Total rainfall (mm)	19.2	149.4	59.0	8.0	5.2	14.4	10.6	7.8	17.6	108.6	39.4	44.4	483.6

Figure 5.1

A **B** **C**

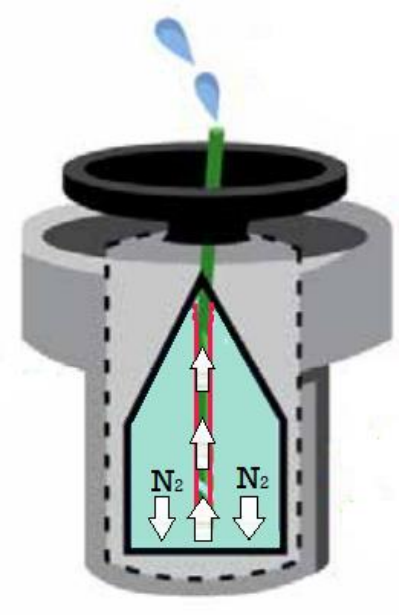


Figure 5.2

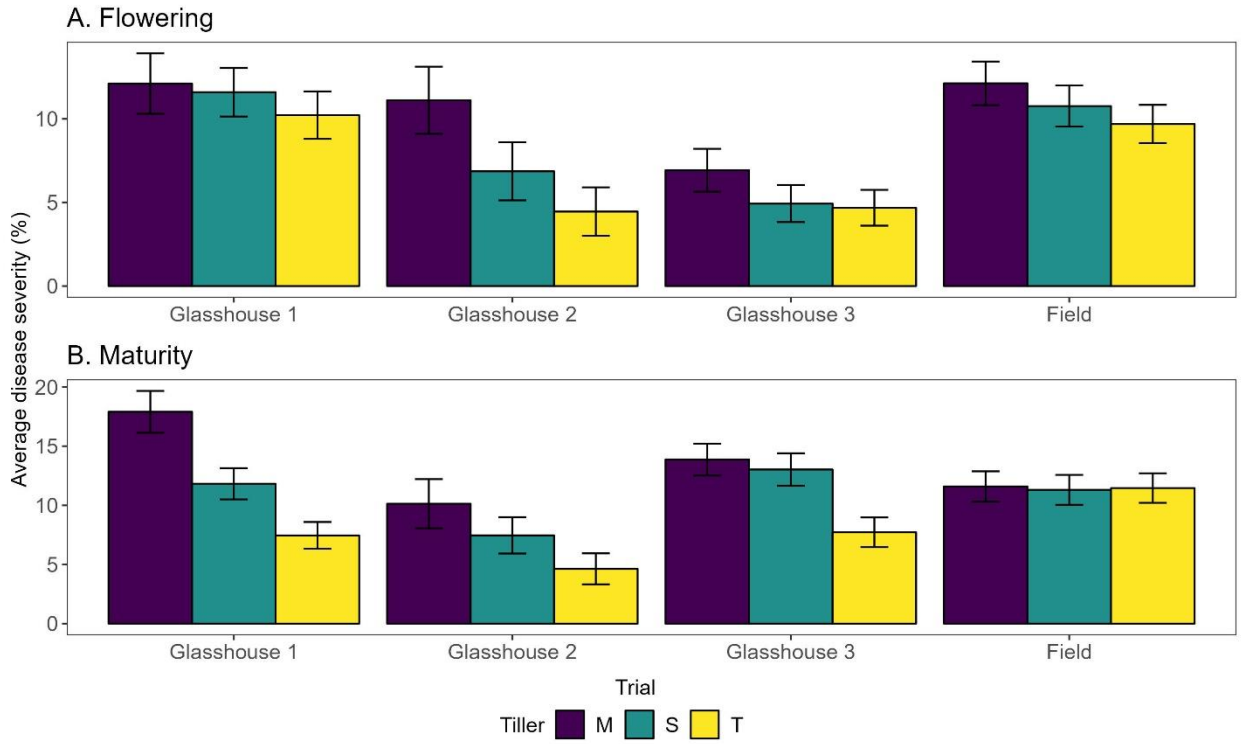

A. Flowering
Average disease severity (%)
0
5
10
Glasshouse 1
Glasshouse 2
Glasshouse 3
Field
B. Maturity
0
5
10
15
20
Glasshouse 1
Glasshouse 2
Glasshouse 3
Field
Trial
Tiller
M
S
T

Figure 5.3

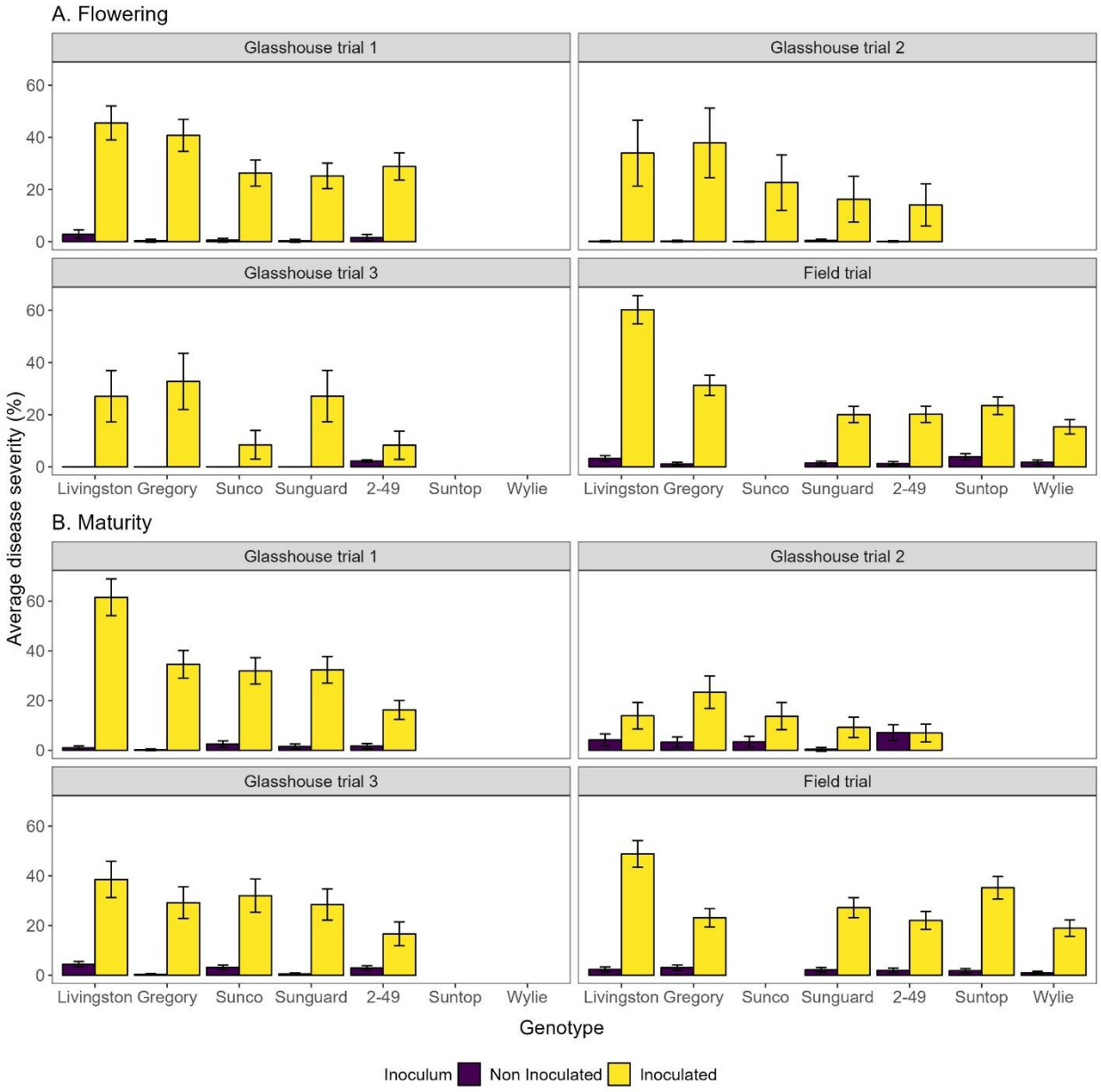

A. Flowering
Glasshouse trial 1
Glasshouse trial 2
Glasshouse trial 3
Field trial
B. Maturity
Glasshouse trial 1
Glasshouse trial 2
Glasshouse trial 3
Field trial
Average disease severity (%)
0
20
40
60
Livingston
Gregory
Sunco
Sunguard
2-49
Suntop
Wylie
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 5.4

