RANGE OF WINTER CEREAL REACTIONS TO ROOT AND CROWN ROT PATHOGENS

A Thesis submitted by

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ABSTRACT

Winter cereals can be colonised by a number of different soil-borne organisms, including crown rot and common root rot pathogens which can have a devastating economic impact on associated industries. These diseases damage the tissues of the sub-crown internode (SCI) and the stem, and result in subsequent yield losses. In Australia, crown rot disease is predominantly caused by *Fusarium pseudograminearum* and *Fusarium culmorum*. In different parts of the world, such as the United States of America (USA), *Fusarium graminearum* has also been associated with crown rot. In Australia, *Bipolaris sorokiniana* is the soil-borne pathogen causing common root rot. The current study aims to examine the ability of these four pathogens to induce crown rot and common root rot symptoms in five winter cereals in the glasshouse and field.

Seedling inoculation tests were conducted, in the glasshouse, to examine disease severity on leaf sheaths and SCIs, specifically the impacts on shoot length and shoot dry weight. Two strains each of *F. pseudograminearum, F. culmorum, F. graminearum,* and *B. sorokiniana* were inoculated across a single cultivar of a range of winter cereals: barley (*Hordeum vulgare*), bread wheat (*Triticum aestivum*), durum wheat (*Triticum durum*), oat (*Avena sativa*), and triticale (x*Triticasecale*). A significant variation in disease severity ($p < 0.05$) among the four crown rot and common root rot pathogens was observed, including variation between pathogen strains. Significant differences amongst the cereal cultivars were observed in the disease severity of the leaf sheaths and SCIs. Furthermore, a significant reduction in the shoot length of all cultivars was observed when inoculated with *F. pseudograminearum* ($p < 0.05$), while dry shoot weight was only significantly reduced ($p < 0.05$) in the barley variety Grimmett.

Field inoculation trials using three strains of crown rot pathogens and one strain of common root rot pathogen were conducted to compare the visual disease symptoms on the stems and SCIs of all five winter cereals at three harvest stages (tillering, flowering, and maturity). Significant differences in the disease severities on the SCI ($p < 0.001$) and stems were observed ($p = 0.002$) for the crown rot and common root rot pathogens across the five winter cereals. Variation in disease severity was observed among the cultivars depending on the pathogen inoculum. There was a significant effect for pathogens on plant height ($p < 0.001$) for both years. A significant difference was observed in plant weight between harvest time and pathogen for 2016 trial ($p < 0.05$), and significant impact between the pathogens in plant weight for 2017 experiment ($p < 0.001$). *Fusarium pseudograminearum* and *F. culmorum* DNA were most

frequently detected in all cereal cultivars, while *F. graminearum* and *B. sorokiniana* DNA were detected the least. Oat had low or no disease severity, although the DNA of each pathogen was detected.

Oat is a potential host to crown rot pathogens and is considered an asymptomatic crop. The reaction of forage oat cultivars to inoculation with a range of *F. culmorum* strains has not previously been examined in Australia. The virulence of eight strains of *F. culmorum* collected from different regions of Australia (northern, southern, and western) across five forage oat cultivars and one bread wheat cultivar were tested. A positive control of mixed inoculum of *F. pseudograminearum* was included in this experiment, along with a negative/non-inoculated control for each cultivar. A significant interaction between strains and cultivars ($p < 0.001$) was observed for the disease severity on the leaf sheaths. One *F. culmorum* strain caused greater disease severity on forage oat cultivars Coolabah, Genie, and Drover compared to the other strains.

Yield losses of bread wheat due to crown rot can be more severe when drought conditions occur during grain fill. Studies have shown that drought alters the root architecture of bread wheat resulting in changes to the root system including root angle, length and biomass which have a negative impact on crop yield. The impact of crown rot infection on the root architecture of bread wheat has not been investigated. Root angle, length, number, fresh weight, and root dry weight of a bread wheat cultivar inoculated with the crown rot pathogens *F. pseudograminearum* and *F. culmorum* were examined in a controlled environment. Fresh shoot and dry weight, leaf area of the oldest and the youngest fully-formed leaf, leaf number, and disease severity of the first leaf sheath were recorded. All aspects of the root system were reduced when inoculated with *F. pseudograminearum*, excluding root angle which exhibited no difference between the inoculated and non-inoculated treatments. In contrast, no impact was observed on the root characteristics of bread wheat when inoculated with *F. culmorum*.

In conclusion, this research explored the host reaction of different winter cereals species inoculated with four crown rot and common root rot pathogens at different stages of development. In this study, *F. pseudograminearum* was generally more pathogenic on barley, bread wheat, durum wheat and triticale cultivars than on oat. Whilst oat was considered to be more tolerant to crown rot and common root rot pathogens, some *F. culmorum* strains caused infection on a range of oat cultivars. This suggests that oats may not be suitable in a crop rotation system to reduce crown rot pathogens in the field. This study has led to a better understanding of the infection potential of some crown rot and common root rot pathogens across a range of winter cereal species. The results of this study will provide valuable

information for management strategies targeting crown rot and common root rot diseases in crop rotations.

CERTIFICATION OF THESIS

This thesis is the work of Ahmed Saad except where otherwise acknowledged, with the majority of the authorship of 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

Principal Supervisor: Dr. Anke Martin

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Student and supervisors' signatures of endorsement are held at the University.

STATEMENT OF CONTRIBUTION

The following detail is the agreed share of contribution for candidate and co-authors in the presented publications in this thesis:

Chapter 2: Comparison of disease severity caused by four soil-borne pathogens in winter cereal seedlings. **Saad, A**, Macdonald, B, Martin, A, Knight, NL, Percy, C. To be submitted to **European Journal of Plant Pathology.**

Ahmed Saad (AS) contributed 60% towards the concept of the manuscript, design the experiments, inoculum preparation and collecting the data. Cassandra Percy (CP) 20% towards the concept and experiment design, critical revision of the manuscript and final editorial input. Bethany Macdonald (BM) 10% towards data analysis and interpretation of data. Anke Martin (AM) and Noel Knight (NK) contributing 5% each towards the concept, drafting and final important editorial input.

Chapter 3: Winter cereal responses to common root rot and crown rot pathogens in the field. **Saad, A**, Macdonald, B, Martin, A, Knight, NL, Percy, C. To be submitted to **Crop and Pasture Science.**

AS contributed 60% towards writing and drafting the manuscript, inoculum preparation, data collecting, DNA extraction and PCR analyse of the field samples. CP 20% towards the concept and design, critical revision of the manuscript and final editorial input. Bethany Macdonald (BM) 10% towards data analysis and interpretation of data. AM contributing 5% towards the concept, drafting and final important editorial input and PCR test data analysing. NK 5% towards critical revision of the manuscript and final editorial input.

Chapter 4 Crown rot of Australian forage oat cultivars caused by *Fusarium culmorum* in glasshouse tests. **Saad, A**, Martin, A, Knight, NL, Percy, C. To be submitted to **European Journal of Plant Pathology**

AS contributed 65% towards the concept of manuscript and experiments design, inoculum preparation, data collecting, analysis and interpretation. CP 20% towards the concept, inoculum preparation, critical revision of the manuscript and final editorial input and data analysis. AM contributing 10% towards the concept, drafting, final important editorial input and PCR test data analysing. NK 5% towards critical revision of the manuscript and final editorial input and PCR test data analysing.

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LIST OF CONFERENCES ATTENDED AND CONFERENCE PAPERS

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TABLE OF CONTENTS

List of Abbreviations and Units:

AFLP – Amplified Fragment Length Polymorphism

CLA – Carnation Leaf Agar

CAPS – Cleaved Amplified Polymorphic Sequences

SNP – Single Nucleotide Polymorphism

cm – centimetre

cv – cultivar

CZA – Czapek-Dox Agar

g – gram

h – hour

ha – hectare

ISSR – Inter-Simple Sequence Repeat

ITS – Internal Transcribed Spacers

kg – kilogram

L – litre

m – meter

mm – millimetre

MPa – megapascal

mt – million tons

NSW – New South Wales

PCR – Polymerase Chain Reaction

PDA – Potato Dextrose Agar

QLD – Queensland

RAPD – Random Amplified Polymorphic DNA

RFLP – Restriction Fragment Length Polymorphism

SA – South Australia

SCI – sub-crown internode

t/ha – tonne per hectare

VIC – Victoria

WA – West Australia

°C – degree Celsius

μL – microlitre

μM – micromolar

CHAPTER 1: LITERATURE REVIEW

Crops such as maize, wheat, and rice, are vital for feeding the ever-increasing global population and play a major role in the agriculture sector, contributing to economies worldwide. Many countries rely on grain cereals such as wheat, rice, barley, maize, sorghum, rye and triticale for food consumption (Pitts et al. 2017). World grain production in 2018/19 including rice, wheat and coarse grain was 2,625.4 mt (USDA 2019). Recently, the global production of grains, specifically wheat, has been decreasing partly due to the significant increase in global temperature and drought that has occurred in several regions of the world (FAO 2018). Crop diseases are also major contributors to the reduction in crop production (FAO 2018). As a result, the 2017/18 wheat production estimate of 761.9 mt was reduced by 31 mt during the 2018/19 season (USDA 2019).

1.1 GRAINS INDUSTRY IN AUSTRALIA

In Australia, grain production including rice, wheat and coarse grain totalled 78.9 mt in 2018 which is considered small in comparison to world production (USDA 2019). However, due to Australia's relatively small population, grain crops can be used both for local consumption, as well as contributing a significant portion to the global trade (approximately 77% of Australian grain production is exported) (Pitts et al. 2017; White et al. 2018). In 2017 a report from the Department of Agriculture and Water and the Australian Bureau of Agricultural and Resource Economics and Sciences Resources (ARESR) showed that grains (wheat, barley, sorghum, oat and coarse grains) worth $17.313 billion and oilseeds and pulses worth $6.688 billion are Australia's largest category of food production, representing 26% ($24 billion) of the Australian agricultural production (McKeon et al. 2016). Australia produced up to 63 mt of grain (wheat, coarse grains and oilseeds) in 2015/16 (White et al. 2018), with an average 14 mt consumed domestically and 49 mt exported (White et al. 2018). Across Australia, on average 20 to 22 million ha are planted annually to commercial grains (McKeon et al. 2016).

Geographically, the grains industry is located in the 'cropping belt' of Australia and is defined by three regions: the northern, southern and western regions (Murray & Brennan 2009; McKeon et al. 2016; GRDC 2019a) (Fig.1). The northern region encompasses Queensland (QLD) through to New South Wales (NSW) (GRDC 2019a) (Fig.1). Most rainfall occurs over the summer months. The high moisture storing capacity of the clay-based soils of this region, enhanced by some winter rainfall, allow winter crops to be grown (Backhouse & Burgess 2002;

Murray & Brennan 2009; GRDC 2019a). In winter, this region produces barley (*Hordeum vulgare* L.), canola (*Brassica Napus*), chickpeas (*Cicer arietinum*), faba beans (*Vicia faba*), field peas (*Pisum sativum*), linseed (*Linum usitatissimum*), lupine (*lupins*), millet/panicum (*Pennisetum glaucum*), oat (*Avena sativa* L.), safflower (*Carthamus tinctorius*), triticale (x *Triticosecale* wittmack) and wheat (*Triticum aestivum* L.). In summer it produces cotton (*Gossypium*), maize (*Zea mays*), mung beans (*Vigna radiata*), peanuts (*Arachis hypogaea*), sorghum (*Sorghum bicolor*), soybeans (*Glycine max*) and sunflowers (*Helianthus*) (McKeon et al. 2016). The southern region covers south-eastern Australia including Victoria (VIC), South Australia (SA) and Tasmania (GRDC 2019a) (Fig.1) and has an even rainfall pattern through summer to winter (Backhouse & Burgess 2002; Murray & Brennan 2009; GRDC 2019a). This region produces barley, canola, cereal rye (*Secale cereal*), chickpeas, faba beans, field peas, lupins, oat, safflower, triticale, vetch (*Vicia*), lentils and wheat in the winter and maize and irrigated rice (*Oryza sativa*) in the summer (McKeon et al. 2016). The western region is located in the south-western corner of Western Australia (WA), where soil fertility is generally low to very low (GRDC 2019a) (Fig.1). This region has a moderate climate with very dry summers and reliable winter rainfall (Backhouse & Burgess 2002; Murray & Brennan 2009; GRDC 2019a) and barley, canola, lupins and wheat are the most common grains grown in winter (McKeon et al. 2016).

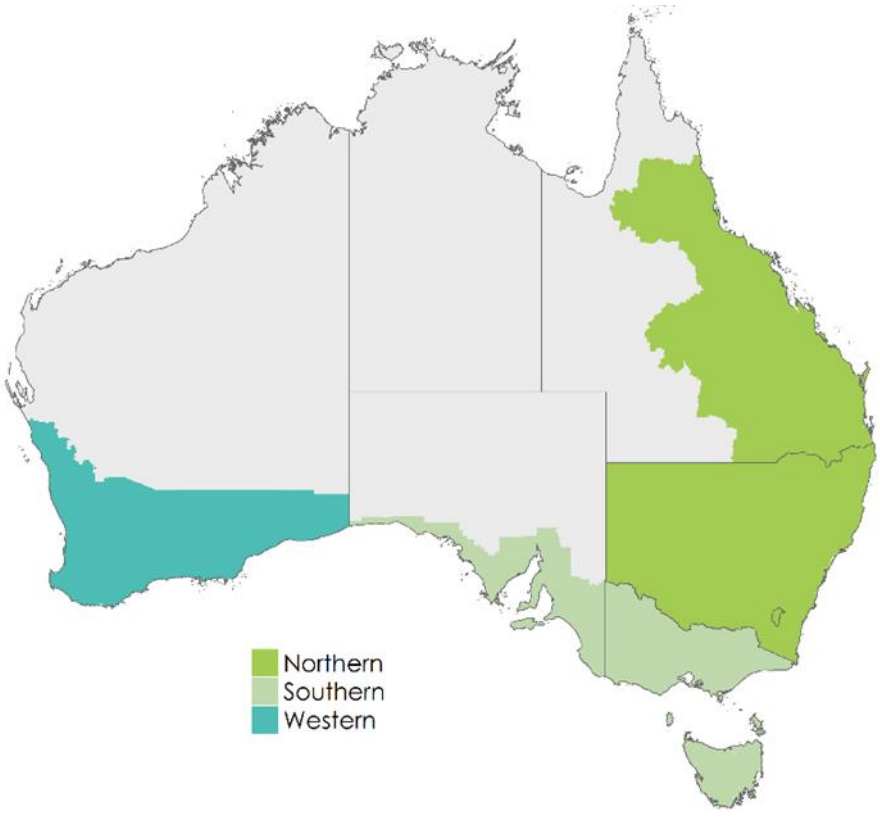

Figure 1. Australian grains regions including the Northern region (QLD and NSW), Southern region (VIC, SA and Tasmania) and the Western region (South west of WA) (GRDC 2019a).

1.1.1 Economically important winter cereals in Australia

Winter cereals have many industrial uses and provide nutrition for humans and animals. The most important winter cereals in Australia include wheat, barley, durum wheat, triticale, rye, and oat. The following sections will describe the major uses of winter cereals in Australia.

1.1.1.1 Bread wheat (*Triticum aestivum* L.)

Bread wheat (*Triticum aestivum*) is considered one of the most important grain crops in Australia, with 21.2 mt produced in 2018/19 (ABARES 2019). Bread wheat is processed to produce bread, flour and pastries. Commercially, the Australian wheat industry accounts for 10 to 15% of the world's 100 mt annual global wheat trade (AEGIC 2015).

1.1.1.2 Barley (*Hordeum vulgare* L.)

Barley (*Hordeum vulgare*) is the second most important grain crop in Australia. More than 8.9 mt of barley were produced in 2018/19, with almost 3.4 t/ha of barley grown across the southern region of Australia (ABARES 2019). Barley is used in livestock feed, malt and alcoholic beverages (ABARES 2019).

1.1.1.3 Durum wheat (*Triticum turgidum* L. var. *durum*)

In 2005/06, durum wheat production in Australia was 0.5 mt (Kneipp 2008). NSW accounted for around 56% and SA 41% of production (Kneipp 2008). Durum wheat is used to produce pasta. The Australian durum grain is known to be of high quality and is exported across the world (McKeon et al. 2016).

1.1.1.4 Oat (*Avena sativa* L.)

Australia is a world leader in the production of milling oats and they are considered an important product for the international market with 1 mt produced in 2018/19 (ABARES 2019). Oat is used as animal feed, but also for human consumption in cereals, like oat flour, oatmeal and bread.

1.1.1.5 Triticale (x *Triticosecale* Wittmack)

Triticale is a cereal crop that was artificially created in the late 19th century from crosses between bread wheat (hexaploid) and rye (Castleman 1996). In Australia, in 2018/19, 0.255 mt of triticale was produced. Triticale is often used as livestock feed because the soft grain makes it more palatable than wheat and barley. Small amounts of triticale are also used for human consumption (Castleman 1996).

1.2 WINTER CEREALS DISEASES

Winter cereals may be infected with different root and foliar diseases, which can cause severe losses to the Australian economy. Diseases and pests that attack the roots of the plant are called soil-borne diseases. Root lesion nematode (*Pratylenchus neglectus)* is a significant soil-borne pest, which can cause great losses to the Australian wheat and barley industry with average losses of $73 million recorded for wheat and $21 million for barley (Murray & Brennan 2009; Murray & Brennan 2010). Other important soil-borne diseases which cause significant damage to the Australian wheat and barley industry are crown rot, common root rot and take-all (Murray & Brennan 2009; Murray & Brennan 2010).

1.3 CROWN ROT: THE DISEASE

In Australia, crown rot caused by *Fusarium* species is a significant disease of winter cereals such as wheat and barley, annually costing the wheat industry $79 million and the barley industry $18 million in lost yield (Murray & Brennan 2009; Murray & Brennan 2010). Crown rot was first reported in QLD in 1951 (McKnight & Hart 1966) and in VIC in 1965 (Purss 1969). Burgess et al. (2001), however, suggested the disease could have been in Australia before this time. It is also an important disease worldwide, causing significant losses in North America (Smiley et al. 2005), South Africa (Lamprecht et al. 2006), West Asia and North Africa (Ammar 2004; Saremi 2011).

1.3.1 Crown rot symptoms

In seedlings, visual symptoms appear as small necrotic lesions on the coleoptile, followed by brown discolouration on the sub-crown internode (SCI), leaf sheath and stem tissues (Burgess et al. 2001; Percy et al. 2012; Kazan & Gardiner 2018). Brown discolouration on roots is less severe than that found on the SCI and leaf sheaths (Knight & Sutherland 2013b). The symptoms of crown rot disease on a mature plant are a honey-brown discolouration on the base of the

stem and discolouration of the SCI (Burgess et al. 2001; Knight & Sutherland 2015). Brown lesions develop on the stem from the crown of the plant and can be seen up to the fifth node under drought condition (Burgess et al. 2001). At maturity, under moist conditions, a pink colour often occurs on diseased tissue, particularly around the nodes, and the lumina inside the stems can be filled with hyphae of the fungus (Purss 1969). Crown rot can lead to the formation of white heads, which have few or no grains, and sometimes the disease can become severe enough to cause premature death of the plant (Burgess et al. 2001). White heads develop during water stress, usually after flowering (Moore et al. 2005; Hollaway et al. 2013), and are hypothesised to occur due to disturbances in the vascular translocation system at the base of the plant (Burgess et al. 2001; Hollaway et al. 2013). Hollaway et al. (2013) indicated that the level of *F. pseudograminearum* and *F. culmorum* DNA was positively correlated with crown rot expression including the brown stem discolouration and the formation of white heads in barley, durum wheat, and spring wheat. Even when white heads do not occur, yield loss due to crown rot can still be observed (Moore et al. 2005; Hollaway et al. 2013).

1.3.2 Crown rot: pathogens

Several *Fusarium* species have been associated with crown rot on winter cereals, including *Fusarium pseudograminearum, F. culmorum* and *F. graminearum* (Backhouse & Burgess 2002; Akinsanmi et al. 2004; Backhouse et al. 2004). During the 1950s a complex of fungal species was believed to be associated with crown rot and foot rot in Australia, with *F. culmorum,* considered the most important species (Butler 1961). Magee (1957) reported that crown rot caused by *F. pseudograminearum* was firstreported in QLD in 1951 and in NSW in 1955. *F. pseudograminearum* is now the dominant pathogen responsible for crown rot disease, with other *Fusarium* species, such as *F. avenaceum, F. crookwellense, F. culmorum* and *F. poae* infrequently isolated from crown rot diseased tissue (Wearing & Burgess 1977b; Burgess et al. 2001; Backhouse et al. 2004; Obanor et al. 2013). *Fusarium pseudograminearum* has been found throughout the main cereal growing regions and its occurrence did not appear to be limited by climate within the Australian grain belt (Backhouse & Burgess 2002; Backhouse et al. 2004). However, the fungus has not been found in some areas including the Eyre Peninsula in South Australia, the western margins of the grain belt in central New South Wales, and an area north-east of Charleville in Queensland (Backhouse & Burgess 2002). The distribution of *F. culmorum* on cereals is more limited than that of *F. pseudograminearum*. This fungus most frequently occurs in colder high rainfall areas of SA, VIC and scattered localities of WA in

Australia and also, in the south-eastern Darling Downs, QLD (northern summer rainfall zone) (Backhouse & Burgess 2002; Backhouse et al. 2004). This suggests that there factors other than climate are responsible for the distribution of *Fusarium* species such as stubble retention practices in farming systems (Burgess et al. 2001).

Fusarium graminearum (previously *Fusarium roseum*) is an important pathogen causing *Fusarium* head blight in several parts of the world including Asia, Canada, Europe, America, and Australia (Goswami & Kistler 2004; Akinsanmi et al. 2006; Hogg et al. 2010). This fungus has been reported to cause crown rot of wheat in southern Chile in South America (Moya-Elizondo et al. 2015) North America (Smiley et al. 2005; Dyer et al. 2009) and Australia (Akinsanmi et al. 2004; Akinsanmi et al. 2006). The importance of *F. graminearum* as a crown rot pathogen is not fully understood.

1.3.3 Morphology and characteristics of *Fusarium* spp.

For many years, a lot of confusion surrounded the identification of *F. graminearum.* Francis and Burgess (1975) divided *F. graminearum* into two groups: Group 1, the pathogen responsible for crown rot, and Group 2, the pathogen responsible for Fusarium head blight. Many other research groups tried to further subdivide *F. graminearum* based on pathogenicity/ virulence, culture morphology and zearalenone toxin production (Cullen et al. 1982; Carter et al. 2002; Desjardins et al. 2004). Cullen et al. (1982) divided *F. graminearum* into two groups termed 'A' and 'B', and also, Carter et al. (2002) and Desjardins et al. (2004) who identified three groups A and B and C in Europe, Nepal and the United States based on the same characteristics.

Aoki and O'Donnell (1999b), stated that "It is hard to differentiate between the two groups morphologically, but there are important ecological and pathological differences". They concluded that the two pathogens could be distinguished based on the conidial shape and intensity of sporulation and absence of homothallic production of perithecia. The morphological differences between some *Fusarium* species are difficult to determine and cultures of *F. graminearum* can be easily confused with other *Fusarium* species such as *F. pseudograminearum* and *F. crookwellense* (Leslie & Summerell 2008).

1.3.3.1 *Fusarium pseudograminearum* Aoki & O'Donnell

F. pseudograminearum (previously known as *Fusarium graminearum* Group 1) is a heterothallic pathogen (the condition of having male and female reproductive structures in different thalli) but predominantly reproduces asexually. Its sexual stage (or teleomorph) is

known as *Gibberella coronicola* (Aoki & O'Donnell 1999b). Both teleomorph and anamorph stages typically produce well developed branched, septate hyphae (Aoki & O'Donnell 1999b). *Fusarium pseudograminearum* produces macroconidia in pale orange sporodochia on Carnation Leaf Agar (CLA) (Leslie & Summerell 2008). On Potato Dextrose Agar (PDA), the colony colour ranges from a red, brownish yellow to white (Leslie & Summerell 2008). The macroconidia are falcate to fusiform and sometimes relatively slender, curved to almost straight with a curved apical cell and a foot-shaped basal cell (Fig. 2a) (Leslie & Summerell 2008). *Fusarium pseudograminearum* produces a thick-walled hyphal cell (chlamydospore) which functions as a spore and usually develops within four weeks on CLA culture (Leslie & Summerell 2008). Chlamydospores are rarely produced and when they occur usually are a pale, yellowish-grey (Aoki & O'Donnell 1999b). *Fusarium pseudograminearum* has been associated with winter cereals such as barley, bread wheat, rye, durum wheat, triticale, oat and grassy weeds (Burgess et al. 2001; Leslie & Summerell 2008).

1.3.3.2 *Fusarium culmorum* (W.G. Smith) Saccardo

The sexual stage (teleomorph) of *F. culmorum* has not been found and it is still unknown if the fungus is heterothallic or homothallic (Mishra et al. 2003; Scherm et al. 2013). The macroconidia are formed in large quantities in orange sporodochia on CLA. On PDA, *F. culmorum* grows quickly creating numerous sporodochia in a large central spore mass (1 to 2 cm diameter) (Leslie & Summerell 2008). Sporodochia are pale orange but become brown to dark brown with age. Most strains produce red pigments, but some can have olive-brown mycelium and pigment in the agar (Leslie & Summerell 2008). The macroconidia are short and falcate to fusiform and thick-walled, usually 3 to 5 septate (Fig. 2b) (Leslie & Summerell 2008; Scherm et al. 2013). The basal cell is foot-shaped or just notched; the apical cell is blunt or slightly papillate (Scherm et al. 2013). The chlamydospores usually take 3 to 5 weeks to form on CLA (Leslie & Summerell 2008). *F. culmorum* has a large host range including barley, maize, oat, rye, sorghum, wheat and various grasses and has also been isolated from asparagus, bean, carnation, flax, pea, red clover and sugar beet (Scherm et al. 2013).

1.3.3.3 *Fusarium graminearum* Schwabe

The sexual spores of *F. graminearum* (*Gibberella zeae* Schwein*)* (previously known as *F. graminearum* Group 2) are produced in small flask-shaped bodies called perithecia that enclose the asci. The fungus is homothallic and thus perithecia can be formed without the need to cross isolates (Leslie & Summerell 2008). Macroconidia are slim, thick-walled with 5 to 6-septate,

evenly curved to straight and the ventral side is straight, and the backside smoothly bent (Fig. 2c) (Leslie & Summerell 2008). The sporodochia are faint orange on CLA (Leslie & Summerell 2008). On PDA, the colonies grow quickly, producing large hyphae usually white to faint orange to yellow in colour (Leslie & Summerell 2008). *F. graminearum* has been found primarily in maize, wheat and barley, but also can be found in a variety of other crops (Leslie & Summerell 2008).

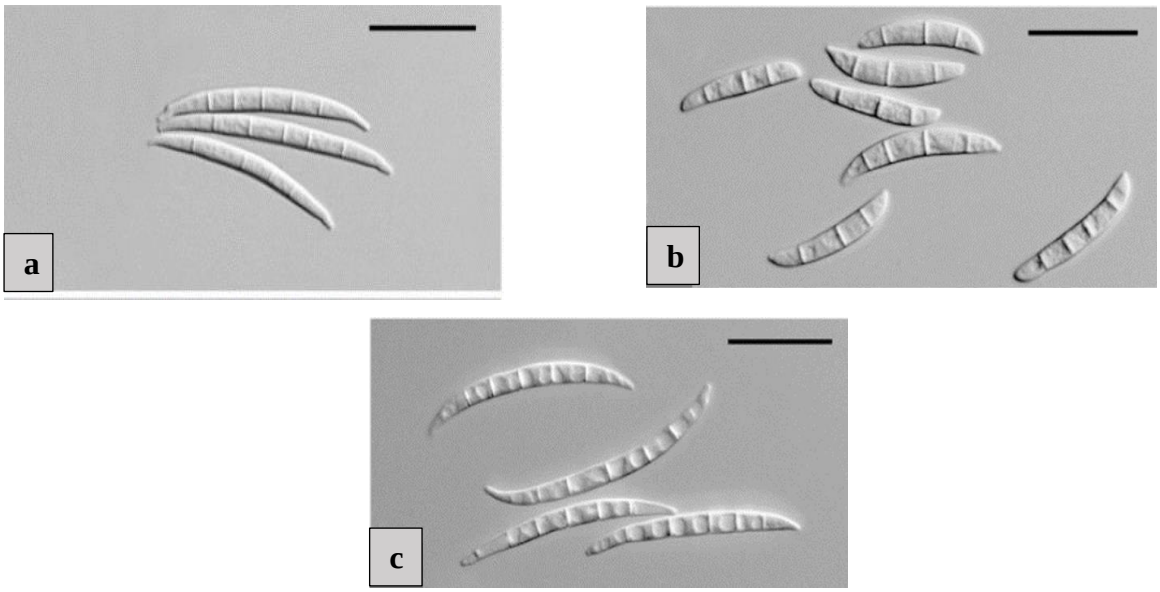

Figure 2. Macroconidia of *Fusarium pseudograminearum* (a), *Fusarium culmorum* (b), *Fusarium graminearum* (c). Scale bar = 25 μm (Leslie & Summerell 2008).

1.3.4 Life cycle of *Fusarium* species

The life cycles of *F. pseudograminearum* and *F. graminearum* are similar, whereby, both fungi can reproduce through sexual and asexual sporulation (Ma et al. 2013) (Fig. 3). While *Fusarium* species can survive for many years in the soil as chlamydospores, *F. pseudograminearum* predominantly survives as hyphae in the soil and on stubble remaining from the previous crops, which germinate to produce asexual macroconidia (Fig. 3) (Wearing & Burgess 1977a; Burgess et al. 2001; Ma et al. 2013; Kazan & Gardiner 2018). The stubble of the previous year acts as a source of inoculum for the next season (Burgess et al. 2001; Kazan & Gardiner 2018). Direct contact between host and stubble fragments has been shown to be necessary for infection to occur in *F. pseudograminearum* (Burgess et al. 2001; Ma et al. 2013).

Initial infection of *F. pseudograminearum* occurs through the crown region of the host including coleoptile, SCI, lower leaf sheaths, and subsequently moves into the basal internode of the stem (Purss 1966; Burgess et al. 2001; Kazan & Gardiner 2018). The pathogen then

frequently persists to the stem epidermal tissue via stomatal openings, then moves into the hypodermis to induce typical browning discolouration on the stem and vascular tissues (Knight & Sutherland 2013a, 2013b; 2016). Both hyphae and spores can survive for years in the stubble residue in soil (Burgess et al. 2001). Environmental conditions and cultural practices play an important role in the survival of the inoculum in the field such as soil moisture, and cool conditions, in which the spores can survive longer (Burgess et al. 2001; Kazan & Gardiner 2018). High temperatures between 25°C to 31°C are favourable for disease development in the field (Burgess et al. 2001; Backhouse & Burgess 2002).

Fusarium culmorum can survive in the soil for up to three years as chlamydospores (Bockus et al. 2010). However, the life cycle of *Fc* has not been fully examined.

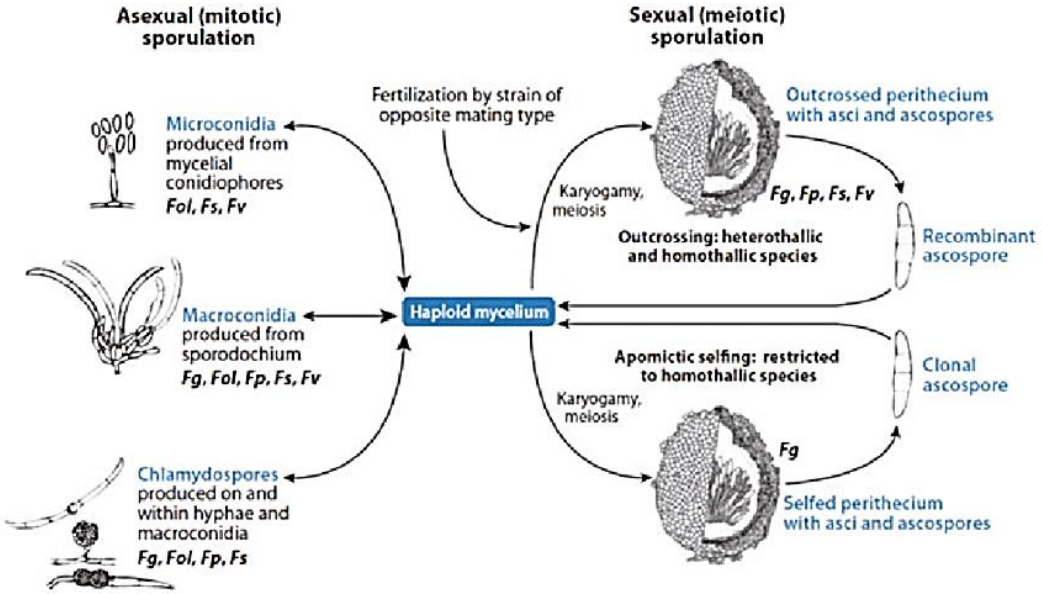

Figure 3. Generalised life cycle of *Fusarium* species (Ma et al. 2013). Abbreviations: *Fg* (*F. graminearum*)*; Fol* (*F. oxysporum* f. sp. *lycopersici*); *Fp* (*F. pseudograminearum*); *Fs* (*F. 'solani'* f. sp. *pisi*); *Fv* (*F. verticillioides*).

1.3.5 *Fusarium* species identification and genetic diversity

Comparing morphological characteristics is one method used to distinguish between *Fusarium* species (Leslie & Summerell 2008), however, this method is not always accurate. As mentioned previously, *F. graminearum* and *F. pseudograminearum* were considered to be one species until 1975, when *F. graminearum* was split into Group 1 and Group 2 by Francis and Burgess (1975) based on geographic distributions and pathological symptoms. These two groups are morphologically and culturally difficult to distinguish (Aoki & O'Donnell 1999b). In molecular studies, Random Amplified Polymorphic DNAs (RAPDs) were used to

differentiate and identify Group 1 and Group 2 strains of *F. graminearum* (Schilling et al. 1996). Several Polymerase Chain Reaction (PCR) based assays were developed for the specific detection and identification of several *Fusarium* species using a unique marker for each species, including *F. culmorum*, *F. graminearum*, and *F. pseudograminearum* (Schilling et al. 1996; Niessen & Vogel 1997). Furthermore, molecular methods were used to identify the genetic diversity amongst the strains within the pathogen (O'Donnell & Cigelnik 1997; Aoki & O'Donnell 1999a; Aoki & O'Donnell 1999b). Aoki and O'Donnell (1999b) identified two groups of *F. graminearum* based on morphological, physiological characters and their molecular phylogenetic relationship by analysing 17 Group 1 (*F. pseudograminearum*) and 15 Group 2 (*F. graminearum*) strains. In this assay, the DNA sequence data from the *β*-tubulin gene was employed to examine the systematic and phylogenetic relationships of the two species. They found that *F. pseudograminearum* represents a phylogenetically distinct species but is related to the *F. graminearum* monophyletic groups.

A high level of genetic variability has been reported within *Fusarium* populations (Mishra et al. 2003; Akinsanmi et al. 2004; Akinsanmi et al. 2006; Khudhair et al. 2019). These studies were carried out in different geographical ranges, or at a single field level, and have reported a wide genetic variability within a field, whereas relatively modest differences were detected among populations selected from different climatic regions (Miedaner et al. 2001; Akinsanmi et al. 2006; Chakraborty et al. 2006; Bentley et al. 2008). Akinsanmi et al. (2006) employed Amplified Fragment Length Polymorphism (AFLP) analysis to assess 72 *F. pseudograminearum* strains and 59 *F. graminearum* strains collected from QLD and NSW, Australia from crown rot (77) or head blight (52) affected plant materials. The ALFP analysis showed that 70 of the 72 *F. pseudograminearum* strains and 56 of the 59 *F. graminearum* strains had different haplotypes. An additional 27 *F. graminearum* strains collected from a single location revealed 18 different AFLP haplotypes. The results suggest that there is significant genetic diversity amongst *F. pseudograminearum* and *F. graminearum* strains collected from different geographical areas or from the same field (Akinsanmi et al. 2006). In another study, the AFLP analysis within and between eight *F. pseudograminearum* populations (217 strains) from north-eastern (south QLD and north NSW), south-central (central and south NSW and VIC), and south-western (SA and WA) regions of the Australian grain belt were collected (Bentley et al. 2008). The results revealed high levels of genetic diversity with 176 haplotypes identified. The analysis of the molecular variation of Bentley et al. (2008)'s population study indicated 50% differences within the population of *F. pseudograminearum* strains from the Australian grain belt, and 32% between the populations and a further 18%

among the populations within regions. One cluster contained isolates from the north-eastern Australian populations and the other cluster contained isolates from the south-central and south-western Australian population. Within each cluster, isolates shared at least 78% similarity. In a recent study, Khudhair et al. (2019) investigated the genetic diversity of 297 *F. pseudograminearum* strains in years 2008 and 2015 from a total of seven different sites. In 2008, three geographically distinct location sites were surveyed including Tammin, Karlgarin and Jerramungup in WA. In 2015, four other sites were surveyed including Merredin, Corrigin, Kondinin and Ongerup, WA. Twenty-one Cleaved Amplified Polymorphic Sequence (CAPS) primers were designed targeting potential Single Nucleotide Polymorphisms (SNPs). A high level of genotypic variation was observed within *F. pseudograminearum* populations, but a low level was observed between years. An analysis of molecular variance indicated that over 91% of the variation in the SNP data was within the *F. pseudograminearum* 2008 and 2015 populations, less than 1% accounted for differences in years and 8% accounted for the variation among populations.

The genetic variability within a global collection of *F. culmorum* strains was determined using Inter-Simple Sequence Repeat (ISSR) analysis, along with an assessment of aggressiveness using three ISSR markers (Mishra et al. 2003). The ISSR analysis in this assay observed a high degree of intra-specific polymorphisms within the *F. culmorum* strains. The research further went on to test the aggressiveness of the *F. culmorum* strains, which was conducted on seeds of the wheat cv. Armada (moderately susceptible to *F. culmorum*). The analysis of variance revealed that there was a non-significant difference ($P = 0.081$) in the overall effect of ISSR clusters/groups for aggressiveness to wheat cultivar Armada. However, there was a significant variation ($P < 0.001$) in the aggressiveness of the strains within a particular ISSR clade, with 59 different strains clustering into seven distinct clades amongst 75 isolates. For instance, the isolates belonging to ISSR clade C4 caused 76.1% mean root infection, comparatively higher than the isolates of any other ISSR clusters. Additionally, the analysis of variance showed that the geographical origins of the strains had a significant ($P = 0.013$) effect on the diversity. This indicates that the effect of geographical origins of the isolates was highly significant for pathogenicity to the wheat cultivar (Mishra et al. 2003).

1.3.6 Crown rot inoculation and screening methods

Visual disease screening of adult plants in the field is challenging due to cultivar environment interactions, the long growing season, the requirement for laborious hand-harvesting, cleaning

and rating of individual field-inoculated plants. Therefore, screening methods conducted first in a glasshouse provide a useful tool to study early symptom development and potential resistance in winter cereal genotypes. In Australia, many studies have utilised different methods for visually screening germplasm for assessing crown rot disease in a controlled environment, such as the layered pot design method (Wildermuth & McNamara 1994). Wildermuth and McNamara (1994) were the first to report the layered pot design as a seedling inoculation technique that could be used as an efficient method in breeding programs for screening resistance to crown rot. The layered pot design contains three layers of soil. In the first layer, the seeds are planted on top of moistened sterilised soil and covered with sieved soil. Next ground colonised grain inoculum of *F. pseudograminearum* is applied and additional sieved soil placed on top of the inoculum. The inoculum is activated after seven days by watering each pot to field capacity then watering pots daily, to field capacity. After 21 days the three-leaf sheaths are rated for disease severity using a 0 to 4 scale where 0= healthy and 4 is greater than 75% diseased. The result of the disease severity in the glasshouse of this method was positively correlated to the disease severity in the field (Wildermuth & McNamara 1994). Wallwork et al. (2004), tested the disease severity of bread wheat and durum wheat infected with *F. pseudograminearum* and *F. culmorum* using a terrace screening system to pursue alternative sources of resistance. In this system, plants are grown in open-ended tubes set into galvanised baskets which are then placed on sand in outdoor terraces. The seeds are planted in the surface of potting mix and covered with another layer of the potting mix. The inoculum in this system is applied as small propagules of inoculated wheat chaff with the potting mix in the last layer. This system can be used to screen large numbers of cultivars at a low cost and has the ability to detect adult plant resistance with reasonable reliability without conducting a field trial (Wallwork et al., 2004). In both the Terrace system and the layered pot design, the inoculum is applied as a colonised grain added to the soil in a layer above the seeds. Thus, the coleoptile grows through the soil and the inoculum, similar to a natural field infection. However, the Terrace method cannot distinguish the very high level of susceptibility observed in durum in the field (Wallwork et al., 2004). Purss (1966) also reported that there was no correlation between the seedling in the glasshouse and field reaction for the adult plants. Furthermore, Wildermuth et al. (2001) investigated sources of partial resistance to crown rot caused by *F. pseudograminearum* in wheat grown in artificially inoculated soil in the glasshouse and the field. The disease resistance could not be detected in all cultivars at the seedling stage. In this case, the depth of crown formation was determined to test the partial resistance of 13 cultivars/lines of wheat. There was a correlation between the depth of crown

formation in wheat cultivars and their reaction to crown rot in seedlings and mature plants. With the exception of cultivar Sunco, cultivars and lines with deeper crowns were less resistant than those with shallow crowns (Wildermuth et al., 2001).