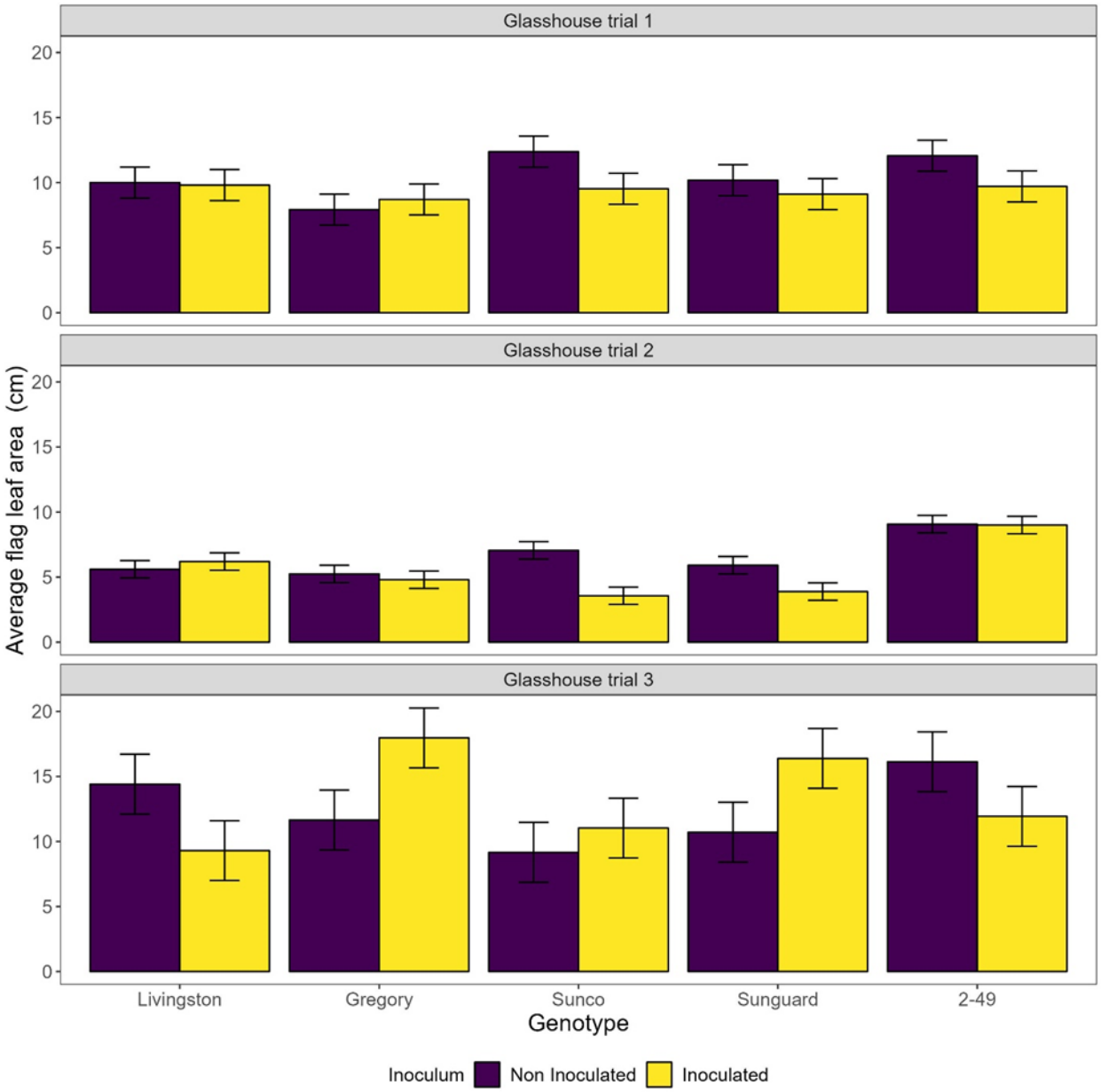

Glasshouse trial 1
Glasshouse trial 2
Glasshouse trial 3
Average flag leaf area (cm)
0
5
10
15
20
Livingston
Gregory
Sunco
Sunguard
2-49
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 5.5

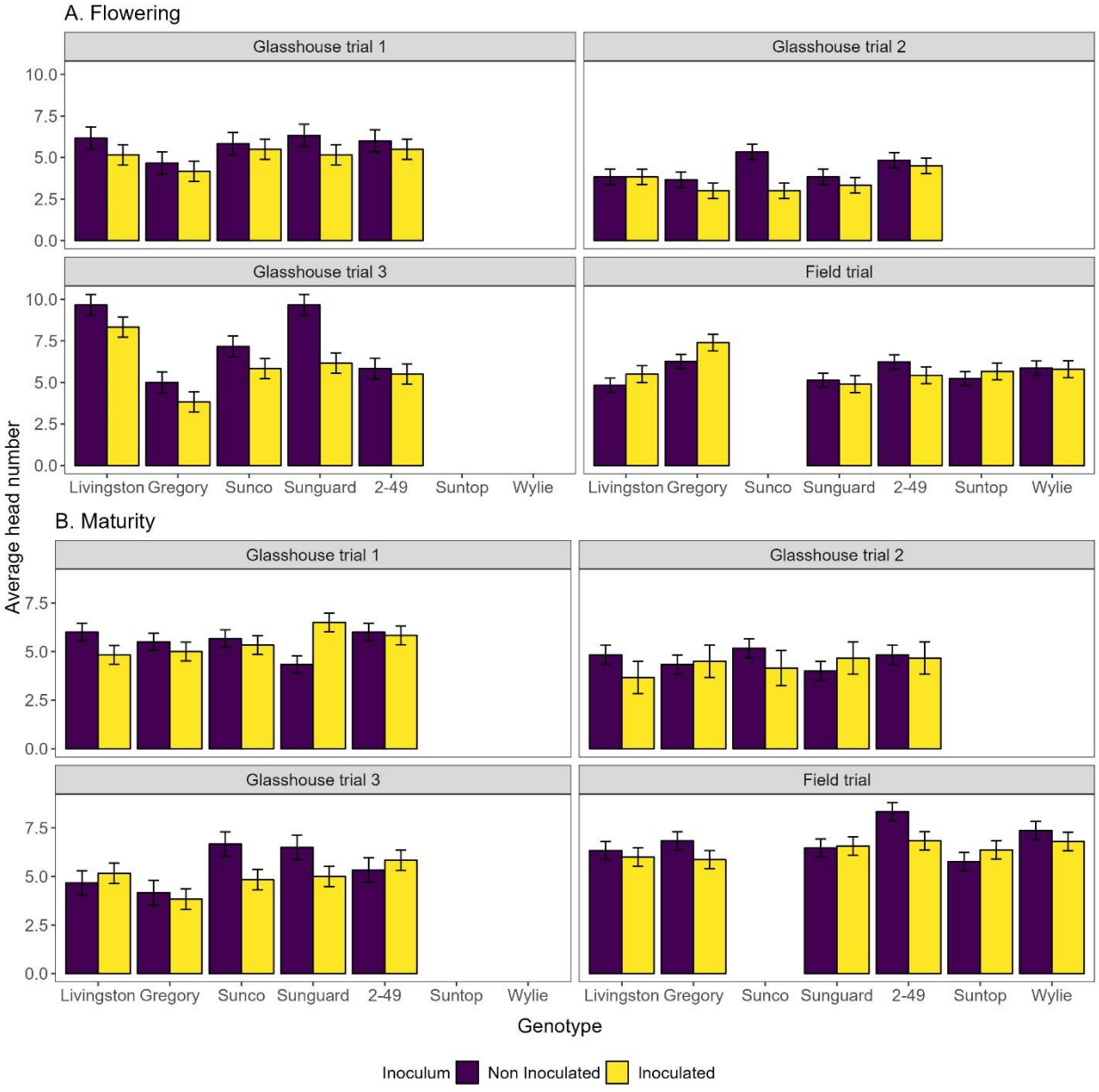

A. Flowering
Glasshouse trial 1
Glasshouse trial 2
Glasshouse trial 3
Field trial
B. Maturity
Average head number
10.0
7.5
5.0
2.5
0.0
Livingston
Gregory
Sunco
Sunguard
2-49
Suntop
Wylie
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 5.6

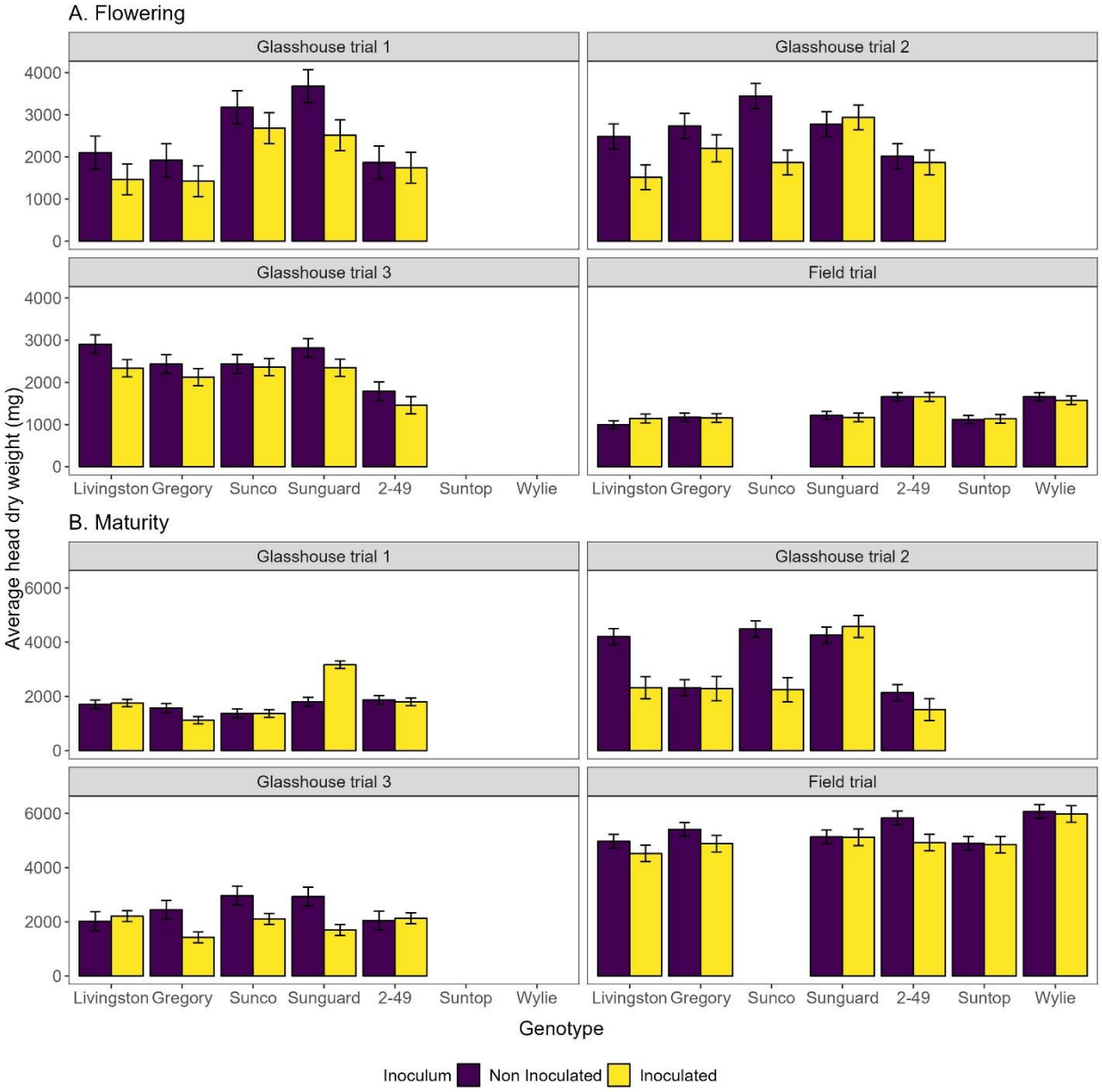

A. Flowering
Glasshouse trial 1
Glasshouse trial 2
Glasshouse trial 3
Field trial
B. Maturity
Glasshouse trial 1
Glasshouse trial 2
Glasshouse trial 3
Field trial
Average head dry weight (mg)
0
1000
2000
3000
4000
6000
Livingston
Gregory
Sunco
Sunguard
2-49
Suntop
Wylie
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 5.7

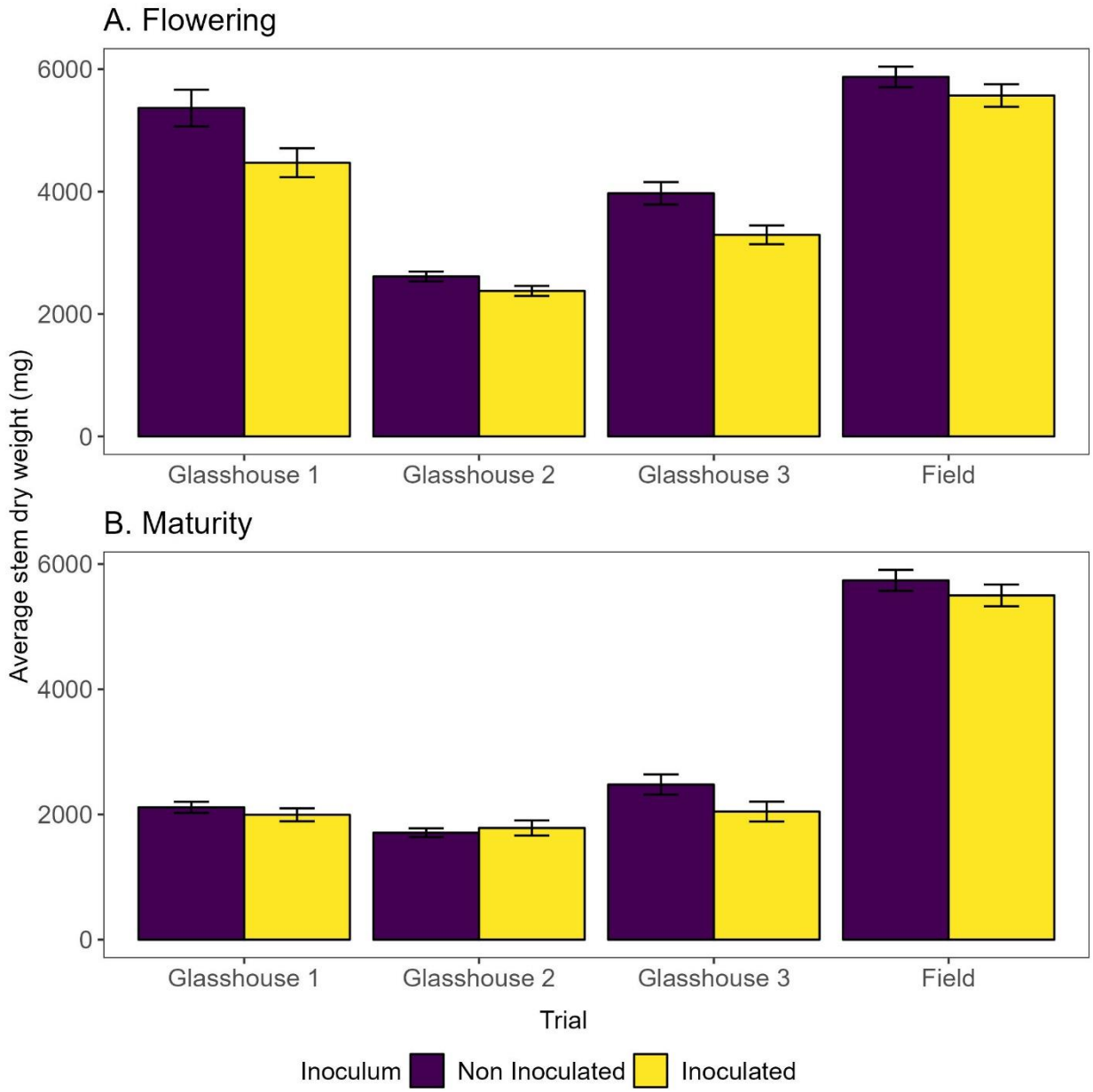

A. Flowering
6000
4000
2000
0
Glasshouse 1
Glasshouse 2
Glasshouse 3
Field
B. Maturity
Average stem dry weight (mg)
Trial
Inoculum
Non Inoculated
Inoculated