Mitter et al. (2006) developed a high-throughput germplasm screening method which inoculates the stem base of a wheat seedling with a macroconidia suspension of *F. pseudograminearum*. Mitter et al. (2006) used the application of a 10µl fungal inoculum to the stem base of a wheat seedling. The seedlings are held in a horizontal position for 2 days in 100% humidity in the dark before being returned to normal growth conditions in the glasshouse. The disease measured was scored using the lesion length using the formula of Crown rot severity index = (length of stem discolouration/seedling height) × (number of leaf sheath layers with necrosis) (Mitter et al. 2006; Li et al. 2008). This method is useful for evaluating a large number of cultivars in breeding programs (Mitter et al. 2006; Li et al. 2008). To inoculate crown rot trials in the field the inoculum is added to a plot by either applying cereal seed inoculated with a macroconidia suspension of *Fusarium* species (Hollaway et al. 2013), or by applying colonised grain inoculum such as colonised millet (Smiley et al. 2005) or colonised wheat/barley grain (Malligan 2009) into the farrow. Recently, a study by Smiley (2019) was conducted to assess the influences of *Fusarium* inoculation procedures on the development of crown rot under field conditions. They used a mechanized system to place exact inoculum units above the wheat seed. Two methods of inoculum were utilised in this study including a ground *Fusarium*-colonised (*F. pseudograminearum* or *F. culmorum*) wheat and oat mixture and a colonised whole millet. Wheat seeds and the inoculum were dropped from separate tubes at different depths in the soil. Both inoculation systems increased the incidence and severity of crown rot. The millet seed inoculation system was preferred for wheat production systems in the semi-arid Pacific Northwest, USA, because it was much safer to use than the ground inoculum placed with the wheat seed, and the number of inoculum units dispensed per unit area was much easier to quantify.

1.3.7 Crop root system characteristic

Drought is one of the principal factors that have an impact on plant growth and contribute to yield losses (Bray 1997; Manschadi et al. 2006; Palta & Watt 2009). A crop root system that is deep and abundant at depth contributes to maintaining yield stability in dry seasons and dry environments, particularly where periods of drought occur during the season (Palta & Watt 2009; Palta & Yang 2014). Drought also has an impact on water efficiency and osmotic adjustment (Munns 1988). Root system characteristics are of fundamental importance to soil

exploration and below-ground resource possession which can be the key to improve crop adaptation to drought (Manschadi et al. 2006; Manschadi et al. 2008; Christopher et al. 2013). A large root system, classified by root dry weight, length, density and root angle, plays a major role in adapting grain crops to dry environments (Manschadi et al. 2006; Manschadi et al. 2008; Palta et al. 2011). Root characteristic formation can be reflected to some extent in the genetic background and the environmental adaptation of genotypes. For instance, Manschadi et al. (2008) indicated that wheat cultivars that grow in the southern and western regions generally had a wider growth angle and lower number of seminal roots. However, wheat cultivars with higher performance on deep clay soils in the northern region revealed a narrower root angle. In addition, Manschadi et al. (2008) also suggested that some cultivars grown in the northern region of Australia expressed conflicting combinations of seminal root angle and number of the seminal roots. That may be due to other factors such as resistance to various diseases and improved grain quality along with possessing drought-adaptive root systems (Manschadi et al. 2008).

1.3.7.1 Crown rot and root characteristic interaction

The incidence of crown rot disease is greater in areas with high levels of soil moisture at the start of the season (Burgess et al. 2001; Kazan & Gardiner 2018). During the season, the increased growth of the plants depletes the sub-soil moisture, making the plant more susceptible to water stress late in the season and more vulnerable to disease infection (Burgess et al. 2001; Kazan & Gardiner 2018). Thus, yield losses due to crown rot pathogens are intensified by limited rainfall and drought conditions at the end of the season (Burgess et al. 2001; Hollaway et al. 2013). Drought conditions may have an impact on water efficiency, osmotic adjustment and the root architecture of the plant. Several studies have inspected the colonisation of crown rot pathogens on the roots (Knight & Sutherland 2013b; Knight & Sutherland 2017; Xu et al. 2018), with a focus on examining the visual symptoms and the presence of DNA of crown rot pathogens (Knight & Sutherland 2013b; Knight & Sutherland 2017). However, there has been no research published which has examined the root physiology in plants affected by crown rot.

1.3.8 Disease control and management

Crown rot is a difficult disease to manage, as the fungus can survive for years in soil and stubble residues (Wildermuth et al. 1997; Kazan & Gardiner 2018). For many years, the disease has become more widespread in Australian cereal growing environments because of the adoption

of minimum tillage and stubble retention practices in farming systems (Burgess et al. 2001; Bovill et al. 2006; Kazan & Gardiner 2018). Susceptible cultivars, duration of cropping and favourable environmental conditions are other factors associated with an increase in crown rot in the field. Furthermore, stubble burning or incorporating stubble into the soil may reduce inoculum levels (Burgess et al. 1996), but this practice may have a negative effect on the yield by removing moisture that would otherwise be available to the crop, especially during early development stages (Burgess et al. 2001). Crop rotation is another method to reduce the inoculum levels of the disease (Burgess et al. 2001; Kazan & Gardiner 2018). A non-host crop such as chickpea, canola, and sorghum can be helpful in reducing crown rot levels in subsequent plantings (Purss 1966; Burgess et al. 1996). A study by Burgess et al. (1996) indicated that the inoculum levels of *F. pseudograminearum* were reduced in sorghum (non-crown rot host)/wheat rotations compared to continuous wheat rotations.

Early studies showed that seed treatments with fungicides or the application of fungicides to stem bases did not seem to provide sufficient protection from crown rot (Serafin et al. 2011). However, more recently it has been reported that fungicides have been used effectively against *F. culmorum* by reducing incidence by 29 to 96% in glasshouse experiments, either as a seed treatment (Moya-Elizondo & Jacobsen 2016) or as a foliar spray (applied twice at Zadoks growth stages 31 and 45 with either fluquinconazole or tebuconazole, or with epoxiconazole and carbendazim) (Akgül & Erkilic 2016). It is not clear whether these fungicides are suitable for crown rot caused by *F. pseudograminearum*.

Agronomic practices are useful tools to reduce crown rot in the field, however, they are not always compatible with economical and practical considerations. Hence, improving the genetic resistance of cereal cultivars to crown rot is an important breeding objective. To date cultivars with complete resistance to this disease for both wheat and barley are not available. Currently, in Australia, varieties are moderately susceptible (MS), which is a measurement of visual disease symptoms and the fungal biomass (Zaicou-Kunesch et al. 2017; GRDC 2018, 2019b). In Queensland (QLD), Australia, the wheat cultivars most resistant to crown rot are Mitch, Sunguard, Baxter, and LongReach Spitfire which are moderately susceptible. For barley, the cultivars most resistant are RGT Planet, Rosalind, and Shepherd, which are moderately susceptible to susceptible (MSS) to crown rot (GRDC 2019b). Durum wheat cultivars are considered susceptible to very susceptible to crown rot (GRDC 2019b).

In Australia, quantifying the level of fungal DNA in the soil has become possible due to access to the commercial PreDicta B test (Ophel-Keller et al. 2008). This test is an important tool for growers to assist with managing the risk of crown rot pathogens in the paddock. This assay

uses a quantitative real-time polymerase chain reaction (qPCR) technology to determine DNA concentrations of different pathogens, including crown rot pathogens (Hollaway et al. 2013). In Australia, a low risk category for crown rot are reported at 0.6 – 1.4 log *Fusarium* DNA/g soil, medium and high levels are reported as 1.4 – 2.0 and >2.0 log *Fusarium* DNA/g soil, respectively (Simpfendorfer & McKay 2015).

1.3.9 Resistance to crown rot and the host responses

Crown rot research in Australia is most frequently carried out using *F. pseudograminearum* due to the widespread distribution of *F. pseudograminearum* across Australian cereal growing regions (Burgess et al. 2001; Backhouse et al. 2004). Barley and wheat cultivars have been assessed for crown rot reactions in most of these studies, in which their reaction to *F. pseudograminearum* ranged from partially resistant to very susceptible (Wildermuth & McNamara 1994; Percy et al. 2012; Knight & Sutherland 2017; Kazan & Gardiner 2018). The first study on host responses to crown rot was conducted in glasshouses by Purss (1966), with the crown rot reactions of wheat, oat, barley and maize assessed after inoculation with *F. graminearum* (at which time *F. graminearum* was not divided into two groups but presumably *F. pseudograminearum* was the pathogen that was causing the disease in this study). The general conclusion reached from this study was that bread wheat was less resistant to crown rot and oat showed high levels of resistance. Since then extensive research has focused on the identification of genetic resistance to this pathogen, particularly in bread wheat (Wildermuth & McNamara 1994; Wildermuth et al. 1999; Bovill et al. 2006; Collard et al. 2006; Bovill et al. 2010; Percy et al. 2012). Wildermuth and McNamara (1994), examined wheat cultivars and breeders' lines for potential resistance to crown rot caused by *F. pseudograminearum* in both field and glasshouse trials. Initial results indicated that Puseas was more susceptible then Vasco, Hartog, Gala, and 2-49, respectively. Further experimentation on 28 wheat cultivars/lines, tested in both the field and glasshouse, demonstrated that cultivars/lines Gluyas Early, Mexico 234, and IRN497 showed partial resistance to *F. pseudograminearum*, while Puseas, King, and Vasco indicated high susceptibility. Nelson and Burgess (1994) also assessed the inoculation of *F. pseudograminearum* on bread wheat, barley, oat, and durum wheat cultivars in both field and glasshouse experiments. Each host exhibited varying resistance to *F. pseudograminearum*, with lower visual symptoms observed in the oat cultivars compared to wheat and barley cultivars. Recently, however, oat plants suffering severe crown rot disease were recorded on the Darling Downs QLD (Personal communication Percy C.), with *F.*

culmorum isolated from the diseased stem bases. While *F. culmorum* has been shown to cause crown rot in winter cereals (Backhouse et al. 2004; Scherm et al. 2013), there is only one study that has reported yield losses to this pathogen in Australia (Hollaway et al. 2013). Hollaway et al. (2013) found that *F. culmorum* caused less or similar yield losses compared to *F. pseudograminearum* in bread wheat and barley. In this study, *F. pseudograminearum* caused greater disease severity in barley, bread wheat, and durum wheat than *F. culmorum*. The *F. culmorum* DNA concentrations in the soil correlated positively with the crown rot disease discolouration and whiteheads incidence and negatively related to grain yield of barley, bread wheat, and durum wheat.

1.4 COMMON ROOT ROT: THE DISEASE

Common root rot caused by *Bipolaris sorokiniana* (Sacc. in Sorok) Shoem [(teleomorph *Cochliobolus sativus* (Ito and Kurib. Drechsel. ex Dastur)] is a significant soil-borne disease in Australia which cost the wheat and barley industry $30 and $13 million, respectively (Murray & Brennan 2009; Murray & Brennan 2010). Common root rot can be found in any winter cereal growing region in Australia and worldwide (Wildermuth 1986; Kumar et al. 2002). In Australia, particularly in NSW, common root rot was severe during the 1920s and 1930s due to wheat monoculture practises (Hynes 1932). The first recorded incident of common root rot in QLD was in 1964 (Simmonds 1966). Disease incidences were reduced when ley farming was introduced, in which the paddock is used for pasture or left fallow for a period between cropping (Butler 1961). A survey was conducted by Wildermuth (1986) in 1978, 1979, and 1980 indicating that common root rot was found in all areas of QLD (Darling Downs, Western Downs, South-Western Downs Dawson, Callide Central Highlands, Maranoa, and far West QLD) and was most severe on the Darling Downs and least severe in the Central Highlands of QLD, Australia. The combined incidence of all areas studied changed from 77% in 1978, to 65% in 1979, and to 57% in 1980. The reduction of common root rot incidence observed in 1980 was more likely due to the lower rainfall in that year (Wildermuth 1986).

1.4.1 Common root rot: symptoms

The symptoms of common root rot can be similar to crown rot and it is difficult to distinguish the two diseases without pathogen isolation and identification tests being conducted. Symptoms first appear as small brown necrotic lesions on the coleoptile and roots (Kumar et al. 2002), then lesions also develop on the SCI and lower parts of the leaf sheaths. *B. sorokiniana* infections in seedlings can lead to the death of tissues in the roots, crown and the

base of the leaf sheaths. In the mature plant, brown discolouration may also be observed on stem bases. Severely infected plants are stunted and have fewer tillers, and produce pinched grain at harvest (Kumar et al. 2002; Moore et al. 2005).

1.4.2 Common root rot: pathogens

The fungus *B. sorokiniana* is distributed worldwide causing common root rot, leaf spots, and spot blotch (Kumar et al. 2002; Bockus et al. 2010). In Australia, common root rot is caused by *B. sorokiniana* and is associated with some *Fusarium* species, including *F. pseudograminearum* and *F. culmorum* (Purss 1970; Wildermuth et al. 1997). In dryland regions in North America, the pathogens most frequently associated with common root rot are *B. sorokiniana* and *F. culmorum* (Hill & Meshane 1983; Smiley et al. 2005).

1.4.2.1 *Bipolaris sorokiniana*: morphology and characteristics

B. sorokiniana is a heterothallic pathogen. The fruiting body (perithecium) of the sexual stage is round and 300–400μm in diameter. A small aperture allows sexual spores (ascospores) to be released (Mathre 1982). The mycelium of *B. sorokiniana* on PDA culture varies from olive-brown colour to white thick growth. This has been shown to depend on the aggressiveness of the strain (Jaiswal et al. 2007). The conidiophores are olive-brownish in colour and at the early development stage, they have a light grey colour (Fig. 4a) (Acharya et al. 2011). The conidiophores are 6-10 × 110-220 μm in size. The conidia are 15-28 × 40-120 μm in size, falcate to fusiform, with a slight curve, and pointed at the basal and apical cell (Acharya et al. 2011).

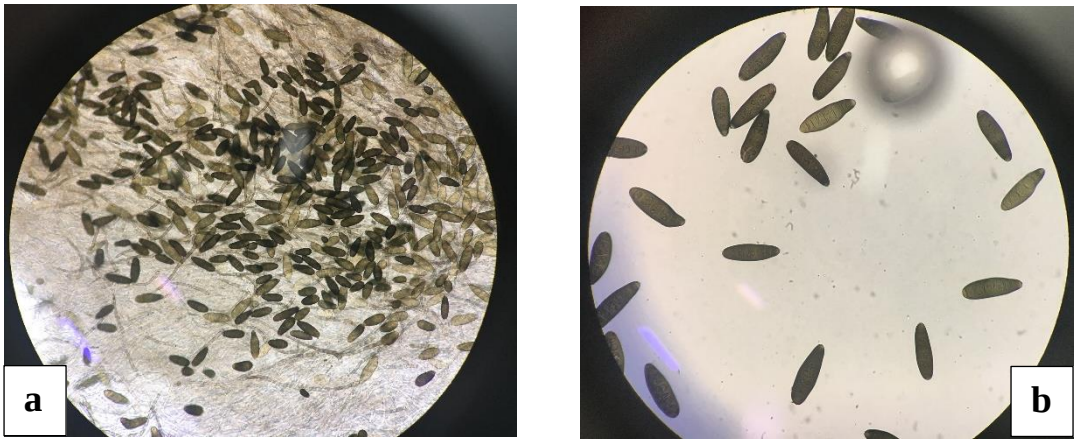

Figure 4. (a) Conidiophores and conidia of *Bipolaris sorokiniana* (100X); (b) a single conidia (400X).

1.4.3 Lifecycle of *Bipolaris sorokiniana*

Bipolaris sorokiniana survives in the form of mycelia and asexual conidia which can be found within the top 10 cm of soil (Wildermuth et al. 1997). This fungus can also be associated with seed infections (Kumar et al. 2002). This pathogen occurs as mycelium, and colonisation of live tissue is important in the disease cycle, while the saprophytic stage has not been shown to be important (Bockus et al. 2010). The conidia produced by the anamorphic stage can also survive for years in the soil or as mycelia in the host residues (Bockus et al. 2010). The first step of infection on the host root tissue is adhesion which occurs with the germination of the conidia within one hour of watering (Apoga et al. 2001). The fungus then grows and colonises on the plant surface and invades the epidermal cells of the host tissue. It colonises the host tissue intracellularly by forming a specialised structure called an appressorium, which is the swelling of a hyphal tip pressed firmly against the tissue surface (Horne, 2015; Kumar et al., 2002). The appressorium enables invasion by forming a penetration peg, an outgrowth at the base of the appressorium, that penetrates the cuticle and the epidermal cells, parenchyma cells and both the external and internal cortex of root or leaf tissue (Han et al. 2010; Horne 2015). First infections occur on coleoptiles, SCI and primary and secondary roots (Bockus et al. 2010).

1.4.4 *Bipolaris sorokiniana* genetic diversity

High levels of genetic variability were observed among *B. sorokiniana* populations (Kumar et al. 2002; Oliveira et al. 2002; Arabi & Jawhar 2007; Knight et al. 2010). Most of these studies investigated the genetic variability in *B. sorokiniana* populations causing spot blotch disease (Zhong & Steffenson 2001; Oliveira et al. 2002; Knight et al. 2010). To our knowledge, in Australia, there is no study to date that has examined the genetic diversity in *B. sorokiniana* populations that cause common root rot. Using RAPDs and ITS-RFLP Arabi and Jawhar (2007) assessed the pathogenicity of 22 *B. sorokiniana* strains causing common root rot and originating from diverse geographical sources within Syria. The results of this study showed a high level of variability within *B. sorokiniana* strains identifying 11 unique haplotypes. The strains clustered into three groups due to their different haplotypes detected by a similarity index of 0.56 for RAPD and 0.47 for ITS-RFLP. There was no relationship observed between the genetic profiles, pathogenicity and geographic origin. The pathogenicity test on barley cultivars ranged from susceptible to resistant, to these *B. sorokiniana* strains.

1.4.5 Inoculation and screening methods

To determine the disease severity caused by the common root rot pathogen, the degree of lesion development on the SCI in both glasshouse and field trials was measured (Wildermuth et al. 1992). Wildermuth and McNamara (1987) developed a screening method to test the susceptibility of winter and summer crop cultivars infected with *B. sorokiniana* in glasshouse experiments. The inoculum used in this test is soil amended with wheat and barley grain colonised by *B. sorokiniana*. The amended soil is added to clay pots and seeds are planted on the top of the soil surface. The soil is watered to the field capacity 39% and subsequently rewatered every 2-3 days. All plants are harvested after flowering. After harvesting, the roots of five plants per treatment are washed and the SCIs removed. Roots are plated onto CZA for seven days. The growth of *B. sorokiniana* from all roots is recorded. The SCIs are rated individually for disease severity using a 1 to 5 scale (Wildermuth 1986). In this study, there was a negative correlation between the SCI lesions and the infection of the root. In general, recovery of *B. sorokiniana* was higher from SCIs than from roots (Wildermuth 1986). Syrian researchers Arabi and Jawhar (2001, 2013) proposed multiple methods in separate studies including in-vitro experiments, in which Gibberellins hormone (GA3) was employed to enhance elongation of the SCI to measure the degree of common root rot severity on barley cultivars. The advantage of this method is that it can be harvested in two weeks (Arabi & Jawhar 2001). In the sandwich filter paper method that was conducted by Arabi and Jawhar (2013) ten SCIs per genotype were inoculated with a mixture of five strains of *B. sorokiniana* and the seedlings incubated for five days in sandwich filter paper placed into polyethylene transparent envelopes. After 48hrs, the initial disease symptoms were detected. In the pot assay that was conducted in the same study of Arabi and Jawhar (2013), the seeds were inoculated by mixing them thoroughly with a conidial suspension of *B. sorokiniana*. After inoculation, seeds were grown in pots and incubated in a growth chamber at 22 ± 1 ℃ (day) and 17 ± 1℃ (night) with a day length of 12 h. Plants harvested after seven weeks and the SCI for each seedling was rated using the 0−5 scales. A positive correlation was identified in the sandwich filter paper method and the pot assays, that were conducted, indicating that this testing procedure was reliable.

1.4.6 Disease control and management

In Australia, *B. sorokiniana* can be found in black earths, grey, brown clays and red-brown earths or wherever wheat and barley are grown (Wildermuth et al. 1992). Common root rot can

cause the most damage to plants at higher temperatures (above 29°C) and when plants are suffering water stress conditions (Wildermuth, 1986, Hill and Blunt, 1994). Similar to crown rot, common root rot needs wet conditions to initiate the infection, while drier conditions towards the end of the season lead to severe disease (Purss, 1970). Common root rot is a difficult disease to manage as the pathogen can survive in the soil for years (Wildermuth 1986; Wildermuth et al. 1997). Several methods have been used to try to manage common root rot in Australia including ley farming with a non-host crop (Butler 1961; Wildermuth & McNamara 1991) stubble management (Wildermuth et al. 1997), and use of fungicides (Murray & Brennan 2010). Wildermuth et al. (1997) reported a reduction in the disease severity of common root rot in wheat when the stubble was removed and also lower in a no-tillage treatment in Billa Billa in southern QLD. Ley farming or crop rotation is a useful tool to reduce the level of *B. sorokiniana* in the field (Wildermuth & McNamara 1991). Lower levels of *B. sorokiniana* have been recorded in buffel grass, cocksfoot, lucerne, mung bean, snail medic, sorghum, sunflower and White French millet-wheat rotation (Wildermuth & McNamara 1991).

Testing the DNA concentration of *B. sorokiniana* in the soil before planting utilising the PreDicta B testing service is a useful tool for the growers to identify the risk of the CRR pathogen in the field (McKay et al. 2018). Risk categories for *B. sorokiniana* are still under development. In Australia, preliminary results have indicated 1 log *B. sorokiniana* DNA/g soil represents a low risk while >2 log *B. sorokiniana* DNA/g soil considered to be a high risk (McKay et al. 2018).

Breeding for resistance is the most effective method of *B. sorokiniana* disease control. However, to date, there are no available wheat and barley cultivars highly resistant to common root rot. Currently, varieties only have moderate resistance to this disease (DPIRD 2017; Zaicou-Kunesch et al. 2017; GRDC 2018, 2019b). The wheat cultivars grown in QLD with the highest level of resistance to common root rot are Strzelecki and Lang which are Moderately Resistant – Moderately Susceptible (MRMS) (GRDC 2019b). The durum cultivar Jandaroi is Moderately Resistant (MR) to common root rot (GRDC 2019b) and barley cultivars Compass, Scope CL, and Spartacus CL are MS (GRDC 2019b).

1.4.7 Resistance to common root rot/host response

In Australia, Wildermuth and McNamara (1987) examined the susceptibility of 24 winter and 17 summer crops to *B. sorokiniana* infection in glasshouse and field experiments. Infections of both lesioned and randomly selected root segments of crowns were confirmed by plating them

on CZA. For this test, the infection in winter cereals was higher than in summer crops. Among the summer crops, the perennial grass Cocksfoot had high levels of infection in the roots (85%), while white panicum had high levels of infection on the crowns (32%). Among the winter crops, wheat, barley, oat, rye and canary grass had the greatest infection on roots and crowns (44 to 100%) in seedling assays. The recovery of *B. sorokiniana* was higher from crowns and stems than from the roots. For the field trials, wheat (36.8 to 93.8%), barley (84.2 to 87.1%) and triticale (53.1 to 83.8 %) were equally susceptible, while oat was more resistant (17.7 to 32.2%) to common root rot.

Wildermuth et al. (1992), reported significant yield losses when examining disease infection of wheat cultivars inoculated with common root rot. The Australian wheat varieties Timgalen, Songlen, and Hartog were susceptible to common root rot with yield losses ranging between 23.9 and 40% whereas yield loss in the partially resistant lines 1008 C16 and ISWYN 32 ranged between 6.8 and 13%.

1.5 SIGNIFICANCE OF THE STUDY

Multiple fungal species can be associated with crown rot and common root rot, including *F. pseudograminearum, F. culmorum, F. graminearum,* and *B. sorokiniana*. Barley, bread wheat, durum wheat, oat, and triticale have been identified as potential hosts of these fungal species. The comparative ability of *F. pseudograminearum*, *F. culmorum*, *F. graminearum* and *B. sorokiniana* to cause significant crown and common root rot disease in these winter cereal species has not been examined in detail. Extensive research on crown rot has focused on *F. pseudograminearum* including host response, genetic resistance, and yield loss mostly on wheat and barley, while there are few studies on *F. culmorum* and *F. graminearum* causing crown rot.

The rankings of *F. pseudograminearum, F. culmorum, F. graminearum*, and *B. sorokiniana* on different hosts and the ranking of barley, bread wheat, durum wheat, oat, and triticale according to visual discolouration of each pathogen have not been thoroughly compared. Knight and Sutherland (2017) and Hollaway et al. (2013) compared the disease symptoms of a single strain each of *F. pseudograminearum* and *F. culmorum* in a range of winter species. In these studies, *F. culmorum* showed the same or less disease severity on winter cereals as *F. pseudograminearum*. Furthermore, *F. graminearum* has not been considered an important pathogen causing crown rot in Australia. However, with a changing climate, *F. graminearum* could be a significant pathogen causing crown rot on a large number of hosts such as barley,

bread wheat, durum wheat, oat and triticale. This knowledge is critical to designing effective disease management strategies to crown rot and common root rot.

In Australia, oat has been associated with crown rot and has been described as either a completely resistant or symptomless host. However, the capability of *F. culmorum* strains to cause crown rot on Australian forage oat cultivars has not been investigated. The result of this study will extend our knowledge to understand the role of oat cultivars in crop rotation to crown rot.

Crown rot disease occurs under water stress conditions (drought) which can lead to yield loss and death of the host (Burgess et al. 2001). Characteristics such as increased transpiration efficiency, osmotic adjustment and adapted roots may assist in reducing the disease severity of the host. Investigating the root health system under crown rot disease pressure may be useful to understand some of the mechanisms involved in disease resistance. Breeding for these characteristics may minimise disease susceptibility and thus yield losses. To the best of our knowledge, no study to date has investigated the effect of crown rot pathogens on the root architecture of wheat in a controlled environment.

1.6 FOCUS AND AIMS OF THE STUDY

This study will conduct detailed experimentation to measure disease development and impacts caused by the crown rot and common root rot pathogens *F. pseudograminearum, F. culmorum, F. graminearum,* and *B. sorokiniana* on bread wheat, barley, durum wheat, oat, and triticale.

Aim 1: Examine the host responses to *F. pseudograminearum, F. culmorum, F. graminearum,* and *B. sorokiniana* and the disease development at the seedling stage in glasshouse tests.

The disease severity will be recorded by scoring the brown discolouration on the SCI and three-leaf sheaths. The host reaction to the four pathogens will be examined by measuring the shoot height and dry weight.

Aim 2: Determine the differences in crown rot and common root rot disease severity and host response across a range of winter cereals in field trials.

The rate of infection spread through the plants will be examined by rating the lesions on the SCI, leaf sheaths and stem tissue. Differences in disease reactions between cultivars and species will be described to determine potential resistance to crown rot and common root rot.

Aim 3: Assess the susceptibility of forage oat cultivars to different *F. culmorum* strains in a controlled environment.

The visual discolouration will be recorded by rating the browning discolouration on first leaf sheath. The presence and absence of the *Fusarium* DNA will be identified using PCR assay.

Aim 4: Examine the effect of crown rot disease on the root development of a susceptible bread wheat cultivar in a controlled environment.

The impact of *F. pseudograminearum* and *F. culmorum* on root system characteristics will be examined by measuring the root angle, root length, root number and root fresh and dry weights. Above ground disease reaction will be described to determine whether there is an association between the above ground and below ground response during crown rot infection.

CHAPTER 2

COMPARISON OF DISEASE SEVERITY CAUSED BY FOUR SOIL-BORNE PATHOGENS IN WINTER CEREAL SEEDLINGS

In this study, two strains each of *Fusarium pseudograminearum*, *F. culmorum*, *F. graminearum*, and *Bipolaris sorokiniana* were compared across a range of winter cereal species: barley, bread wheat, durum wheat, oat, and triticale in glasshouse tests for visual symptoms on the leaf sheaths and sub-crown internode . The host reaction including shoot height and dry weight of the five winter cereals infected with the crown rot and common root rot pathogens was recorded.

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Comparison of disease severity caused by four soil-borne pathogens in winter cereal seedlings

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Abstract

In Australia, crown rot of cereals is predominantly caused by *Fusarium pseudograminearum* and *Fusarium culmorum,* and common root rot by *Bipolaris sorokiniana*. *Fusarium graminearum* is an important pathogen causing Fusarium head blight worldwide and has also been reported to cause crown rot of wheat. The comparative ability of *F. pseudograminearum, F. culmorum, F. graminearum* and *B. sorokiniana* to cause crown rot and common root rot across a range of winter cereal species requires investigation. One cultivar each of barley, bread wheat, durum wheat, oat, and triticale were inoculated with two strains of each of the four pathogens in glasshouse trials. At 21 days after inoculation, the sub-crown internode and leaf sheaths of each plant were visually rated for disease severity. Shoot length and dry weight of inoculated plants were compared to non-inoculated controls. Barley and bread wheat had the greatest disease severity ratings on leaf sheaths and sub-crown internode (64.7 to 99.6%) whereas oat had the lowest disease severity ratings across all pathogens (less than 5%). The shoot length of all cultivars was significantly reduced (12.2 to 55%, $p < 0.05$) when exposed to *F. pseudograminearum*, while only barley dry shoot weight was significantly reduced (45.1 to 57%, $p < 0.05$) when inoculated with *F. pseudograminearum*. This study provides a comparison of pathogenicity of crown rot and common root rot pathogens and demonstrates significant variation in disease severity and host response across a range of winter cereals. These results will contribute to the search for resistance in these cereal species.

Keywords: *Bipolaris sorokiniana, Fusarium culmorum, F. graminearum, F. pseudograminearum,* winter cereals

Introduction

Crown rot and common root rot of cereals are of key economic significance worldwide (Kazan and Gardiner 2018; Kumar et al. 2002). In Australia, crown rot results in estimated annual

losses of $21 and $79 million for the barley and bread wheat industries, respectively (Murray and Brennan 2009; Murray and Brennan 2010). In comparison, common root rot resulted in estimated annual losses of $13 and $30 million for the barley and bread wheat industries, respectively (Murray and Brennan 2009; Murray and Brennan 2010).

Fusarium pseudograminearum is the predominant fungus associated with crown rot of cereals in Australia (Backhouse et al. 2004; Burgess et al. 1975). *Fusarium culmorum* also occurs in all regions but has been described as the dominant crown rot pathogen in cooler, high rainfall areas of South Australia and Victoria (Backhouse and Burgess 2002; Backhouse et al. 2004).

Fusarium graminearum is considered the most important Fusarium head blight pathogen worldwide (Obanor et al. 2013), and has been associated with crown rot in the USA (Dyer et al. 2009), South America (Moya-Elizondo et al. 2015) and China (Zhang et al. 2015). In Australia, *F. graminearum* has been reported to cause epidemics of Fusarium head blight (Burgess et al. 1975; Obanor et al. 2013), and *F. graminearum* strains have been shown to cause crown rot of wheat following artificial inoculation, however, they were less aggressive than *F. pseudograminearum* strains (Akinsanmi et al. 2004). Other *Fusarium* species associated with winter cereals include *F. avenaceum, F. crookwellense,* and *F. poae*, however, these fungi are infrequently isolated from crown rot diseased tissue (Backhouse et al. 2004; Obanor and Chakraborty 2014).

The fungus *Bipolaris sorokiniana* causes seedling blight and the disease common root rot (Wildermuth 1986). *Bipolaris sorokiniana* is often associated with *Fusarium* species such as *F. pseudograminearum, F. culmorum* and *F. graminearum* as part of a general dryland root rot complex. This complex has been given several other common names including dryland foot rot, Fusarium foot rot, crown rot, and common root rot (Smiley et al. 2005) and is viewed as one disease affecting the same plant (Smiley et al. 2005; Moya-Elizondo et al. 2011).

Crown rot and common root rot pathogens have been isolated from all small grain and winter cereals including barley (*Hordeum vulgare*), bread wheat (*Triticum aestivum*), durum wheat (*Triticum turgidum* var. *durum*), oat (*Avena sativa*), and triticale (× *Triticosecale*) (Burgess et al. 2001; Kumar et al. 2002; Backhouse and Burgess 2002). Bread wheat and barley cultivars range from moderately susceptible to very susceptible to crown rot (Lush et al. 2018; Sturgess 2014), while durum wheat is considered susceptible to very susceptible (Lush et al. 2018). Klein et al. (1989) included one triticale genotype in their study, which was considered susceptible to *F. pseudograminearum*. Oat exhibited low levels of discolouration following infection with *F. pseudograminearum* (Burgess et al. 2001; Nelson and Burgess 1994; Percy et al. 2012).

Barley and bread wheat cultivars have varying levels of resistance to the common root rot pathogen *B. sorokiniana* (Wildermuth et al. 1992), where bread wheat cultivars range from moderately resistant to very susceptible (Lush et al. 2018). In Australia, Wildermuth et al. (1992) reported significant yield losses associated with common root rot infection of bread wheat cultivars ranging from susceptible to partially resistant. The yield losses in the susceptible cultivars ranged between 13.9 and 23.9%, whereas those in partially resistant cultivars ranged from 6.8 to 13.6% depending on the cultivar. Wildermuth and McNamara (1991) reported a significant increase in *B. sorokiniana* levels in wheat that followed plantings of barley, oat, and triticale in the field ($p<0.05$) (naturally field infection), thus, increasing the disease severity in wheat.

The symptoms of crown rot and common root rot are similar, and it is, therefore, difficult to distinguish between the two diseases without conducting pathogen isolation and identification tests. Crown rot symptoms caused by different *Fusarium* species are also indistinguishable. The symptoms of crown rot begin as small necrotic lesions on the coleoptile, followed by a browning of the sub-crown internode and leaf sheath tissue (Burgess et al. 2001). The first

obvious symptom of crown rot in the field is browning of stem bases, which is usually observed after flowering (Burgess et al. 2001). Subsequent discolouration can reach up to the fifth node in stem tissue (Butler 1961; Burgess et al. 2001). Similar to crown rot, common root rot symptoms first appear as small brown necrotic lesions on the coleoptile and roots (Wegulo and Klein 2010). As the disease progresses, lesions also develop on the sub-crown internode and lower parts of the leaf sheaths and the stem (Burrage and Tinline 1960). The sub-crown internode has typically been used for rating common root rot disease, while leaf sheaths and stems have been used for rating crown rot (Wildermuth et al. 1992; Burgess et al. 2001). A strong association occurs between sub-crown internode browning and resistance to common root rot (Wildermuth et al. 1992) however, this has not been demonstrated for crown rot (Percy et al. 2012; Wildermuth and McNamara 1994).

The aim of the current study was to determine and compare the abilities of *F. pseudograminearum, F. culmorum, F. graminearum* and *B. sorokiniana* to cause crown rot and common root rot diseases in a single commercially important cultivars of barley (cv. Grimmett), bread wheat (cv. Livingston), durum wheat (cv. Hyperno), oat (cv. Genie), and triticale (cv. Endeavour). The response to inoculation with the four pathogens was assessed using disease severity, shoot length and dry weight measurements of each cereal species. Knowledge of the disease-causing abilities of each pathogen species informs strategies for disease management and future breeding goals.

Materials and methods

Strains and inoculum preparation

Two strains of each pathogen (*F. pseudograminearum, F. culmorum, F. graminearum,* and *B. sorokiniana*) were used for inoculations (Table 1). Colonised grain inoculum was produced using a modified method described by Malligan (2009) and Percy et al. (2012). A single spore

from each strain was grown on Czapek-Dox Agar (CZA) (Leslie and Summerell 2008) and incubated for seven days at 25℃ for *Fusarium* species and 22℃ for *B. sorokiniana*. Mycelium was scraped off two CZA plates for each strain and mixed into 1kg bags of sterilised (twice autoclaved) bread wheat (650g) and barley (350g) grains and incubated at 25℃ in the dark. After seven days, the bags were shaken manually every two to three days over 21 days to encourage uniform colonisation of the grain. After the 28-day period, colonised grain was air-dried between sheets of blotting paper and subsequently kept in the dark at 25℃ for a further 14 days and stirred every two days. Individual inoculums were then ground using an electric 8-inch Laboratory Mill (Christy & Norris Ltd) to pass through a 2mm sieve. All inoculum bags were sealed and stored at 4℃ for future use.

Plant growth and inoculation

Two replicated seedling tests were conducted in a glasshouse at the Leslie Research Facility Department of Agriculture and Fisheries (LRF-DAF-Qld), Toowoomba, Australia. The plant growth medium consisted of self-mulching black Vertosol of the Irving clay soil association, obtained from the Darling Downs in Queensland, Australia (Thompson and Beckmann 1959), mixed with river sand (50% sand:50% soil). This mixture was steam-sterilised at 80℃ for 40 minutes and air-dried for seven days. No fertiliser was added to the mix. The two seedling tests were planted on 5th April 2016 and 5th May 2016, respectively (Australian autumn). Three replicate pots each of barley (cv. Grimmett) (moderately susceptible to *F. pseudograminearum*) (GRDC 2018), bread wheat (cv. Livingston) (susceptible to *B. sorokiniana* and *F. pseudograminearum*) (Lush et al. 2018), durum wheat (cv. Hyperno) (moderately resistant to moderately susceptible to *B. sorokiniana* and susceptible to very susceptible to *F. pseudograminearum*) (Lush et al. 2018)., oat (cv. Genie), and triticale (cv. Endeavour) were inoculated individually with two strains of each of the four pathogens, along with a non-inoculated control treatment. The two experiments were arranged as a randomised complete

block design, where each treatment (combination of pathogen, strain, and cultivar) was randomly allocated to a pot within each replicate block. The seedling inoculation method described by Wildermuth and McNamara (1994) was used with slight modifications. Briefly, 280g of moist soil (38% moisture content) was added to 5×5×10 cm pots. Fifteen seeds were planted at a depth of 5.5 cm from the top of the pot and covered with a layer of sieved dry soil (160g). Inoculum (0.45g) was applied in an even layer to the soil surface of all pots excluding the non-inoculated control. The inoculum was covered with 40g of dry soil. All pots were placed in a water bath at 25^{o}C with natural day lengths in a glasshouse. The inoculum was activated after seven days by watering each pot to field capacity (38% moisture content) by weight, after which the pots were watered daily up to field capacity. Plants were harvested 21 days after planting, up to ten plants from each pot were rated for disease severity and assessed for shoot length and shoot dry weight.

Disease severity ratings

Disease severity was assessed using a 0 to 100% rating scale based on the visual discolouration of the sub-crown internode and the first three leaf sheaths. Rating of each tissue occurred in 5% increments where 0 = no discolouration and 100% = completely discoloured tissue. The disease symptoms of the inoculated plants were assessed relative to the background discolouration of the non-inoculated control plants of the respective cultivars. Following disease severity ratings, all roots and sub-crown internode were removed, and shoot length of each plant was measured from the base of the crown to the tip of the longest leaf. Individual shoots were placed in paper bags and dried in a 65°C oven (UF160, Memmert) for 48 h, after which dry weights were recorded.

Data analysis

The percentage of disease severity on the first three leaf sheaths were totalled and divided by three to give a combined leaf sheath percentage. To ensure homogeneity of variance an arcsine

square root transformation was applied to the sub-crown internode and the combined leaf sheath rating data. The analysis of each variable was performed using a linear mixed model. The model included fixed effects for pathogen, strain within pathogen, cultivar, experiment, and their interactions. Terms to account for the replicate blocks, plots, and plants within plots were included as random effects, with these variances estimated separately for each experiment. Estimates of variance parameters were generated using Residual maximum likelihood (REML) estimation (Patterson and Thompson 1971). Predictions for each trait were generated from their respective models as empirical Best Linear Unbiased Estimators (eBLUEs). Where a transformation had been used, predicted means were back transformed to the original scale, and approximate standard errors were calculated using the Taylor series approximation. All analyses were performed using ASReml-R (Butler et al. 2009) in the R software environment (R Core Team 2016). Significance of fixed effects were assessed using a Wald test with a significance level of 0.05.

Data for disease severity of the leaf sheaths and the sub-crown internode have each been presented graphically in two ways to allow comparison of significant differences detected between cultivars and strains in this study.

Results

Comparison of leaf sheath disease severity

The appearance of visual symptoms caused by the four pathogens on the first three leaf sheaths of each symptomatic cultivar were similar (Fig. 1). A significant interaction between experiment, pathogen, strain and cultivar (p = 0.017) was observed in the leaf sheath ratings (Supplementary Table 1). This interaction meant the two experiments could not be combined. *Fusarium pseudograminearum* caused significantly greater disease severity than *F. culmorum*, *F. graminearum* and *B. sorokiniana* in barley (cv. Grimmett), triticale (cv. Endeavour) and

durum wheat (cv. Hyperno) (Fig. 2). The greatest disease severity rating was observed for *F. pseudograminearum* in Grimmett (99.6% disease severity). Significant differences were observed between some strains of the same pathogen, for example, strain *Fc*2 and *Fg*2 had a significantly higher leaf sheath rating than strain *Fc*1 and *Fg*1, respectively, for Grimmett, Livingston, and Endeavour and strain *Fp*1 had a greater leaf sheaths rating than *Fp*2 in Livingston (Fig. 2).

For most of the strains, the disease severity on Grimmett leaf sheath tissue was significantly greater than Livingston, Hyperno, Endeavour and oat cultivar Genie, respectively, ranged from 0.33 to 99.6%, yet Livingston was greater than Grimmett for *B. sorokiniana* (19.6%) (Fig. 3). Hyperno (ranged from 0.09 to 30%) and Endeavour (ranged from 0.002 to 30%) had lower disease severity when infected by all pathogens compared to Grimmett (ranged from 0.33 to 99.6%) and Livingston (ranged from 1.14 to 51.53 %). Oat cultivar Genie exhibited significantly lower disease severity ($p > 0.05$) across all pathogens (0.03 to 2.5%) (Fig 3).

Comparison of sub-crown internode disease severity

There was a significant pathogen by strain by cultivar interaction for the sub-crown internode rating ($p < 0.001$) (Supplementary Table 2). The highest disease severity ratings were observed in Grimmett infected with *F. pseudograminearum* (100%), followed by *F. culmorum* (97.6%) and one strain of *F. graminearum* (59.8%) (Fig. 4a). The sub-crown internode disease severity in Livingston infected with *F. pseudograminearum* strains ranged from 32.3 to 64.7%, *B. sorokiniana* strains from 16.5 to 58.2% and a 31% disease severity rating was observed with *F. culmorum* strains (Fig. 4a). Significant variation between strains was observed in the sub-crown internode disease severity of Grimmett inoculated with *F. culmorum* and *F. graminearum*, Livingston inoculated with *F. pseudograminearum*, and Genie inoculated with *F. culmorum* (Fig. 4a).

Grimmett (0.5 to 100%) and Livingston (0.9 to 64.7%) had the greatest disease severity on the sub-crown internode, whereas Genie (0 to 12%), Endeavour and Hyperno (0 to 13.7%) had low levels of sub-crown internode disease severity (Fig. 4b).

Shoot length

Shoot length of cultivars varied significantly ($p < 0.001$) (Supplementary Table 3) in response to pathogen inoculation (Fig. 5). In most instances, the inoculated treatments had a reduced shoot length compared to the controls. The greatest reduction in shoot length occurred in all cultivars inoculated with *F. pseudograminearum,* where shoot length was reduced by 12% for Genie, 13% for Hyperno, 20% for Endeavour, 34.3% for Livingston, and 55% for Grimmett compared to the control (Fig. 5). Oat cultivar Genie had the lowest levels of reduction in shoot length across all pathogens (6 to 12%) (Fig. 5).

Shoot dry weight

A significant interaction between cultivars and strains within the pathogens was observed for shoot dry weight ($p = 0.035$) (Supplementary Table 4). The reduction of dry shoot weight between the control and inoculated plants was significant for Grimmett infected with both *F. pseudograminearum* strains (45.1 to 57%), *Fc*2 (11.6%) and *Fg*2 (10.7%) (Fig. 6). Genie seedlings inoculated with strain *Fg*1 also had a significant 11% decrease in dry shoot weight compared to the control. The dry shoot weight of Livingston was significantly lower (17.2%) than the control when inoculated with *Fp*1.

Discussion

In Australia, extensive research on crown rot has focused on *F. pseudograminearum*. In these studies, host response, genetic resistance and yield loss were assessed mostly on bread wheat and barley cultivars (Percy et al. 2012; Wildermuth and McNamara 1994; Burgess et al. 1975).

In the present study, the focus was expanded to assess the pathogenicity of four crown rot and common root rot pathogens across a range of a single cultivar of five winter cereals species. *Fusarium pseudograminearum* strains caused greater disease symptoms than any of the other pathogens in Grimmett, Livingston, Hyperno and Endeavour. Similar results to our study were observed by Knight and Sutherland (2017), who reported a comparison of visual disease symptoms on the leaf sheaths and fungal biomass of a single strain of *F. pseudograminearum* and *F. culmorum* in seedlings of six winter cereals and three summer cereals. *Fusarium pseudograminearum* caused greater discolouration than *F. culmorum* in all the cereals, except oat, rye, maize, and rice, where the only minimal disease was reported by Knight and Sutherland (2017).

Greater leaf sheaths discolouration was observed on barley cultivar Grimmett (15 to 25%) and bread wheat cultivar Livingston (22.4 to 23.5%) when infected with *Fg*2. In addition, the disease severity on the sub-crown internode of Grimmett was also high with this strain (59.8%). *Fusarium graminearum* has not historically been considered an important crown rot pathogen in Australia (Obanor & Chakraborty 2014). However, Dyer et al. (2009) and Obanor & Chakraborty (2014) suggested that the ability of *F. graminearum* to cause crown rot might increase in areas where Fusarium head blight is more common. In Australia, the fungus has been associated with crown rot in areas where head blight occurred in northern NSW and in the warm to subtropical areas with moderate to high rainfall in Queensland (Akinsanmi et al. 2004; Backhouse and Burgess 2002). The *F. graminearum* strains that were used in our glasshouse experiments were isolated from Fusarium head blight affected grain. This suggests that *F. graminearum* may contribute to crown rot disease in Australia under favourable conditions.

Low disease severity ratings (0.07 to 9%) were observed on the leaf sheaths of most hosts inoculated with *B. sorokiniana* strains, except for Livingston where *B. sorokiniana* strains were

equivalent to *F. culmorum* strains in disease severity. High levels of disease severity (58.2%) were only observed on the sub-crown internode of Livingston infected with strain *Bs*2. This finding confirms previous studies that suggested that *B. sorokiniana* is more effective at causing disease on the lower part of the plant (Burrage and Tinline 1960; Wildermuth et al. 1992).

Variation between strains within the pathogen species was observed for combined leaf sheath and sub-crown internode ratings in some of the cultivars. *Fusarium culmorum* strain *Fc*2 caused greater disease severity on the leaf sheaths than *Fc*1 on Endeavour, Grimmett and Livingston. Also, strain *Fg*2 frequently caused greater disease severity than *Fg*1 across the cultivars. *Fusarium pseudograminearum* has been described to vary in aggressiveness between strains, depending on several factors including, farming system, geographical factors, and the genetic diversity of each strain (Akinsanmi et al. 2004).

Low levels of disease were observed in durum wheat cultivar Hyperno on the leaf sheaths and the sub-crown internode after inoculation with each of the pathogens. Wallwork et al. (2004) reported lower than expected disease severity in durum wheat cultivars Gundaroi, Tamaroi and Yallaroi with a terrace system. These results are in contrast to the result of Knight and Sutherland (2017), where durum wheat cultivars EGA Bellaroi and Jandaroi had similar disease severity ratings to the most diseased cultivars of barley. This could be due to some cultivars exhibiting resistance in the early stages of growth, with the disease symptoms becoming more pronounced in the advanced stages. Yang et al. (2010) suggested that different genes can be responsible for crown rot resistance at early developmental stages of wheat and barley cultivars and this resistance might disappear throughout growing stages. In addition, the inoculation techniques could have had an impact on disease progression. Knight and Sutherland (2017) applied 6µL of a 10^6 conidia/mL to the coleoptile, which may have elevated the disease severity. In both our system and that of Wallwork *et al*. (2004), the inoculum was applied as a

colonised grain added to the soil in a layer above the seeds. Thus, the coleoptile grows through the soil and the inoculum, similar to a natural field infection.

Triticale cultivar Endeavour had low levels of visual symptoms on the leaf sheaths (0 to 30%) and sub-crown internode (0 to 13.7%) compared to Grimmett (0.33 to 99.6%) and Livingston (1.1 to 51.5%). Knight and Sutherland (2017) reported that triticale cultivars Hawkeye and Berkshire had a high level of disease severity with similar responses to *F. pseudograminearum* and *F. culmorum* as spring wheat, barley and durum wheat cultivars. This difference may be due to the different inoculation methods used or genetic variation between cultivars.

Oat is considered a resistant or an asymptomatic host of *F. pseudograminearum* (Percy *et al*. 2012; Knight and Sutherland 2017). Low levels of disease, significantly greater than the controls, were observed on the sub-crown internode of Genie when inoculated with one strain of *F. culmorum* (12%) and on the leaf sheaths when inoculated with strains of each pathogen, with the exception of *F. pseudograminearum* strain *Fp*1 (2.5%). While disease levels were low, the capacity of oats to host all of these pathogens was confirmed and further supports recommendations that oat should not be used as a rotational crop for crown rot (Nelson and Burgess 1994) nor common root rot management (Wildermuth and McNamara 1991).

The physiological impact of disease caused by these four crown rot and common root rot pathogens has not extensively been detailed. *Fusarium pseudograminearum* resulted in the greatest reduction of shoot length across all cultivars. Grimmett and Livingston had significant shoot length reductions across all pathogens, except *B. sorokiniana* with Grimmett. While oat cultivar Genie had low or no symptoms, there was a significant reduction in the shoot length of Genie across all pathogens ($p < 0.05$). The reduction in the shoot length indicates that each pathogen had a negative effect on the development of the host. Similarly, Smiley et al. (2005) indicated that disease severity was negatively correlated with plant height for *F. pseudograminearum, F. culmorum,* and *F. graminearum* but not for *B. sorokiniana.*

Differences in host shoot dry weight were observed but varied according to pathogen or strain. Strain *Fp*1 significantly reduced the dry shoot weight in Grimmett and Livingston while *Fg*1 significantly decreased the dry shoot weight in Genie ($p < 0.05$). This outcome is in contrast to Knight et al. (2012), who indicated a significant increase in dry weight of the individual leaf sheath up to the fourth leaf for four bread wheat cultivars colonised by *F. pseudograminearum*, compared to non-inoculated controls. This difference between the two studies could be due to the dry shoot weight of the entire seedling, including leaf blade, being included in our study. A negative impact on the dry shoot weight of plant tissue indicates that each pathogen has a detrimental effect on plant growth, however, this level in reduction was not as significant as that for shoot length. Further assessment of plant height and weight in the field is crucial for investigating the physiological impact of these pathogens.

This study identified significant differences in disease severity caused by infection with *F. pseudograminearum*, *F. culmorum*, *F. graminearum*, and *B. sorokiniana* in five winter cereals species. *Fusarium pseudograminearum* caused the greatest disease severity on both the sub-crown internode and leaf sheaths followed by *F. culmorum*, *B. sorokiniana*, and *F. graminearum*. The most severe disease symptoms were observed on Grimmett and Livingston, while Genie showed low or no symptoms. Significant differences were observed in the host response (shoot dry weight and shoot length) to all pathogens with the reduction in the shoot length being more significant than the shoot weight. The reactions observed across the cereal hosts demonstrate the comparative disease impacts of each of these fungi, which will inform improved management strategies for crown rot and common root rot diseases by crop rotation. A field test will facilitate further investigation of the impact of these four pathogens on the five winter cereals at different stages of plant growth.

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Compliance with Ethical Standards

Disclosure of potential conflict of interest

The authors declare no conflict of interest.

Research involving Human Participants and/or Animals

Not applicable

Informed consent

Not applicable

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Table Heading

Table 1 Location and source of each strain of *Fusarium pseudograminearum*, *F. culmorum*, *F. graminearum*, and *Bipolaris sorokiniana*.

Figure Headings

Fig. 1 Example of disease symptoms observed on the first leaf sheaths and sub-crown internode of triticale (cv. Endeavour) after inoculation. Treatments include non-inoculated control, *Bipolaris sorokiniana* (*Bs*), *Fusarium culmorum* (*Fc*), *F. graminearum* (*Fg*), and *F. pseudograminearum* (*Fp*). This is representative of symptoms observed on barley, bread wheat, durum wheat, and oat.

Fig. 2 Mean combined leaf sheath disease severity ratings for each experiment by strain by cultivar interaction. Treatments include *Fusarium pseudograminearum* (strains *Fp*1 and *Fp*2), *F. culmorum* (strains *Fc*1 and *Fc*2), *F. graminearum* (strains *Fg*1 and *Fg*2), *Bipolaris sorokiniana* (strains *Bs*1 and *Bs*2), and non-inoculated control for each host. Different letters represent significant differences within a cultivar and experiment at $\alpha < 0.05$.

Fig. 3 Mean combined leaf sheath disease severity ratings for each experiment by cultivar by strain interaction. Treatments include *Fusarium pseudograminearum* (strains *Fp*1 and *Fp*2), *F. culmorum* (strains *Fc*1 and *Fc*2), *F. graminearum* (strains *Fg*1 and *Fg*2), *Bipolaris sorokiniana* (strains *Bs*1 and *Bs*2), and non-inoculated control for each host. Different letters represent significant differences within a strain and experiment at $\alpha < 0.05$.

Fig. 4 Mean values of the sub-crown internode disease severity rating for the pathogen by cultivar interaction (a) and the cultivar by pathogen interaction (b). Treatments include *Fusarium pseudograminearum* (strains *Fp*1 and *Fp*2), *F. culmorum* (strains *Fc*1 and *Fc*2), *F. graminearum* (strains *Fg*1 and *Fg*2), *Bipolaris sorokiniana* (strains *Bs*1 and *Bs*2), and non-inoculated control for each host. Different letters indicate significant differences between strains within a cultivar (a) and between cultivars for a strain (b) at $\alpha < 0.05$.

Fig. 5 Mean values of shoot length for the pathogen by cultivar interaction. Treatments include *Fusarium pseudograminearum* (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *Bipolaris sorokiniana* (*Bs*), and non-inoculated control. Different letters indicate significant differences within a cultivar at $\alpha < 0.05$.

Fig. 6 Mean values of cultivar dry shoot weight for the pathogen by strain by cultivar interaction. Treatments include *Fusarium pseudograminearum* (strains *Fp*1 and *Fp*2), *F. culmorum* (strains *Fc*1 and *Fc*2), *F. graminearum* (strains *Fg*1 and *Fg*2), *Bipolaris sorokiniana* (strains *Bs*1 and *Bs*2), and non-inoculated control for each host. Different letters indicate significant differences within a cultivar $\alpha < 0.05$.

Supporting Information Headings

Supplementary Table 1 ANOVA table for analysis of combined leaf sheath disease severity ratings of five winter cereals colonised by the four crown rot and common root rot pathogens used for this study. Mean separations were described using $\alpha < 0.05$.

Supplementary Table 2 ANOVA table for analysis of sub-crown internode rating of five winter cereals colonised by the four crown rot and common root rot pathogens used for this study. Mean separations of $\alpha < 0.05$.

Supplementary Table 3 ANOVA table for analysis of shoot length of five winter cereals colonised by the four crown rot and common root rot pathogens used for this study. Mean separations of $\alpha < 0.05$.

Supplementary Table 4 ANOVA table for analysis of dry shoot weight of five winter cereals colonised by four crown rot and common root rot pathogens used for this study. Mean separations of $\alpha < 0.05$.

Table and Figure Headings

Table 1 Location and source of each strain of *Fusarium pseudograminearum*, *F. culmorum*, *F. graminearum*, and *Bipolaris sorokiniana*.

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Figure 2 Mean combined leaf sheath disease severity ratings for each experiment by strain by cultivar interaction. Treatments include *Fusarium pseudograminearum* (strains *Fp*1 and *Fp*2), *F. culmorum* (strains *Fc*1 and *Fc*2), *F. graminearum* (strains *Fg*1 and *Fg*2), *Bipolaris sorokiniana* (strains *Bs*1 and *Bs*2), and non-inoculated control for each host. Different letters represent significant differences within a cultivar and experiment at $\alpha < 0.05$.

Figure 3 Mean combined leaf sheath disease severity ratings for each experiment by cultivar by strain interaction. Treatments include *Fusarium pseudograminearum* (strains *Fp*1 and *Fp*2), *F. culmorum* (strains *Fc*1 and *Fc*2), *F. graminearum* (strains *Fg*1 and *Fg*2), *Bipolaris sorokiniana* (strains *Bs*1 and *Bs*2), and non-inoculated control for each host. Different letters represent significant differences within a strain and experiment at $\alpha < 0.05$.

Figure 4 Mean values of the sub-crown internode disease severity rating for the pathogen by cultivar interaction (a) and the cultivar by pathogen interaction (b). Treatments include *Fusarium pseudograminearum* (strains *Fp*1 and *Fp*2), *F. culmorum* (strains *Fc*1 and *Fc*2), *F. graminearum* (strains *Fg*1 and *Fg*2), *Bipolaris sorokiniana* (strains *Bs*1 and *Bs*2), and non-inoculated control for each host. Different letters indicate significant differences between strains within a cultivar (a) and between cultivars for a strain (b) at $\alpha < 0.05$.

Figure 5 Mean values of shoot length for the pathogen by cultivar interaction. Treatments include *Fusarium pseudograminearum* (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *Bipolaris sorokiniana* (*Bs*), and non-inoculated control. Different letters indicate significant differences within a cultivar at $\alpha < 0.05$.

Figure 6 Mean values of cultivar dry shoot weight for the pathogen by strain by cultivar interaction. Treatments include *Fusarium pseudograminearum* (strains *Fp*1 and *Fp*2), *F. culmorum* (strains *Fc*1 and *Fc*2), *F. graminearum* (strains *Fg*1 and *Fg*2), *Bipolaris sorokiniana* (strains *Bs*1 and *Bs*2), and non-inoculated control for each host. Different letters indicate significant differences within a cultivar $\alpha < 0.05$.

Table 1 Location and source of each strain of *Fusarium pseudograminearum*, *F. culmorum*, *F. graminearum*, and *Bipolaris sorokiniana*.

Species	Strain designation	BRIP accession no.[a]	Collection year	Collection location	Source of strain
F. pseudograminearum	*Fp*1	64949	2009	Emerald, Queensland (Qld)	Crown rot affected stem
	*Fp*2	64952	2012	Irvingdale, Qld	
F. culmorum	*Fc*1	64973	2010	Unknown location, New South Wales	Crown rot affected stem
	*Fc*2	64974	2010		
F. graminearum	*Fg*1	64975	2010	Tolga, Qld	Fusarium Head blight affected grain
	*Fg*2	64976	2010	Clifton, Qld	
B. sorokiniana	*Bs*1	64970	2005	Moonie, Qld	Common root rot affected sub-crown internode
	*Bs*2	64972	2006	Wallumbilla, Qld	

[a] BRIP: Queensland Plant Pathology Herbarium (Australia)

Figure 1

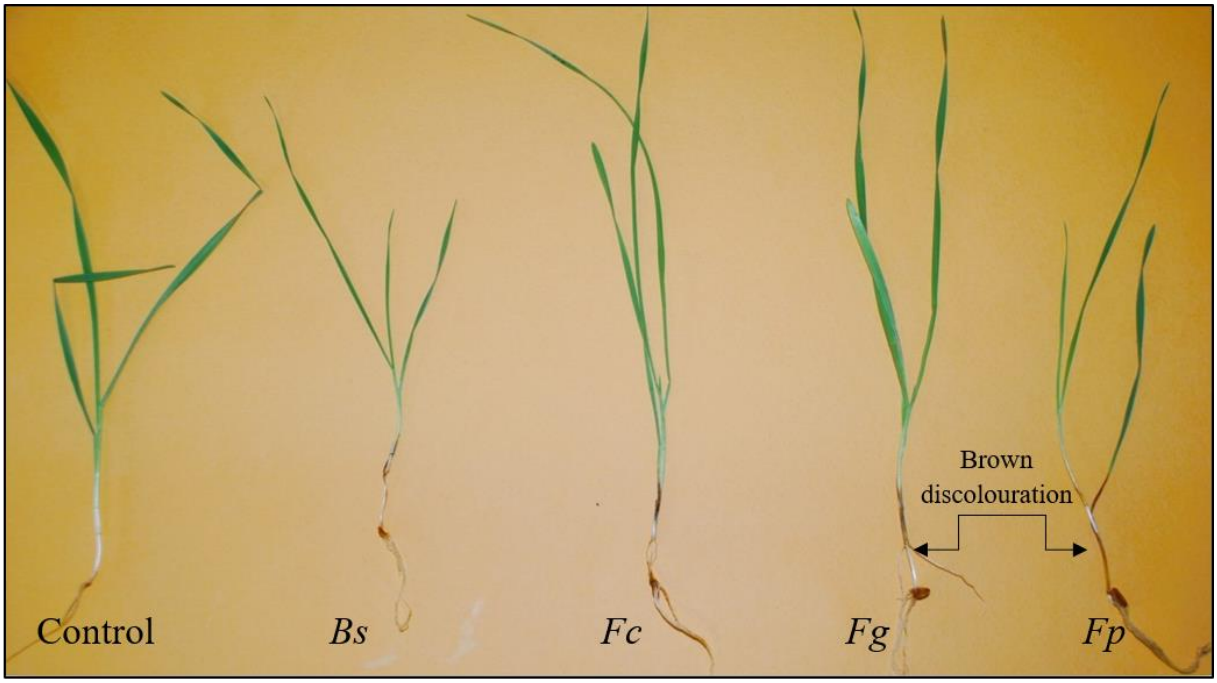


Figure 2

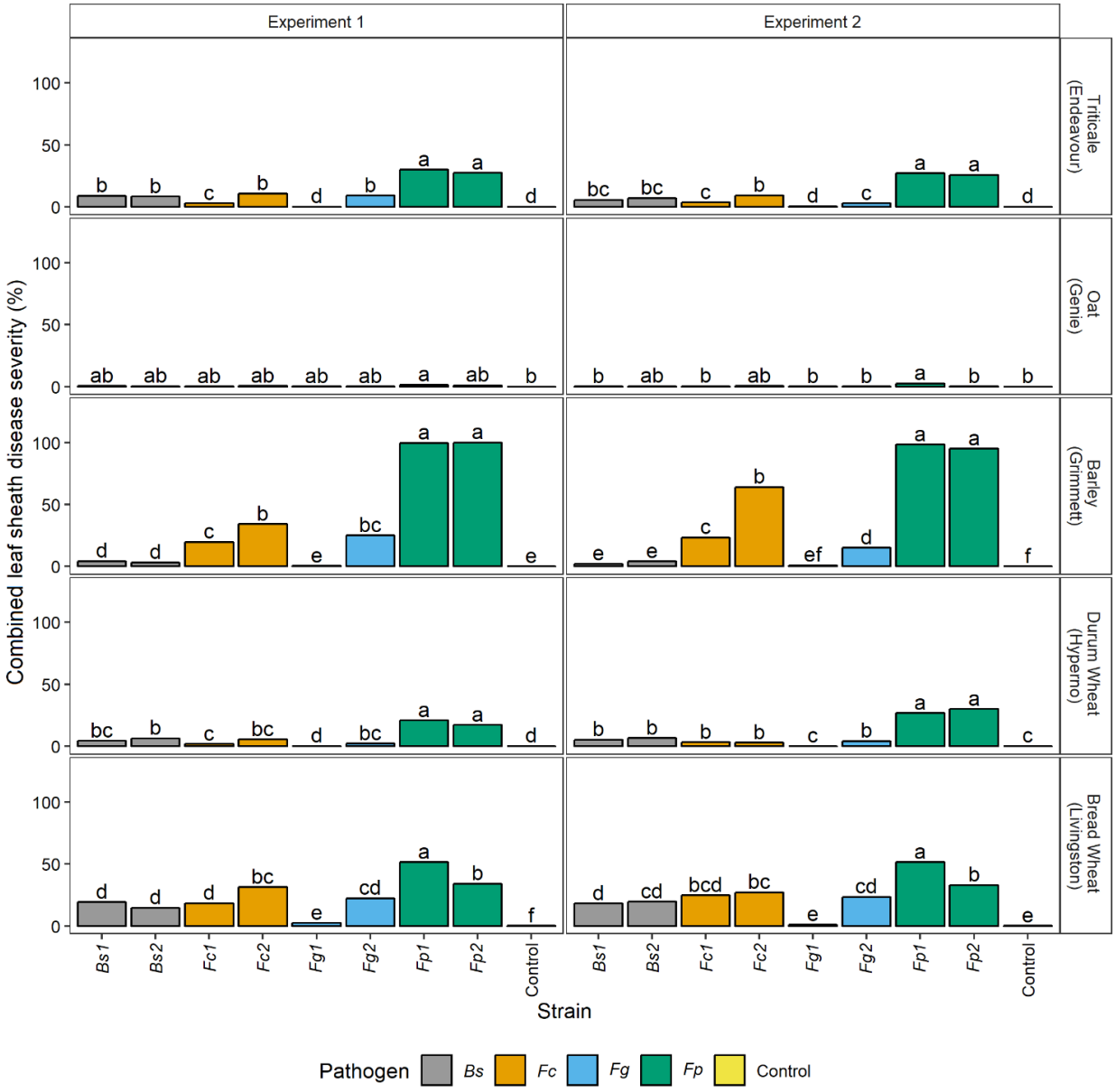

Experiment 1
Experiment 2
Triticale (Endeavour)
Oat (Genie)
Barley (Grimmett)
Durum Wheat (Hyperno)
Bread Wheat (Livingston)
Combined leaf sheath disease severity (%)
0
50
100
Bs1
Bs2
Fc1
Fc2
Fg1
Fg2
Fp1
Fp2
Control
Strain
Pathogen
Bs
Fc
Fg
Fp
Control

Figure 3

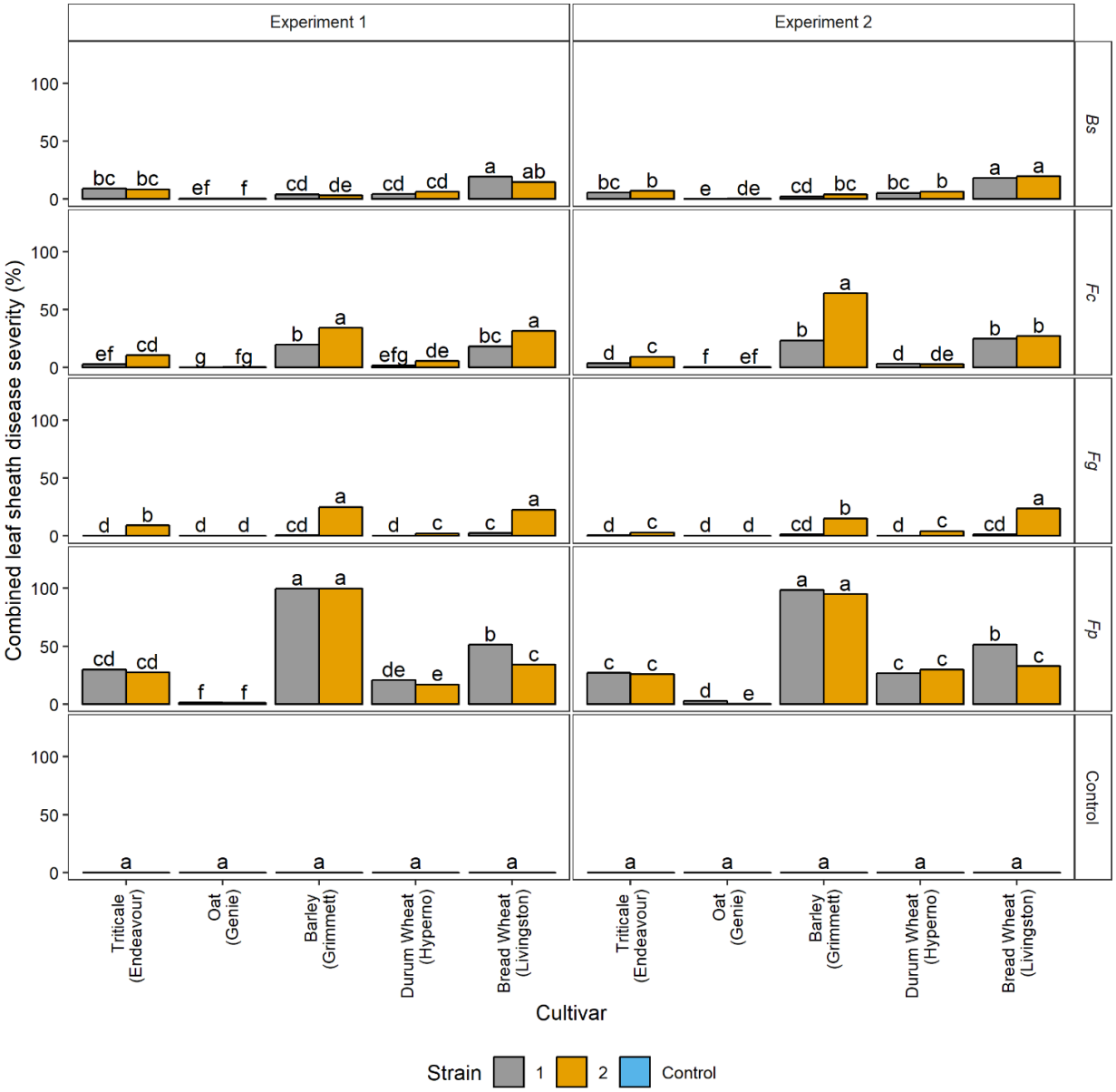

Experiment 1
Experiment 2
Bs
Fc
Fg
Fp
Control
Combined leaf sheath disease severity (%)
0
50
100
Triticale (Endeavour)
Oat (Genie)
Barley (Grimmett)
Durum Wheat (Hyperno)
Bread Wheat (Livingston)
Cultivar
Strain
1
2
Control

Figure 4

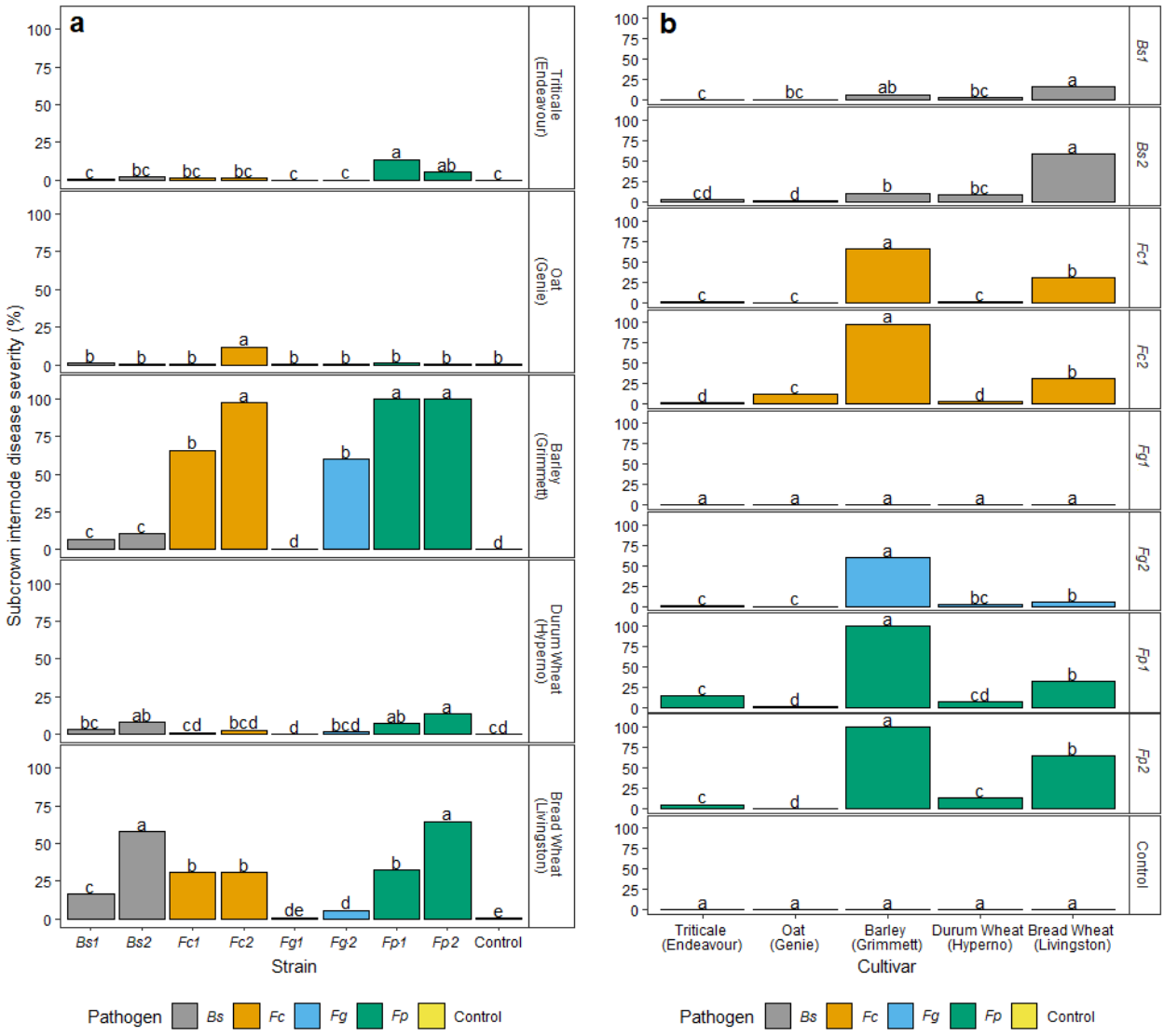

a
b
Subcrown internode disease severity (%)
Triticale (Endeavour)
Oat (Genie)
Barley (Grimmett)
Durum Wheat (Hyperno)
Bread Wheat (Livingston)
Bs1
Bs2
Fc1
Fc2
Fg1
Fg2
Fp1
Fp2
Control
Strain
Cultivar
Pathogen
Bs
Fc
Fg
Fp
Control

Figure 5

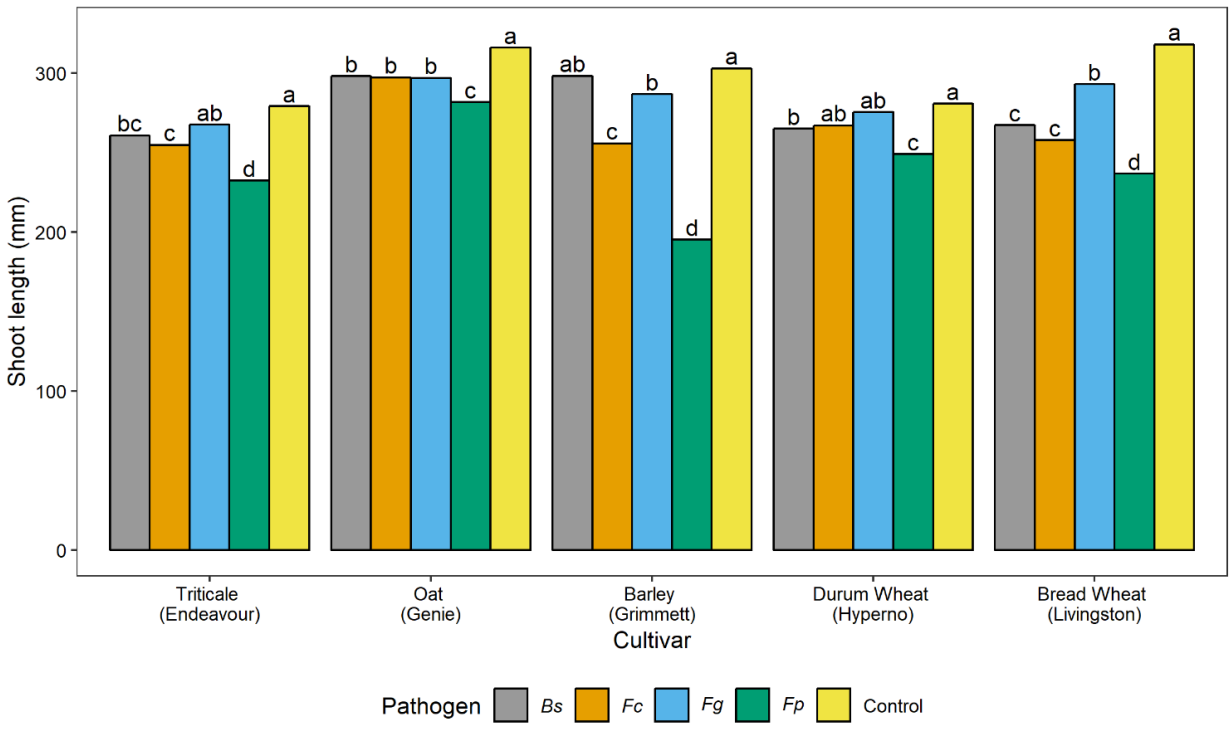
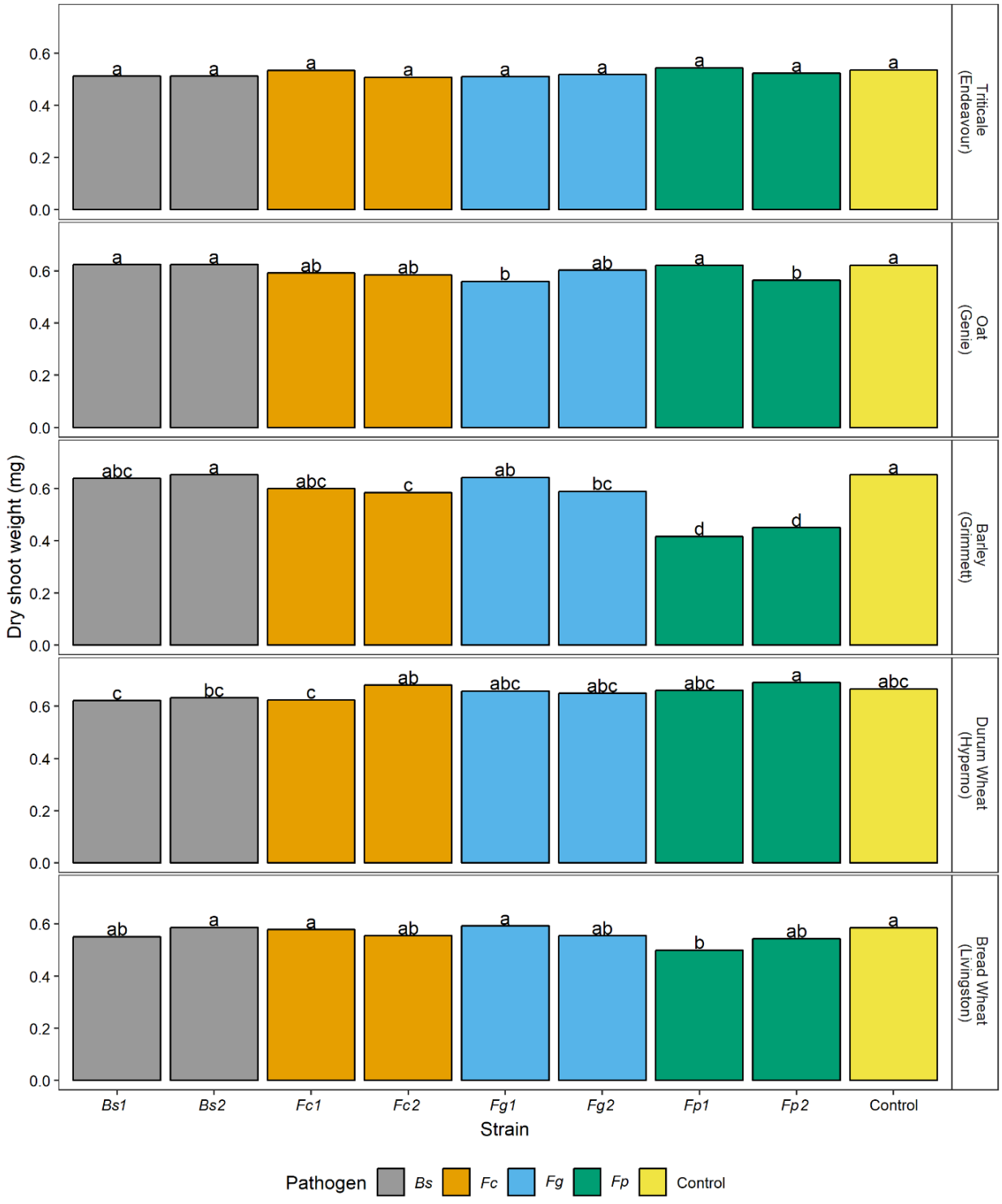

Shoot length (mm)
300
200
100
0
bc c ab d a
b b b c a
ab c b d a
b ab ab c a
c c b d a
Triticale (Endeavour)
Oat (Genie)
Barley (Grimmett)
Durum Wheat (Hyperno)
Bread Wheat (Livingston)
Cultivar
Pathogen
Bs
Fc
Fg
Fp
Control

Figure 6


Dry shoot weight (mg)
0.0
0.2
0.4
0.6
Triticale (Endeavour)
Oat (Genie)
Barley (Grimmett)
Durum Wheat (Hyperno)
Bread Wheat (Livingston)
a a a a a a a a a
a a ab ab b ab a b a
abc a abc c ab bc d d a
c bc c ab abc abc abc a abc
ab a a ab a ab b ab a
Bs1 Bs2 Fc1 Fc2 Fg1 Fg2 Fp1 Fp2 Control
Strain
Pathogen Bs Fc Fg Fp Control

Supplementary Table 1 ANOVA table for analysis of combined leaf sheath disease severity ratings of five winter cereals colonised by the four crown rot and common root rot pathogens used for this study. Mean separations were described using $\alpha < 0.05$.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept) (grand mean)	1	7.4	5588.00	<0.001
Experiment	1	8.3	0.00	0.975
Pathogen	4	176.7	645.10	<0.001
Cultivar	4	176.8	467.90	<0.001
Pathogen: Strain	4	176.9	55.20	<0.001
Experiment: Pathogen	4	177.2	1.52	0.197
Experiment: Cultivar	4	177.3	1.42	0.228
Pathogen: Cultivar	16	176.7	106.60	<0.001
Experiment: Pathogen: Strain	4	177.4	1.36	0.248
Pathogen: Strain: Cultivar	16	177.0	7.27	<0.001
Experiment: Pathogen: Cultivar	16	177.2	2.19	0.007
Experiment: Pathogen: Strain: Cultivar	16	177.4	1.98	0.017

[a] Num.: They stand for numerator degrees of freedom

[b] Den. DF: Denominator degrees of freedom

Supplementary Table 2 ANOVA table for analysis of sub-crown internode rating of five winter cereals colonised by the four crown rot and common root rot pathogens used for this study. Mean separations of $\alpha < 0.05$.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	11.2	1356.00	<0.001
Experiment	1	5.6	0.46	0.524
Pathogen	4	176.8	131.40	<0.001
Cultivar	4	176.9	244.20	<0.001
Pathogen: Strain	4	177.0	18.95	<0.001
Experiment: Pathogen	4	176.2	0.53	0.714
Experiment: Cultivar	4	176.2	0.37	0.831
Pathogen: Cultivar	16	176.8	36.87	<0.001
Experiment: Pathogen: Strain	4	176.3	1.34	0.258
Pathogen: Strain: Cultivar	16	177.0	8.55	<0.001
Experiment: Pathogen: Cultivar	16	176.1	1.26	0.226
Experiment: Pathogen: Strain: Cultivar	16	176.3	1.11	0.344

[a] Num.: They stand for numerator degrees of freedom

[b] Den. DF: Denominator degrees of freedom

Supplementary Table 3 ANOVA table for analysis of shoot length of five winter cereals colonised by the four crown rot and common root rot pathogens used for this study. Mean separations of $\alpha < 0.05$.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	177.4	96700.00	<0.001
Experiment	1	177.4	79.81	<0.001
Pathogen	4	177.5	122.20	<0.001
Cultivar	4	177.4	57.29	<0.001
Pathogen: Strain	4	177.4	9.79	<0.001
Experiment: Pathogen	4	177.5	1.25	0.291
Experiment: Cultivar	4	177.4	3.85	0.005
Pathogen: Cultivar	16	177.5	13.72	<0.001
Experiment: Pathogen: Strain	4	177.4	2.22	0.069
Pathogen: Strain: Cultivar	16	177.4	1.65	0.060
Experiment: Pathogen: Cultivar	16	177.5	1.00	0.454
Experiment: Pathogen: Strain: Cultivar	16	177.4	1.65	0.061

[a] Num.: They stand for numerator degrees of freedom

[b] Den. DF: Denominator degrees of freedom

Supplementary Table 4 ANOVA table for analysis of dry shoot weight of five winter cereals colonised by four crown rot and common root rot pathogens used for this study. Mean separations of $\alpha < 0.05$

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	2.7	5168.00	<0.001
Experiment	1	2.7	3.34	0.174
Pathogen	4	163.9	9.95	<0.001
Cultivar	4	163.8	56.66	<0.001
Pathogen: Strain	4	163.7	0.35	0.843
Experiment: Pathogen	4	164.5	1.76	0.139
Experiment: Cultivar	4	164.4	1.41	0.234
Pathogen: Cultivar	16	163.9	8.25	<0.001
Experiment: Pathogen: Strain	4	164.4	1.36	0.251
Pathogen: Strain: Cultivar	16	163.7	1.80	0.035
Experiment: Pathogen: Cultivar	16	164.5	1.72	0.048
Experiment: Pathogen: Strain: Cultivar	16	164.4	1.46	0.123

[a] Num.: They stand for numerator degrees of freedom

[b] Den. DF: Denominator degrees of freedom

CHAPTER 3

WINTER CEREAL RESPONSES TO COMMON ROOT ROT AND CROWN ROT PATHOGENS IN THE FIELD

This study presents the first direct comparison of three crown rot pathogens (*Fusarium pseudograminearum, F. culmorum,* and *F. graminearum*) and one common root rot pathogen (*Bipolaris sorokiniana*) across a single cultivar of five Australian commercial winter cereals (barley, bread wheat, durum wheat, oat, and triticale) in two field experiments throughout three harvest stages (tillering, flowering, and maturity). The visual symptoms on the incidence of diseased tillers, disease severity on the stem and sub-crown internode were recorded. The impact of each pathogen on these five winter cereals species including plant dry weight and height was measured. Species-specific PCR was used to detect the *Fusarium pseudograminearum, F. culmorum, F. graminearum* or *Bipolaris sorokiniana* DNA using a specific marker for each pathogen across the cultivars.