Figure 5.8

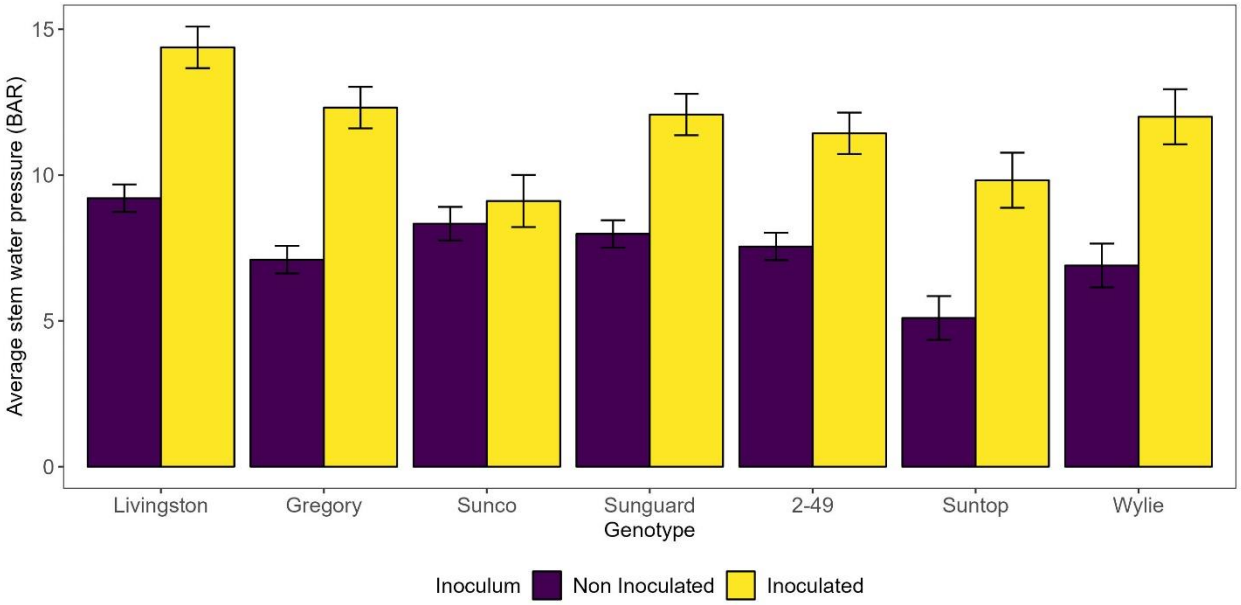

Average stem water pressure (BAR)
15
10
5
0
Livingston
Gregory
Sunco
Sunguard
2-49
Suntop
Wylie
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 5.9

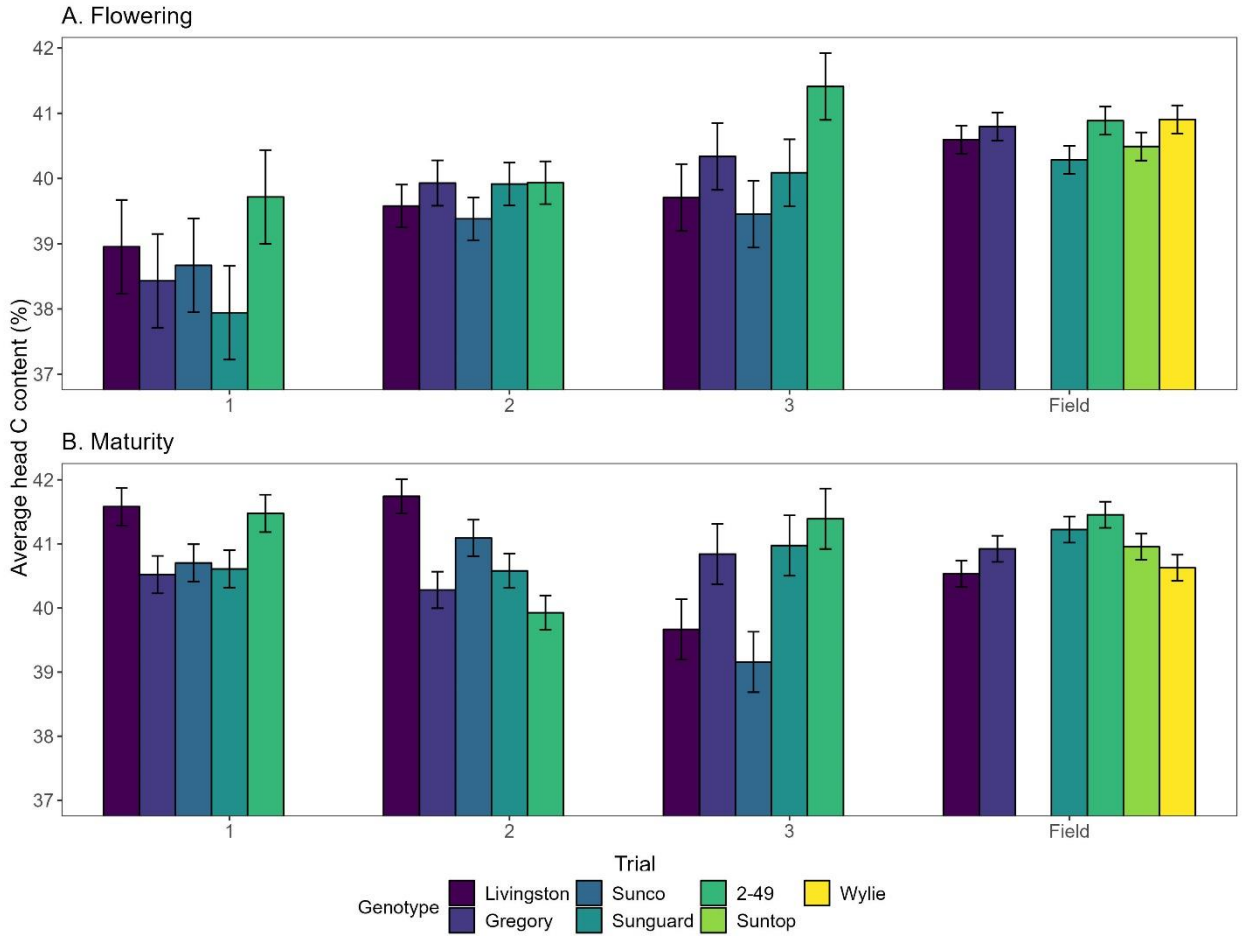

A. Flowering
B. Maturity
Average head C content (%)
42
41
40
39
38
37
1
2
3
Field
Trial
Genotype
Livingston
Gregory
Sunco
Sunguard
2-49
Suntop
Wylie