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Winter cereal responses to common root rot and crown rot pathogens in the field

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Abstract

Crown rot and common root rot are significant diseases of wheat and barley worldwide. In Australia, *Fusarium pseudograminearum* and *F. culmorum* are the two main fungi causing crown rot, while *Bipolaris sorokiniana* is associated with common root rot. *Fusarium graminearum* is typically linked with Fusarium head blight; however, it has been associated with crown rot in Australia and other parts of the world. Here we investigate the importance of each of these pathogens to cause crown rot and common root rot across a range of winter cereals in Australian cropping systems. A single cultivar of bread wheat, barley, durum wheat, oat, and triticale was each inoculated with two strains of *F. pseudograminearum, F. culmorum, F. graminearum* and *B. sorokiniana* in two field experiments. Plants were harvested at tillering, flowering and maturity, and disease incidence, disease severity of the sub-crown internode (SCI) and stem tissue, plant dry weight and plant height were determined. *Fusarium pseudograminearum* and *F. culmorum* treatments exhibited greater disease severity than *F. graminearum* and *B. sorokiniana* on both stems and SCI. Oat exhibited a significantly lower disease severity compared to the other cultivars; however, DNA from all pathogens was detected in oat stems. The specific infection of each fungus and the host responses in these

field experiments improves our understanding of disease development in these crops and their subsequent importance in Australian winter cereal growing regions.

Keywords: *Bipolaris sorokiniana, Fusarium culmorum, F. graminearum, F. pseudograminearum,* winter cereals

Introduction

Crown rot and common root rot are devastating soil-borne diseases of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) worldwide including Australia, Canada, China, New Zealand, North Africa, South Africa, and the USA (Kumar *et al.* 2002; Kazan and Gardiner 2018). In Australia, annual crown rot losses and common root rot losses in wheat and barley were estimated at $97 million and $43 million, respectively (Murray and Brennan 2009; Murray and Brennan 2010).

Crown rot has been associated with a number of *Fusarium* species including *F. pseudograminearum, F. culmorum, F. graminearum, F. avenaceum, F. crookwellense* and *F. poae* (Wearing and Burgess 1977; Burgess *et al.* 2001; Backhouse *et al.* 2004; Dyer *et al.* 2009). In Australia, *F. pseudograminearum* and *F. culmorum* are the predominant pathogens causing crown rot of barley and wheat (Backhouse and Burgess 2002; Backhouse *et al.* 2004; Obanor and Chakraborty 2014). *Fusarium pseudograminearum* occurs across all wheat growing regions and crown rot can be severe under the hot, dry conditions found in Australia (Burgess *et al.* 2001; Backhouse and Burgess 2002). *Fusarium culmorum* is more widely distributed in the high rainfall cooler regions of Victoria, South Australia and in the summer rainfall regions of the northern region [eastern Darling Downs, in southern Queensland (QLD)] (Backhouse and Burgess 2002; Backhouse *et al.* 2004). In Australia, *F. graminearum* has been reported to cause localised Fusarium head blight epidemics in the subtropical areas with high summer rainfall (Backhouse and Burgess 2002). In different parts of the world, *F. graminearum* is a potential crown rot pathogen and can be significant in some areas including

China and the USA (Francis and Burgess 1975; Wu *et al.* 2005; Dyer *et al.* 2009). In Australia, *F. graminearum* has been associated with crown rot under wet conditions, but to date, it is not considered to be a significant crown rot pathogen (Akinsanmi *et al.* 2004; Obanor and Chakraborty 2014).

Bipolaris sorokiniana is a soil-borne pathogen causing common root rot and seedling blight (Purss 1970; Wildermuth 1986). *Bipolaris sorokiniana* occurs in most regions where barley and wheat are grown (Wildermuth 1986). Common root rot spreads widely and causes the most damage to plants in both warmer areas with high rainfall (Wildermuth 1986) and hot dry regions (Hill and Blunt 1994).

Both *B. sorokiniana* and *Fusarium* species can affect the SCI, crown, leaf base, coleoptile and roots of plants (Wildermuth *et al.* 1992; Burgess *et al.* 2001; Kumar *et al.* 2002; Kazan and Gardiner 2018). The first symptoms of crown rot and common root rot are lesions on the coleoptile, SCI, leaf sheaths contiguous stems and nodal tissues. These symptoms are often difficult to distinguish from crown rot, however, the discolouration caused by *B. sorokiniana* does not extend as high up the stem as lesions that are caused by *F. pseudograminearum* (Burrage and Tinline 1960; Burgess *et al.* 2001; Bockus *et al.* 2010). During crown rot infection, under drought conditions, the visual symptoms can progress up the stem, sometimes as high as the fifth node (Burgess *et al.* 2001). At flowering, infected stems may undergo premature senescence, resulting in heads containing no or shrivelled grain (Burgess *et al.* 2001; Malligan 2009; Kazan and Gardiner 2018). There are no reports suggesting that *B. sorokiniana* is associated with whiteheads (Tunali *et al.* 2008). A positive correlation has been reported between the visual discolouration on the stem tissue and the severity of discolouration of the SCI caused by *B. sorokiniana* (Purss 1970; Wildermuth *et al.* 1992). The SCI tissue discolouration has been adopted as a standard method to measure common root rot severity,

while stem tissue browning has been used to measure crown rot disease infection (Wildermuth and McNamara 1994; Percy *et al.* 2012).

Crown rot and common root rot pathogens have been associated with an extensive range of hosts including barley, bread and durum wheat, rye, triticale, oat, maize, and sorghum (Burgess *et al.* 2001; Kumar *et al.* 2002; Paterson *et al.* 2005; Kazan and Gardiner 2018). The incidence and severity of these diseases can vary significantly within the host, field, and season (Burgess *et al.* 2001; Kumar *et al.* 2002). Thus, the importance of these host-pathogen associations in Australian cropping systems is unclear.

Crown rot research in Australia has focussed on *F. pseudograminearum* as the dominant pathogen due to the large distribution of the *F. pseudograminearum* strains in this country (Burgess *et al.* 2001; Backhouse *et al.* 2004). Most of the bread wheat cultivars and all of the durum wheat cultivars are very susceptible to susceptible to crown rot (Percy *et al.* 2012; Kunesch *et al.* 2017; Lush *et al.* 2018). Barley cultivars exhibit a high level of disease severity to crown rot, similar or greater than bread wheat cultivars (Percy *et al.* 2012; Sturgess 2014; Knight and Sutherland 2017) (Chapter 2). However, estimated yield losses of barley are less than those in bread wheat and the formation of whiteheads is less common (Wallwork *et al.* 2004).

Bipolaris sorokiniana has a varied range of disease severity depending on the host (Purss 1970; Wildermuth *et al.* 1992; Lush *et al.* 2018). In Australia, bread and durum wheat cultivars range from susceptible to moderately resistant. (GRDC 2018; Lush *et al.* 2018; GRDC 2019) and barley cultivars range from susceptible to moderately susceptible to common root rot. (GRDC 2019).

Oats have been shown to exhibit a low level of discolouration when they are inoculated with crown rot (*F. pseudograminearum* and *F. culmorum*) and common root rot pathogens (Nelson

and Burgess 1994; Percy *et al.* 2012; Knight and Sutherland 2017) (Chapter 2). However, the role of oat cultivars as a rotation crop to crown rot and common root rot is not clear.

Long-term management and control of crown rot and common root rot pathogens can be difficult as the fungus can survive in the soil for several years, particularly in the presence of a host (Wildermuth *et al.* 1997; Burgess *et al.* 2001). To manage inoculum levels in the field different strategies may be applied including stubble management, crop rotations with at least two years of resistant crops to reduce the fungal populations in the soil, as well as using resistant cultivars (Wildermuth and McNamara 1991; Burgess *et al.* 2001). Unfortunately, levels of resistance in bread wheat, durum and barley are still not suitable for reducing inoculum for either of the diseases.

Previous studies have examined a range of cereal seedlings for crown rot and common root rot pathogens reactions under glasshouse conditions (Knight and Sutherland 2017) (Chapter 2). However, disease reactions in the field are essential for a robust understanding of host response to crown rot and common root rot pathogens. The current study was designed to examine the importance of host reactions of crown rot and common root rot pathogens under field conditions. Thus, the primary aim of this study was to compare the host response of barley, bread wheat, durum wheat, oat, and triticale infected by *F. pseudograminearum, F. culmorum, F. graminearum*, and *B. sorokiniana* in two field experiments. Disease severity including assessment of SCI and stem tissues and the impact of each pathogen on different host cultivars are compared by reporting differences in plant height and plant dry weight. The comparison of cereal reactions to these pathogens in the field will provide valuable information for management strategies targeting crown rot and common root rot diseases. In addition, this research will improve the understanding of pathogen impacts on different hosts which may help to improve resistance in these cereals to crown rot and common root rot.

Materials and methods

Strains and inoculum preparation

Colonised grain inoculum was prepared for two strains of each pathogen species (*F. pseudograminearum, F. graminearum, F. culmorum,* and *B. sorokiniana*) (Table 1) following the method described in Chapter 2. Inoculum was stored at 4°C prior to application in the field.

Field site characteristics

Two field experiments were conducted in 2016 and 2017 at the Wellcamp Field Station, Department of Agriculture and Fisheries, Queensland, Australia (coordinates: 27°33'54" S 151°51'51" 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). In each year, urea (100 kg N/ha) was applied three weeks before sowing at a depth of 50 mm.

In both years, the DNA levels in the paddock of a range of soilborne pathogens, including *B. sorokiniana, F. culmorum, F. graminearum,* and *F. pseudograminearum* were determined by using the PreDicta B testing service which uses quantitative real-time PCR technology (Hollaway *et al.* 2013). Furthermore, *Gaeumannomyces graminis* var. *avenae, Macrophomina phaseolina, Phoma Koolunga, Phoma rabiei, Pratylenchus quasitereoides, Pratylenchus penetrans, Pratylenchus thornei* and *Rhizoctonia solani* AG were determined using the PreDicta B test. Three samples were taken from the site before planting and twenty soil samples were taken from the non-inoculated control at post-harvest using an Accucore sampler at depth 30 cm (15 to 20 cores were included for each sample).

In both years, experiments were planted in July and plants at the tillering and flowering stages were harvested in September and November, respectively. Mature plants were harvested in December of each year.

The minimum and maximum temperature and rainfall were collected for both years from the Moyola weather station, 8.1 km from the site of the experiments, (site number 041369, coordinates: 27.52°" S 151.88°" E).

Field experiments

Experimental design

The two experiments were designed as randomised completed block designs, where each treatment (combination of harvest time, pathogen, strain, and cultivar) was randomly allocated to a plot within each replicate block (three replicates for each harvest time). Five cultivars [barley (cv. Grimmett), bread wheat (cv. Livingston), durum wheat (cv. Hyperno), oat (cv. Genie), and triticale (cv. Endeavour)] were exposed to the four crown rot and common root rot pathogens. Two individual strains were used for each pathogen. In both years, twenty seeds were sown in 1m rows, then covered with soil. In 2016, treatments consisted of individual plots inoculated with individual strains of each pathogen species. In 2017, the individual strains were combined to inoculate each plot. Control treatments consisted of non-inoculated plants. Two grams of ground colonised grain inoculum was applied along the furrow above the seed. In 2016 seed was hand planted into furrows. In 2017 the seed was machine planted. Inoculum was applied manually in both years to avoid any contamination. Three replicates of each treatment were harvested at three harvest times, tillering (Zadoks growth stage GS20 – 29), flowering (Zadoks growth stage GS60 – 69), and maturity (Zadoks growth stage GS90 – 99).

Disease severity rating and host response

At each harvest, ten plants were collected from each plot. At tillering, flowering and maturity, the tiller number was counted and the disease incidence was evaluated by counting the number of diseased tillers of each plant. The disease severity was measured as a honey brown to dark brown discolouration of tissue using a 0-100% rating scale where 0 = no discolouration and 100% = completely discoloured tissue (Fig. 1). This scale was applied to both SCI and all stems of each plant. After the disease rating had been taken, the roots and the SCI were removed, and plant height of each plant was taken by measuring from the base of the tiller to the tip the longest leaf at tillering stage and from the base of the stem to the tip of the longest head at

flowering and maturity stages. All heads were removed and the individual plants (leaves and stems) were dried in a 60°C dehydrator (Wessberg Martin Engineering Pty Ltd, dryer/oven, Germany) for three days. Following drying, the individual plants were weighed.

Data analysis of disease severity and host response

The incidence of disease tillers (%) (disease incidence) was calculated by dividing the number of diseased tillers by the total number of tillers per plant. To ensure the assumptions of normality were not violated, disease severity of stems and SCIs, and tiller number and tiller incidence were logit transformed, and the plant dry weight and stem length were log transformed. The analysis of each variable was performed using a linear mixed model. The model included fixed effects for pathogen, harvest time, cultivars, and their interactions. Terms to account for such as the replicate blocks, plots, and plants within plots were included as random effects. Estimates of variance parameters were generated using Residual maximum likelihood (REML) estimations (Patterson and Thompson 1971). Predictions for each trait were generated from their respective models as empirical Best Linear Unbiased Estimators (eBLUEs). Where a transformation had been used, predicted means were back-transformed to the original scale, and approximate standard errors were calculated using the Taylor series approximation. All analyses were performed using ASReml-R (Butler *et al.* 2009), in the R software environment (R Core Team 2016).

Tissue processing and DNA extraction

The fungal DNA from each strain was extracted using the mycelium, which was grown on Potato Dextrose Agar (PDA) at 25°C for *Fusarium* species and 20°C for *B. sorokiniana* under 24 hours darkness for 14 days. Two metal beads 2 mm (Winchester Australia limited, VIC, Australia) were placed into each 2mL centrifuge tube and each sample was ground twice using a FastPrep-24 instrument (MP Biomedicals, California, USA) at 6.5m/s for 15s. The extraction

was conducted using the Wizard® Genomic DNA Purification Kit for plant tissue (Promega, Sydney, NSW, Australia) according to the manufacturer's recommended procedure.

In 2017, DNA was extracted from one random stem from each of five randomly selected plants from each plot, resulting in a total of 15 stems from each of the four pathogens treatments, along with the non-inoculated control treatments collected at flowering stage. The basal 3cm of each stem was cleaned and stored separately in 5 cm tubes. Stems were dried at 60°C in a dehydrator (Wessberg Martin Engineering Pty Ltd, dryer/oven, Germany) for three days and stored in a -80°C freezer. The basal 3cm portion of the stem was cut into approximately 2mm lengths and placed in 2 ml tubes. Prior to the extraction, each sample was dried overnight at 65°C in a dehydrator (UF160-en, Memmert, Germany). Four metal beads of 2mm (Winchester Australia limited, VIC, Australia) were placed into the 2ml tube and each stem was ground twice using a FastPrep-24™ instrument (MP Biomedicals, Valiant Co., Ltd., Santa Ana, California, USA) at 6.5 m/s for 60 s. Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Sydney, Australia). The protocol provided by the manufacturer was used with the exception of incubation time of 60 minutes at 65°C after the Nuclei Lysis Solution was added. DNA was resuspended in 100µl of autoclaved deionised water and stored at -20°C until required.

Species-specific PCR assays

PCR primers specifically amplifying DNA of *F. pseudograminearum* (Knight *et al.* 2012), *F. culmorum* (Knight and Sutherland 2017), *F. graminearum* (Nicholson *et al.* 1998), and *B. sorokiniana* (Horne 2015) were used to detect *F. pseudograminearum, F. culmorum, F. graminearum,* and *B. sorokiniana* DNA (Table 2). Pure genomic DNA standards of each pathogen strain were used as a positive control in this test.

Each 10ul PCR reaction consisted of 0.5 Units Immolase DNA polymerase (Bioline Pty Ltd., Australia), 100µM dNTPs, 1× Immolase buffer, 2.5 mM $MgCl_2$, 0.25mM of forward and

reverse primers (Invitrogen), and 3µl of DNA template. Thermal cycling conditions for Fc_K13B-P_F & Fc_K13B-P_R and Fg16NF & Fg16NR were 95°C for 7 minutes followed by 40 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 10 minutes. Thermal cycling conditions For Fp_TEF1α.2Fa & Fp_TEF1α.2Ra and CosA_F_519 & CosA_R_248 were 7 minutes at 95°C, followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 10 minutes. DNA amplicons were separated by agrose gel (1% agrose) electrophoresis with 5µl GelRed and visualised using a Fusion FX (Vilber Lourmat, Fisher Biotec, Australia). The specific PCR primers of *F. pseudograminearum, F. culmorum, F. graminearum,* and *B. sorokiniana* were amplified across all of the samples.

Results

In both experiments, the harvest of tillering plants was conducted nine weeks after planting. The flowering harvest varied depending on each host. Bread and durum wheat cultivars were harvested 13 weeks and barley, oat, and triticale cultivars 15 weeks after planting. Mature plants were harvest 17 weeks after planting for barley, bread and durum wheat cultivars and 18 weeks after planting for oat and triticale cultivars.

Variation in the rainfall was observed in the two seasons. In 2016 high rainfall occurred early in the season (tillering) and early spring (flowering), with a dry end (maturity). In contrast, in 2017, dry condition occurred at the beginning and the end of the season and high rainfall in the middle of the season (flowering) (Supplementary Table 1).

PreDicta B result

The PreDicta B result of the pre-sowing soil samples showed 0pg DNA/g Sample of *Fusarium* species and *B. sorokiniana* DNA at the site. For the post-harvesting soil samples, there were low levels of *F. culmorum, F. graminearum, F. pseudograminearum* and *B. sorokiniana* DNA detected in the non-inoculated control plots (0 to 4pg DNA/g Sample) for both years.

Incidence of disease tillers

A significant interaction was observed in the incidence of diseased tillers between harvest time, cultivars, and pathogens in both 2016 (p = 0.008) and 2017 (p = 0.001) trials. Differences between strains were not significant in this trial. In general, the number of tillers infected per plant increased in each cultivar at each harvest time, with the highest incidence of diseased tillers at maturity (Fig. 2a & b). In 2016 the incidence of diseased tillers was higher than expected in the non-inoculated control plots, and *F. pseudograminearum* incidence of diseased tillers were significantly greater in durum wheat (cv. Hyperno) (48.2%) and bread wheat (cv. Livingston) (51.5%) at flowering and in barley (cv. Grimmett) at maturity (90.2%) (Fig 2a). *Fusarium culmorum* disease incidence was higher in Hyperno at flowering (34.2%) and triticale (cv. Endeavour) (74.4%) and Grimmett (87.7%) at maturity than the non-inoculated control plots (Fig. 2a). In 2017, greater differences were observed between the controls and inoculated plots in all pathogens at each harvest time (Fig.2b). At flowering time *F. pseudograminearum* and *F. culmorum* were significantly higher than the controls in all cultivars except oat (cv. Genie), where only *F. pseudograminearum* was significantly greater than the non-inoculated control (ranged from 81.8 to 92.6%) (Fig. 2b). *Bipolaris sorokiniana* was also significantly higher than the non-inoculated controls in Hyperno (84.8%) and Livingston (77.3%), while *F. graminearum* had a significantly higher incidence of diseased tillers in Hyperno (64.5%) at flowering. At maturity, the incidence of diseased tillers was only different from the non-inoculated controls in Endeavour when infected with *F. pseudograminearum* (92.6%) (Fig 2b).

Disease severity on stems

There was a significant interaction between harvest time, cultivar, pathogen and strain within the pathogen (p = 0.002) for the disease severity on the stems in the 2016 trial (Fig. 3a). Low levels of disease severity (less than 20%) were recorded on the stems of all treatments in all

cultivars at flowering with the exception of one *F. graminearum* inoculated Endeavour (27.4%) (Fig. 3a and 4a). At maturity, cultivars inoculated with *F. pseudograminearum* strains had the greatest disease severity on stems (ranged from 18.1 to 54.8%), significantly higher than the non-inoculated controls with the exception of oat cultivar Genie (Fig. 3a and 4a). One strain of *F. culmorum* inoculated Grimmett (22.4%) and both strains of *F. culmorum* inoculated Endeavour (22.2 and 37.9%) and Hyperno (15.1 to 32.5%) were significantly higher than the non-inoculated controlled plots at maturity (Fig. 3a and 4a). Significant differences between the strains occurred for *F. pseudograminearum* strains on Hyperno and Endeavour (ranged between 22.2 to 54.8%) at maturity (Fig. 3a and 4a).

In the 2017 trial, the two strains of each pathogen were combined to inoculate each plot. A significant interaction between harvest time, cultivar, and pathogen ($p = 0.003$) was detected for disease severity on the stems in 2017. At flowering, disease severity of plots inoculated with *F. Pseudograminearum* was significantly higher than the non-inoculated controls in Grimmett, Livingston and Hyperno cultivars (ranging from 11.7 to 68.5%) (Fig 3b and 4b). Consistent with 2016, the highest level of disease on the stems was recorded in *F. pseudograminearum* inoculated plots with the disease severity significantly greater than the non-inoculated controls in all cultivars (23.4 to 61%) except Genie at maturity (Fig 3b and 4b). Disease severity in *F. culmorum* inoculated plots was also significantly higher than the non-inoculated controls in Grimmett (13.2 and 28.7%) and Hyperno (32.7 and 36.1%) at both flowering and maturity (Fig 3b and 4b).

Sub-crown internode disease severity

There was a significant interaction between cultivar and pathogen ($p < 0.001$) for the SCI disease severity for both the 2016 and 2017 experiments (Fig. 5a and b). There was no interaction between the harvest time and strain and data for disease severity of the SCI has thus been combined across tillering, flowering, and maturity for each of these trials. The disease

severity of the SCI of the *F. pseudograminearum* and *F. culmorum* plots were significantly higher than the non-inoculate controls in Grimmett and Livingston in both the 2016 and 2017 trials (ranged from 50.6 to 65.9% for *F. pseudograminearum*, and from 46.5 to 47.4% for *F. culmorum*) (Fig. 5a and b and 6a and b) and also in Endeavour and Hyperno in the 2017 trial (ranged from 69.2 to 87.3% for *F. pseudograminearum*, and from 32.3 to 76.3% for *F. culmorum*). The SCI disease severity for the *B. sorokiniana* and *F. graminearum* inoculated treatments was not significantly different from the non-inoculated control except for Genie in 2016 (Fig. 5a) and Endeavour in 2017.

Plant Height

There was significant interaction between harvest time and cultivars (p <0.001) and significant effect for pathogens for the plant height (p <0.001) in 2016 and 2017 experiment. In both seasons *F. pseudograminearum* caused the greatest reduction (5 to 7% reduction) in the plant height compared to the non-inoculated control and the other pathogens (Fig 7a and b).

In 2017 *F. culmorum* caused 3% reduction in plant height compared to the non-inoculated control (Fig 7b). A significant plant height interaction was observed between harvest time and cultivars (p < 0.001) for both the 2016 and 2017 seasons (Supplementary Fig. 1).

Plant dry weight

In the 2016 experiment, there was a significant interaction between harvest time and pathogen for plant dry weight (p = 0.008). There was an increase in the plant dry weight at flowering compared to maturity for all pathogens (ranged from 20.1 to 24.8% at flowering and 15.4 to 19% at maturity) (Fig. 8). *Fusarium pseudograminearum* then *F. culmorum* had the greatest increase in the plant dry weight at flowering (7 to 7.9% increase) and maturity (3.3 to 4.2% increase) compared to the non-inoculated control.

In 2017, a significant interaction was observed between harvest time and cultivar (Supplementary Fig. 2) and significant impact for pathogen for the plant dry weight (p < 0.001)

(Fig. 9). *Fusarium pseudograminearum* caused a significant reduction in the plant dry weight ($p < 0.05$) compared to the non-inoculated control (Fig 9).

Tiller number

In 2016, there was significant interaction between harvest time, pathogen, and isolate within pathogen ($p = 0.021$) for the tiller number (Fig 10). There were no differences between the strains within the pathogens and the non-inoculated control for the tiller number across the harvest time with the exception of *F. pseudograminearum* and one *F. culmorum* strain (*Fc*2) at maturity (3.9% increase). Cultivars inoculated with *Fp*2 of *F. pseudograminearum* had significant 3.7% increase in the tiller number at tillering compared to non-inoculated control. For both flowering and maturity, both strains of *F. pseudograminearum* caused a significant rise in the tiller number of the cultivars ranged between 3.2 to 4.9% compared to the non-inoculated control (Fig 10).

In 2017, a significant difference was observed between cultivars and pathogen for tiller number ($p = 0.010$). The data was consistent across different harvest times. There were no differences between all cultivars across pathogens compared to the non-inoculated control except for barley cultivar Grimmett (Fig 11). There was a 4 to 5.6% reduction in the tiller number of Grimmett when inoculated with *F. pseudograminearum* and *F. culmorum* compared to the non-inoculated control (Fig. 11).

PCR assay

PCR assay of F. pseudograminearum, F. culmorum, F. graminearum, and B. sorokiniana treatments

PCR assays were conducted on all plots (*F. pseudograminearum, F. culmorum, F. graminearum, B. sorokiniana* and the non-inoculated control treatments) for the 2017 experiment at flowering. A large number of plants had symptoms, but the pathogen-specific fragment was not amplified for *F. graminearum, B. sorokiniana* treatments, while, *F.*

pseudograminearum and *F. culmorum* DNA were detected the most across the cultivars (Table 3). The PCR assay detected the *F. pseudograminearum* DNA fragment most frequently in Grimmett (80%), Livingston (73%), Endeavour (67%), and Hyperno (53%), respectively (Table 3). The *F. culmorum* DNA was detected in Hyperno and Genie (73%), Livingston (67%), Endeavour (50%) and Grimmett (40%) (Table 3). *Fusarium graminearum* DNA was identified the least across all *F. graminearum* treatments ranging from 0 to 13% (Table 3). Most of *B. sorokiniana* and *F. graminearum* treatments that had symptoms but did not amplify with the fungal fragments had 5% or lower disease severity. Inconsistent with the low level of disease severity on the stem in Genie, *F. culmorum* (73%), *F. pseudograminearum* (47%), *F. graminearum* (13%), and *B. sorokiniana* (7%) DNA was detected (Table 3).

Comparison of disease incidence and pathogen DNA detection

An association between the disease incidence and the presence of PCR fragments of *F. pseudograminearum* and *F. culmorum* for most cultivars was observed (Table 4). Grimmett (80%), Livingston (71.4%), Endeavour (66.7), and Hyperno (60.0%) had the greatest number of the plants with symptoms and with DNA of *F. pseudograminearum* (Table 4). Livingston (73.3%), Hyperno (73.3%), and Genie (66.7%) had the highest proportion of samples with plants that have symptoms and with DNA of *F. culmorum* (Table 4). *Fusarium graminearum* DNA was detected the least across all cultivars (Table 4). All pathogens' DNA were detected in most of the asymptomatic plants of Genie (Table 4).

Discussion

In Australia, research on crown rot has focussed on inoculations with *F. pseudograminearum* (Burgess *et al.* 2001; Percy *et al.* 2012; Kazan and Gardiner 2018) and to a lesser extent *F. culmorum* (Wallwork *et al.* 2004; Hollaway *et al.* 2013; Knight and Sutherland 2017). Apart from a limited number of studies this research has included only bread wheat and barley. In the current study, the focus has been expanded to make direct comparisons in disease development

between *F. pseudograminearum* and *F. culmorum* and include two other crown and common root rot pathogens *F. graminearum* and *B. sorokiniana* on a single cultivar each of barley, bread wheat and durum wheat, oat, and triticale. *Fusarium pseudograminearum* followed by *F. culmorum* showed significant disease severity on both stem and SCI tissues for all cultivars excluding Genie across each harvest time in the two seasons. This is consistent with previous research which suggests *F. pseudograminearum* is the dominant pathogen causing crown rot on barley and wheat cultivars in Australia and other parts of the world (Burgess *et al.* 2001; Dyer *et al.* 2009; Chakraborty *et al.* 2010; Knight and Sutherland 2017). The dominance of *F. pseudograminearum* over *F. culmorum* could be due to different factors such as the aggressiveness of each individual strain population, the genetic diversity for each strain within the pathogen and the geographic origin of each strain (Obanor *et al.* 2010; Khudhair *et al.* 2019).

In Australia, crown rot has been induced in wheat under wet conditions in the field following artificial inoculation with *F. graminearum.* (Akinsanmi *et al.* 2004). However, the level of disease produced was not considered to be high enough to consider the isolates used as important or aggressive crown rot pathogens (Akinsanmi et al. 2004; Obanor and Chakraborty 2014). Akinsanmi *et al.* (2004) and Obanor and Chakraborty (2014) indicated that the capacity of *F. graminearum* to become a crown rot pathogen may increase in regions where Fusarium head blight is epidemic but less aggressive than *F. pseudograminearum*. Given that *F. graminearum* strains in the present study came from Fusarium head blight affected grain, *F. graminearum* had a low level of disease severity on both stem (ranged from 3.6 to 24.6% at maturity) and SCI (ranged from 8.7 to 37%) across all cultivars compared to the other pathogens. This result suggests that these strains of *F. graminearum* are not important crown rot pathogens under the conditions of these experiments. A low level of disease severity in one *F. graminearum* strain across most of the cultivars was reported in Chapter 2. The second strain

of *F. graminearum,* however caused 60% disease severity on SCI of barley cultivar Grimmett. The outcome of this result slightly differed with the current study which showed a low level of disease severity across all cultivars that were infected with *F. graminearum* strains.

In our study, durum wheat cultivar Hyperno was observed to have the greatest disease severity on the stem in both years (ranged from 54.8 to 68.5 %) compared to Grimmett (18.1 to 50.4%), Livingston (35.8 to 41%) and Endeavour (5.6 to 37.9%) when inoculated with *F. pseudograminearum*. Our result agrees with other reports that have indicated that durum wheat cultivars are very susceptible to susceptible to *F. pseudograminearum* (Percy *et al.* 2012; Knight and Sutherland 2017; GRDC 2019). A high level of disease severity was observed in triticale cultivar Endeavour on the stem and SCI when infected with *F. pseudograminearum*. Similarly, previous reports have shown that triticale may be susceptible to crown rot pathogen *F. pseudograminearum* (Klein *et al.* 1989; Knight and Sutherland 2017). A study by Klein *et al.* (1989) reported a significant disease severity ($p < 0.05$) for *F. pseudograminearum* infection on triticale cultivar (Ningadhu) (ranged from 85 to 91%). The findings of our study are in contrast with the results in Chapter 2 where Hyperno and Endeavour seedlings showed a low level of disease severity on both the SCI and the leaf sheaths when inoculated with *F. pseudograminearum*. This suggests that different genes are responsible for crown rot resistance at early developmental stages of durum wheat and triticale cultivars Hyperno and Endeavour, in which case, this resistance may not be expressing at the later stages (Yang *et al.* 2010).

Low disease severity was exhibited on the stem of oat cultivar Genie compared to the non-inoculated control for both years' experiments. In the 2016 field trial, there was a low level of disease severity significantly greater than the non-inoculated control on the SCI of Genie when inoculated with *F. pseudograminearum* (32.5%), and *F. culmorum* (29.1%). These findings are consistent with the seedling tests in Chapter 2, whereas *F. pseudograminearum* and *F. culmorum* had higher than control disease severity on the SCI of oat cultivar Genie. Oat is

considered to be a resistant or an asymptomatic host to the crown rot (Nelson and Burgess 1994; Percy *et al.* 2012; Knight and Sutherland 2017) (Chapter 2). and common root rot (Wildermuth and McNamara 1991) pathogens; however, other studies have also recommended that oat should not be employed in crop rotation for crown rot (Nelson and Burgess 1994) nor common root rot management (Wildermuth and McNamara 1991).

The symptoms of crown rot and common root rot pathogens might differ in the same field on the same host (Wildermuth 1986; Burgess *et al.* 2001). Initially, the browning discolouration that is associated with crown rot and common root rot manifest as small necrotic lesions on the coleoptile tissue followed by brown discolouration on the SCI and the first leaf sheaths. The discolouration then develops on the base of the first internode of stem tissue (Burrage and Tinline 1960; Burgess *et al.* 2001; Kazan and Gardiner 2018). In our study, across harvest time, the brown discolouration was observed mostly at the base of the stem and moving up the stem. However, in some cases, at flowering, the discolouration had only developed on the second node. Knight and Sutherland (2016) indicated that the fungal invasion of internodes above ground level was mainly via stomata, with penetration hyphae growing across from the infected subtending leaf sheaths in close contact with the stem. This may indicate that while the fungus colonised on the first leaf sheath tissue, penetration hyphae did not invade the second or the third leaf sheath tissue until late in the development of the plant more likely after or during stem extension.

Significant differences in plant height of the cultivars were observed between the pathogens treatments, with *F. pseudograminearum* resulting in the greatest reduction in the plant height of the cultivars compared to the other pathogens and the non-inoculated control. This indicates *F. pseudograminearum* has a negative impact on the development of the cultivars. Similarly, the results in Chapter 2 suggested that the disease severity was inversely correlated with plant height for crown rot and common root rot pathogens in seedlings.

An increase in the plant dry weight was observed at flowering and maturity for the cultivars when inoculated with *F. pseudograminearum* and *F. culmorum* strains in the 2016 experiment. This may be due to higher tiller numbers observed at flowering and maturity for the treatments that were inoculated with *F. pseudograminearum* and *F. culmorum* strains in the same season. This result is in contrast with that in Chapter 2 which found that there was a decrease in the plant dry weight in seedlings across the cultivars when infected with *F. pseudograminearum*, *F. culmorum* and *F. graminearum* and there were no differences across cultivars when infected with *B. sorokiniana*. Further research is needed to examine the impact of crown rot pathogens on the physiological response of the host at different stages of growth. Plant dry weight was significantly greater at flowering than at maturity for all inoculated treatments compared to the non-inoculated control in 2016 trial. The dry weight differences between flowering and maturity may be due to loss in the dry material during maturity harvest, transport and processing. In the 2017 season, the significant interaction was among the pathogens for the plant dry weight. *Fusarium pseudograminearum* and *F. culmorum* were observed to have a reduction in the plant weight compared to the non-inoculated control.

The PCR assay showed that the DNA of *F. pseudograminearum* and *F. culmorum* (40 to 73%) were detected the most frequently across the cultivars, whilst *B. sorokiniana* DNA (ranged from 13 to 40%) and *F. graminearum* (ranged from 0 to 13%) were the least frequently detected. Additionally, there was a high level of disease incidents on all non-inoculated controls treatments (78 to 97%). However, none of the pathogens' DNA was detected. That could be due to physiological or other biological impacts which cause similar browning discolouration. The oat cultivar Genie was observed to have little or no disease severity across all pathogens. However, the PCR assay detected the DNA of all pathogens in Genie. Furthermore, *F. culmorum* had greater levels of DNA amplified on Genie cultivars (73%) followed by *F. pseudograminearum* (47%), suggesting that Genie cultivars may be susceptible to different *F.*

culmorum strains. Oat may, therefore, escalate the population of crown rot pathogens in the field. This requires further investigation using qPCR assay to identify the fungal biomass of these fungi in oat.

In conclusion, this research has identified significant differences in the disease severity of three *Fusarium* species and *B. sorokiniana* in a single cultivar each of five winter cereals in field trials over two years. In particular, *F. pseudograminearum* caused the greatest disease severity on the stem and SCI followed by *F. culmorum*, *B. sorokiniana*, and *F. graminearum*. *Fusarium pseudograminearum* had a negative impact on the host height and the dry weight in both 2016 and 2017 experiments. In 2016, a significant disease effect was observed on plant dry weight of the cultivars across harvest times. Hyperno, Grimmett, Livingston, and Endeavour, respectively had the highest disease severity in both stem and SCI ratings. Oat cultivar Genie exhibited a low level of disease severity. However, DNA of all pathogens was detected from Genie stems. Therefore, oat may not be recommended for crop rotation as it may host crown rot and common root rot pathogens. Knowing the ranking of each pathogen on a variety of hosts will lead to a better understanding of each fungus disease severity pattern which will, in turn, inform breeding strategies and the search for resistance in these different cereal species.

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Tables and Figures Headings:

Table 1. The location and source of each strain of *Fusarium pseudograminearum* (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *and Bipolaris sorokiniana* (*Bs*).

Table 2. Primer pairs (forward, F and reverse, R) for specific amplification of *Fusarium pseudograminearum* (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *and Bipolaris sorokiniana* (*Bs*) DNA.

Table 3. Comparison of disease incidence (%) and DNA detection frequency (%) of *Bipolaris sorokiniana*, *Fusarium culmorum*, *F. graminearum*, *F. pseudograminearum* and non-inoculated control treatments (n= 15 of each the four inoculation treatments and one non-inoculated control).

Table 4. Comparison of disease incidence and DNA detection frequency of *Bipolaris sorokiniana*, *Fusarium culmorum*, *F. graminearum*, *F. pseudograminearum* and non-inoculated control treatments (%) (n= 10 to 15 for each cultivar by inoculum combination).

Fig. 1. Example of disease symptoms and different discolouration observed on bread wheat stems using 0 to 100 % scale where 0= no discolouration and 100= complete discoloured tissue. This is representative of symptoms observed on barley, bread wheat, durum wheat, and oat.

Fig. 2. Average percentage of diseased tillers per plant between pathogen within a cultivar and harvest time interaction in 2016 (a) and 2017 (b). Different letters represent significant differences between pathogens within a cultivar and harvest time at $\alpha < 0.05$**.** Treatments include *Fusarium. pseudograminearum* strains (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *Bipolaris sorokiniana* (*Bs*), and one non-inoculated control for each host.

Fig. 3. Average percentage of disease severity on the stems of each cultivar infected with two strains of each pathogen at flowering and maturity in the 2016 field trial (pathogen and strain within a cultivar and harvest times interaction) (a), and pathogen (combined strains) within a cultivar and harvest times interaction (b) in 2017. Different letters indicate significant differences between pathogens and strains within a cultivar and harvest time (a) and between pathogens within a cultivar and harvest time at $\alpha < 0.05$. Treatments include *Fusarium. pseudograminearum* strains (*Fp1* and *Fp2*), *F. culmorum* (*Fc1* and *Fc2*), *F. graminearum* (*Fg1* and *Fg2*), *Bipolaris sorokiniana* (*Bs1* and *Bs2*), and one non-inoculated control for each host. X = data sets excluded from the analysis due to storage damage to the plant.

Fig. 4. Average percentage of disease severity on the stems between cultivars within a pathogen by strain by harvest time interaction (a) and between cultivars within a pathogen (combined strains) and harvest time interaction (b) in 2017. Different letters indicate significant differences between cultivars within a pathogen and strain and harvest time (a) and between cultivars within a pathogen and harvest time (b) at $\alpha < 0.05$. Treatments include *Fusarium. pseudograminearum* strains (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *Bipolaris sorokiniana* (*Bs*), and one non-inoculated control for each host. X = data sets excluded from the analysis due to storage damage to the plants.

Fig. 5: Average percentage of SCI disease severity between pathogens within a cultivar interaction for 2016 (a) and 2017 (b) experiments. Different letters represent significant differences between pathogens within a cultivar at $\alpha < 0.05$. Treatments include *Fusarium.*

pseudograminearum strains (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *Bipolaris sorokiniana* (*Bs*), and one non-inoculated control for each host.

Fig. 6: Average percentage of SCI disease severity between cultivars within a pathogen interaction for 2016 (a) and 2017 (b) experiments. Different letters represent significant differences between cultivars within a pathogen at $\alpha < 0.05$. Treatments include *Fusarium. pseudograminearum* strains (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *Bipolaris sorokiniana* (*Bs*), and one non-inoculated control for each host.

Fig. 7. Average value of plant height for each pathogen effect for 2016 (a) and 2017 (b). Treatments include *Fusarium. pseudograminearum* strains (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *Bipolaris sorokiniana* (*Bs*) and non-inoculated control. Different letters represent significant differences between pathogens at $\alpha < 0.05$.

Fig. 8 Average value of plant dry weight for the pathogen within harvest time interaction in 2016. Treatments include *Fusarium. pseudograminearum* strains (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *Bipolaris sorokiniana* (*Bs*). Different letters represent significant differences pathogens within harvest time at $\alpha < 0.05$.

Fig. 9. Average value of plant dry weight for each pathogen effect for 2017. Treatments include *Fusarium. pseudograminearum* strains (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *Bipolaris sorokiniana* (*Bs*) Different letters represent significant differences between pathogens at $\alpha < 0.05$.

Fig. 10. Average value of tiller number for pathogens and strains at different harvest times for 2016. Treatments include *Fusarium. pseudograminearum* strains (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *Bipolaris sorokiniana* (*Bs*). Different letters represent significant differences between pathogens within harvest time at $\alpha < 0.05$.

Fig. 11. Average tiller number for pathogens by cultivars interaction for 2017. Treatments include *Fusarium. pseudograminearum* strains (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *Bipolaris sorokiniana* (*Bs*). Different letters represent significant differences between pathogens within cultivars at $\alpha < 0.05$.

Table. 1

Species	Strain designation	BRIP accession no.[a]	Collection year	Collection location	Source of strain
F. pseudograminearum	*Fp*1	64949	2009	Emerald, Queensland (Qld)	Crown rot affected stem
	*Fp*2	64952	2012	Irvingdale, Qld	
F. culmorum	*Fc*1	64973	2010	Unknown location, New South Wales	Crown rot affected stem
	*Fc*2	64974	2010		
F. graminearum	*Fg*1	64975	2010	Tolga, Qld	Fusarium Head blight affected grain
	*Fg*2	64976	2010	Clifton, Qld	
B. sorokiniana	*Bs*1	64970	2005	Moonie, Qld	Common root rot affected sub-crown internode
	*Bs*2	64972	2006	Wallumbilla, Qld	

[a] BRIP: Queensland Plant Pathology Herbarium (Australia)

Table. 2.

Primer	Sequence (5′→3′)	Target	Fragment (bp)	Reference
Fp_TEF1α.2Fa	ATCATTCGAATCGCTCGACG	*Fp*	82	Knight *et al.* (2012)
Fp_TEF1α.2Ra	AAAAATTACGACAAAGCCGTAAAAA	*Fp*		Knight and Sutherland (2017)
Fc_K13B-P_F	ATGACCGAAGACTCGGAGAA	*Fc*	112	
Fc_K13B-P_R	ACCTTGTGCTGAGCTCGTCT	*Fc*		Nicholson *et al.* (1998)
Fg16NF	ACAGATGACAAGATTCAGGCACA	*Fg*	280	
Fg16NR	TTCTTTGACATCTGTTCAACCCA	*Fg*		Horne (2015)
CosA_F_519	TCAAGCTGACCAAATCACCTTC	*Bs*	248	
CosA_R_248	AATGTCGAGCTTGCCAAAGT	*Bs*		

Table 3.

Average *Fusarium* and *B. sorokiniana* DNA detection (%) [a] / Average Disease incidence (%) [b]										
Treatment	**Endeavour**		**Genie**		**Grimmett**		**Hyperno**		**Livingston**	
Fp **[c]**	**67.0**	100	**47.0**	73	**80.0**	100	**53.0**	100	**73.0**	100
Fc **[d]**	**50.0**	100	**73.0**	93	**47.0**	100	**73.0**	100	**67.0**	100
Fg **[e]**	**0.0**	93	**13.0**	80	**0.0**	100	**0.0**	100	**0.0**	93
Bs **[f]**	**13.0**	93	**7.0**	73	**13.0**	93	**47.0**	100	**33.0**	100
Control [g]	**0.0**	96	**0.0**	78	**0.0**	97	**0.0**	96	**0.0**	97

[a] The percentage frequency of *Bipolaris sorokiniana, Fusarium culmorum, F. graminearum, F. pseudograminearum* DNA in winter cereals cultivars (average = the total number of samples for one treatment (15)/ the number of the incidence of DNA fragment in this treatment)

[b] Percentage disease incidence of the plant that was used in the PCR assay (average = the total number of samples of one treatment (15)/ the number of the incidence of disease in this treatment)

[c] *Fusarium pseudograminearum* strain inoculated treatment

[d] *Fusarium culmorum* strains inoculated treatment

[e] *Fusarium graminearum* strain inoculated treatment

[f] *Bipolaris sorokiniana* strain inoculated treatment

[g] Non-inoculated control treatments

Table 4.

Isolate	Cultivars	Symptoms (+) DNA (+) % [a]	Symptoms (-) DNA (-) % [b]	Symptoms (+) DNA (-) % [c]	Symptoms (-) DNA (+) % [d]	Total samples number	Notes
[e]***Fp***	**Endeavour**	66.7	0.0	33.3	0.0	15	
[f]***Fc***	**Endeavour**	50.0	0.0	50.0	0.0	10	
[g]***Fg***	**Endeavour**	0.0	8.3	91.7	0.0	12	Most samples have 5% disease severity (DS)
[h]***Bs***	**Endeavour**	13.3	6.7	80	0.0	15	Most samples have 5%DS
[i]**Control**	**Endeavour**	0.0	6.7	93.3	0.0	15	Most samples have 5%DS
Fp	**Genie**	33.3	13.3	40.0	13.3	15	Most samples have 5%DS
Fc	**Genie**	66.7	0.0	26.7	6.7	15	Most samples have 5%DS
Fg	**Genie**	0.0	13.3	73.3	13.3	15	Most samples have 5%DS
Bs	**Genie**	0.0	20.0	73.3	6.7	15	Most samples have 5%DS
Control	**Genie**	0.0	26.7	73.3	0.0	15	Most samples have 5%DS
Fp	**Grimmett**	80.0	0.0	20.0	0.0	15	
Fc	**Grimmett**	40.0	0.0	60.0	0.0	15	
Fg	**Grimmett**	0.0	0.0	100	0.0	15	Most samples have 5%DS
Bs	**Grimmett**	13.3	6.7	80.0	0.0	15	Most samples have 5%DS
Control	**Grimmett**	0.0	6.7	93.3	0.0	15	Most samples have 5%DS
Fp	**Hyperno**	60.0	0.0	40.0	0.0	15	
Fc	**Hyperno**	73.3	0.0	26.7	0.0	15	
Fg	**Hyperno**	0.0	0.0	100	0.0	15	All samples have 5%DS
Bs	**Hyperno**	40.0	0.0	60.0	0.0	15	
Control	**Hyperno**	0.0	20.0	80.0	0.0	15	Most samples have 5%DS
Fp	**Livingston**	71.4	0.0	28.6	0.0	14	
Fc	**Livingston**	73.3	0.0	26.7	0.0	15	
Fg	**Livingston**	0.0	6.7	93.3	0.0	15	Most samples have 5%DS
Bs	**Livingston**	33.3	0.0	66.7	0.0	15	Most samples have 5%DS
Control	**Livingston**	0.0	6.7	93.3	0.0	15	Most samples have 5%DS

[a] Percentage of the plants with visual symptoms and DNA of *Fusarium* Species and *Bipolaris sorokiniana* in five winter cereal species cultivars

[b] Percentage of the plants without visual symptom or DNA of *Fusarium* Species and *Bipolaris sorokiniana* in five winter cereal species cultivars

[c] Percentage of the plants with the visual symptom but no DNA of *Fusarium* Species and *Bipolaris sorokiniana* in five winter cereal species cultivars

[d] Percentage of the plants without visual symptom but with DNA of *Fusarium* Species and *Bipolaris sorokiniana* in five winter cereal species cultivars

[e] *Fusarium pseudograminearum* strain inoculated treatment

[f] *Fusarium culmorum* strains inoculated treatment

[g] *Fusarium graminearum* strain inoculated treatment
[h] *Bipolaris sorokiniana* strain inoculated treatment
[i] Non-inoculated control treatment

Fig. 1.

Fig. 2.

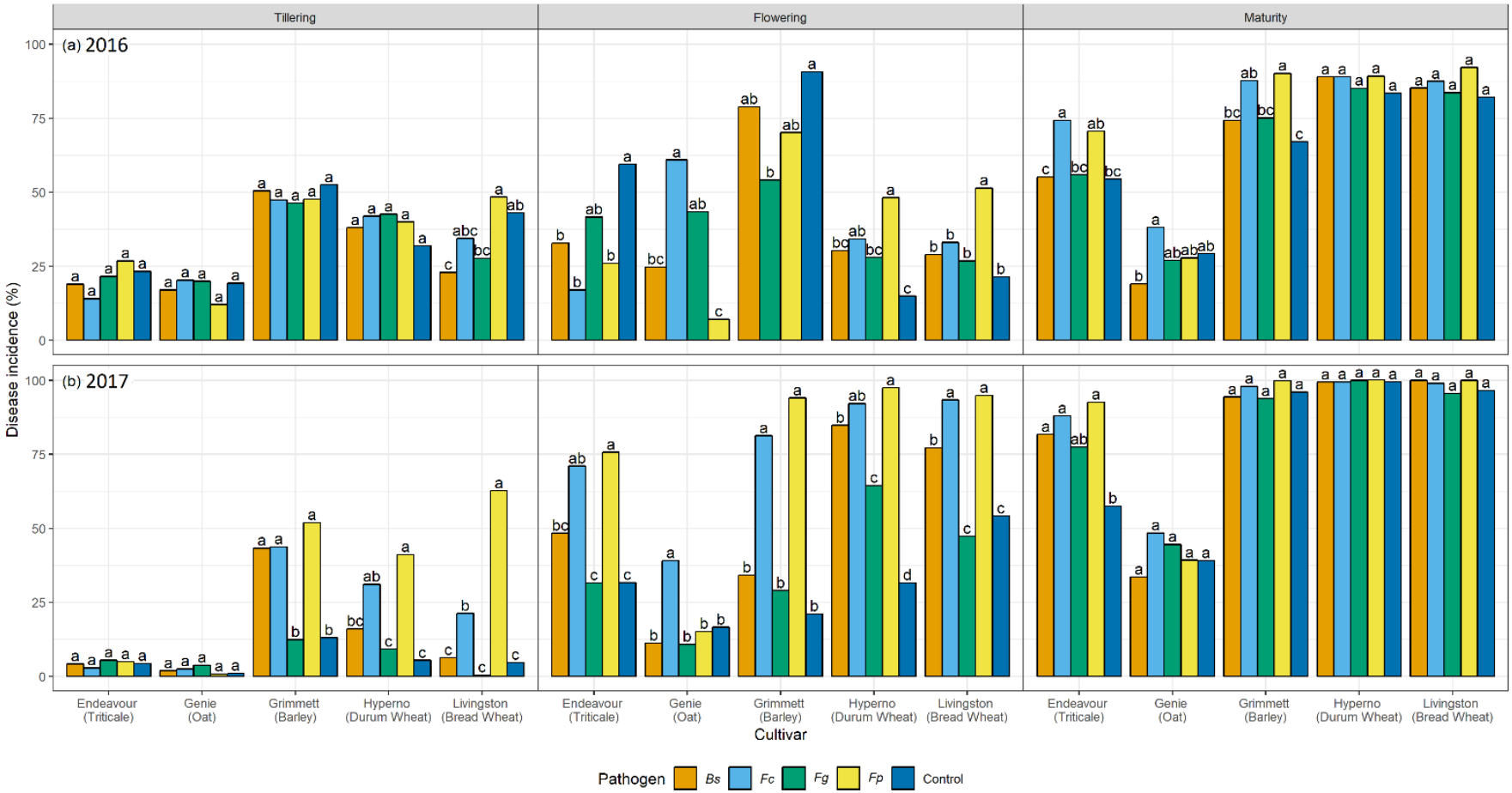
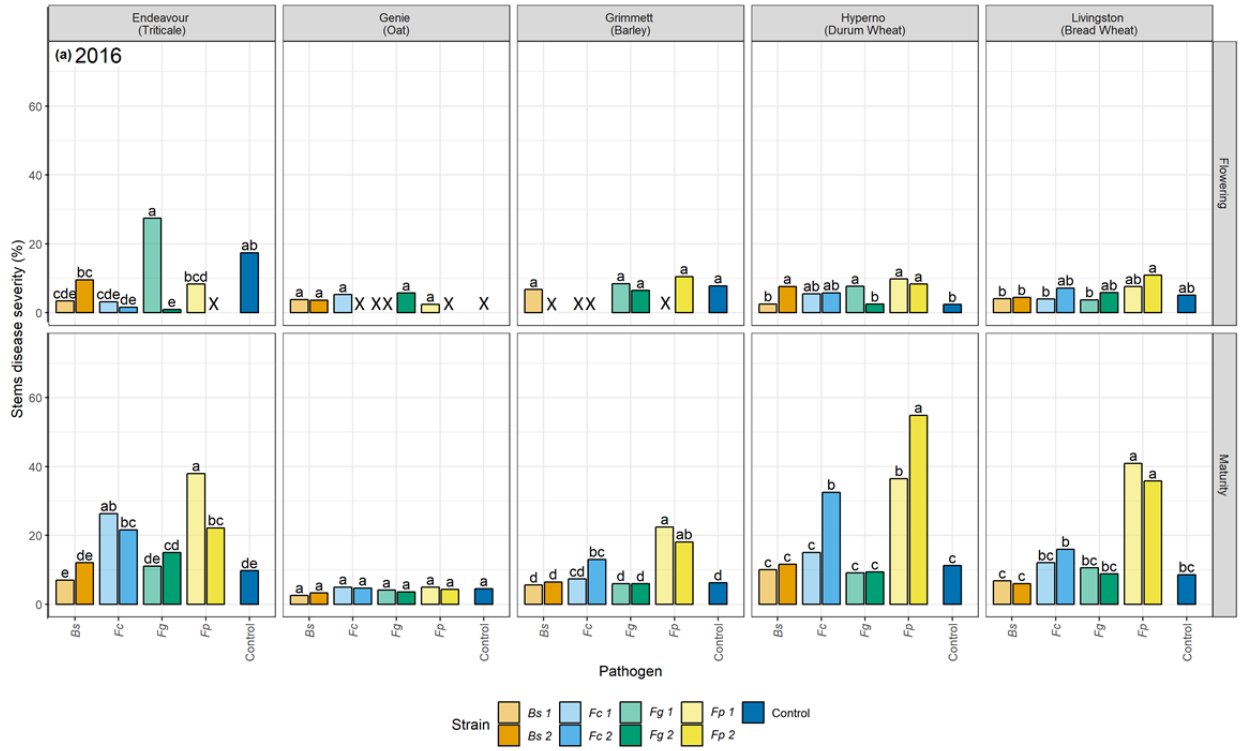

Tillering
Flowering
Maturity
(a) 2016
(b) 2017
Disease incidence (%)
0
25
50
75
100
Endeavour (Triticale)
Genie (Oat)
Grimmett (Barley)
Hyperno (Durum Wheat)
Livingston (Bread Wheat)
Cultivar
Pathogen
Bs
Fc
Fg
Fp
Control

Fig. 3.


Endeavour (Triticale)
Genie (Oat)
Grimmett (Barley)
Hyperno (Durum Wheat)
Livingston (Bread Wheat)
(a) 2016
Flowering
Maturity
Stems disease severity (%)
Bs
Fc
Fg
Fp
Control
Pathogen
Strain
Bs 1
Bs 2
Fc 1
Fc 2
Fg 1
Fg 2
Fp 1
Fp 2
Control

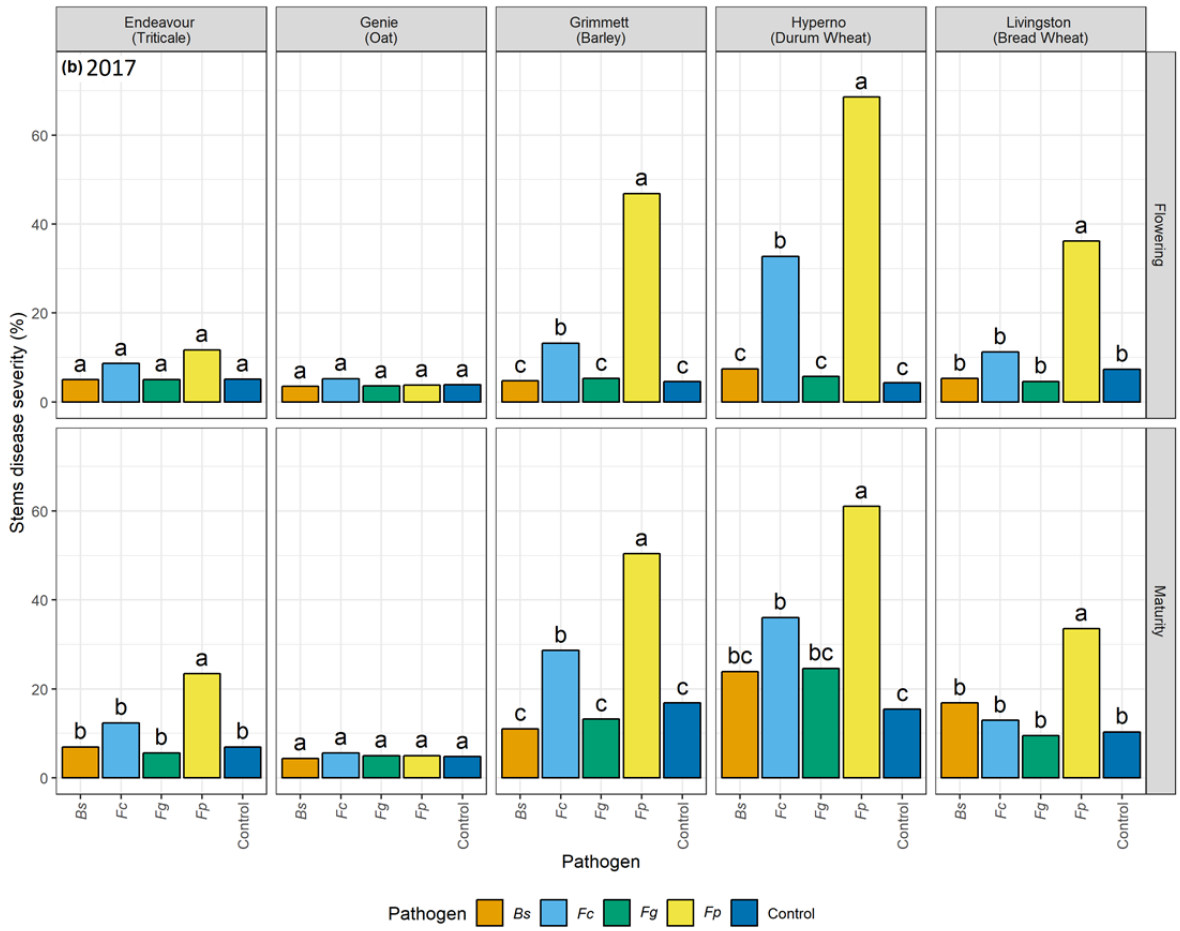

Endeavour (Triticale)
Genie (Oat)
Grimmett (Barley)
Hyperno (Durum Wheat)
Livingston (Bread Wheat)
(b) 2017
Flowering
Maturity
Stems disease severity (%)
Bs
Fc
Fg
Fp
Control
Pathogen
Pathogen
Bs
Fc
Fg
Fp
Control

Fig. 4.

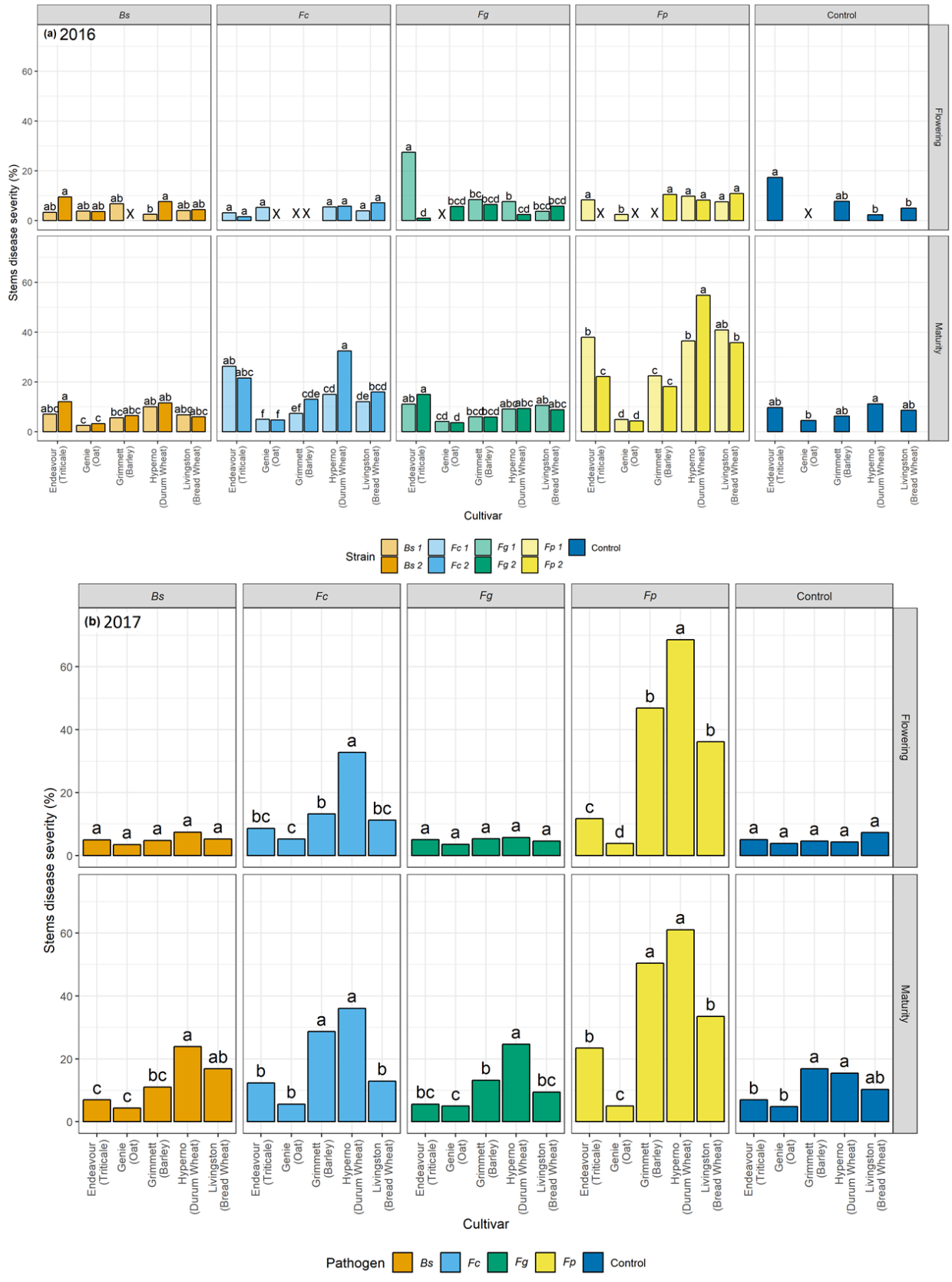

(a) 2016
(b) 2017
Bs
Fc
Fg
Fp
Control
Flowering
Maturity
Stems disease severity (%)
Cultivar
Endeavour (Triticale)
Genie (Oat)
Grimmett (Barley)
Hyperno (Durum Wheat)
Livingston (Bread Wheat)
Strain
Bs 1
Bs 2
Fc 1
Fc 2
Fg 1
Fg 2
Fp 1
Fp 2
Control
Pathogen
Bs
Fc
Fg
Fp
Control

Fig. 5:

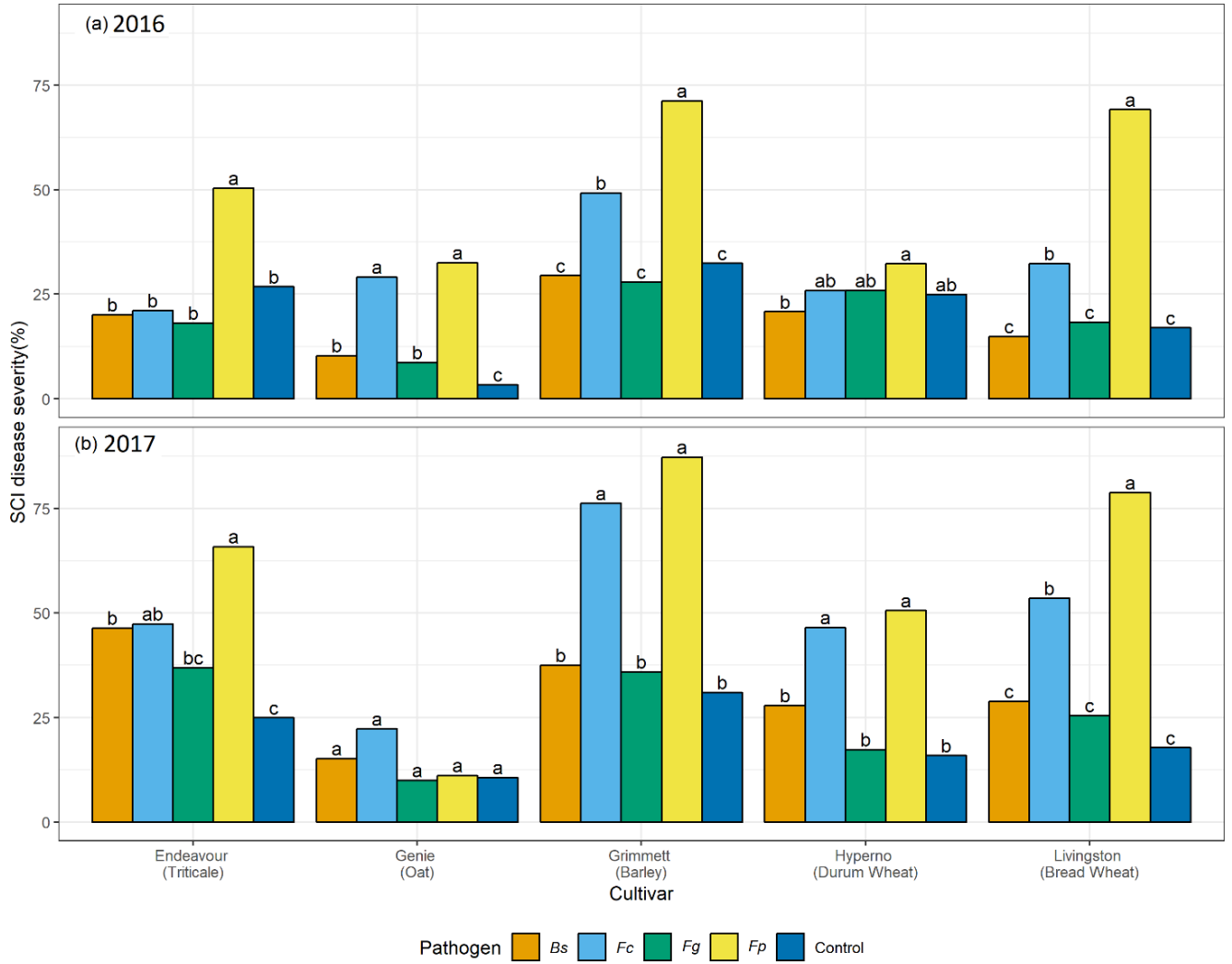

(a) 2016
(b) 2017
SCI disease severity(%)
0
25
50
75
Endeavour (Triticale)
Genie (Oat)
Grimmett (Barley)
Hyperno (Durum Wheat)
Livingston (Bread Wheat)
Cultivar
Pathogen
Bs
Fc
Fg
Fp
Control

Fig. 6:

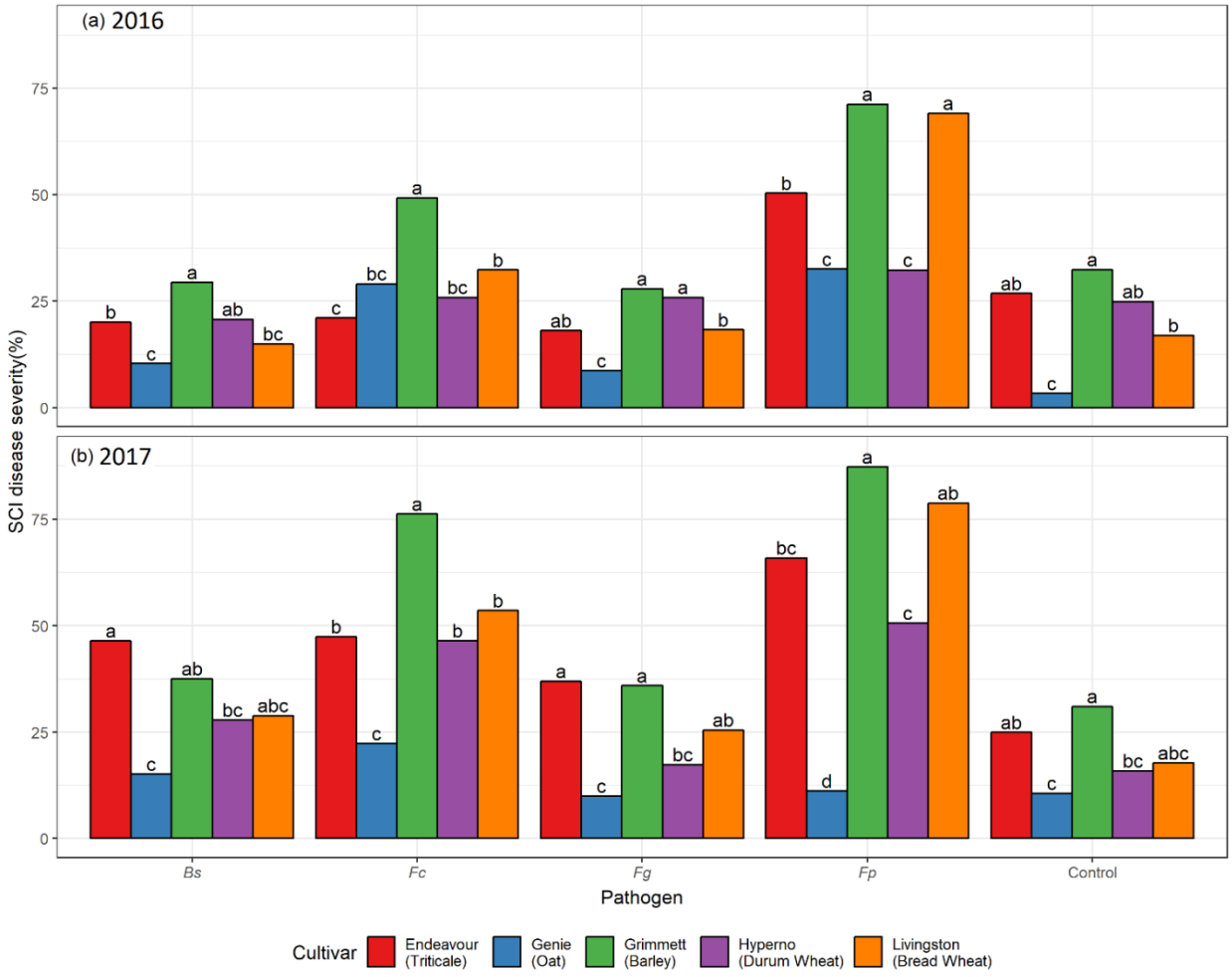

(a) 2016
(b) 2017
SCI disease severity(%)
0
25
50
75
Bs
Fc
Fg
Fp
Control
Pathogen
Cultivar
Endeavour (Triticale)
Genie (Oat)
Grimmett (Barley)
Hyperno (Durum Wheat)
Livingston (Bread Wheat)

Fig. 7.

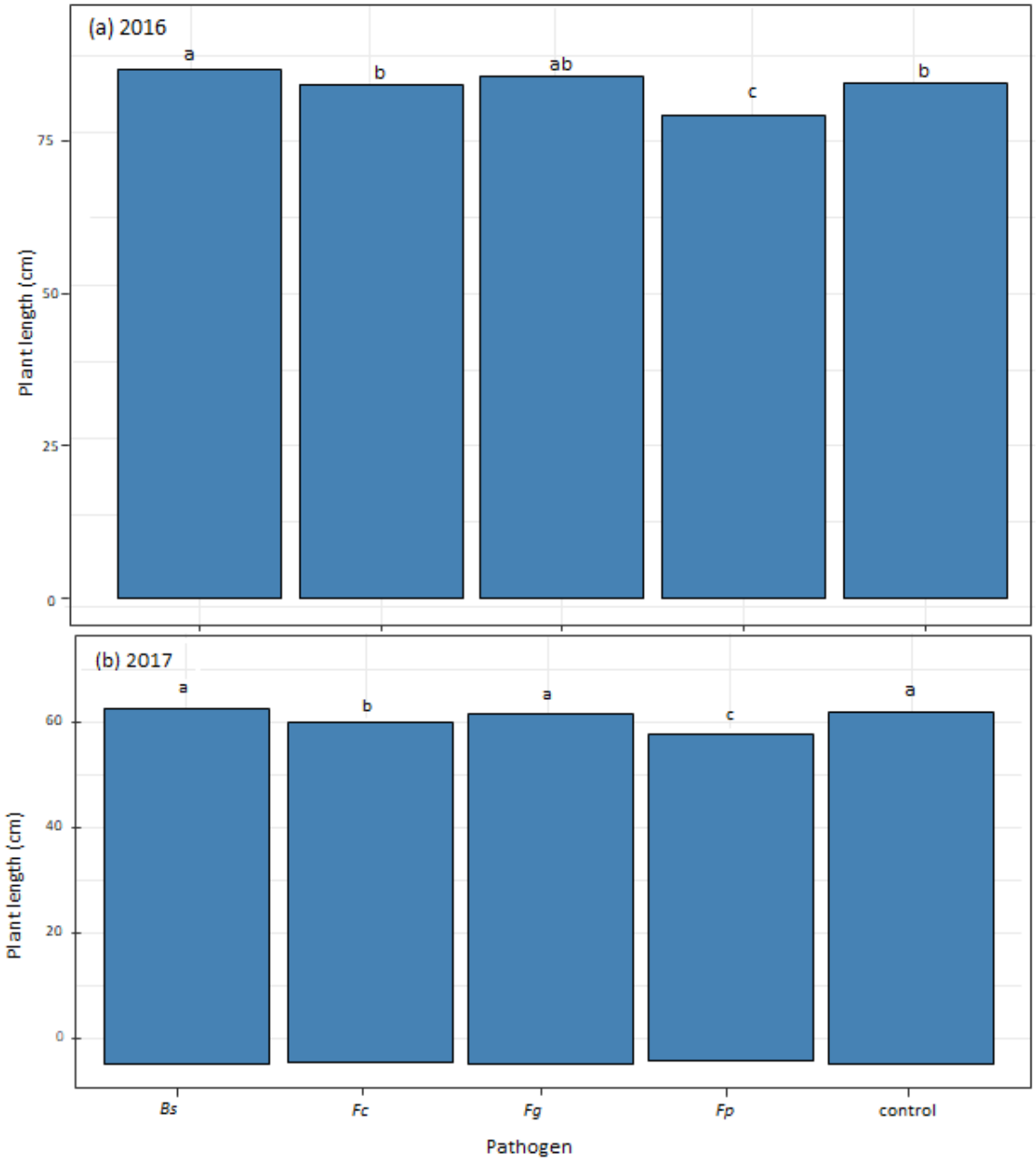

(a) 2016
a
b
ab
c
b
75
50
25
0
Plant length (cm)
(b) 2017
a
b
a
c
a
60
40
20
0
Plant length (cm)
Bs
Fc
Fg
Fp
control
Pathogen

Fig. 8

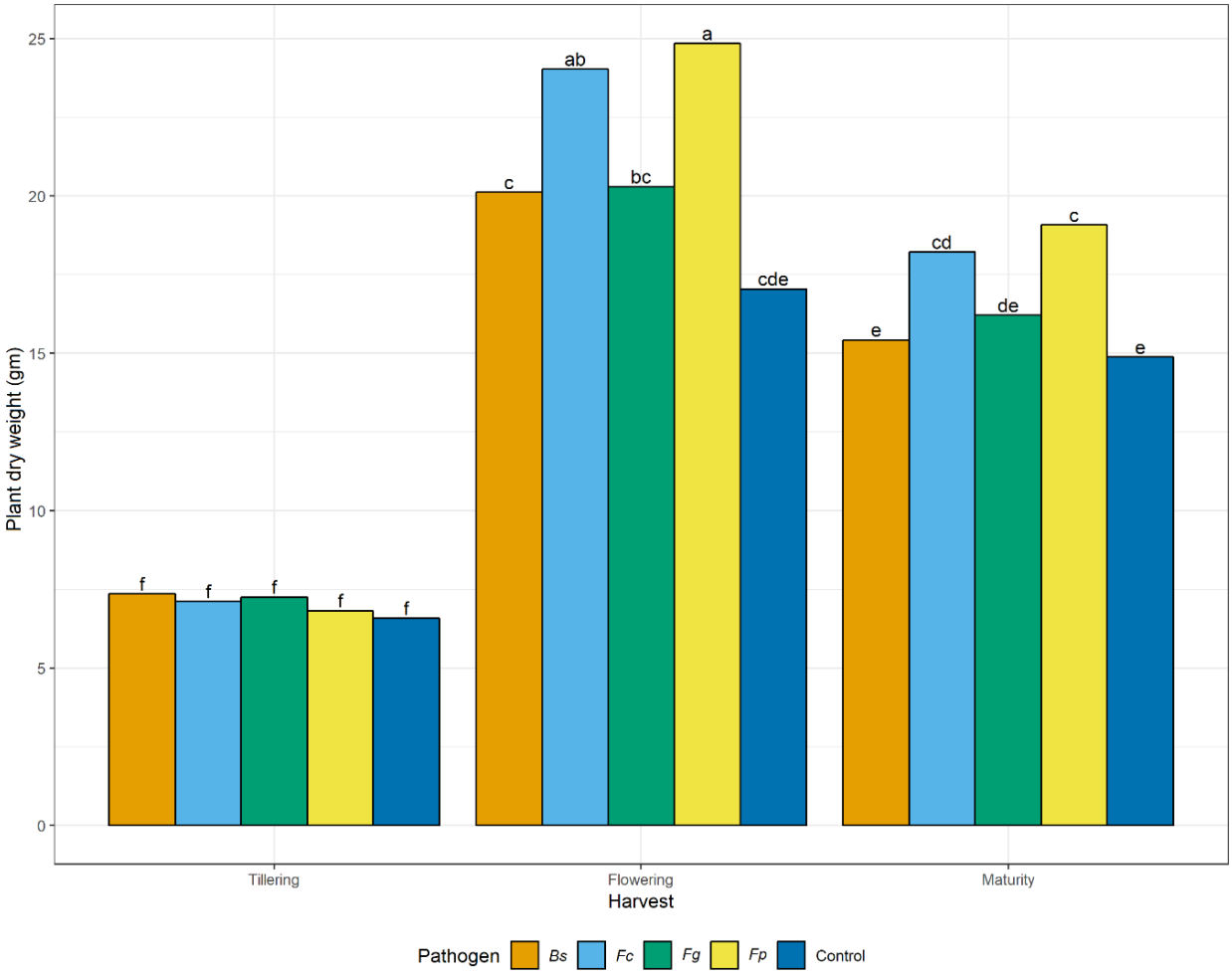

Plant dry weight (gm)
25
20
15
10
5
0
f
f
f
f
f
c
ab
bc
a
cde
e
cd
de
c
e
Tillering
Flowering
Maturity
Harvest
Pathogen
Bs
Fc
Fg
Fp
Control

Fig. 9.