Figure 5.10

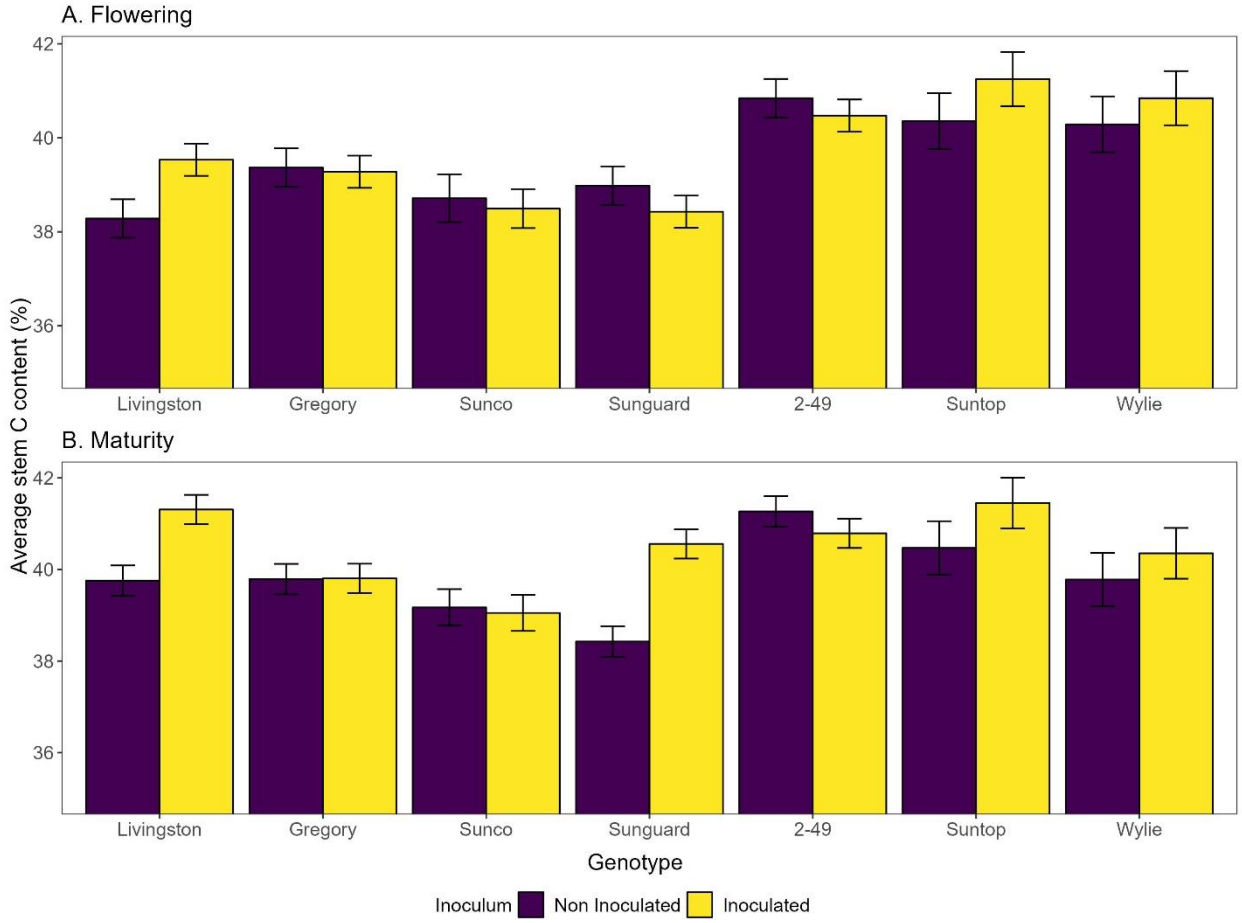

A. Flowering
B. Maturity
Average stem C content (%)
42
40
38
36
Livingston
Gregory
Sunco
Sunguard
2-49
Suntop
Wylie
Genotype
Inoculum
Non Inoculated
Inoculated

Figure 5.11

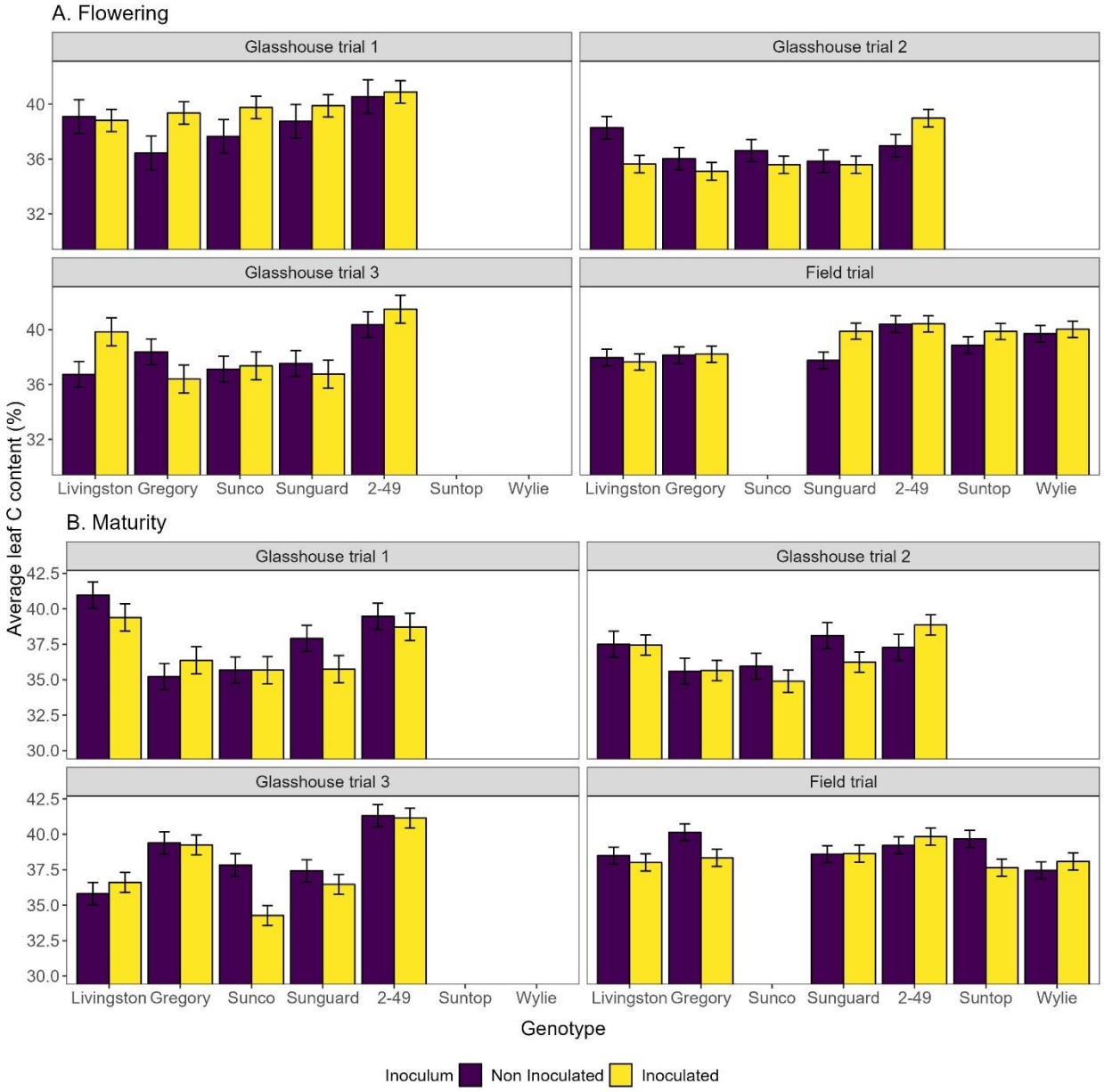

A. Flowering
Glasshouse trial 1
Glasshouse trial 2
Glasshouse trial 3
Field trial
40
36
32
Livingston Gregory Sunco Sunguard 2-49 Suntop Wylie
B. Maturity
42.5
40.0
37.5
35.0
32.5
30.0
Average leaf C content (%)
Genotype
Inoculum Non Inoculated Inoculated

Figure 5.12

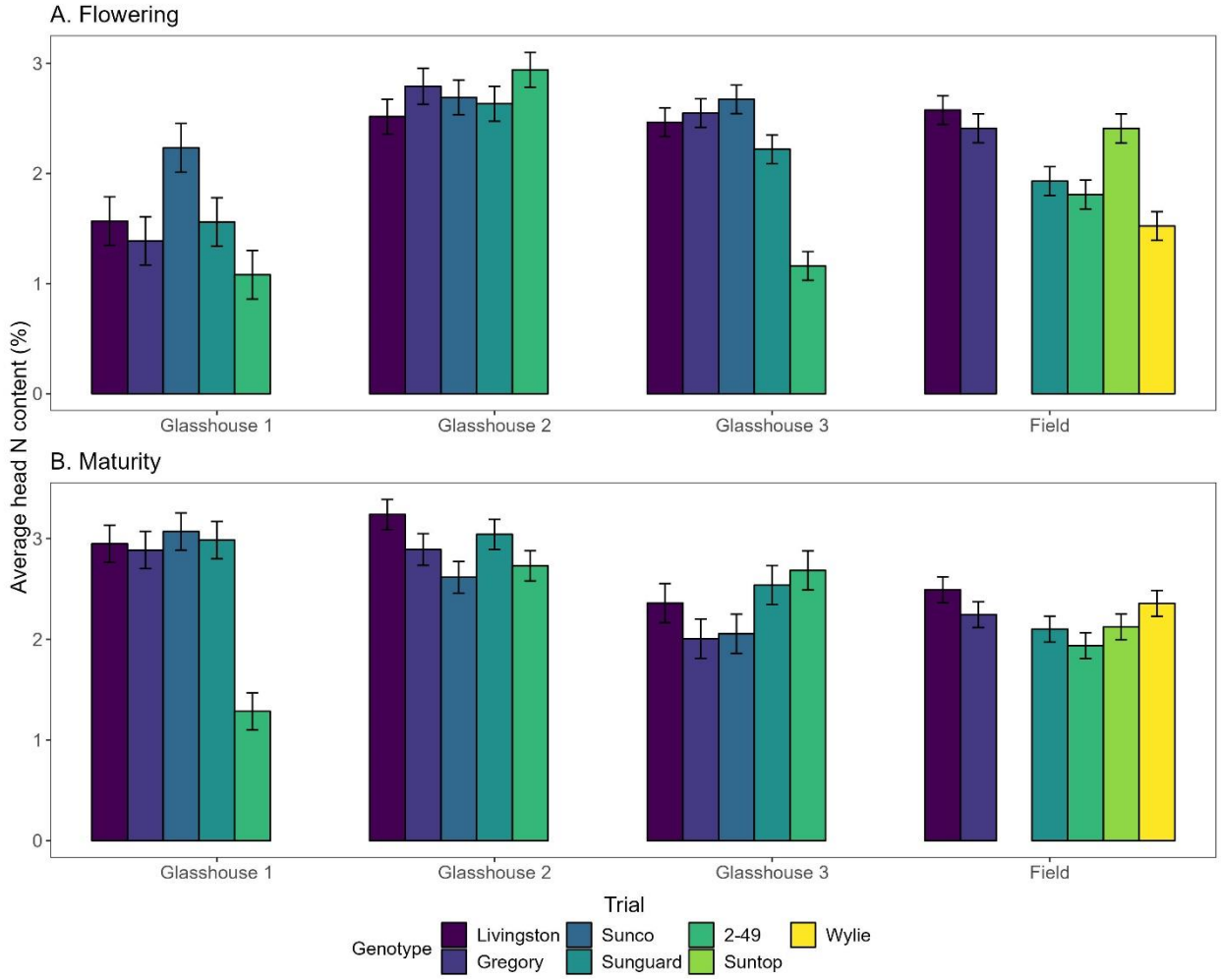

A. Flowering
B. Maturity
Average head N content (%)
0
1
2
3
Glasshouse 1
Glasshouse 2
Glasshouse 3
Field
Trial
Genotype
Livingston
Gregory
Sunco
Sunguard
2-49
Suntop
Wylie

Figure 5.13

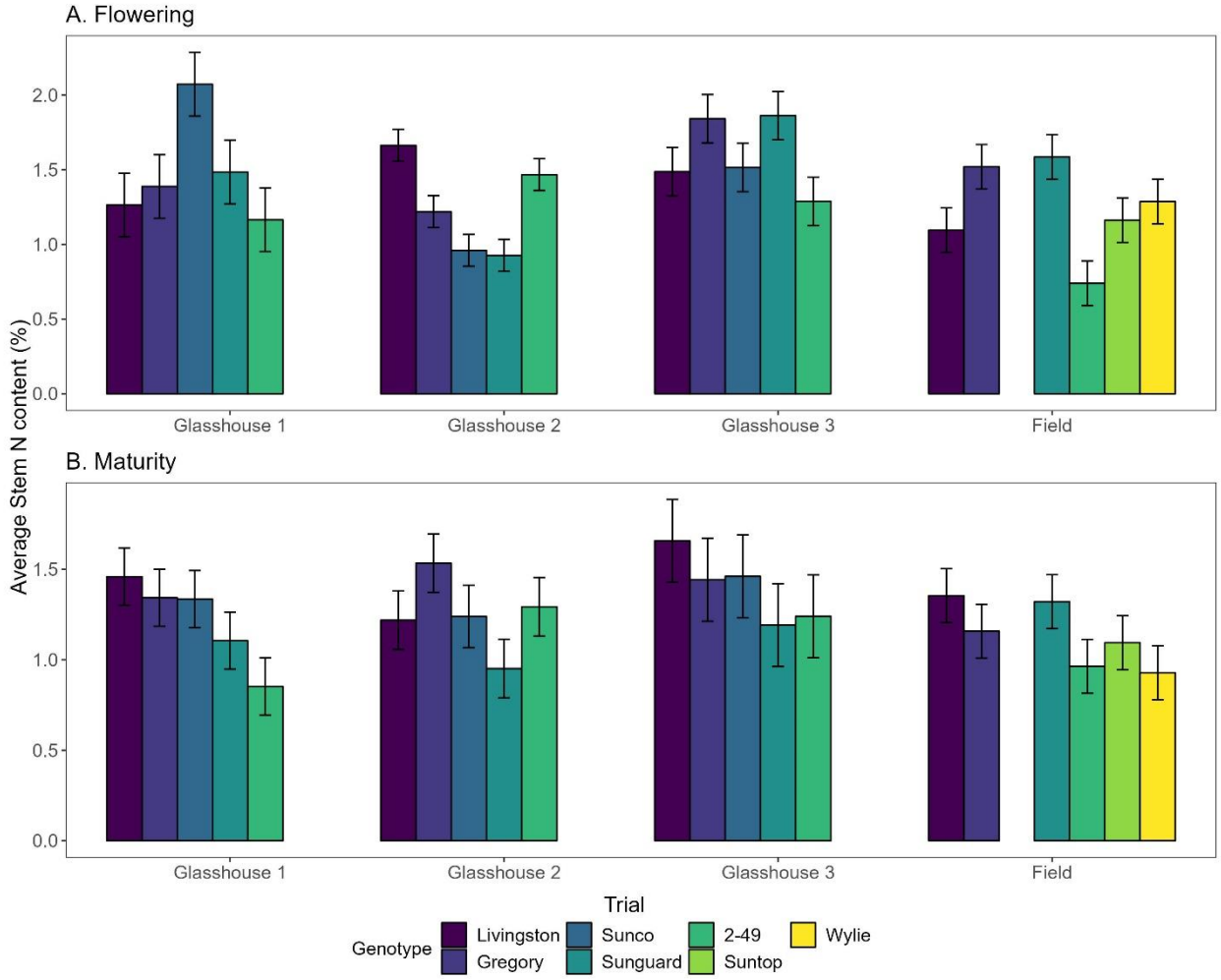
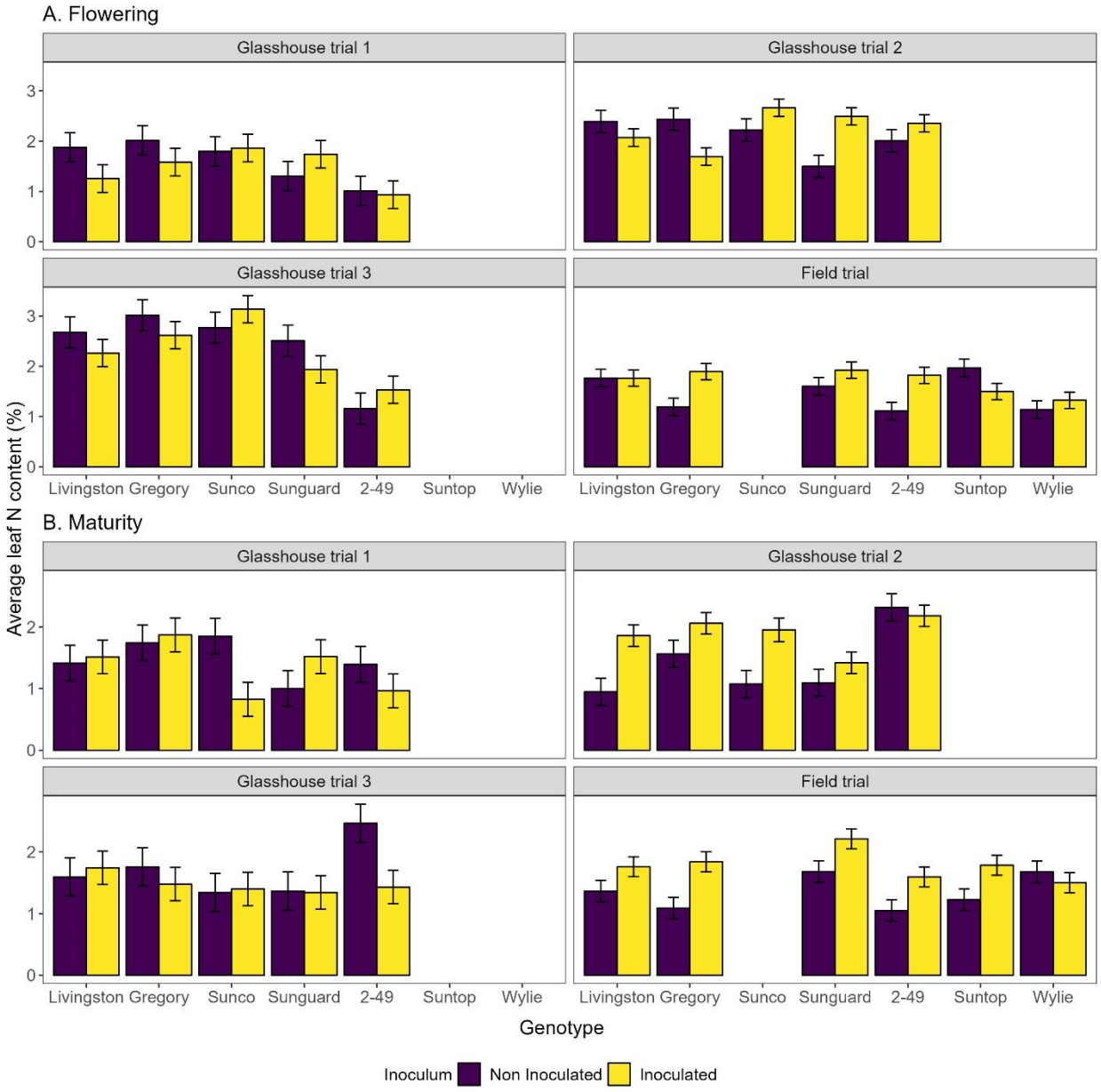