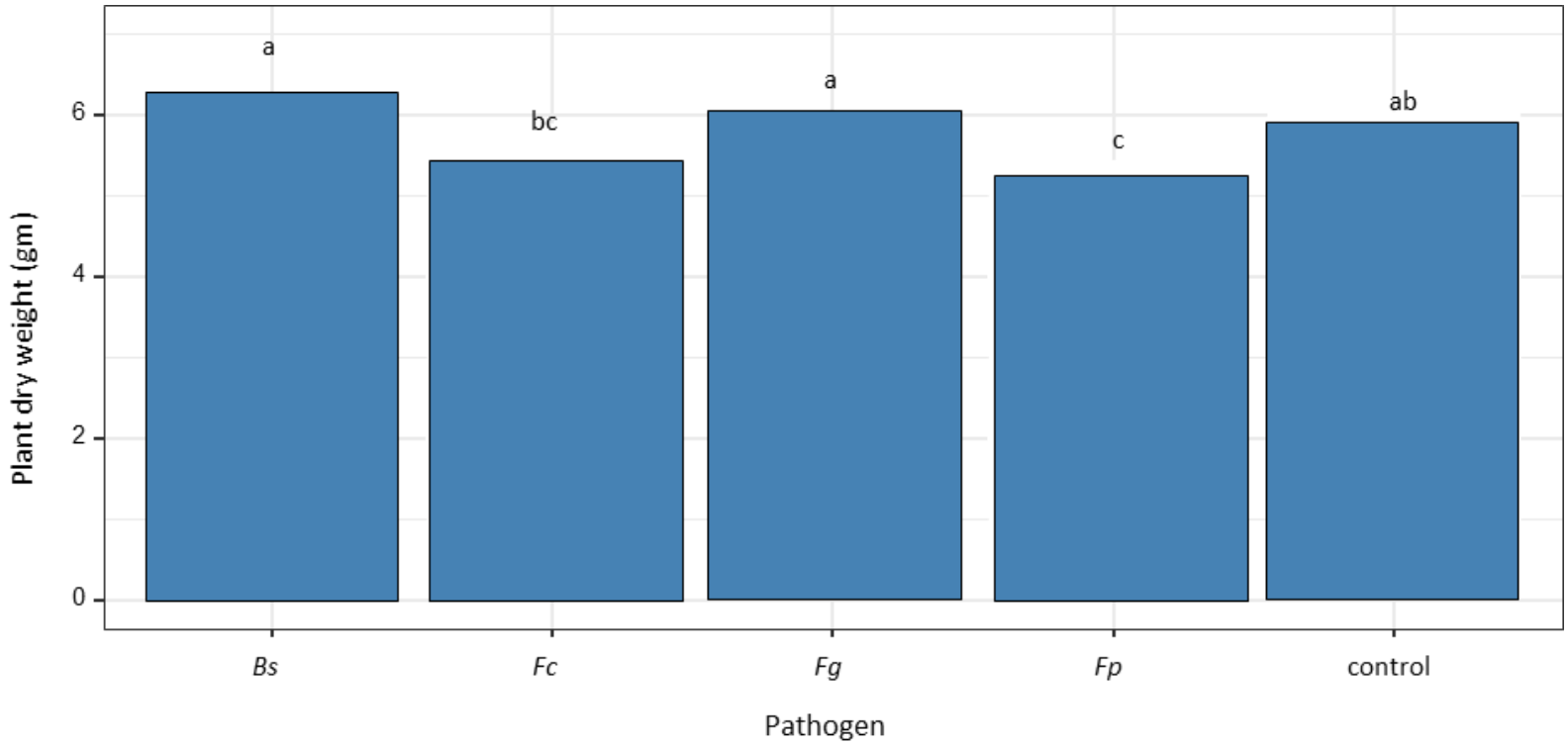

a
bc
a
c
ab
6
4
2
0
Plant dry weight (gm)
Bs
Fc
Fg
Fp
control
Pathogen

Fig. 10.

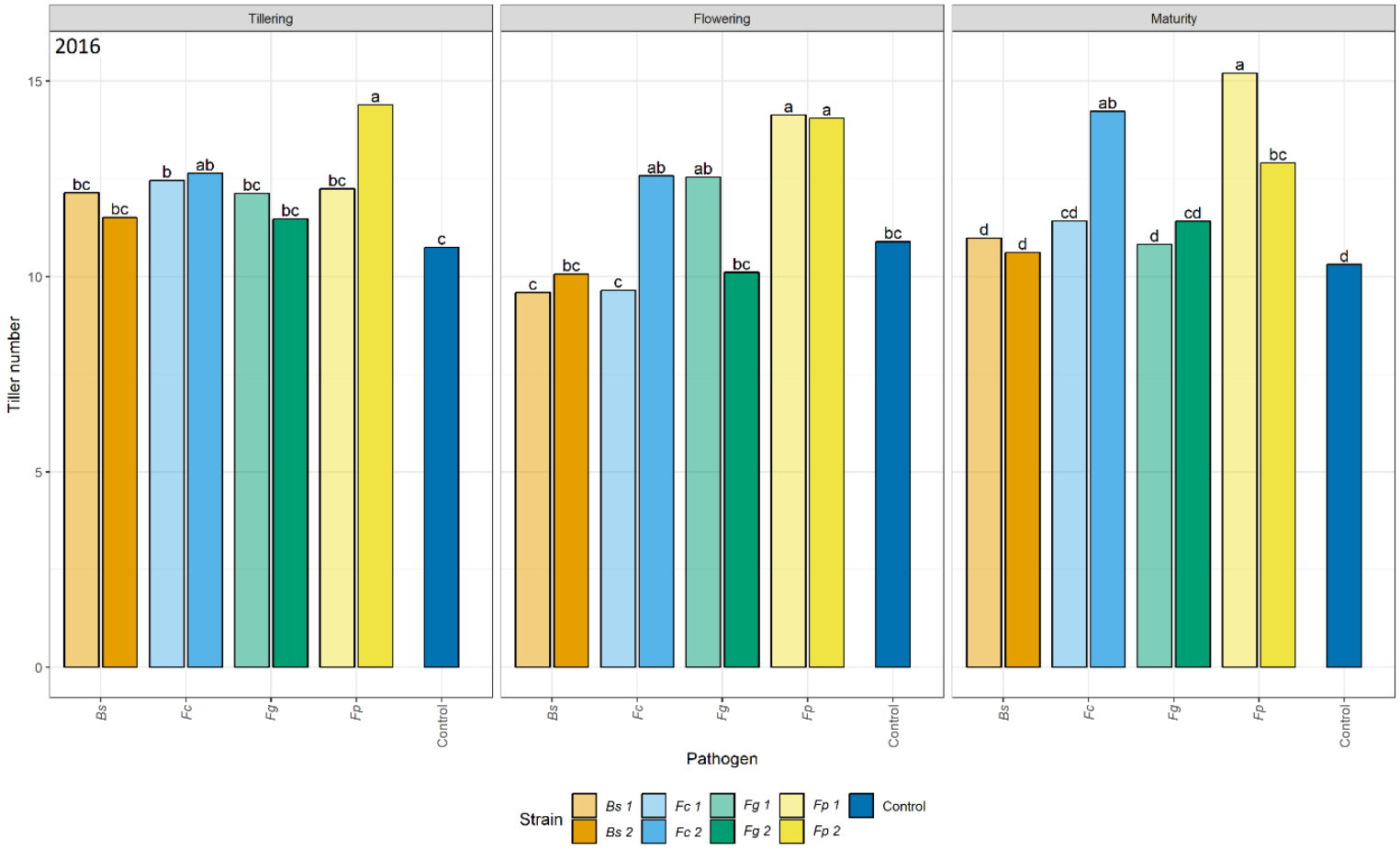
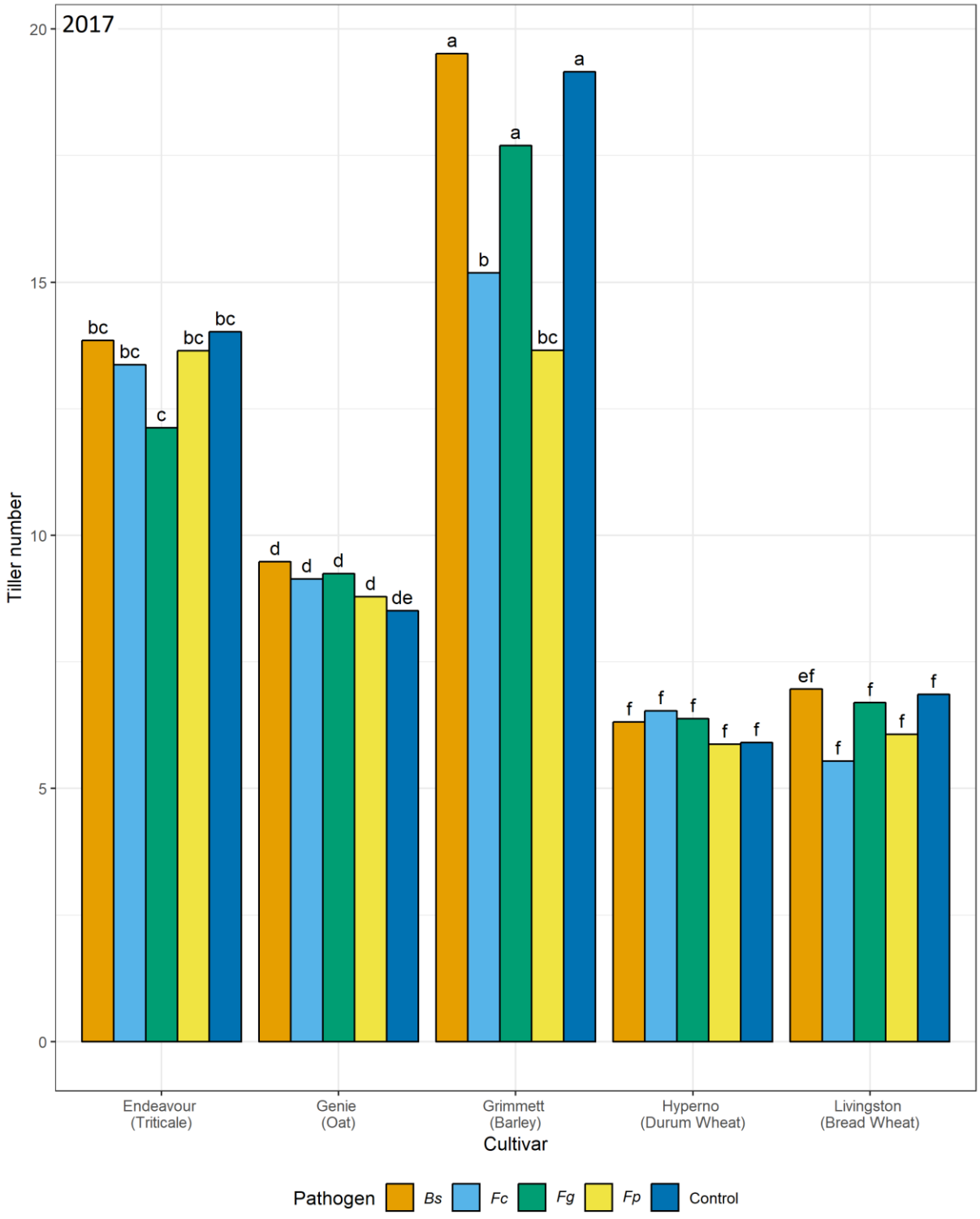

Tillering
Flowering
Maturity
2016
Tiller number
0
5
10
15
bc bc b ab bc bc bc a c
c bc c ab ab bc a a bc
d d cd ab d cd a bc d
Bs
Fc
Fg
Fp
Control
Pathogen
Strain
Bs 1
Bs 2
Fc 1
Fc 2
Fg 1
Fg 2
Fp 1
Fp 2
Control

Fig. 11.

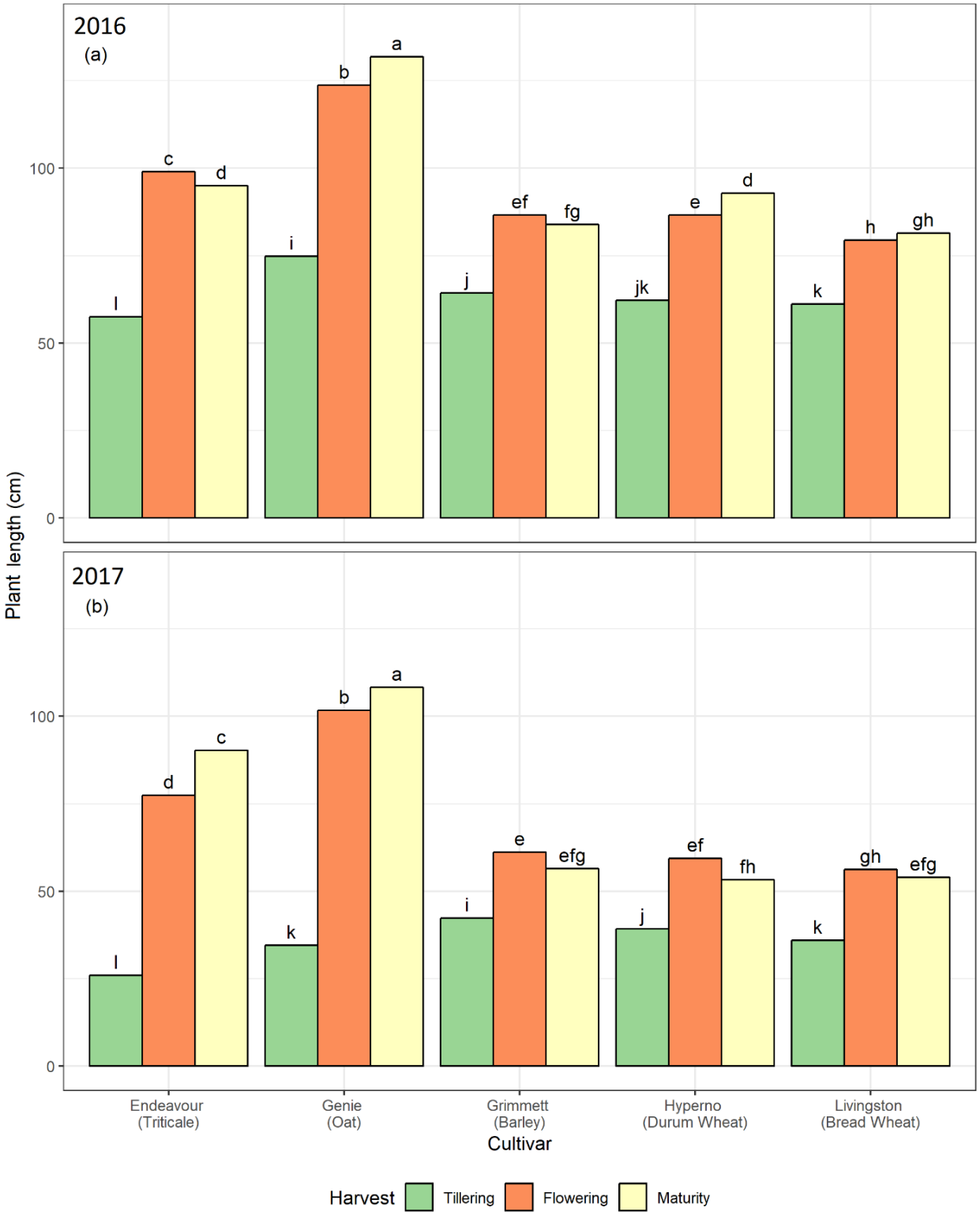

2017
Tiller number
20
15
10
5
0
bc
bc
c
bc
bc
d
d
d
d
de
a
b
a
bc
a
f
f
f
f
f
ef
f
f
f
f
Endeavour
(Triticale)
Genie
(Oat)
Grimmett
(Barley)
Hyperno
(Durum Wheat)
Livingston
(Bread Wheat)
Cultivar
Pathogen
Bs
Fc
Fg
Fp
Control

Supplementary

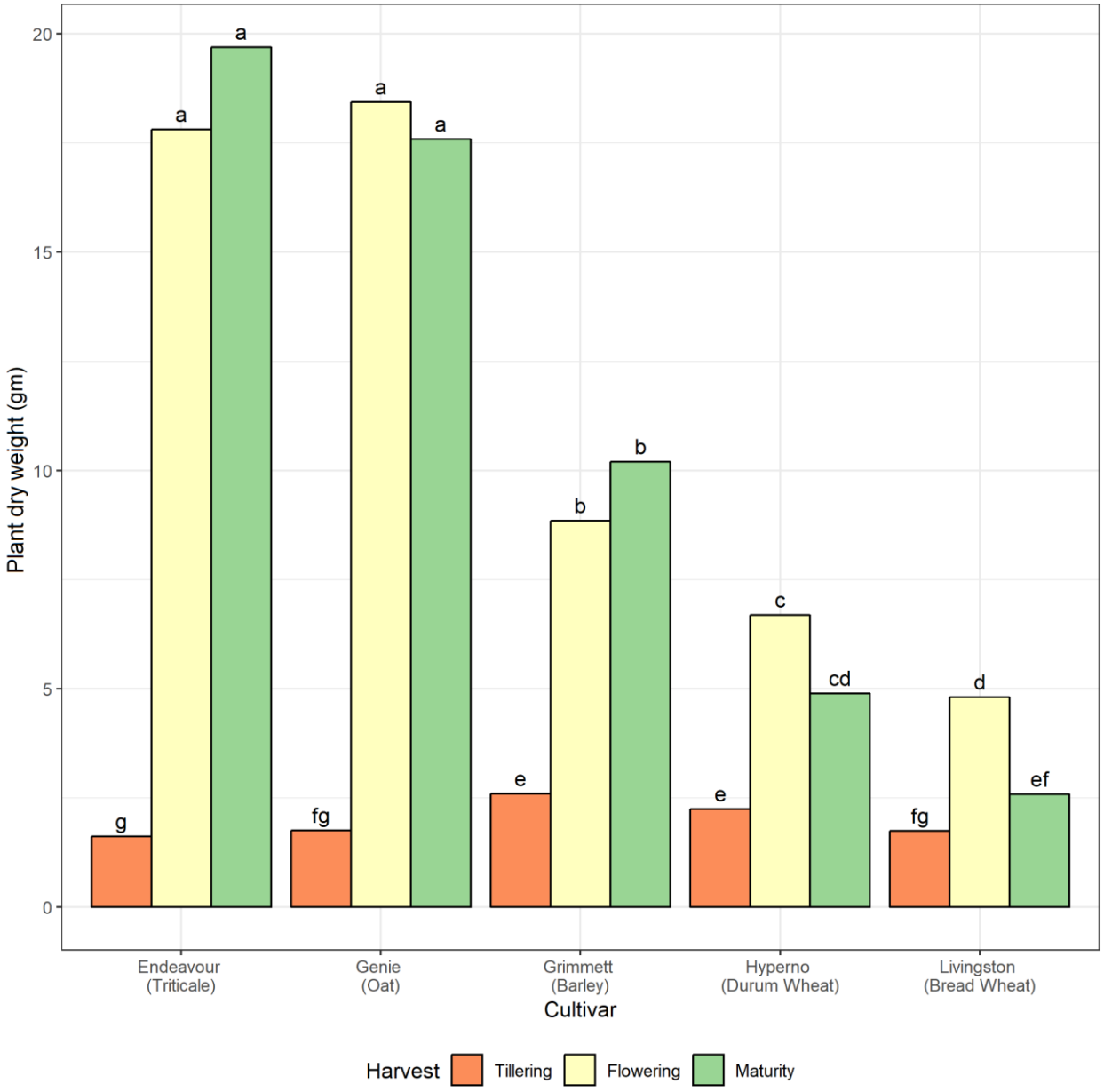
Supplementary Table 1 Monthly rainfall (mm) for 2016/2017 Moyola station http://www.bom.gov.au/climate/data
Station Number: 041369; State: QLD; Opened: 1972; Latitude: 27.52°S; Longitude: 151.88°E; Elevation: 559 m.

2016	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
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
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

Supplementary Figure. 1. Average value of plant Length for cultivar at each harvest time for 2016 (a) and 2017 (b). Different letters represent significant differences between cultivar within harvest time at $\alpha < 0.05$.



Supplementary Figure. 2. Average value of plant dry weight for cultivar at each harvest time for 2017. Different letters represent significant differences cultivars within harvest time at $\alpha < 0.05$.



Supplementary Table 1 ANOVA table for analysis diseased tillers per plant of five winter cereals colonised by the four crown rot and common root rot pathogens in the 2016 field trial. Mean separations were described using α <0.05.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	2.0	247.40	0.004
Harvest	2	224.0	315.00	<0.001
Cultivar	4	221.0	88.22	<0.001
Pathogen	4	220.8	5.16	0.001
Harvest: Cultivar	8	224.4	21.66	<0.001
Pathogen: Strain	4	222.2	2.28	0.061
Harvest: Pathogen	8	223.7	1.11	0.360
Cultivar: Pathogen	16	220.5	2.09	0.010
Harvest: Pathogen: Strain	8	225.6	1.93	0.056
Cultivar: Pathogen: Strain	16	221.5	0.65	0.841
Harvest: Cultivar: Pathogen	30	224.1	1.82	0.008
Harvest: Cultivar: Pathogen: Strain	25	223.5	1.25	0.197

Supplementary Table 2 ANOVA table for analysis diseased tillers per plant of five winter cereals colonised by the four crown rot and common root rot pathogens in the 2017 field trial. Mean separations were described using α <0.05.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	2.0	0.14	0.741
Harvest	2	146.1	710.90	<0.001
Cultivar	4	146.3	134.80	<0.001
Pathogen	4	146.1	45.88	<0.001
Harvest: Cultivar	8	146.4	12.39	<0.001
Harvest: Pathogen	8	146.2	7.52	<0.001
Cultivar: Pathogen	16	146.4	3.61	<0.001
Harvest: Cultivar: Pathogen	32	146.5	2.20	0.001

[a]Num.: They stand for numerator degrees of freedom

[b]Den. DF: Denominator degrees of freedom

Supplementary Table 3 ANOVA table for analysis of disease severity on the stems ratings of five winter cereals colonised by the four crown rot and common root rot pathogens in the 2016 field trial. Mean separations were described using α <0.05.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	1.9	1148.00	0.001
Harvest	1	127.6	139.70	<0.001
Cultivar	4	127.1	40.98	<0.001
Pathogen	4	126.9	46.93	<0.001
Harvest: Cultivar	4	127.1	12.45	<0.001
Pathogen: Strain	4	128.6	3.10	0.018
Harvest: Pathogen	4	127.9	22.13	<0.001
Cultivar: Pathogen	16	127.1	5.06	<0.001
Harvest: Pathogen: Strain	4	130.9	2.21	0.071
Cultivar: Pathogen: Strain	16	128.6	1.63	0.070
Harvest: Cultivar: Pathogen	14	128.5	2.52	0.003
Harvest: Cultivar: Pathogen: Strain	9	126.8	3.04	0.002

Supplementary Table 4 ANOVA table for analysis of disease severity on the stems ratings of five winter cereals colonised by the four crown rot and common root rot pathogens in the 2016 field trial. Mean separations were described using α <0.05.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	144.4	7528.00	<0.001
Harvest	2	144.4	173.50	<0.001
Cultivar	4	144.6	53.20	<0.001
Pathogen	4	144.4	60.57	<0.001
Harvest: Cultivar	8	144.7	7.82	<0.001
Harvest: Pathogen	8	144.5	11.45	<0.001
Cultivar: Pathogen	16	144.7	6.91	<0.001
Harvest: Cultivar: Pathogen	32	144.7	2.01	0.003

[a]Num.: They stand for numerator degrees of freedom

[b]Den. DF: Denominator degrees of freedom

Supplementary Table 5 ANOVA table for analysis of sub-crown internode rating of five winter cereals colonised by the four crown rot and common root rot pathogens in the 2016 field trial. Mean separations of α <0.05.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	1.8	5650.00	<0.001
Harvest	2	223.8	121.10	<0.001
Cultivars	4	210.4	26.11	<0.001
Pathogen	4	211.9	69.64	<0.001
Harvest: Cultivars	8	225.1	25.46	<0.001
Pathogen:Strain	4	214.5	3.67	0.006
Harvest: Pathogen	8	226.6	1.85	0.068
Genotype: Pathogen	16	211.1	4.38	<0.001
Harvest: Pathogen: Strain	8	229.2	1.11	0.360
Cultivar: Pathogen: Strain	16	213.3	1.36	0.162
Harvest: Cultivars: Pathogen	30	226.7	1.31	0.136
Harvest: Cultivars: Pathogen: Strain	25	227.4	0.64	0.908

Supplementary Table 6 ANOVA table for analysis of sub-crown internode rating of five winter cereals colonised by the four crown rot and common root rot pathogens in the 2017 field trial. Mean separations of α <0.05.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	2.0	116.40	0.009
Harvest	2	143.2	179.00	<0.001
Cultivar	4	143.1	36.74	<0.001
Pathogen	4	143.1	37.47	<0.001
Harvest: Cultivar	8	143.7	5.24	<0.001
Harvest: Pathogen	8	143.6	1.19	0.307
Cultivars: Pathogen	16	143.6	3.16	<0.001
Cultivar: Pathogen: Strain	32	144.2	0.87	0.664

[a]Num.: They stand for numerator degrees of freedom

[b]Den. DF: Denominator degrees of freedom

Supplementary Table 7 ANOVA table for analysis of plant height of five winter cereals colonised by the four crown rot and common root rot pathogens in the 2016 field trial. Mean separations of α <0.05.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	1.9	52190.00	<0.001
Harvest	2	206.1	2120.00	<0.001
Cultivar	4	203.2	588.50	<0.001
Pathogen	4	202.8	25.03	<0.001
Harvest: Cultivar	8	206.4	96.01	<0.001
Pathogen: Strain	4	204.4	1.97	0.100
Harvest: Pathogen	8	205.5	0.87	0.546
Cultivar: Pathogen	16	202.6	1.49	0.108
Harvest: Pathogen: Strain	8	207.6	0.42	0.908
Cultivar: Pathogen: Strain	16	203.9	1.62	0.066
Harvest: Cultivar: Pathogen	30	206.0	1.12	0.309
Harvest: Cultivar: Pathogen: Strain	25	206.4	0.83	0.699

Supplementary Table 8 ANOVA table for analysis of plant height of five winter cereals colonised by the four crown rot and common root rot pathogens in the 2017 field trial. Mean separations of α <0.05.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	1.9	23370.00	<0.001
Harvest	2	120.7	2271.00	<0.001
Cultivar	4	117.9	227.10	<0.001
Pathogen	4	117.2	6.43	<0.001
Harvest: Cultivar	8	132.9	143.40	<0.001
Harvest: Pathogen	8	119.0	1.47	0.177
Cultivar: Pathogen	16	116.8	1.23	0.253
Harvest: Cultivar: Pathogen	22	118.2	1.38	0.138

[a]Num.: They stand for numerator degrees of freedom

[b]Den. DF: Denominator degrees of freedom

Supplementary Table 9 ANOVA table for analysis of plant dry weight of five winter cereals colonised by the four crown rot and common root rot pathogens in the 2016 field trial. Mean separations of α <0.05.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	214.9	44170.00	<0.001
Harvest	2	223.7	732.70	<0.001
Cultivar	4	214.0	88.65	<0.001
Pathogen	4	214.4	4.37	0.002
Harvest: Cultivar	8	224.7	20.99	<0.001
Pathogen: Strain	4	216.4	1.44	0.223
Harvest: Pathogen	8	223.6	2.65	0.008
Cultivar: Pathogen	16	213.4	0.42	0.976
Harvest: Pathogen: Strain	8	226.6	1.08	0.376
Cultivar: Pathogen: Strain	16	214.6	1.03	0.421
Harvest: Cultivar: Pathogen	30	224.3	0.69	0.884
Harvest: Cultivar: Pathogen: Strain	25	222.3	0.88	0.633

Supplementary Table 10 ANOVA table for analysis of plant dry weight of five winter cereals colonised by the four crown rot and common root rot pathogens in the 2017 field trial. Mean separations of α <0.05.

Term	Num.[a]	Den. DF[b]	f-value	p-value
(Intercept)	1	2.0	11550.00	<0.001
Harvest	2	143.3	939.00	<0.001
Cultivar	4	143.6	178.60	<0.001
Pathogen	4	143.2	4.78	0.001
Harvest: Cultivar	8	143.8	63.00	<0.001
Harvest: Pathogen	8	143.4	1.13	0.349
Cultivar: Pathogen	16	143.8	0.92	0.549
Harvest: Cultivar: Pathogen	32	144.0	1.01	0.465

[a]Num.: They stand for numerator degrees of freedom

[b]Den. DF: Denominator degrees of freedom

CHAPTER 4

CROWN ROT OF AUSTRALIAN FORAGE OAT CULTIVARS CAUSED BY *FUSARIUM CULMORUM* IN GLASSHOUSE TESTS

In this study, we have tested the capacity of *Fusarium culmorum* strains to cause crown rot on forage oat cultivars in seedling tests. Eight *F. culmorum* strains collected from four regions of Australia and a mixture of *F. pseudograminearum* were used to colonise five forage oat and one bread wheat cultivars. At 21 days after inoculation, the first leaf sheath of each plant was visually rated for disease severity. Species-specific PCR was used to detect the *F. culmorum* or *F. pseudograminearum* DNA in oat treatments.

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Crown rot of Australian forage oat cultivars caused by *Fusarium culmorum* in glasshouse tests

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Abstract

Fusarium culmorum is a widely distributed soil-borne pathogen causing crown rot of cereals, including wheat and barley. In Australia, oat has been examined for crown rot disease caused by *F. pseudograminearum* but is generally considered an asymptomatic host. Recently, *F. culmorum* has been isolated from crown rot affected oat in Queensland, Australia. However, the ability of *F. culmorum* strains to cause crown rot in Australian oat cultivars has not been thoroughly investigated. In this study, the incidence of infection by *F. culmorum* and *F. pseudograminearum* strains was compared among five cultivars of forage oat and one bread wheat cultivar in glasshouse tests. Eight individual strains of *F. culmorum* from four regions of Australia and a mixture of six strains of *F. pseudograminearum* were used for inoculation. At 21 days after inoculation, the first leaf sheath of each plant was visually rated for disease severity. Forage oat cultivars Coolabah (19.8%), Genie (18.1%), and Drover (14.8%) had a significant disease severity ($p < 0.001$) when inoculated with *F. culmorum* strain *Fc*6 compared to non-inoculated control. The *F. pseudograminearum* mixture caused a similar level of disease severity ($p < 0.001$) on forage oat cultivars Drover (13.8%) and Coolabah (11.3%). Three strains of *F. culmorum* (*Fc*2, *Fc*3, and *Fc*5) had low levels of disease severity (ranging from 1.8 to 7.3%) ($p > 0.05$) on bread wheat cultivar Livingston compared to the other *F. culmorum* strains. These results indicate the virulence of *F. culmorum* strains and *F. pseudograminearum* on oat cultivars. This study will contribute to understanding the disease responses of Australian forage oat cultivars to *F. culmorum* strains and the role of oat cultivars in crop rotations to manage crown rot.

Introduction

In Australia, crown rot is predominantly caused by the stubble-borne fungi *Fusarium culmorum* and *F. pseudograminearum* (Backhouse et al. 2004; Burgess et al. 2001). Both pathogens are also associated with crown rot disease globally (Scherm et al. 2013; Kazan and Gardiner 2018).

Annual grain yield losses due to crown rot in bread wheat (*Triticum aestivum* L.) have been estimated to be $38 million in the northern grain growing region of Australia [Queensland (QLD) and North New South Wales (NSW)] and $36 million in the southern grain growing region of Australia [Central NSW, Victoria (VIC), and South Australia (SA)] (Murray and Brennan 2009).

Fusarium culmorum and *F. pseudograminearum* also cause wheat and barley (*Hordeum vulgare* L.) seedling blight before or after emergence (Scherm et al. 2013; Kazan and Gardiner 2018). The visual symptoms caused by *F. culmorum* and *F. pseudograminearum* cannot be distinguished once the disease is established (Chapter 2). Brown discolouration of the coleoptile is the first symptom associated with crown rot at the seedling stage. The fungus initially colonises the coleoptile and the hyphae then grow from the coleoptile across the abaxial surface of the leaf sheaths (Knight and Sutherland 2013). This is followed by discolouration of the leaf sheaths and sub-crown internode (Scherm et al. 2013; Kazan and Gardiner 2018). Penetration of internode for the tissue above ground is mainly via stomata. This penetration hyphae grows from infected subtending leaf sheaths in close contact with the stem (Knight and Sutherland 2016). At flowering, hyphal growth extends vertically through the hypoderm and pith cavity of the stem and can reach up to the fifth internode under drought conditions (Knight and Sutherland 2016; Burgess et al. 2001). Whiteheads, which may contain shrivelled or no grains can also develop during water stress (Burgess et al. 2001; Scherm et al. 2013; Hollaway et al. 2013).

The distribution of *F. culmorum* varies between countries and between regions within countries (Scherm et al. 2013). This distribution has been associated with climate variation (Backhouse et al. 2004; Backhouse and Burgess 2002). In the USA, Cook (1981) reported that *F. culmorum* mainly occurs in low-temperature areas, whereas *F. pseudograminearum* predominately formed in areas with a high temperature. In the Australian southern winter

rainfall region, including southern NSW, VIC, SA, and Western Australia (WA), *F. culmorum* is mainly confined to sites with mean summer temperatures of less than 22°C up to 31.9°C and annual rainfall greater than 350mm based on climate surfaces (Backhouse and Burgess 2002). In the northern summer rainfall region including central NSW and Queensland, it occurs in areas with mean summer temperatures between 22.6°C to 34.8°C and annual rainfall greater than 625mm based on climate surfaces (Backhouse and Burgess 2002).

Fusarium culmorum has been associated with a wide range of cereal species, including bread wheat, barley, durum wheat (*Triticum turgidum* L. var. *durum*), rye (*Secale cereale* L.), oat (*Avena sativa* L.), and triticale (× *Triticosecale*) (Scherm et al. 2013; Backhouse et al. 2004; Balmas et al. 2006) (Chapter 2). *Fusarium culmorum* strains differ in their pathogenicity/virulence across different winter cereals species. A variation in the disease severity between two strains of *F. culmorum*, in a single cultivar of five winter cereals species including barley, bread wheat, durum wheat, triticale, and oat was described in Chapter 2. *F. culmorum* strain *Fc*2 had a maximum leaf sheath percentage disease severity rating of 64.8% while strain *Fc*1 had a maximum rating of 34.1% on the leaf sheaths of barley (p <0.05). In addition, *Fc*2 caused greater disease severity on the sub-crown internode, with a maximum of 97.6% on barley compared to 65.9% with *Fc*1. No differences were found between the *F. culmorum* for the other host (Chapter 2).

In Australia, oat is considered an asymptomatic host of *F. pseudograminearum* (Knight and Sutherland 2017; Nelson and Burgess 1995; Percy et al. 2012) (Chapter 2), and some reports suggest oat may be useful as a rotation crop of wheat (Knight and Sutherland 2017; Nelson and Burgess 1994). *Fusarium culmorum* DNA has been detected in low frequency in oat plants which had low disease severity scores (less than 3%) in the field and glasshouse (Knight and Sutherland 2017) (Chapter 3) which suggests that *F. culmorum* can colonise oat. Nelson and Burgess (1994) reported the incidence of infection of *F. pseudograminearum* on oat cultivars

in the glasshouse and field to have a low or zero symptoms on sub-crown (20 to 32%) internode and stem (0.0%) when inoculated with *F. pseudograminearum*.

Knight and Sutherland (2017) ascertained that oat cultivars Taipan and Quoll had low levels of visual disease severity (ranging from 0.1 to 0.3%) compared to other winter cereals when inoculated with a single strain each of *F. culmorum* and *F. pseudograminearum* in glasshouse tests. In addition, low levels of both *Fusarium* species DNA was obtained from oat cultivars (ranging from 40 to 56ng/g) compared to other winter cereals (ranging from 91 to 765ng/g). Hüberli et al. (2018) conducted two inoculated field experiments in WA in 2016 and 2017 to determine the effect of *F. pseudograminearum* and *F. culmorum* inoculation on the yield of six oat cultivars and two wheat cultivars. The results revealed that oat cultivars were more tolerant to *F. pseudograminearum* than the wheat varieties. Oat and wheat had lower levels of yield loss when infected with *F. culmorum* than for *F. pseudograminearum*, especially for wheat. The average yield loss of oat (4%) was about four times lower than those measured in wheat (17%).

In 2016 it was reported in the Darling Downs, QLD region that oat plants had significant crown rot disease severity and *F. culmorum* was isolated from the infected stem bases. Thus, the objective of the current study was to assess the ability of *F. culmorum* strains to cause crown rot on forage oat cultivars. Variation in the ability of *F. culmorum* strains to cause crown rot on a host, which was previously considered asymptomatic, was investigated. Determining whether oat is resistant or tolerant to crown rot infection may lead to a better understanding of the potential role of oat cultivars in the management of this disease, which will advise for future breeding objectives.

Materials and methods

Strains and inoculum preparation

Eight strains of *F. culmorum* were collected from QLD, NSW, VIC, and WA were used for inoculation trials in this study (Table 1). Inoculum of a mixture of six aggressive strains of *F. pseudograminearum*was provided and used as a positive control. It had been collected from crown rot affected bread wheat and tested for pathogenicity and is routinely used in screening by the crown rot research group at University of Southern Queensland (Table 1). A single spore from each strain was grown on Czapek-Dox Agar (CZA) and incubated for seven days at 25℃ in the dark (Chapter 2). The mycelium of each strain was harvested and used to produce colonised grain inoculum for inoculum production following the method described in Chapter 2. *Fusarium pseudograminearum* strains were combined (1:1 ratio) and ground up, to be used as a positive inoculated control. All inoculums were stored at 4℃ prior to application.

Plant growth and inoculation

Two replicated seedling experiments were conducted in a glasshouse at the Leslie Research Facility, Department of Agriculture and Fisheries, Toowoomba, Australia. The plant growth medium consisted of self-mulching black Vertosol of the Irving clay soil association, obtained from the Darling Downs in Queensland, Australia (Thompson and Beckmann 1959), mixed with river sand (50% sand to 50% soil) (Chapter 2). The two seedling experiments were planted on the 20th of September and 18th of October 2018. Each experiment included three replicated pots inoculated with eight individual strains of *F. culmorum*, one of *F. pseudograminearum* disease control and a non-inoculated/negative control. Each inoculation treatment was applied across three replicate pots of each of the five forage oat cultivars (Algerian, Coolabah, Drover, Genie, and Nugene) and the crown rot susceptible bread wheat cultivar (Livingston) (Lush et al. 2018). Thirteen seeds of each cultivar were planted per pot. For each experiment, the pots were positioned in a randomised complete block design, where each treatment/cultivar combination was randomly allocated to a pot within separate replicate blocks, ' following the layered pot design [modified method of Wildermuth and McNamara (1994)]. Briefly, 280g of

moist soil (38%) was added to 5×5×10 cm pots. The seeds were planted at a depth of 5.5 cm from the top of the pot and covered with a layer of sieved dry soil (160g). Inoculum (0.45g) of ground colonised grain was applied in an even layer to the soil surface of all pots excluding the non-inoculated control. The inoculum was covered with 40g of dry soil. All pots were placed in a water bath at 23ºC with natural day lengths in a glasshouse. The inoculum was activated after seven days by watering each pot to field capacity (38% moisture content) by weight, after which the pots were watered daily up to field capacity.

Disease severity ratings

At 21 days after planting a maximum of ten plants from each pot were rated for disease severity. Disease severity was assessed using a 0 to 100% rating scale based on the visual brown discolouration of the first leaf sheath. Rating of the leaf tissue occurred in 5% increments where 0 = no discolouration and 100% = completely discoloured tissue. The disease symptoms of the inoculated plants were assessed relative to the background colour of the plants of the non-inoculated control of the respective cultivars.

Disease severity data analysis

The analysis of each variable was performed using the two-way ANOVA model. The model included fixed effects for the strain, cultivar, experiment and their interactions. Terms to account for the replicate blocks, pots, and plants within pots were included as random effects, with these variances estimated separately for each experiment. Estimates of variance parameters were generated using residual maximum likelihood (REML) estimation (Patterson & Thompson, 1971). The standard error of difference (SED) values were calculated by taking the square root of both standing tests errors of the mean square (SE^2) of the cultivars (c) and the strains (s) using the formula of $\sqrt{(SE^2_{(c)} + SE^2_{(s)})}$. All analyses were performed in Genstat 18th Edition (VSN 2015) (VSN International Limited, Hemel Hempstead, UK).

Tissue processing and DNA extraction

Each of the eight *F. culmorum* and one *F. pseudograminearum* strain were grown on CZA at 25°C under 24h darkness. After ten days, mycelium was harvested into 2mL tubes. Two metal beads 2mm (Winchester Australia limited, VIC, Australia) were placed into each 2mL tube and each sample was ground twice using a FastPrep-24 instrument (MP Biomedicals, California, USA) at 6.5m/s for 15s. DNA was extracted using the Wizard® Genomic DNA Purification Kit for plant tissue (Promega, Sydney, NSW, Australia) according to the manufacturer's recommended procedure.

Two shoots of each cultivar were randomly obtained from each pot of an inoculation replicate across both experiments, resulting in a total of six from each experiment from each *F. culmorum* and *F. pseudograminearum* inoculation treatment along with the non-inoculated control treatments (total of 12 samples). Three centimetres of the shoot base (from the top of the sub-crown internode) from each plant was collected into 2mL tubes for DNA extraction. The plant tissue was dried in an oven (UF160, Memmert, Germany) at 60°C for three days and then stored at -80^{o}C. Prior to tissue grinding, each sample was again placed in an oven at 60°C (UF160, Memmert, Germany) overnight. Three metal beads were placed into each tube and each sample was ground twice using a FastPrep-24 (MP Biomedicals, California, USA) at 6.5m/s for 30s. DNA was extracted using the Wizard® Genomic DNA Purification Kit for plant material (Promega, Sydney, NSW, Australia) according to the manufacturer's recommended procedure. Samples were eluted into 100µL of autoclaved high purity water and stored at -80°C until required.

PCR detection of *F. culmorum* and *F. pseudograminearum*

DNA extracted from each of the samples across the treatments was assessed for the presence or absence of *F. culmorum* or *F. pseudograminearum* DNA using species-specific PCR. *Fusarium culmorum* and *F. pseudograminearum* DNA was amplified using the specific PCR primers *Fc*_K13B-P_F and Fc_K13B-P_R (Knight and Sutherland 2017) and *F.*

pseudograminearum PCR primers TEF1α.2F and TEF1α.2R (Knight et al. 2012), respectively. Reactions were performed in 10μL volumes containing 2μL dH_2O, 5μL SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, Hercules, USA), 1μM each of the forward and reverse primers for *F. culmorum* or *F. pseudograminearum,* and 2μL of DNA template. Amplification was performed in a CFX384 Touch Real-time system (Bio-Rad, Hercules, USA). Thermal cycling conditions were 3 min at 98°C followed by 35 cycles of 98°C for 15 s and 60°C for 30s. A melt curve analysis was performed by ramping the temperature from 65°C to 95°C, rising by 0.5°C each step with continuous acquisition of fluorescence. The CFX Maestro™ Software (Bio-Rad, Hercules, USA) was used to collect fluorescence data. All samples that used in this assay were run singly.