A. Flowering
B. Maturity
Average Stem N content (%)
0.0
0.5
1.0
1.5
2.0
Glasshouse 1
Glasshouse 2
Glasshouse 3
Field
Trial
Genotype
Livingston
Gregory
Sunco
Sunguard
2-49
Suntop
Wylie

Figure 5.14


A. Flowering
Glasshouse trial 1
Glasshouse trial 2
Glasshouse trial 3
Field trial
B. Maturity
Glasshouse trial 1
Glasshouse trial 2
Glasshouse trial 3
Field trial
Average leaf N content (%)
0
1
2
3
Livingston Gregory Sunco Sunguard 2-49 Suntop Wylie
Genotype
Inoculum
Non Inoculated
Inoculated

CHAPTER 6: DISCUSSION

The first purpose of this study is to provide a detailed information to improve our knowledge and understanding of the physiological mechanism of crown rot (CR). Our study was to investigate the underlying physiological mechanism of CR disease development in relation to the fundamental physiological processes. To examine the differences in gas exchange, structural and morphological characteristics among various wheat genotypes, including tiller number, shoot length, leaf size, stem dry weight, head dry weight, amount of any alterations in the stem water potential of infected wheat, as well as the carbon (C) and nitrogen (N) content of the head, leaf and stem.

In seedling trials, Chapter 3, we investigated the relationship between water availability, plant physiology and *Fp* infection in six wheat genotypes. We conducted a study to investigate the impact of water deficit on CR. In Chapter 4 we examined the response of twelve wheat genotypes to CR in three different field trials conducted during the flowering and maturity stages. In these trials, we assessed the occurrence of infected tillers the severity of disease on stems, and the visual symptoms on sub-crown internodes. In addition, Chapter 4 included three glasshouse trials and one field study, which revealed the negative effects of crown rot disease severity on important physiological parameters in seven different bread wheat varieties. These studies included detailed evaluations of disease severity and physiological traits.

Disease levels in non-inoculated genotypes remained minimal across all experiments, including seedling, field and glasshouse trials, as well as during the flowering and maturation stages of growth. In all experiments the severity of disease presented a clear increase, particularly among the very susceptible variety Livingston, followed by the susceptible EGA Gregory and moderately susceptible genotypes, including Sunco (GRDC and DAFF. 2014). Significantly decreased average of visual discoloration was observed in genotypes exhibiting partial resistance, including IRN497, W21MMT70, and 2-49, which exhibited the highest resistance (Collard et al. 2005, Bovill et al., 2006., Wildermuth et al. 2001). When comparing the outcomes of the reduced water treatment to those of the field capacity treatment, this decrease became more evident. The findings of this study agree with the initial hypothesis that suggested *Fp* resistance is largely varied between cultivars and the disease development is influenced by water supply.

This is the first study to address a long held supposition about the role of that water deficit plays in the exacerbation of CR. Following our findings, Buster et al. (2022) documented a

yield decline in bread and durum wheat varieties under both field and controlled settings, yet in the absence of water scarcity. This suggests that the detrimental effects on *Fp* infection on yield is not exclusively confined to the situations of water limitations.

Disease severity in the inoculated genotypes in the field experiments varied between years at both the flowering and maturity development stage. Disease severity was much higher in the 2018 experiment compared to the 2016 and 2017 experiments. However, when disease severity was measured at maturity, the 2017 experiment had the highest disease severity in the infected genotypes, followed by the 2018 experiment and the 2016 experiment. These temporal fluctuations in disease severity raise intriguing questions about the underlying factors influencing *Fp* infection in wheat fields. The observed variation in disease severities may be attributed to the rainfall patterns observed during the trials. In the 2016 experiment fairly consistent (above average) rainfall was received each month throughout the experiment. In the 2017 experiment relatively dry conditions were presented during the seedling, tillering, and flowering stages, with less than 3 mm received during August and September. This was followed by significant rainfall post anthesis. In the 2018 experiment low levels of rainfall were received during the seedling and tillering stages with significant rainfall during flowering. This pattern highlights the potential impact of dry conditions throughout the season, followed by a surge in rainfall post anthesis on disease severity and wheat health. The level of symptoms of CR in the glasshouse trials differed from CR in the field; however, they consistently revealed significant disease severity in the inoculated very susceptible and susceptible genotypes.

The variability in rain condition have experienced across the three experimental years, as discussed in the previous paragraph, play a crucial role in understanding the consistent differences observed in stem water pressure between inoculated and non-inoculated, as elaborated in the subsequent findings. We assume that plant genotypes with higher resistance to CR will demonstrate improved stem water pressure compared to susceptible genotypes. The results consistently demonstrated that stem water pressure was higher in treatments where inoculated compared to non-inoculation. In this study the susceptible genotypes, Livingston and Gregory, were found to have the highest stem water pressures. In contrast, the genotype Sunco, which is known for its tolerance, exhibited the lowest stem water pressure. Other partial resistance genotypes also indicated the requirement for higher stem water presser when inoculated.

It has been reported that *Fp* has a complex lifestyle in an agricultural setting, which encompasses characteristics of both hemibiotrophic and necrotrophic behaviours (Kazan et al., 2012). The dynamics of pathogenic interactions in plants are complex and can be influenced by various factors (Rajarammohan 2021). One such factor is the presence of an initial biotrophic phase, which is followed by necrosis. Interestingly, even within hemibiotrophic pathogens, the duration of the biotrophic phase can vary, adding further intricacy to the overall dynamics of the interaction (Hane et al. 2020). The influence of environmental factors on *Fp*'s behaviour is multifaceted and intricate. The correlation between *Fp*'s survival and lifestyle and the availability or scarcity of water is apparent based on the aforementioned observations. It is possible that *Fp* has developed adaptations to cope with the unique conditions of the environment. This research further highlights the importance of conducting additional biological research to understand the mechanisms behind *Fp*'s response to different water conditions.

A second objective of this study was to examine the fundamental physiological mechanism of CR disease development in association with gas exchange. The present study reveals significant variations in important gas exchange parameters, including photosynthesis (A), internal CO_2 concentration (C_i), stomatal conductance (G_s), and transpiration (E), between inoculated and non-inoculated genotypes in three separate field trials. Additionally, differences in these parameters were particularly pronounced in the seedling trial, while the data regarding C_i in the field trial was not provided. The impact of CR infection on gas exchange parameters was observed in all genotypes, although the extent of the response varied among them. The observation of increased gas exchange parameters in non-inoculated genotypes is present. The findings of our study present evidence of a significant and negative effect on A, G_s, and E in various wheat genotypes when infected with *Fp*. However, it is important to note that the C_i genotype exhibited an increase in these parameters after inoculation during the seedling trial. The findings highlight the distinct extent of reduction in gas exchange parameters among genotypes categorized as partially resistant, tolerant, susceptible, or very susceptible to CR. This emphasizes the differential responses to CR infection within this diverse group of genotypes. There were obvious differences in gas exchange parameters between seedling and field trials. In terms of the seedlings, it was observed that the inoculated genotypes exhibited lower values of A. On the other hand, the field experiments demonstrated a wider range of A values. The gas exchange data frequently was within a specific range, indicating a certain level of consistency in the measurements. Especially comparing gas exchange averages between

seedlings and field trials, despite the variations in rainfall during the field experiments. However, overall, the data stayed within a certain scope. Interestingly, the *A* parameter in 2017 showed a noticeable deviation from the values seen in 2016 and 2018. In the experiment conducted in 2017, under arid conditions and across various growth stages, the *A* values displayed significant variability. Furthermore, there were significant differences noted between the field and seedling trials, especially in relation to parameter G_s. Seedlings that were inoculated consistently showed G_s values that fell below a specific threshold, while field experiments displayed a broader range of results. Interestingly, a conspicuous congruity emerged between seedling and field trials in terms of *E.* All the genotypes that were inoculated showed consistent trends within a certain range, suggesting a consistent pattern without providing specific numerical values. These results are consistent with the hypothesis that different genotypes will reflect different structural and morphological characteristics in response to *Fp* inoculation.

There are several factors that can contribute to differences in gas exchange parameters between field and seedling trials, which are related to the experimental conditions. There are significant differences in environmental variations between controlled seedling environments and open-field conditions, including light intensity, temperature, humidity and soil moisture. In addition, variations in plant development stages and reactions to diverse growth environments may play a role in these differences. The intricate interaction of these elements frequently results in differences in gas exchange parameters observed between controlled seedling experiments and the ever-changing field conditions (Keller et al., 2019; Flood et al., 2016). Further investigation is required for a comparative genetic investigation between field and seedling.

A third objective of this study was to investigate the effect of *Fp* infection on growth parameters in wheat genotypes. The findings presented in this study highlight the negative impact of *Fp* infection on the growth and development of wheat. Through three comprehensive field and three glasshouse experiments, the results consistently revealed the negative impact of *Fp* inoculation on various growth parameters. The impact of *Fp* inoculation on genotypes was observed to result in reduced shoot lengths, head numbers, and head and stem dry weights when compared to non-inoculated treatments.

In the 2016 field experiment, several genotypes displayed decreased head weights at maturity, with the exception of Syn110, IRN497, W21MMT70, and 2-49. In 2017, there were no significant differences between the inoculated and non-inoculated genotypes during flowering.

However, at maturity, it was observed that Livingston, Mace, Gregory, Suntop, AUS29529, and IRN497 revealed reductions in head weight. In the 2018 experiment, there were no significant distinctions observed during flowering. However, at maturity, the inoculated genotypes of Livingston, Gregory, and 2-49 provided significantly lower head weights. The glasshouse trials conducted in this study demonstrated significant reductions in head dry weight among susceptible genotypes such as Gregory and Livingston. In the glasshouse trials, it was observed that the moderate resistance/tolerance genotype such as Sunco and Sunguard also exhibited significantly reductions in head dry weight.

In this investigation we have hypothesised that partially resistant wheat genotypes will exhibit superior biomass production, including leaf area and dry weight. However, this study found that there were differences in head dry weight between inoculated and non-inoculated genotypes. This suggests that the growth parameters of head dry weight and leaf area may not be strongly influenced by *Fp* infection. The study demonstrated a significant decline in stem weights among the genotypes that were inoculated. The 2016 experiment had higher stems than 2017 and 2018. In 2016, inoculated Syn110, Sunco AUS29529, and GW95-703*C15 had lower stem weights at flowering and Livingston, Sunco, and IRN497 at maturity. Except for Gregory, Syn110, Sunco, and IRN497, all inoculation genotypes had reduced flowering stem weights in 2017. Inoculated Mace, Gregory and Suntop have lighter stems at maturity. In 2018, inoculated and non-inoculated genotypes made no difference during flowering and maturity. Moreover, no difference in leaf area was found between infected and uninoculated samples. The infection in the lower stem did not influence the leaf area, therefore no alterations were visible.

We have hypothesised genotypes with partial resistance to CR will display higher levels of carbon (C) and nitrogen (N) content compared to the more susceptible genotypes when exposed to *Fp* infection. The investigation on the impact of *Fp* infection on the C and N content in wheat heads, leaves, and stems did not result in statistically significant results. However, the findings of this study raise interesting considerations for further study. This discussion focuses on the subtle differences observed between inoculated and non-inoculated genotypes, which can be attributed to the hemibiotrophic lifestyle of the fungus. The potential role of a plant's ability to maintain its C and N content in its survival during an infection, contrasting with the thriving nature of the fungus. The interesting aspect discussed in this statement applies to the complexities of the interaction between plants and pathogens. In this dynamic relationship, both plants and pathogens undergo adaptations in order to secure their own survival. The

findings of this study contradict the initial hypothesis that suggested genotypes would display differences in structural and morphological traits when exposed to *Fp* infection. This study aimed to investigate the underlying physiological mechanisms of CR disease development and analyze the changes in C and N content. However, the expected outcomes were not obtained, highlighting the necessity for additional research to better understand the intricate nature of this dynamic interaction.

It is evident that further research is needed in the field of molecular and microbiological investigation on genotypes exhibiting partial resistance, tolerance, and susceptibility to CR. This will contribute to a more accurate and comprehensive understanding of the interactions between wheat and *Fp*. The findings of this study contribute to the existing body of knowledge on the role of generating better varieties with resistance to CR. The demonstration of the significant negative impact of *Fp* on all tested genotypes across several physiological traits further emphasises the need for the identification of further sources of resistance and tolerance for breeding programs. Continued research in this area will be crucial in expanding our understanding and ultimately improving the resilience of crop populations against this devastating disease.

The findings of this study hold significant implications for the industry of grains production in Australia and worldwide. Opposing to the initial hypothesis, the lack of substantial differences in structural and morphological traits between inoculated and non-inoculated wheat genotypes, despite their varying levels of CR resistance, provides novel and unexpected insights into the complex plant-pathogen interactions involved in this disease. This challenges the succeeding assumption that resistant genotypes would demonstrate clear physiological advantages over susceptible genotypes when infected with *Fp*. The constancy of C and N content observed across inoculated and non-inoculated genotypes further suggests that wheat plants may employ sophisticated adaptation strategies to maintain essential nutrient levels in the face of *Fp* infection. This points to the potential for genotypes to enhance resistance mechanisms that allow them to better withstand the attacks of CR, beyond just reducing disease severity.

The insights from this research can significantly influence the development of more effective CR management strategies and breeding of wheat genotypes with improved resistance. The detailed physiological data, in particular the gas exchange measurements, provide breeders with valuable information to guide the selection of parental lines and the evaluation of advanced breeding materials. For instance, the observed reductions in *A*, *Gs*, and *E* in

inoculated genotypes could be used as early-stage screening criteria to identify lines with greater tolerance with traditional disease severity assessments could develop the efficiency of breeding programs targeting CR resistance. In Addition, the data of the impact of the *Fp* infection of the stem water pressure suggest the potential for using these parameters as a proxy for evaluating the integrity of the vascular system and water transport dynamics in wheat genotypes. This finding could inform the development of novel management practice, such as the use of interventions to maintain water flow and alleviate the detrimental effects CR on yield.

The information of this study advances the current understanding of the complex plant-pathogen interactions involved in CR diseases development. The unexpected findings regarding the lack of distinct structural and morphological difference between inoculated and non-inoculated cultivars, despite their differences in resistance level, challenge the prevailing assumptions in the field. These results underscore the need for more nuanced and comprehensive investigations into wheat-Fusarium interactions, encouraging researchers to explore alternative physiological and adaptive mechanisms that may contribute to CR resistance, beyond the traditional focus on disease severity and biomass reduction. By providing deeper understanding of the dynamic balance between the wheat plant and the *Fp* pathogen, this investigation lays the foundation for the development of more effective management strategies and the identification of the novel sources of resistance. This data can ultimately aid in improving the resilience of wheat crops to these devastating diseases, benefiting growers and broader grains industry.

In conclusion this study presents significant knowledge on the complex relationship between *Fp* infection and many growth parameters in wheat genotypes. The study repeatedly shows the negative impact of *Fp* infection on the growth and development of wheat. Gas exchange measurements highlight significant differences between inoculated and non-inoculated, in multiple seedling and field trials. The differences were apparent in the seedling experiment. An interesting component of this study is the modest disparities in C and N levels between genotypes. Although these variations did not achieve statistical significance, we suggest the complex relationship between the host plant and the pathogen. The observed constancy of C and N levels in the presence of infection implies the utilization of an adaptation strategy by both the wheat plant and the fungus.

In this study, we have examined the impact of *Fp* infection on different growth parameters in wheat genotypes. Our findings offer significant insights into the understanding of this infection and its effects on wheat. The significance of genotype-specific responses and resilience mechanisms in influencing wheat's response to CR disease is highlighted by these findings. The complexity of the plant-pathogen interaction is evident in the variations observed in different growth parameters. This highlights the importance of conducting further research to gain a better understanding of this crucial disease in wheat cultivation and develop effective management strategies. The study aims and results are consistent with the hypothesis that different genotypes will exhibit different structural and morphological characteristics in response to *Fp* inoculation. The aims of the study were to identify the differences in structural and morphological characteristics across wheat genotypes and investigate the basic physiological mechanisms of CR disease development.

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