For samples that had symptoms but did not show amplification in the PCR assay, the PCR run was repeated (47 samples).

Pure DNA from each *Fusarium* strain was amplified to make sure the PCR worked. Four tenfold serial dilutions of genomic DNA from each of the eight *F. culmorum* strains and one *F. pseudograminearum* were included to assess the range of DNA quantities which could be amplified. Each run contained DNA from each *F. culmorum* and *F. pseudograminearum* strains used as positive controls, and DNA from each oat cultivars along with DNA from wheat cultivar used as a negative control, along with non-templates for each run.

In order to determine the presence or absence of *F. culmorum* or *F. pseudograminearum* DNA, endpoint analysis was performed using the CFX Maestro™ Software (Bio-Rad, Hercules, USA). This method uses the total Relative Fluorescence Units (RFU) of each sample, usually after 30 to 40 PCR cycles. A baseline level of fluorescence was set using the RFU of a negative control (non-inoculated oat DNA). A cut-off value was calculated using the formula: Cut-off value = Negative Control Average + Tolerance. A Tolerance value (a percentage of the RFU range) of 10% was used. Positive samples had an RFU value that was equal to or greater

than the cut-off value. The results of each treatment were confirmed by using the melt-curve value of each strain which was performed using the CFX Maestro™ Software (Bio-Rad, Hercules, USA).

The percentage of disease incidence and incidence of DNA detection of the 12 plants of the *F. culmorum* or *F. pseudograminearum* treatments that were used in the PCR assay was calculated.

Result

First leaf sheath disease severity

A significant interaction between strains and cultivars ($p < 0.001$) and significant variation between strains ($p < 0.001$) was observed for the disease severity of the first leaf sheath. *Fusarium culmorum* strain *Fc*6 caused the greatest disease severity on oat cultivars Coolabah (19.8%), Genie (18.1%), and Drover (14.8%), whereas *Fc*7 caused the greatest disease severity on cultivars Genie (9.2%) and Coolabah (8.0%) (Fig. 1).

Of the *F. culmorum* strains, *Fc*6 caused the greatest disease severity on the bread wheat cultivar Livingston (64.09%) followed by *Fc*8 (60.9%) and *Fc*7 (57.4%) (Fig.1). Three of the eight *F. culmorum* strains (*Fc*2, *Fc*3, and *Fc*5) caused minimal disease severity on bread wheat cultivar Livingston (range of 1.8 to 7.3%) (Fig. 1).

The *F. pseudograminearum* mixture caused the greatest disease severity on oat cultivars Drover (13.8%) and Coolabah (11.3%), while also causing high levels of disease severity on Livingston (80.3%) (Fig. 1).

PCR detection of *F. culmorum* and *F. pseudograminearum*

PCR amplification of the DNA of each of the 12 samples from every ten treatments of *F. culmorum* and the *F. pseudograminearum* along with the non-inoculated controls on the six cultivars was conducted using species-specific PCR primers.

PCR product melt was between 84.0°C and 85.0°C for *F. culmorum* and 83.5°C for *F. pseudograminearum*. Positive results were set at threshold 300 RFU. All results below the threshold of 300 RFU are considered negative. The CFX Maestro software compared the RFU of the treatment samples to those of the negative controls (non-inoculated treatments) and identified the sample as positive or negative for the target DNA. The cut-off value of the pure fungal DNA was calculated by adding the value of the negative control average (-22.9) to the tolerance value (300) which was 277 RFU. The cut-off value for the first experiment was 309 RFU and was calculated by adding the value of the negative control average (9.41) to the tolerance value (300). For the second experiment, the cut-off point was 312 RFU, which was calculated by adding the value of the negative control average (12.2) to the tolerance value (300). Samples were determined to be positive for *F. culmorum* or *F. pseudograminearum* DNA if the RFU was equal to or greater than the cut-off of 309 or 312 RFU.

Cultivars Nugene and Livingston had 0.0% DNA detection when inoculated with *Fc*2, also, no DNA was detected in cv. Drover when inoculated with *Fc*4 (Table 2). The strain most frequently detected was *Fc*6 (58.4 to 100%) across the six inoculated cultivars (Table 2). The second most frequently detected strains were *Fc*8 (50.0 to 100%) and *Fc*7 (33.4 to 91.7%) across the six inoculated cultivars. The *F. pseudograminearum* DNA fragment was detected across all cultivars in a range from 58.3 to 100% (Table 2).

Comparison of disease incidence and pathogen DNA detection

Data analyses of the PCR results were carried out to determine the impact of the disease incidence and the presence and absence of the strains DNA fragments for each treatment. There was an association between the presence of the PCR fragment of *F. culmorum* and *F. pseudograminearum* and the disease incidence for most cultivars (Table 2). Wheat cultivar Livingston had the highest disease incidence and the highest number of DNA fragments detected across *F. culmorum* strains (Table 2). Other cultivars such as cv. Drover (83.4%) and

cv. Coolabah (66.7%) had a high disease incidence and the pathogen DNA was detected only when infected with *Fc*6. Oat cultivar Algerian had the highest proportion of samples with plants that did not show symptoms but DNA of *Fc*8 (66.7%) and *Fc*6 (58.4%) was present (Table 2). The strain *Fc*3 was detected in cv. Genie (58.4%) also in plants not showing any symptoms (Table 2). *Fusarium pseudograminearum* DNA was positively identified in the cultivars that had symptoms such as Livingston (100%), Drover (58.4%), and Genie (50%). Furthermore, *F. pseudograminearum* DNA was detected in 50% of cv. Genie in the plants without any symptoms (Table 2).

Discussion

This study assessed the virulence of eight *F. culmorum* strains across five forage oat cultivars using seedling inoculation tests. A significant interaction (p <0.001) was observed in the first leaf sheath disease severity amongst the cultivars for the combined leaf sheaths when inoculated with each of the *F. culmorum* strains.

Of the eight strains, only *Fc*6 and *Fc*7 caused high levels of disease severity across the oat and wheat cultivars. *Fusarium culmorum* has been described to vary in aggressiveness and disease severity depending on climate, location, and genetic diversity (Obanor et al. 2010; Çepni et al. 2013; Miedaner et al. 2001). Obanor et al. (2010) suggested that genetic recombination and gene flow between *F. culmorum* populations may lead to the development of highly aggressive and/or toxigenic strains, which remains a major concern for crown rot management. Chapter 2 reported that some *Fusarium* species, including *F. culmorum* strains, caused greater disease severity than others in five winter cereals species. Surprisingly, the QLD strains (*Fc*1 and *Fc*2) that were isolated from the infected plants in Darling Downs, QLD in 2016 that were used in the current report, caused a significantly lower disease severity across oat cultivars. This may indicate background disease (such as viruses or bacteria) may have weaken the oat plants, allowing *F. culmorum* to colonise and cause significant disease severity,

or these two strains of *F. culmorum* may perform differently in field conditions. Further investigation is required to assess these two strains in the field across a range of oat cultivars.

In Australia, crown rot studies on *F. culmorum* or *F. pseudograminearum* have shown that oat is asymptomatic or exhibits low levels of discolouration (Knight and Sutherland 2017; Percy et al. 2012; Nelson and Burgess 1995) (Chapter 2 and 3). In contrast, the results of our study indicated a variation in resistance amongst forage oat species, with three oat cultivars (Coolabah, Drover, and Genie) having greater disease severity ratings when inoculated with two *F. culmorum* strains compared to the other oat cultivars. In the South-Eastern part of Poland, Kiecana et al. (2014) conducted a survey in two field trials under natural inoculation of crown rot pathogens. Six weeks after planting, the incidence of roots and tillers infection was evaluated. *Fusarium culmorum* was isolated from 19.84% of 39 diseased oat genotypes compared to the other *Fusarium* species which were present at lower frequencies, *F. solani* (6.70%), *F. avenaceum* (7.04%), *F. sporotrichioides* (2.22%), *F. equiseti* (2.30%), *F. graminearum* (1.39%), and *F. crookwellense* (0.08%). They suggested that *F. culmorum* can be virulent on oat cultivars under favourable conditions.

In this report, the disease was also observed in the first leaf sheath of oat cultivars infected with *F. pseudograminearum*. Oat cultivars Coolabah and Drover were having a greater level of disease severity compared to the other oat cultivars when inoculated with *F. pseudograminearum*. In Australia, *F. pseudograminearum* may cause low or no disease severity on oat cultivars (Percy et al. 2012, Nelson and Burgess 1995) (Chapter 2 and 3). Percy et al. (2012) reported a category 1 rating score (1-25%) of disease severity on the first leaf sheath in the forage oat cultivar Cleanleaf ' after 21 days of planting when inoculated with *F. pseudograminearum* in glasshouse tests. This indicates different strains of *F. pseudograminearum* may cause disease severity on a variety of oat cultivars depending on the strain aggressiveness.

Bread wheat cultivar Livingston used in this study is considered to be susceptible to *F. pseudograminearum* (Lush et al. 2018) (Chapter 2 and 3). High levels of disease severity were observed on Livingston when infected with *F. pseudograminearum*. *Fusarium culmorum* strains used in the current study exhibited significant variation ($p < 0.05$) in virulence on Livingston. Strains *Fc*2, *Fc*3, and *Fc*5 had the least disease severity on this bread wheat cultivar compared to the other *F. culmorum* strains. Other studies have shown that *F. culmorum* causes high levels of disease severity in bread wheat (Knight and Sutherland 2017; Hollaway et al. 2013) (Chapter 2 and 3). This suggests that the strains of *F. culmorum* differ genetically and that disease severity is dependent on the combination of strain and host species.

In the current study, the DNA of *F. culmorum* and *F. pseudograminearum* strains was detected frequently in the treatments that had high visual symptoms (10 to 100%). Yet, *F. culmorum* and *F. pseudograminearum* strains were also frequently identified in the treatments that expressed few or no visual symptoms. This supports the premise that *F. culmorum* and *F. pseudograminearum* strains may be present in oat crops even though symptoms of the disease are not evident. Chapter 3 indicated that even though the oat cultivar Genie had less disease severity than one cultivar each of barley, bread wheat, durum wheat, and triticale, the fungal DNA of three *Fusarium* species, *F. culmorum*, *F. graminearum*, *F. pseudograminearum*, and *Bipolaris sorokiniana* were frequency detected in this cultivar with *F. culmorum* DNA was the most frequent in Genie. Nelson and Burgess (1995) tested the effect of rotation with barley and oats on crown rot disease in the northern cereal belt of NSW, Australia and found that although oat cultivars did not express any symptoms, the frequency of isolation of *F. pseudograminearum* was high. Furthermore, some of the samples that had symptoms had no fungal DNA. Most of these samples (67%) had very low visual symptoms of between 1 to 5%. This suggests that the fungal DNA present in these samples may have been too low to detect.

To the best of our knowledge, this is the only study to date to assess the ability of different strains of *F. culmorum* to cause crown rot disease on different Australian forage oats. Significant variation in the disease severity across forage oat cultivars was reported when inoculated with eight *F. culmorum* strains and a mixture of *F. pseudograminearum* strains. Further research is necessary to test the ability of multiple strains *F. culmorum F. pseudograminearum* to colonise a range of oat cultivars, particularly in field situations. The results of the current study support that the fungal DNA of both *F. culmorum* and *F. pseudograminearum* can subsist in oat cultivars even though there are no symptoms. This may increase the fungal population in the field. Thus, oat may not be a good choice in crop rotation systems to reduce the crown rot pathogens in the field. This proposition requires further investigation in field trials using a variety of *Fusarium* strains on a range of oat cultivars evaluated as adult plants. Further investigation is required to test the fungal level in post-harvest crop stubble. In addition, a qPCR technique should be employed to determine the level of the fungal biomass in seedling and adult plants. Assessing the susceptibility of oat varieties to *F. culmorum* strains will lead to a greater understanding of the potential impact of this pathogen under favourable environments. The current study contributes towards the development of recommendations for optimised cropping systems that reduce the risk of *F. culmorum* causing crown rot in cereals.

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Table and Figure Headings

Table 1 Location and source of each strain of *F. culmorum* and *F. pseudograminearum*.

Table 2 Percentage of plants showing visual symptoms and having DNA of the *Fusarium culmorum* and *F. pseudograminearum* (n= 12 of each the nine inoculation treatments).

Figure 1 Average percentage of the first leaf sheath disease severity ratings for the experiments by strain by cultivar interaction ($p < 0.05$). Treatments include *Fusarium culmorum* strains *Fc*1, *Fc*2, *Fc*3, *Fc*4, *Fc*5, *Fc*6, *Fc*7, and *Fc8*, and *F. pseudograminearum* mixture strains (*Fp*) along with non-inoculated control (Nil) for each cultivar. Error bars indicate the standard error of difference (+/- s.e.d).

Table 1

Species	Culture collection code	Strain name	Strain location
Fusarium culmorum	BRIP code [a] 64977	*Fc1*	QLD
	BRIP code 64978	*Fc2*	
	14CRS 52	*Fc3*	WA
	14CRS 26-5	*Fc4*	
	FC14.003	*Fc5*	VIC
	FC13-195	*Fc6*	
	BRIP code 64974	*Fc7*	NSW
	BRIP code 64973	*Fc8*	
Fusarium pseudograminearum	BRIP code 64947	*Fp*	NSW
	BRIP code 64948	*Fp*	QLD
	BRIP code 64949	*Fp*	QLD
	BRIP code 64950	*Fp*	QLD
	BRIP code 64951	*Fp*	QLD
	BRIP code 64952	*Fp*	QLD

[a] BRIP: Queensland Plant Pathology Herbarium (Australia)

Table 2.

Strain Cultivar	Symptoms (+) DNA (+) % [a]	Symptoms (-) DNA (-) % [b]	Symptoms (+) DNA (-) % [c]	Symptoms (-) DNA (+) % [d]
Algerian				
Fc1 [e]	**0.0**	**50.0**	**25.0**	**25.0**
Fc2 [e]	**0.0**	**75.0**	**8.4**	**16.7**
Fc3 [e]	**0.0**	**66.7**	**0.0**	**33.4**
Fc4 [e]	**0.0**	**58.4**	**0.0**	**41.7**
Fc5 [e]	**0.0**	**75.0**	**0.0**	**25.0**
Fc6 [e]	**8.3**	**16.7**	**16.7**	**58.4**
Fc7 [e]	**0.0**	**58.4**	**8.4**	**33.4**
Fc8 [e]	**8.3**	**16.7**	**8.4**	**66.7**
Fp [f]	**41.7**	**0.0**	**16.7**	**41.7**
Coolabah				
Fc1	**8.4**	**41.7**	**25.0**	**16.7**
Fc2	**0.0**	**75.0**	**16.7**	**8.4**
Fc3	**8.4**	**41.7**	**16.7**	**33.4**
Fc4	**33.4**	**41.7**	**8.4**	**16.7**
Fc5	**8.4**	**66.7**	**0.0**	**25.0**
Fc6	**66.7**	**0.0**	**25.0**	**8.4**
Fc7	**50.0**	**16.7**	**16.7**	**16.7**
Fc8	**58.4**	**8.4**	**0.0**	**33.3**
Fp	**41.7**	**16.7**	**16.7**	**25.0**
Drover				
Fc1	**0.0**	**83.3**	**8.4**	**8.4**
Fc2	**8.4**	**91.7**	**0.0**	**0.0**
Fc3	**0.0**	**66.7**	**8.4**	**25.0**
Fc4	**0.0**	**91.7**	**8.4**	**0.0**
Fc5	**0.0**	**83.3**	**0.0**	**16.7**
Fc6	**83.4**	**0.0**	**8.4**	**8.4**
Fc7	**66.7**	**0.0**	**8.4**	**25.0**
Fc8	**33.4**	**25.0**	**8.4**	**33.4**
Fp	**58.4**	**8.4**	**25.0**	**8.4**
Genie				
Fc1	**0.0**	**83.3**	**8.4**	**8.4**
Fc2	**8.4**	**83.4**	**8.4**	**0.0**
Fc3	**0.0**	**25.0**	**16.7**	**58.4**
Fc4	**8.4**	**50.0**	**25.0**	**16.7**
Fc5	**0.0**	**58.4**	**41.7**	**16.7**
Fc6	**50.0**	**8.4**	**25.0**	**16.7**
Fc7	**41.7**	**16.7**	**16.7**	**25.0**
Fc8	**50.0**	**25.0**	**8.4**	**16.7**
Fp	**50.0**	**8.4**	**0.0**	**41.7**
Nugene				
Fc1	**8.4**	**75.0**	**8.4**	**8.4**
Fc2	**0.0**	**100.0**	**0.0**	**0.0**
Fc3	**0.0**	**75.0**	**16.7**	**8.4**
Fc4	**0.0**	**58.3**	**8.4**	**33.4**
Fc5	**0.0**	**75.0**	**8.4**	**16.7**
Fc6	**33.3**	**25.0**	**8.4**	**33.4**
Fc7	**25.0**	**58.4**	**8.4**	**8.4**
Fc8	**16.7**	**33.4**	**16.7**	**33.4**
Fp	**41.7**	**8.4**	**33.4**	**16.7**
Livingston				
Fc1	**58.3**	**8.4**	**33.4**	**0.0**
Fc2	**0.0**	**66.7**	**33.3**	**0.0**
Fc3	**25.0**	**25.0**	**41.7**	**8.4**
Fc4	**66.7**	**0.0**	**33.4**	**0.0**
Fc5	**25.0**	**41.7**	**25.0**	**8.4**
Fc6	**100.0**	**0.0**	**0.0**	**0.0**
Fc7	**91.7**	**0.0**	**8.4**	**0.0**
Fc8	**100.0**	**0.0**	**0.0**	**0.0**
Fp	**100.0**	**0.0**	**0.0**	**0.0**

[a] Percentage of the plants with visual symptoms and DNA of *Fusarium culmorum* and *F. pseudograminearum* in oat and wheat cultivars

[b] Percentage of the plants without visual symptom or DNA of *Fusarium culmorum* and *F. pseudograminearum* in oat and wheat cultivars

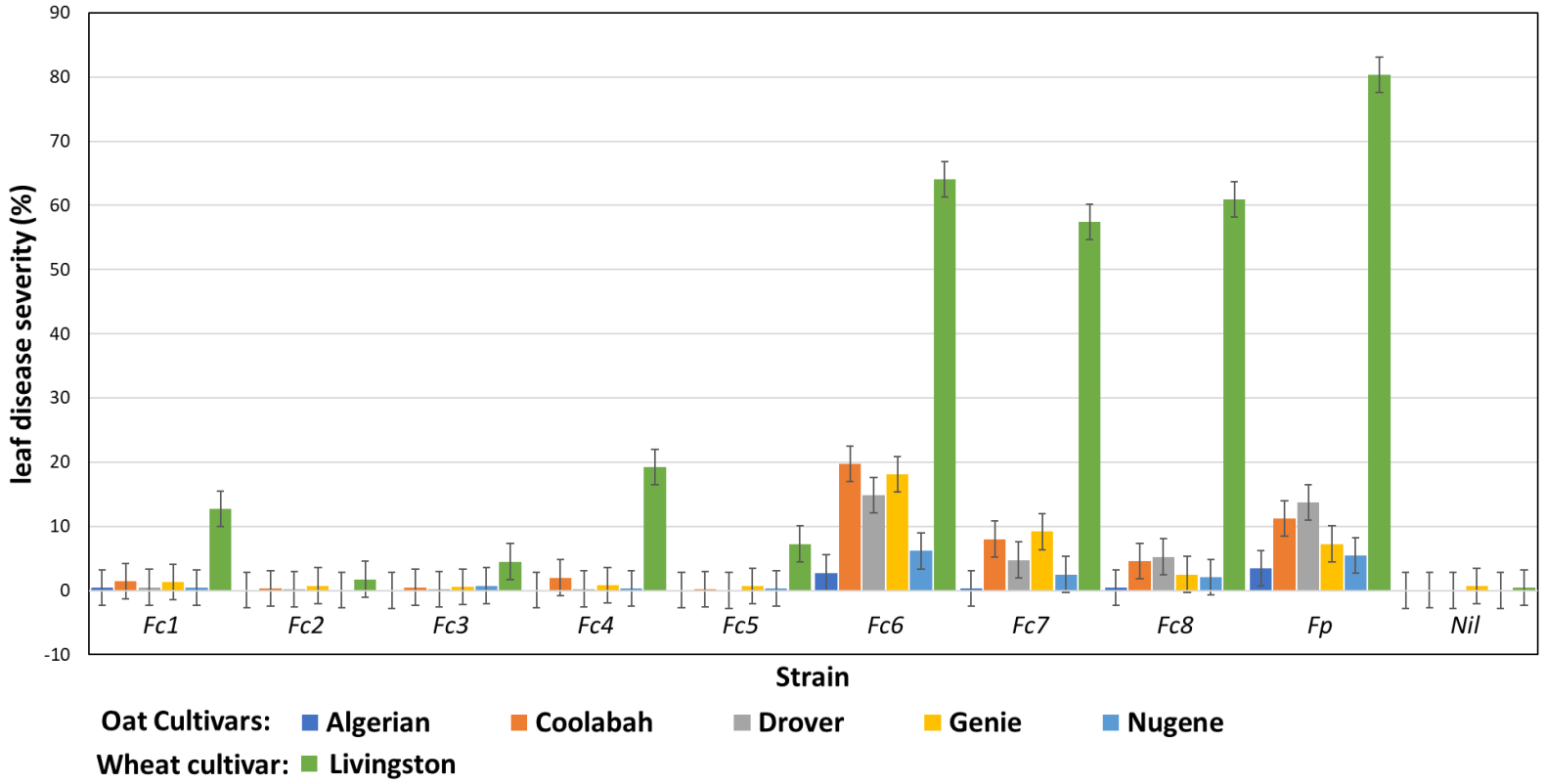
[c] Percentage of the plants with the visual symptom but no DNA of *Fusarium culmorum* and *F. pseudograminearum* in oat and wheat cultivars

[d] Percentage of the plants without visual symptom but with DNA of *Fusarium culmorum* and *F. pseudograminearum* in oat and wheat cultivars

[e] *Fusarium culmorum* strains included treatments

[f] *Fusarium pseudograminearum* strain inculcated treatments

Figure 1


90
80
70
60
50
40
30
20
10
0
-10
leaf disease severity (%)
Fc1
Fc2
Fc3
Fc4
Fc5
Fc6
Fc7
Fc8
Fp
Nil
Strain
Oat Cultivars:
Algerian
Coolabah
Drover
Genie
Nugene
Wheat cultivar:
Livingston

Supplementary Table 1 ANOVA table for analysis of the first leaf sheath disease severity ratings of five oat cultivars and one wheat cultivar colonised by eight *Fusarium culmorum* and *F. pseudograminearum* strains used for this study. Mean separations were described using α <0.05.

Source of variation	d.f.[a]	s.s[b]	m.s.[c]	v.r[d.]	p-Value
Experiment stratum	1	10.502	10.502	1.36	
Experiment. *Units* stratum					
Cultivar	5	13085.17	2617.034	338.99	<.001
Strain	9	7475.868	830.652	107.6	<.001
Cultivar. Strain	45	11900.18	264.448	34.25	<.001
Residual	59	455.481	7.72		
Total	119	32927.2			

[a] d.f: degree of freedom
[b] s.s: sum of squares
[c] m.s.: mean square
[d] v.r: variance ratio

CHAPTER 5

THE IMPACT OF *FUSARIUM PSEUDOGRAMINEARUM* AND *FUSARIUM CULMORUM* ON THE ROOT SYSTEM OF BREAD WHEAT

INTRODUCTION

Drought results in water stress in many regions of Australia and is one of the key factors that contributes to the low yield of wheat in Australia (Christopher et al. 2008; Christopher et al. 2013). The plant root system significantly affects crop growth and yield (Palta & Watt 2009; Palta et al. 2011). Palta et al. (2011) indicated that a deep, wide-spreading and dense root system is essential in the design of drought-tolerant crops. Therefore, root system characteristics are fundamental for soil exploration to forage for water and mineral nutrients such as nitrogen, phosphorus and belowground resource acquisition (Jackson et al. 2000; Palta & Watt 2009; Schlüter et al. 2018).

Genotypic variation in root system characteristics of bread wheat (*Triticum aestivum* L.) cultivars has been identified (Hurd 1968; O'Brien 1979; Nakamoto & Oyanagi 1994; Manschadi et al. 2006; Manschadi et al. 2008; Christopher et al. 2013). Such characteristics include root depth (Hurd 1974), root elongation rate, root distribution at depth (O'Brien 1979), xylem vessel diameter (Richards & Passioura 1989) and root weight (Nakamoto & Oyanagi 1994; Manschadi et al. 2006; Manschadi et al. 2008). The usefulness of each root characteristic in increasing grain yield under water stress is determined by moisture stress during the season (Manschadi et al. 2008). For example, cultivars with superior performance on deep clay soils in the northern region of Australia [Queensland (QLD) and northern New South Wales (NSW)] were clustered into three groups based on their seminal root traits (Manschadi et al. 2008). The QLD adapted lines tended to have a narrower root angle (Manschadi et al. 2008).

Crown rot is a widespread disease of wheat in Australia (Burgess et al. 2001; Kazan & Gardiner 2018). Water availability is a significant factor in crown rot infection (Swan 1998; Burgess et al. 2001). Thus, the level of soil moisture during crown rot infection is a key limiting factor in wheat production in Australia (Liddell & Burgess 1988; Swan 1998; Burgess et al. 2001). Crown rot pathogens occur most commonly in deep clay soils in areas where rainfall is summer dominant, and winter cereals are grown during winter and spring using the sub-soil moisture accumulated during the summer (Liddell & Burgess 1988; Burgess et al. 2001). The optimum water potential to cause crown rot infection is -0.3 to -0.6 MPa (-1 MPa = -10 bars) (Liddell &

Burgess 1988; Burgess et al. 2001). Infection is inhibited during extended dry periods (Swan 1998; Burgess et al. 2001). Root-related characteristics during crown rot infection have not been studied due to limited knowledge of root system growth and technical difficulties in measuring root features. Challenges include root extraction from soil and a lack of simple and effective root screening methods.

The first symptoms of crown rot in winter cereals is at the seedling stage and manifest as necrotic lesions on the coleoptile, sub-crown internode, root emergence points and the basal portion of the leaf sheaths (Purss 1966; Knight & Sutherland 2013a). Penetration of the leaf sheath tissue is initiated through epidermal penetration, most frequently via the stomata aperture (Knight & Sutherland 2013a). In below ground infection, the initial breakthrough of the epidermal layer and adjacent hypoderm appears to be followed by a rapid spread through the central tissues (Knight & Sutherland 2016). Occasionally pith colonisation of the first internode, without hypoderm colonisation, is observed up to the base internode through the nodal tissue (Knight & Sutherland 2016). Under severe conditions such as water stress at the flowering stage, intense colonisation of the fungus occurs in the first three nodes of the stem of very susceptible cultivars, with frequent hyphal obstructions of xylem vessels and phloem tubes (Knight & Sutherland 2016). Under drought, prematurely senescing stems can form whiteheads, which contain shrivelled or no grain (Burgess et al. 2001; Knight et al. 2012; Percy et al. 2012).

Fusarium pseudograminearum and *F. culmorum* are the most common and globally important causal agents of crown rot of grain cereals (Burgess et al. 2001; Beccari et al. 2011; Scherm et al. 2013; Kazan & Gardiner 2018). In Australia, crown rot pathogens occur in many regions where wheat and barley (*Hordeum vulgare* L.) are grown and can cause significant yield losses (Scherm et al. 2013; Kazan & Gardiner 2018). This is predominantly in areas with water stress where minimum tillage, stubble maintenance and similar conservation agricultural practices are used (Wildermuth et al. 1997; Burgess et al. 2001). Infected stubble is the main source of inoculum of the host in the field (Wildermuth et al. 1997; Burgess et al. 2001; Kazan & Gardiner 2018).

Fusarium pseudograminearum and *F. culmorum* have been isolated from a wide range of grain cereals including barley, bread wheat, and durum wheat (*Triticum turgidum* var. *durum*), oat (*Avena sativa* L.), rye (*Secale cereale* L.), and triticale (× *Triticosecale* Wittmack) (Burgess et al. 2001). Bread wheat cultivars differ in disease susceptibility for both *F. pseudograminearum* (Wallwork et al. 2004; Percy et al. 2012; Knight & Sutherland 2013b; Knight & Sutherland 2017) and *F. culmorum* (Beccari et al. 2011; Scherm et al. 2013; Knight & Sutherland 2017),

ranging from highly susceptible to moderately susceptible (Percy et al. 2012; Kunesch et al. 2017; Lush et al. 2018; GRDC 2019). The authors are not aware of any commercial cultivars currently available with strong tolerance or resistance to crown rot.

In Australia, several studies employed wheat cultivars as a host in the assessment of crown rot disease for evaluating potential resistance. This assessment is based on the visual rating of the sub-crown internode, leaf sheaths in seedlings, and brown stem discolouration (Burgess et al. 2001; Percy et al. 2012; Knight & Sutherland 2013b; Scherm et al. 2013). The visual symptoms on the roots are often observed to be significantly less severe (or less obvious) than the leaf sheaths (Knight & Sutherland 2013b; Knight & Sutherland 2017). Knight and Sutherland (2013b) examined the infection of crown rot caused by *F. pseudograminearum* on the roots of wheat cultivars in glasshouse tests using a 0 to 4 scale, where 0 indicated no visual symptoms present and 4, greater than 75 % discolouration. The infection of *F. pseudograminearum* was found on the primary roots at 14 days and after 28 days on the secondary roots (Knight & Sutherland 2013b). *Fusarium culmorum* was generally described as causing more disease on the primary roots than *F. pseudograminearum* (Beccari et al. 2011; Scherm et al. 2013). However, Knight and Sutherland (2017) reported similar levels of colonisation by *F. pseudograminearum* and *F. culmorum* on the primary root of wheat cultivars. Xu et al. (2018) conducted a survey in 104 fields on the North China Plain from 2013 to 2016 to assess the distribution frequency of pathogenic fungi associated with crown rot of wheat. *Fusarium pseudograminearum* was one of the most common pathogens that was identified in this field survey, with 14.9% of *F. pseudograminearum* isolated from roots and 27.8% from stems. In this field survey, *F. culmorum* was observed to have low frequencies in the field (0.1% isolated from the roots and 0.4% isolated from the stem). Limited investigation has been reported in studies to compare the impact of *F. pseudograminearum* and *F. culmorum* on the bread wheat root architecture system in the glasshouse or in the field. Additionally, the root visual assessments are unlikely to be useful for routine crown rot disease rating due to the difficulty of root extraction from soil and the relatively low levels of root discolouration observed (Knight & Sutherland 2017).

Researching root physiology during crown rot infection may prove to be useful to understand the impact of this disease on the root system characteristics. Thus, the objectives of this study were to determine whether the interaction between inoculated *F. pseudograminearum* or *F. culmorum* treatments and non-inoculated plants in a controlled environment differed and to determine if there is variation in the effect of infection between two pathogens of crown rot (*F. pseudograminearum* and *F. culmorum*) on the root system architecture of bread wheat.

MATERIALS AND METHODS

Strains and inoculum preparation

Experiments were performed using the highly aggressive *F. pseudograminearum* strain A09#04 (Chapter 2) collected in Emerald, QLD, Australia, and the highly aggressive strain of *F. culmorum*, Fc 13-195 (Chapter 4) collected in VIC, Australia. Both were isolated from crown rot infected stems. A single spore from each strain was grown on Czapek-Dox Agar and incubated for seven days at 25℃ under 24 hours darkness (Chapter 2). The mycelium of each strain was harvested and used to produce colonised grain inoculum for inoculum production. The inoculum was produced as described in Chapter 2. Sterilised and ground un-inoculated wheat grain was used as a negative control.

Root chamber, plant growth and inoculation

Root chamber tests were conducted as a series of five experiments. In three experiments a single strain of *F. pseudograminearum* was used and in two experiments a single strain of *F. culmorum* was used. The first experiment consisted of six chambers inoculated with the *F. pseudograminearum* strain and six chambers with the non-inoculating control treatment. All other experiments consisted of eight chambers inoculated with either *F. pseudograminearum* or *F. culmorum* and four chambers without inoculum. The bread wheat cultivar (cv.) Livingston was used in all of the experiments. Livingston is classified as susceptible cultivar to both these crown rot pathogens (Lush et al. 2018). Each experiment was designed as a randomised complete block design, where each treatment (combination of strains by cultivar) was randomly allocated to a chamber within each replicate block. All experiments were performed in a growth cabinet at the Leslie Research Facility, Department of Agriculture and Fisheries, Toowoomba, (27°31’58’’S, 151°56’8’’ E), QLD, Australia. The plant growth medium consisted of self-mulching black Vertosol of the Irving clay soil association, obtained from the Darling Downs in QLD, Australia (Thompson & Beckmann 1959), mixed with river sand (50% sand to 50% soil). The soil mixture was pasteurised at 80℃ for 40 min and subsequently air-dried for seven days. No fertiliser was added to the mix. The root chambers were similar to those described by Manschadi et al. (2006). Briefly, the dimensions of the chambers were 38cm wide, 65cm deep and 3.2cm thick and were constructed using steel frames with polycarbonate sides (0.8cm thick).

The plant growth and inoculation method was a simulation of the layer pot design described by Wildermuth and McNamara (1994). Prior to planting, 6.3kg of soil mix was added to each chamber and saturated using distilled water and then allowed to drain overnight. The following

day all chambers were covered with one of the polycarbonate sides and two seeds were placed on top of the soil, using forceps. The seeds were positioned with the embryo facing down and placed close to one side of the chamber to allow good visibility of the primary roots after germination. The seeds were covered with 365g of dry soil. Inoculum (0.30g) was applied in an even layer on top of the soil surface for all inoculated chambers. Sterilised ground grain was applied to each of the negative control treatments. The inoculum was covered with 120g of dry soil. All chambers were placed in a growth cabinet at 20 ^{o}C with a 12 hours day/night cycle. Polycarbonate sides of the chambers were covered with vinyl to exclude light from the root system. A week after planting, one plant was removed to avoid interference between the root systems of both seedlings. Chambers were watered one week after planting to activate the inoculum, and then once weekly to saturation. After 30 days of growth, each chamber was placed horizontally on a nail board and soil washed from the roots. A picture of each plant root system was taken using a Canon EOS 80D DSLR 26 MP camera. Each plant was rated for disease severity as described below. The seminal root angle, longest root length, root and shoot fresh and dry weight, leaf number, and leaf area of the oldest and most recently emerged leaf were also assessed.

Root, shoot, and disease assessment

Disease severity was assessed using a 0 to 100% rating scale based on the visual discolouration of the sheaths of the first three leaves, where 0 = no discolouration and 100% = completely discoloured tissue. Following the disease severity ratings, the intact root system of each plant was removed from the nail board and placed individually into a water bath containing Tetrasodium Pyrophosphate (TSPP) ($Na_4P_2O_7$) (Albright & Wilson Limited, Australia) overnight to clean off any clay left on the roots. The first infection of crown rot on the leaf sheaths occurs in the oldest leaf. Thus, the oldest leaf sheath was examined to determine if crown rot has an impact on its size and whether the effects continue on the youngest fully-formed leaf. The leaf area of the oldest and the youngest fully-formed leaf for each plant was measured using a leaf area meter (LI-3100C Area Meter, Nebraska, USA). The leaf number of youngest fully-formed leaf varied for each individual plant and ranged between leaf number three to leaf number five. On the following day, the fresh root weight and shoot weight of each plant were weighed and placed in a separate paper bag. Individual shoot and root systems were air-dried in a 60°C oven for 36 hours, after which dry weights were recorded.

ImageJ software (https://imagej.nih.gov/ij/) was used to analyse the root angle of the first-pair of seminal roots (inside pair), which are the first pair to emerge after the primary seminal root

(Manschadi et al. 2008), and the outside-pair of seminal roots, which is usually the last pair of seminal roots developed (Fig.1). Each image was also used to score the root number and the maximum depth of the longest root.

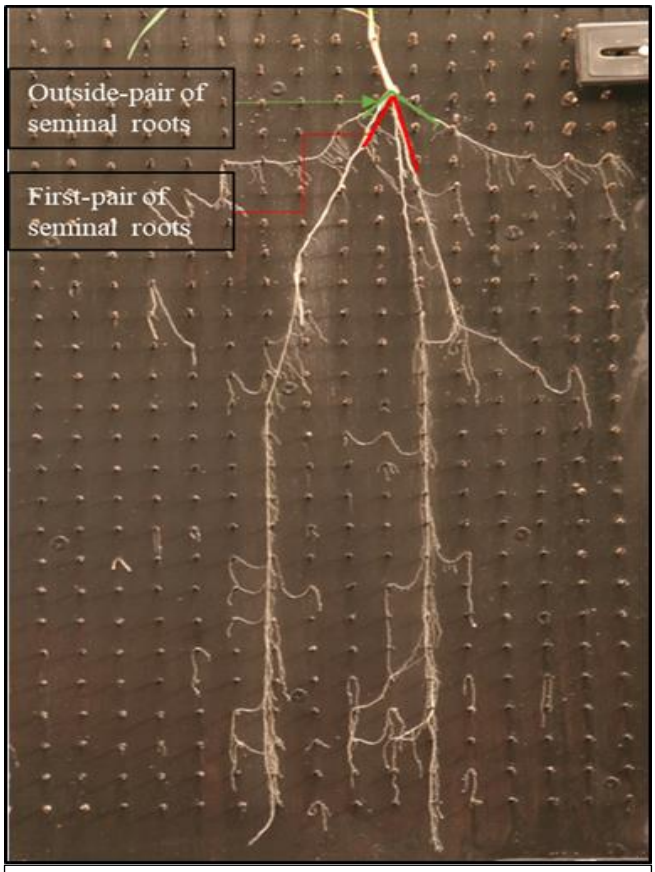


Figure 1. Non-inoculated root system of Livingston on nail board 30 days after emergence.

DATA ANALYSIS

The percentage of disease severity on the sheaths of the first three leaves (combined leaf sheaths) was totalled and divided by three to give a combined leaf sheaths average. There were no significant differences within the three experiments of *F. pseudograminearum* and within the two experiments of *F. culmorum*. Therefore, *F. pseudograminearum* experiments were combined and analysed together and *F. culmorum* experiments were analysed the same. The disease symptoms of the inoculated plants were assessed relative to the background colour of the negative control plant. The analysis of each variable was performed using a one-way ANOVA model. The model included fixed effects for the strain and experiment and their interactions. Terms to account for replicate blocks, chambers, and plants within chambers were included as random effects, with variances estimated separately for each experiment. To test

the distributions for normality for the experiments, Kolmogorov-Smirnov (Conover 1999) and Shapiro-Wilk (Shapiro & Wilk 1965) tests were applied. The standard error of difference (SED) values were calculated by taking the square root of both standard errors of the mean square (SE^2) and the strains (s_1 & s_2) using the formula $\sqrt{(SE^2_{(s1)} + SE^2_{(s2)})}$. All analyses were performed in Genstat 18th Edition (VSN 2015) (VSN International Limited, Hemel Hempstead, UK), using a significance level of 0.05.

RESULTS

Above ground assessments of tissues infected with *F. pseudograminearum*

The infection of the combined leaf sheaths indicated that a significant infection has been achieved by the inoculation and plant culture methods ($p < 0.001$). There were little or no visible symptoms in the negative control while 20.3% of the sheaths were discoloured in the inoculated plants (Fig. 2a). A significant reduction was observed in the leaf area of the oldest leaf (66.2% reduction) and the youngest fully-formed leaf (youngest leaf) (67.2% reduction) of the inoculated plants compared to the negative control ($p < 0.001$) (Fig. 2b and c).

A significant decrease was observed in the fresh (64% decrease) and dry (42.4% decrease) shoot weights of inoculated plants ($p < 0.001$) (Fig. 2d and e). The number of leaves was reduced by 31.2% in the inoculated plants (Fig. 2f).

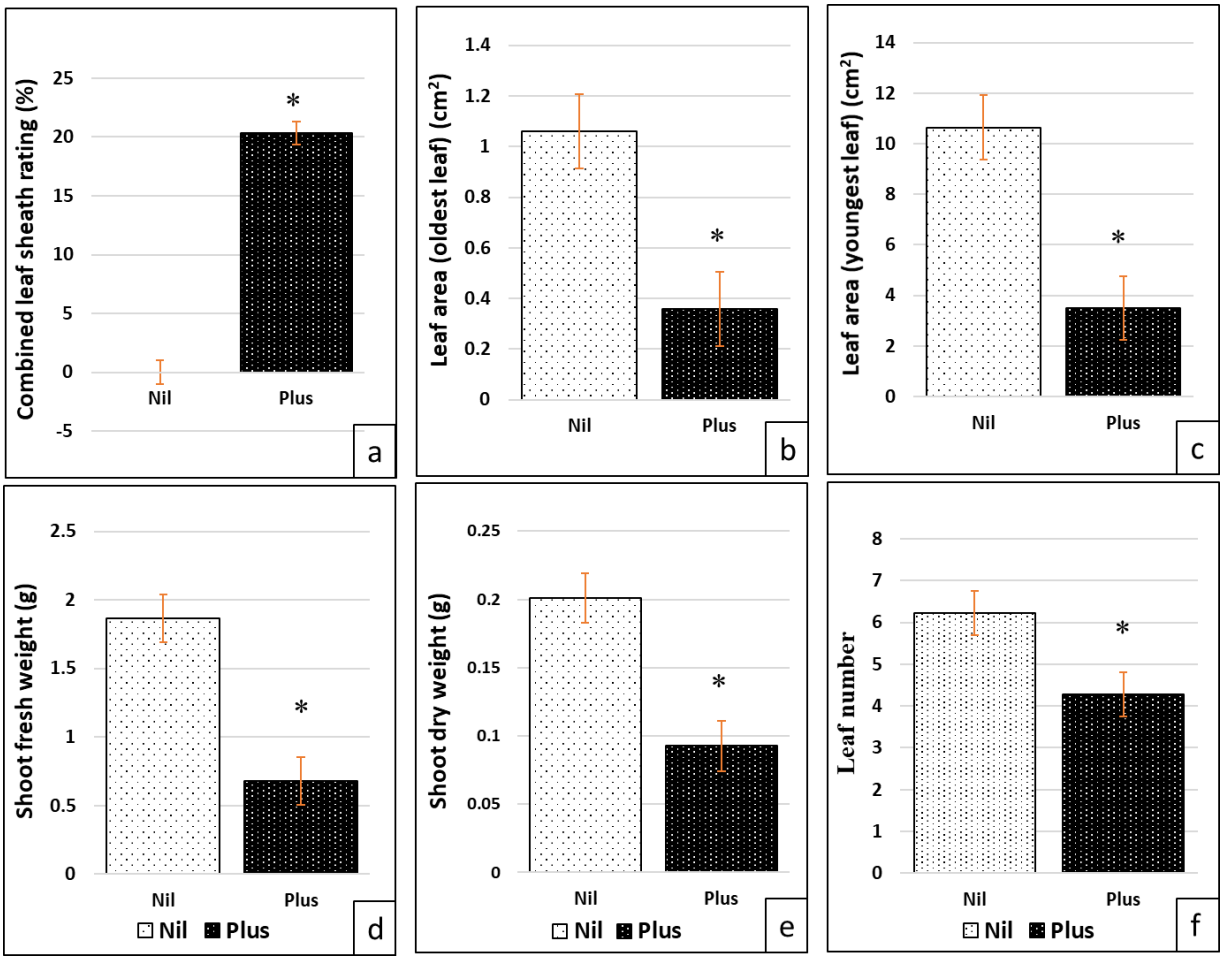


Figure 2. Mean combined leaf sheath disease severity ratings (a), leaf area of oldest leaf (b), leaf area of youngest fully formed leaf (c), shoot fresh weight (d), dry weight (e), and leaf number (f) for the strains interaction. Treatments include one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Error bars indicate the standard error of difference (+/- SED). *= significantly different within treatments ($p < 0.05$).

Assessment of root characteristics infected with *F. pseudograminearum*

There was a significant difference between *F. pseudograminearum* inoculated plants and negative controls for all root characteristic traits measured ($p < 0.05$), with the exception of root angle.

There was a significant decrease in the root fresh weight ($p < 0.001$) (51.5% reduction), and in the root dry weight ($p = 0.037$) (68.1% reduction) of the inoculated plants compared to the negative control (Fig. 3a and b). A significant reduction was observed in the root length ($p = 0.01$) of the inoculated plants, compared to the negative control (Fig. 3c) with a 15.6 % reduction in the root length of the infected plants. The number of roots was reduced significantly for the inoculated plants ($p < 0.001$), with a 36.9% decrease compared to the negative control (Fig. 3d). There was no significant effect of the disease on the root angle of

first-pair of seminal roots (p = 0.378) or the outside-pair (p = 0.076) compared to the negative control (Fig. 3e and f) although the mean angle of treated plants was generally less than control plants for both root types.

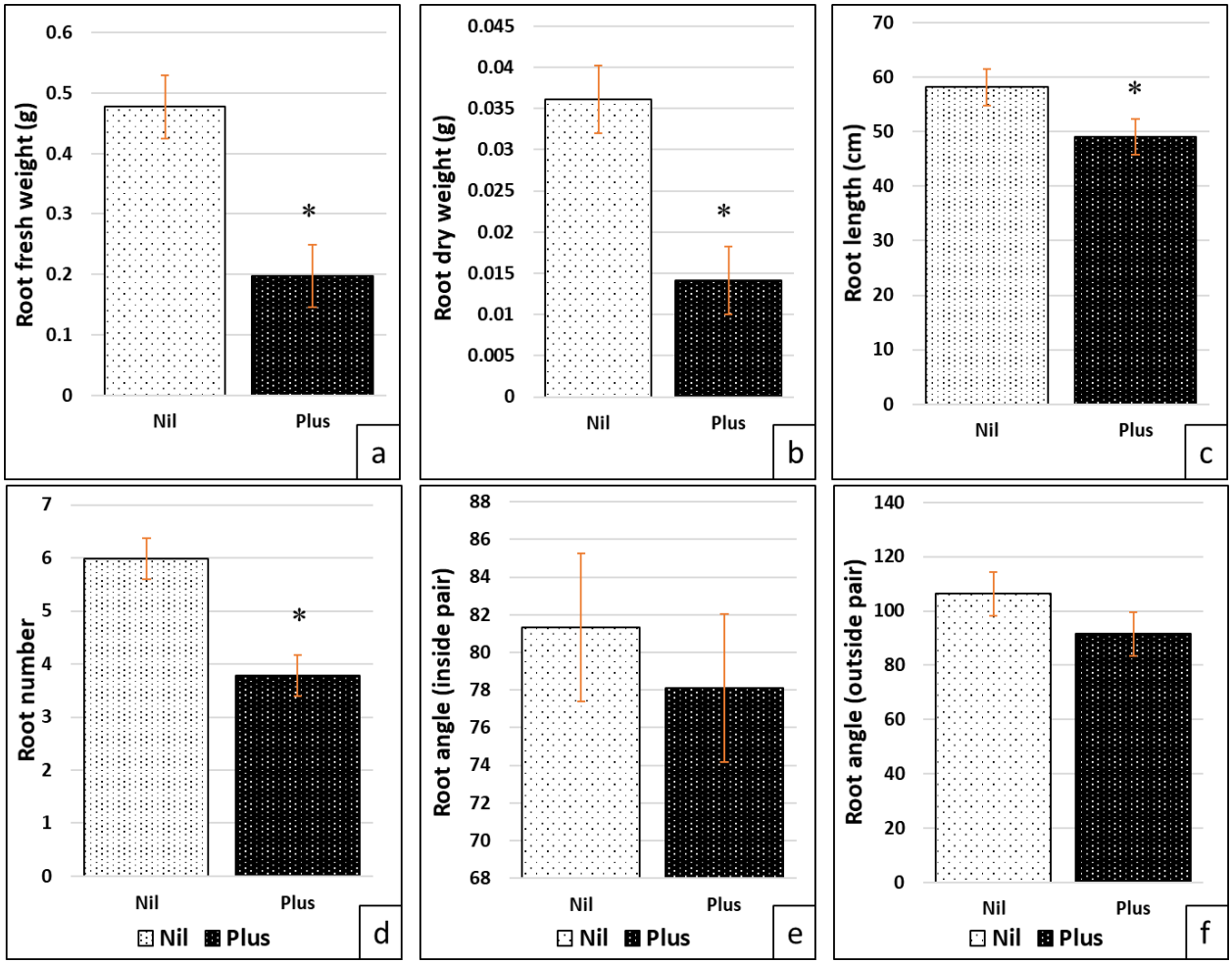


Figure 3. Mean root angle inside (a) and outside pairs (b), root length (c), root fresh weight (d), dry weight (e), and root number (f) for the strains (Plus and Nil) interaction. Treatments include one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Error bars indicate the standard error of difference (+/- SED). *= significantly different within treatments (p < 0.05).

Above ground assessments of tissues infected with *F. culmorum*

A significant interaction was observed for the combined leaf sheaths disease severity (p < 0.001) of Livingston when infected with *F. culmorum*. There was a 21.5% increase in the disease severity of the inoculated plant while there were little or no visual symptoms in the negative control treatments (Fig. 4a). There was no significant effect of the pathogen on the leaf area of the oldest leaf (p = 0.439) (Fig.4b). A significant interaction (p = 0.003) was observed for the leaf area of the youngest fully-formed leaf when comparing the inoculated

plants with the negative controls. The average leaf area of the youngest fully-formed leaf of the inoculated plants was reduced by 25.2% compared to the negative control (Fig.4c). There were significant differences in the shoot fresh weight (p = 0.008) of the inoculated plants, which had a 25.2% reduction compared to the negative control (Fig.4d). No significant interaction was observed in the shoot dry weight (p = 0.057) (Fig.4e) or the leaf number between (p = 0.275) *F. culmorum* inoculated plants and negative controls (Fig.4f).

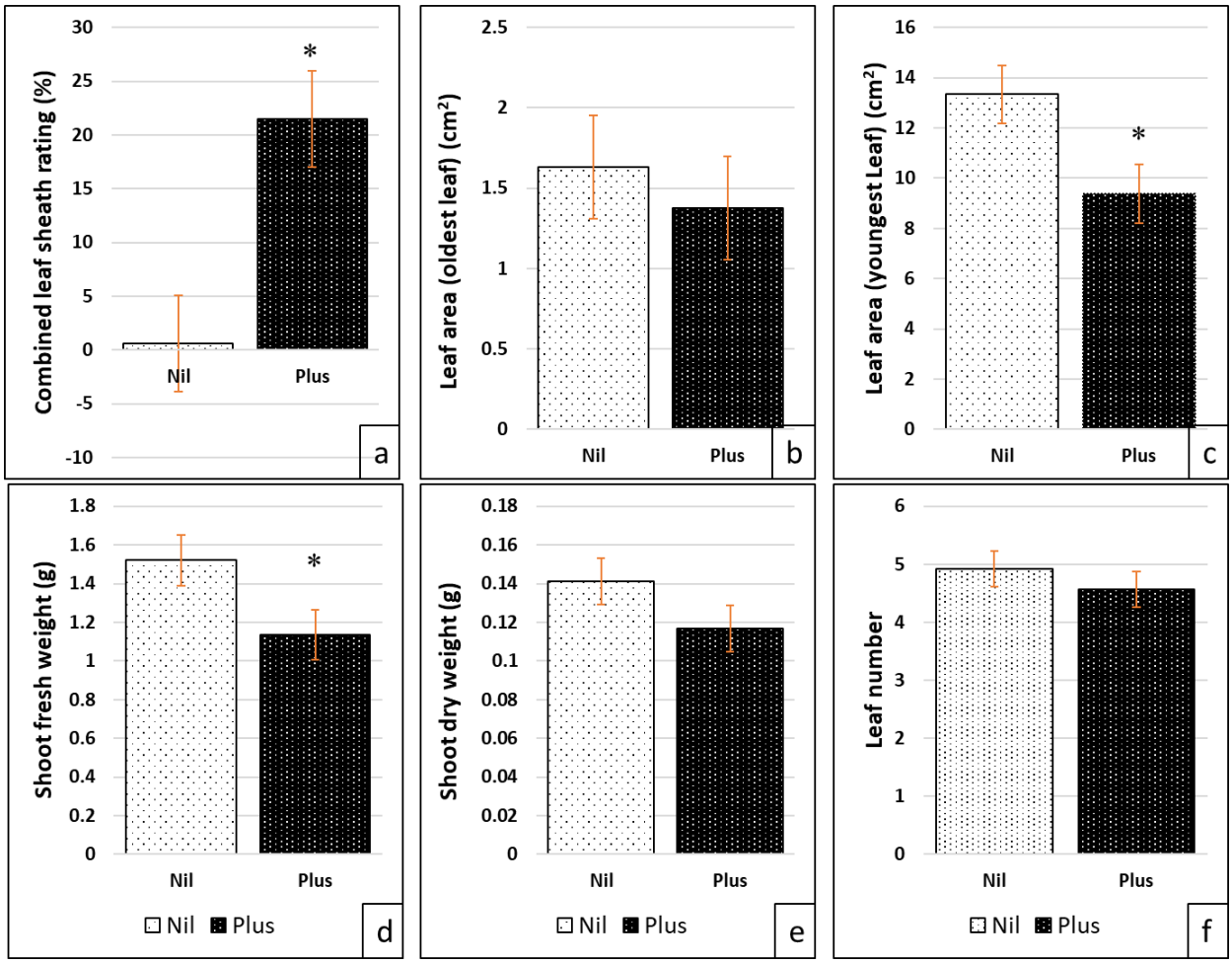


Figure 4. Mean combined leaf sheath disease severity ratings (a), leaf area of oldest (first) leaf (b), leaf area of most recent (last growing) leaf (c), shoot fresh weight (d), dry weight (e), and leaf number (f) for the strains interaction. Treatments include one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Error bars indicate the standard error of difference (+/- SED). *= significantly different within treatments (p < 0.05).

Assessment of root characteristics infected with *F. culmorum*

There were significant differences between the inoculated and non-inoculated plants for fresh (p = 0.02) and dry (p = 0.048) root weight (Fig. 5a and b). Fresh and dry weights of inoculated plants were reduced by 29.6% and 23.1%, respectively, compared to the negative control (Fig. 5a and b). There was no significant interaction between the inoculated and non-inoculated

plants for the root number (p = 0.253) (Fig.5d). There were no major interactions between the pathogen and the root angle measurements (Fig.5e and f) or the root length (p = 0.238) (Fig.5c).

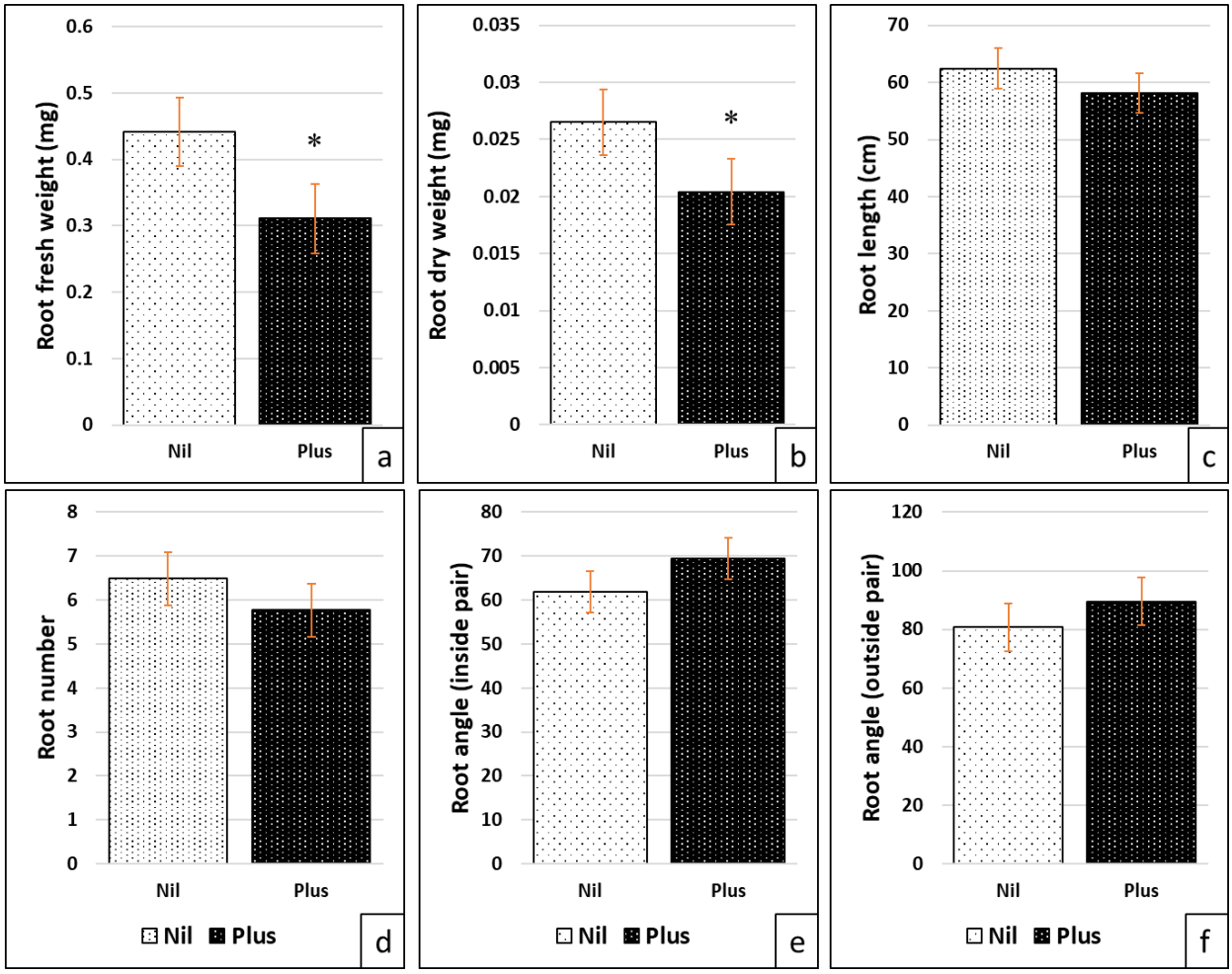


Figure 5. Mean root angle inside (a) and outside pairs (b), root length (c), root fresh weight (d), dry weight (e), and root number (f) for the strains interaction. Treatments include one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Error bars indicate the standard error of difference (+/- SED). *= significantly different within treatments (p < 0.05).

Discussion

Literature searches have failed to identify any previous reports on the effects of crown rot pathogens infection on root architecture and development in bread wheat. Thus, this study may provide the first information on how crown rot affects the ability of plants to explore the soil to access vital resources. Previous studies examining disease infection of crown rot pathogens in the roots focused on the visual symptoms and fungal DNA biomass (Knight & Sutherland 2013b; Knight & Sutherland 2017; Xu et al. 2018).

In this study, *F. pseudograminearum* was shown to cause high levels of disease severity on the combined leaf sheaths and a significant reduction in above ground tissue including shoot fresh and dry weight, leaf area (oldest and most recent growing leaf), as well as leaf number. This confirms that the plant culture and inoculation treatments provided a level of infection sufficient to cause significant adverse effects. Chapter 2 also reported a significant reduction in the shoot weight (45.1 to 57%) and length (12.2 to 55%) of bread wheat cultivar Livingston inoculated with two strains of *F. pseudograminearum* compared to the negative control. This observation indicates the negative impact this fungus has on the above ground tissue resulting in a detrimental effect on plant growth. The *F. pseudograminearum* strain had a significant impact on most of the root system characteristics measured, suggesting that *F. pseudograminearum* may contribute to the inhibition of plant growth by reducing the capture of water and other nutritional requirements during the seedling stage. Palta et al. (2011) indicated that a large and deep root system contributes to adaptation in the dry season. The results of the current study suggest that during infection by this strain of *F. pseudograminearum*, bread wheat cultivar Livingston has the potential to become susceptible to drought, limiting the capacity of root systems to effectively extract water and nutrition out of the soil. Further research is warranted into the effect of *F. pseudograminearum* on the root architecture of bread wheat and its resistant to drought during crown rot infection.

In the current study, a significant difference between the inoculated and un-inoculated plants with *F. culmorum* strain was observed in disease severity of the combined leaf sheaths, the leaf area of the youngest fully-formed leaf and shoot fresh weight, indicating that the *F. culmorum* strain has a detrimental effect on the plant growth. The effect of two different strains of *F. culmorum* on the shoot dry weight in a range of winter cereals in three weeks of glasshouse tests was investigated in Chapter 2. There was no significant impact on the shoot dry weight of Livingston when inoculated with two strains of *F. culmorum* in Chapter 2. Although the mean shoot weight of plants inoculated with *F. culmorum* was less than that of the non-inoculated controls, this difference was not statistically significant as it was for *F. pseudograminearum* inoculated plants in the current study. However, other strains of *F. pseudograminearum* and *F. culmorum* maybe cause different host responses depending on the strain virulence.

Fusarium culmorum in our study showed little effect on the root characteristics of bread wheat cultivar Livingston except for root fresh and dry weight. This strain of *F. culmorum* had little effect on the length of the root, suggesting that the reduction in water uptake under *F. culmorum* infection may be less than *F. pseudograminearum* strain for bread wheat cultivar Livingston.

However, precise measurements of water uptake need to be taken to test this suggestion. It has been proposed that strong and healthy root systems result primarily from more and longer branched roots rather than from roots that are thicker or which have more biomass (Palta & Watt 2009). Manschadi et al. (2006) indicated that greater root-length or density at depth resulted in increased availability of water late in the season, hence, improved wheat grain yields.

Fusarium pseudograminearum strain was observed to have similar levels of disease severity (20.3%) as *F. culmorum* strain (21.5%) on the combined leaf sheaths of the bread wheat cultivar Livingston in the current study. However, differences between pathogens were observed for the effect of disease on root system characteristics and aboveground structures. This suggests that the infection mechanism for each pathogen on the above and below ground physiological traits may differ. In Australia, studies have compared the disease severity on the sheaths of first three leaves or stems of wheat cultivars that are inoculated with *F. pseudograminearum* and *F. culmorum* (Hollaway et al. 2013; Knight & Sutherland 2017) (Chapter 2). Knight and Sutherland (2017) ascertained similar levels of colonisation by a single strain each of *F. pseudograminearum* and *F. culmorum* on the primary roots of bread wheat cultivars. In addition, Chapter 2 indicated that two strains of *F. culmorum* had similar or lower disease severity on the sheaths of first three leaves of bread wheat compared to *F. pseudograminearum* strains. However, there was a significant reduction only in the shoot length of Livingston when colonised with *F. pseudograminearum* compared to *F. culmorum* strains.

The wheat cultivar Livingston used in this study is grown in the northern region of Australia (QLD and northern NSW). The cultivars grown in this region vary in root angle and root number (Manschadi et al. 2008). However, drought-adaptive root system characteristics are not the only traits affecting the adoption of wheat cultivars in a particular environment. Different factors such as resistance to different pathogens and improved grain quality may be equally significant and play an important role in the selection and adoption of cultivars by breeders and growers (Manschadi et al. 2008). In our study, there were no significant differences in the root angles between the inoculated plants and the non-inoculated treatments across the experiments. This may be because the variations in the root angle between the individual plants are greater than between treatments. Further research is needed to investigate the root angle involving a greater number of replicates to improve the measurement of the root angle of the treatments.

All measured above and below ground physiological characteristics were negatively under the influence of *F. pseudograminearum* infection, except for the root angle. In contrast, the disease severity caused by *F. culmorum* did not have a significant impact on most of the above and below ground characteristics. This indicates that future studies are required to identify and characterise the relationship between root traits, aboveground infection and yield under the two pathogens of crown rot in a range of wheat cultivars. *Fusarium pseudograminearum* and *F. culmorum* strains can vary in the virulence, quantification and the rate of the inoculum. However, in our study, the brown discolouration between *F. pseudograminearum* and *F. culmorum* strains were similar, suggesting the virulence of both pathogens was not greatly different. Further research is warranted into direct comparisons of multiple strains of *F. pseudograminearum* and *F. culmorum* for their virulence on the root system characteristics of a variety of wheat cultivars, particularly in adult plants. The findings of this study on root architecture traits during crown rot infection identify it as one of the key attributes necessary for understanding the effect of crown rot disease on the root system characteristics. These findings may assist breeders and researchers to improve the adaptation of wheat to drought to reduce the severity of crown rot and in turn to reduce yield losses.

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Supplementary Table 1 ANOVA table for analysis of root angle outside pair of Livingston colonised by one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Mean separations of $\alpha < 0.05$.

Source of variation	**d.f.[a]**	**s.s[b]**	**m.s.[c]**	**v.r[d.]**	**p-Value**
Experiment stratum					
Strain	1	459.9	459.9	2.34	0.369
Residual	1	196.9	196.9	0.38	
Experiment.*Units* stratum					
Strain	1	1753.4	1753.4	3.36	0.076
Residual	31	16186.2	522.1		
Total	34	18596.4			

Supplementary Table 2 ANOVA table for analysis of root angle inside pair of Livingston colonised by one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Mean separations of $\alpha < 0.05$.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**p-Value**
Experiment stratum					
Strain	1	205.8	205.8	2.2	0.378
Residual	1	93.5	93.5	0.75	
Experiment.*Units* stratum					
Strain	1	82.3	82.3	0.66	0.422
Residual	31	3857.4	124.4		
Total	34	4239.1			

Supplementary Table 3 ANOVA table for analysis of root length of Livingston colonised by one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Mean separations of $\alpha < 0.05$.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**p-Value**
Experiment stratum					
Strain	1	770.85	770.85	0.92	0.512
Residual	1	833.74	833.74	9.51	
Experiment.*Units* stratum					
Strain	1	659.69	659.69	7.53	0.01
Residual	31	2716.82	87.64		
Total	34	4981.1			

[a] d.f: degree of freedom
[b] s.s: sum of squares
[c] m.s.: mean square
[d] v.r: variance ratio

Supplementary Table 4 ANOVA table for analysis of root number of Livingston colonised by one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	**d.f.[a]**	**s.s[b]**	**m.s.[c]**	**v.r[d.]**	**p-Value**
Experiment stratum					
Strain	1	1.533	1.533	0.75	0.545
Residual	1	2.042	2.042	1.68	
Experiment.*Units* stratum					
Strain	1	39.254	39.254	32.39	<.001
Residual	31	37.572	1.212		
Total	34	80.4			

Supplementary Table 5 ANOVA table for analysis of root fresh weight of Livingston colonised by one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**p-Value**
Experiment stratum					
Strain	1	0.04036	0.04036	5.76	0.251
Residual	1	0.007	0.007	0.32	
Experiment.*Units* stratum					
Strain	1	0.62838	0.62838	29.09	<.001
Residual	31	0.66963	0.0216		
Total	34	1.34537			

Supplementary Table 6 ANOVA table for analysis of root dry weight of Livingston colonised by one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**p-Value**
Experiment stratum					
Strain	1	6.15E-05	6.15E-05	0.28	0.688
Residual	1	0.000216	0.000216	1.57	
Experiment.*Units* stratum					
Strain	1	0.0039	0.0039	28.33	<.001
Residual	31	0.004268	0.000138		
Total	34	0.008446			

[a] d.f: degree of freedom
[b] s.s: sum of squares
[c] m.s.: mean square
[d] v.r: variance ratio

Supplementary Table 7 ANOVA table for analysis of combined leaf sheath disease severity ratings of Livingston colonised by one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	**d.f.[a]**	**s.s[b]**	**m.s.[c]**	**v.r[d.]**	**p-Value**
Experiment stratum					
Strain	1	3.36	3.36	0.36	0.656
Residual	1	9.36	9.36	0.23	
Experiment.*Units* stratum					
Strain	1	3372.45	3372.45	82.71	<.001
Residual	31	1264	40.77		
Total	34	4649.18			

Supplementary Table 8 ANOVA table for analysis of leaf area of the oldest leaf of Livingston colonised by one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**p-Value**
Experiment stratum					
Strain	1	1.05	1.05	64.32	0.079
Residual	1	0.0163	0.0163	0.1	
Experiment.*Units* stratum					
Strain	1	3.732	3.732	22.84	<.001
Residual	30	4.9025	0.1634		
Total	33	9.7007			

Supplementary Table 9 ANOVA table for analysis of leaf area of the youngest fully formed leaf of Livingston colonised by one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**p-Value**
Experiment stratum					
Strain	1	18.14	18.14	16.54	0.153
Residual	1	1.1	1.1	0.08	
Experiment.*Units* stratum					
Strain	1	411.77	411.77	31.71	<.001
Residual	31	402.51	12.98		
Total	34	833.52			

[a] d.f: degree of freedom
[b] s.s: sum of squares
[c] m.s.: mean square
[d] v.r: variance ratio

Supplementary Table 10 ANOVA table for analysis of shoot fresh weight of Livingston colonised by one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	**d.f.[a]**	**s.s[b]**	**m.s.[c]**	**v.r[d.]**	**p-Value**
Experiment stratum					
Strain	1	1.656	1.656	3.56	0.31
Residual	1	0.4648	0.4648	1.91	
Experiment.*Units* stratum					
Strain	1	11.3289	11.3289	46.55	<.001
Residual	31	7.5441	0.2434		
Total	34	20.9939			

Supplementary Table 11 ANOVA table for analysis of shoot fresh weight of Livingston colonised by one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**p-Value**
Experiment stratum					
Strain	1	0.010831	0.010831	1.76	0.411
Residual	1	0.006144	0.006144	2.29	
Experiment.*Units* stratum					
Strain	1	0.094484	0.094484	35.18	<.001
Residual	31	0.083253	0.002686		
Total	34	0.194713			

Supplementary Table 12 ANOVA table for analysis of leaf number of Livingston colonised by one strain of *Fusarium pseudograminearum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**p-Value**
Experiment stratum					
Strain	1	1.193	1.193	0.17	0.751
Residual	1	7.042	7.042	3.14	
Experiment.*Units* stratum					
Strain	1	30.332	30.332	13.54	<.001
Residual	31	69.433	2.24		
Total	34	108			

[a] d.f: degree of freedom
[b] s.s: sum of squares
[c] m.s.: mean square
[d] v.r: variance ratio

Supplementary Table 13 ANOVA table for analysis of root angle inside pair of Livingston colonised by one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	d.f.[a]	s.s[b]	m.s.[c]	v.r[d.]	p-Value
Experiment stratum					
Strain	1	222.7	222.7		
Experiment.*Units* stratum					
Strain	1	283.4	283.4	2.69	0.117
Residual	19	2001	105.3		
Total	21	2507			

Supplementary Table 14 ANOVA table for analysis of root angle outside pair of Livingston colonised by one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	d.f.	s.s.	m.s.	v.r.	p-Value
Experiment stratum					
Strain	1	1545.9	1545.9		
Experiment.*Units* stratum					
Strain	1	364	364	1.17	0.293
Residual	19	5909.6	311		
Total	21	7819.5			

Supplementary Table 15 ANOVA table for analysis of root length of Livingston colonised by one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	d.f.	s.s.	m.s.	v.r.	p-Value
Experiment stratum					
Strain	1	0.59	0.59		
Experiment.*Units* stratum					
Strain	1	84.45	84.45	1.49	0.238
Residual	18	1021.95	56.78		
Total	20	1106.99			

[a] d.f: degree of freedom
[b] s.s: sum of squares
[c] m.s.: mean square
[d] v.r: variance ratio

Supplementary Table 16 ANOVA table for analysis of root fresh weight of Livingston colonised by one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	d.f.[a]	s.s[b]	m.s.[c]	v.r[d.]	p-Value
Experiment stratum					
Strain	1	0.02632	0.02632		
Experiment.*Units* stratum					
Strain	1	0.08089	0.08089	6.4	0.02
Residual	19	0.23996	0.01263		
Total	21	0.34717			

Supplementary Table 17 ANOVA table for analysis of root dry weight of Livingston colonised by one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	d.f.	s.s.	m.s.	v.r.	p-Value
Experiment stratum					
Strain	1	0.00007313	0.00007313		
Experiment.*Units* stratum					
Strain	1	0.000155	0.000155	4.48	0.048
Residual	18	0.000622	0.00003458		
Total	20	0.000851			

Supplementary Table 18 ANOVA table for analysis of root number of Livingston colonised by one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	d.f.	s.s.	m.s.	v.r.	p-Value
Experiment stratum					
Strain	1	2.909	2.909		
Experiment.*Units* stratum					
Strain	1	2.393	2.393	1.39	0.253
Residual	19	32.698	1.721		
Total	21	38			

[a] d.f: degree of freedom
[b] s.s: sum of squares
[c] m.s.: mean square
[d] v.r: variance ratio

Supplementary Table 19 ANOVA table for analysis of combined leaf sheath disease severity ratings of Livingston colonised by one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	**d.f.[a]**	**s.s[b]**	**m.s.[c]**	**v.r[d.]**	**p-Value**
Experiment stratum					
Strain	1	244.44	244.44		
Experiment.*Units* stratum					
Strain	1	2070.75	2070.75	21.91	<.001
Residual	19	1795.41	94.5		
Total	21	4110.61			

Supplementary Table 20 ANOVA table for analysis of leaf area of the oldest leaf of Livingston colonised by one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**p-Value**
Experiment stratum					
Strain	1	0.5062	0.5062		
Experiment.*Units* stratum					
Strain	1	0.3048	0.3048	0.62	0.439
Residual	19	9.2808	0.4885		
Total	21	10.0917			

Supplementary Table 21 ANOVA table for analysis of leaf area of the youngest fully formed leaf of Livingston colonised by one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**p-Value**
Experiment stratum					
Strain	1	3.493	3.493		
Experiment.*Units* stratum					
Strain	1	71.79	71.79	11.72	0.003
Residual	18	110.256	6.125		
Total	20	185.539			

[a] d.f: degree of freedom
[b] s.s: sum of squares
[c] m.s.: mean square
[d] v.r: variance ratio

Supplementary Table 22 ANOVA table for analysis of shoot fresh weight of Livingston colonised by one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	d.f.[a]	s.s[b]	m.s.[c]	v.r[d.]	p-Value
Experiment stratum					
Strain	1	0.24728	0.24728		
Experiment.*Units* stratum					
Strain	1	0.60915	0.60915	8.73	0.008
Residual	18	1.25607	0.06978		
Total	20	2.1125			

Supplementary Table 23 ANOVA table for analysis of shoot dry weight of Livingston colonised by one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	d.f.	s.s.	m.s.	v.r.	p-Value
Experiment stratum					
Strain	1	0.001812	0.001812		
Experiment.*Units* stratum					
Strain	1	0.002451	0.002451	4.15	0.057
Residual	18	0.010621	0.00059		
Total	20	0.014885			

Supplementary Table 24 ANOVA table for analysis of leaf number of Livingston colonised by one strain of *Fusarium culmorum* (Plus) and the negative control (Nil). Mean separations of α <0.05.

Source of variation	d.f.	s.s.	m.s.	v.r.	p-Value
Experiment stratum					
Strain	1	3.6818	3.6818		
Experiment.*Units* stratum					
Strain	1	0.5664	0.5664	1.26	0.275
Residual	19	8.5245	0.4487		
Total	21	12.7727			

[a] d.f: degree of freedom
[b] s.s: sum of squares
[c] m.s.: mean square
[d] v.r: variance ratio

CHAPTER 6

CONCLUSION AND FUTURE RECOMMENDATIONS

KEY FINDINGS

The first objective of our research was to compare the ability of two strains each of *Fusarium pseudograminearum, F. culmorum, F. graminearum* and *B. sorokiniana* to cause crown rot and common root rot in a single cultivar of five winter cereals species, including barley (Grimmett), bread wheat (Livingston), durum wheat (Hyperno), oat (Genie) and triticale (Endeavour) in glasshouse and field trials. For both glasshouse and field trials, *F. pseudograminearum* caused the greatest disease severity on the first three leaf sheaths, stem and sub-crown internode (SCI) across the cultivars (excluding Genie), followed by *F. culmorum, B. sorokiniana* and then *F. graminearum*. The greatest levels of disease severity were observed in Grimmett and Livingston leaf sheaths in the seedling test and in Hyperno and Grimmett stems in the field trials when inoculated with *F. pseudograminearum*. This indicates there was no association between the seedling and field reaction of durum wheat and triticale cultivars to crown rot pathogens.

In both glasshouse and field trials, *F. pseudograminearum* caused significant decrease in the plant height of the cultivars compared to the other pathogens and the non-inoculated controls. The reduction in the plant height indicates that *F. pseudograminearum* had a negative effect on the development of the host. A reduction in the plant dry weight of the cultivars in the glasshouse tests and 2017 field trial by *F. pseudograminearum* was observed. In 2016, however, the plant dry weight was greater in *F. pseudograminearum* and *F. culmorum* inoculated treatments than the non-inoculated at flowering and maturity. That could be linked to the increase in the tiller number of the treatments that inoculated with *F. pseudograminearum* and *F. culmorum* at flowering and maturity in the same season. Further investigation is required to test the impact of crown rot pathogens on the plant physiology in a range of winter cereal species. The plant dry weight was greater at flowering than at maturity in the 2016 trial.

This research further highlights the complicated nature of the pathogen by strain by cultivars by the environment interaction. These complex interactions could be one of the reasons that the genetic progress in breeding for crown rot resistance has been slowed down. A comparison

of cultivars indicated that oat cultivar Genie exhibited the least disease severity after inoculation with crown rot and common root rot pathogens compared to the other cereal species in the glasshouse tests. In the glasshouse test, there was also a negative physiological impact (shoot height) of disease caused by crown rot and common root rot pathogens on Genie. In the 2017 field trial, DNA of *F. pseudograminearum* and *F. culmorum* was present at the highest frequency across the cultivars. Although Genie had low or no visual symptoms, DNA of all pathogens were detected in Genie, with *F. culmorum* had the greatest frequency of DNA detection. This suggested that Genie cultivar could be susceptible to some *F. culmorum* strains. This proposal was tested in another study (Chapter 3) by assessing the virulence caused by eight strains of *F. culmorum* on five forage oat cultivars.

In 2016, significant crown rot infection was reported on oat plants in the Darling Downs, QLD, and *F. culmorum* was isolated from these plants. Therefore, the ability of *F. culmorum* strains and *F. pseudograminearum* strains to cause crown rot on five forage oat cultivars and one susceptible bread wheat cultivar was assessed in two glasshouse tests. Eight *F. culmorum* strains collected from different regions around Australia including strains from the Darling Downs were tested, along with a mixture of strains of *F. pseudograminearum* were used as a positive control. The results of this study were consistent with the hypothesis that some *F. culmorum* strains may cause disease on the first leaf sheath of forage oat cultivars. There was also a variation in oat cultivar reactions to the eight *F. culmorum* strains and the *F. pseudograminearum* mixture. Moreover, the presence of DNA of the crown rot pathogens in both shoots that had no visual symptoms and those that had brown discolouration, suggests that oat may be able to support *F. culmorum* growth and might provide a continuing source of inoculum in the field. These results indicate the need for further investigation in a field trial to examine the virulence of *F. culmorum* strains on oat cultivars and to test the role of oat in a crop rotation system for crown rot. In the glasshouse and field trials, the negative impact of the crown rot disease on the above ground tissue was significant, highlighting the need for examining the impact of crown pathogens on the root system of winter cereals.

The third objective was to examine the impact of a single strain of *F. pseudograminearum* and *F. culmorum* on the root characteristics of the crown rot susceptible bread wheat cultivar Livingston. Root characteristics were analysed by measuring the root angle, root length, root number, as well as root fresh and dry weight of one wheat cultivar. Above ground tissue responses were also assessed by scoring the disease severity on the three-leaf sheaths, fresh and dry shoot weight, leaf number along with leaf area of the oldest and youngest fully-formed

leaf. These characteristics were investigated in a series of root chamber tests. While both *F. pseudograminearum* and *F. culmorum* caused similar disease severity on wheat cultivar Livingston, the physiological impact on the above ground and root tissues was different. Significant reduction in the above ground and root system characteristic of Livingston was observed when inoculated with *F. pseudograminearum* (except root angle). In contrast, *F. culmorum* had little impact on the above ground and root tissues of Livingston. This suggests that each pathogen may have a different impact on the plant physiology. The results indicate the need for further study of the root characteristic systems of a range of wheat cultivars inoculated with different *F. pseudograminearum* and *F. culmorum* strains. Further interest would be to investigate the influence of root architecture on the disease severity of crown rot. Variations identified in root architecture among varieties may help in the breeding of crown rot resistant varieties.

FUTURE RECOMMENDATIONS

This is the most extensive study to date reporting a comparative analysis of four crown rot and common root rot pathogens in a single cultivar each of five winter cereal species, which indicated a varied range of disease responses. Variations in cereal species reactions may reveal potential sources of resistance and inform strategies for crop rotations. Hence, future studies should be conducted into direct comparisons that include more cultivars of each crop and multiple strains collected from different regions. This may provide insights into the pathogen diversity of crown rot and common root rot pathogens on different host species.

Crown rot and common root rot caused by *Fusarium* species and *B. sorokiniana* are often associated in the same environment and have been described as part of a root rot complex (Smiley & Patterson 1996; Smiley et al. 2005; Tunali et al. 2008). In Australia, researchers describe the two diseases either as crown rot or common root rot (Wildermuth et al. 1997). Mixtures of multiple strains of these species are required for future research to determine the dominant species in the complex and the adaptation pattern of each species in different environments by employing multiplex qPCR assay.

Oat cultivars in this study exhibited visual symptoms on the first leaf sheath when inoculated with *F. culmorum* or *F. pseudograminearum* strains in 21 days seedling tests. The disease severity could increase as time progresses and become more severe (Percy et al. 2012). Thus, testing the ability of these pathogens to infect oat cultivars in field trials in adult plants will assist in understanding the effect of these pathogens on the plant at different growth stages in natural environments. Furthermore, quantifying the fungal DNA present in oat cultivars will

allow a more accurate measurement of differences in the fungal spread, specifically in the plants that do not exhibit visual symptoms. This would generate further knowledge on the role of oat in crop rotation for crown rot disease.

To our knowledge, the impact of two crown rot pathogens on the root characteristic system has not been investigated prior to this research. In this research one susceptible bread wheat cultivar and a single strain each of *F. culmorum* and *F. pseudograminearum* were tested. In future, a direct comparison between multiple strains of *F. culmorum* and *F. pseudograminearum* is warranted to examine the host response on the root system of a range of wheat cultivars in adult plants. This could be tested in controlled environments using root champers. This study will assist in understanding the variation in the impact of these pathogens on the root system of bread wheat.

CONCLUSION

Some significant goals were achieved in the progression of this study, with room for further investigation. Significant variations in the disease severity of five winter cereal species infected with three *Fusarium* species and *B. sorokiniana* was observed. These differences will have implications on the disease management strategies, including crop rotations undertaken by winter cereal growers. This study assessed the virulence of eight *F. culmorum* strains on five forage oat cultivars in seedling tests. Results from this study suggest that some *F. culmorum* strains could cause significant disease in adult plants. This raises the question about the role of oats in crown rot crop rotation. The variation in the effects of *F. culmorum* and *F. pseudograminearum* on root characteristics may lead to further research to determine if different root systems with specific characteristics perform better under crown rot pressure, thus, improving drought adaptation and minimising yield losses of wheat cultivars due to crown rot.

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