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. In glasshouse trials, we inoculated one cultivar each of barley, bread wheat, durum wheat, oat and triticale with two strains of each of the four pathogens. At 21 days after inoculation, the sub-crown internode and leaf sheaths of each plant were visually rated for brown discoloration. Shoot length and dry weight of inoculated plants were compared with those of non-inoculated controls. Barley and bread wheat had the highest disease severity ratings on leaf sheaths and sub-crown internode (64.7–99.6%), whereas oat had the lowest disease severity ratings across all pathogens (<5%). The shoot length of all cultivars was significantly reduced (by 12.2–55%, $P < 0.05$) when exposed to *F. pseudograminearum*. This study provides a comparison of pathogenicity of crown rot and common root rot pathogens and demonstrates significant variation in visual discoloration and host response across a range of winter cereals.

Keywords: barley, *Bipolaris*, crown rot, *Fusarium*, oat, triticale, wheat.

Introduction

Crown rot and common root rot of cereals are of key economic significance worldwide (Kumar *et al*. 2002; Kazan and Gardiner 2018). In Australia, crown rot results in estimated annual losses of AU$21 million and $79 million for the barley and bread wheat industries, respectively, and common root rot results in estimated annual losses of $13 million and $30 million to these industries (Murray and Brennan 2009, 2010).

Fusarium pseudograminearum is the predominant fungus associated with crown rot of cereals in Australia (Burgess *et al*. 1975; Backhouse *et al*. 2004), whereas *Bipolaris sorokiniana* causes seedling blight and the disease common root rot (Wildermuth 1986). *Fusarium culmorum* also occurs across the Australian grain belt and 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* 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 although *F. graminearum* strains may

cause crown rot of wheat under artificial conditions, they were less aggressive than *F. pseudograminearum* strains (Akinsanmi *et al*. 2004). *Fusarium graminearum* has been divided into 16 phylogenetically distinct species (Aoki *et al*.2012), with *F. graminearum* sensu stricto considered the most important Fusarium head blight pathogen worldwide (Gale *et al*. 2007; Backhouse 2014). 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). *Bipolaris sorokiniana* and *Fusarium* species such as *F. pseudograminearum*, *F. culmorum* and *F. graminearum* have been reported to occur together in the same paddocks (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; Backhouse and Burgess 2002; Kumar *et al*. 2002). In Australia, extensive research on crown rot has focused on

F. pseudograminearum. Commercial bread wheat and barley cultivars range from moderately susceptible to very susceptible to *F. pseudograminearum* (Sturgess 2014; Lush *et al.* 2018). Commercial durum wheat cultivars are considered susceptible to very susceptible to *F. pseudograminearum* (Lush *et al.* 2018). Limited research on varietal rankings has been conducted on other winter cereals. Three triticale genotypes (Ningadhu, Hawkeye and Berkshire) have been reported as susceptible to *F. pseudograminearum* (Klein *et al.* 1989; Knight and Sutherland 2017), and low or zero levels of disease have been measured in oat cultivars following inoculation with *F. pseudograminearum* (Nelson and Burgess 1994; Burgess *et al.* 2001; Percy *et al.* 2012). Further to varietal rankings, research into *F. pseudograminearum* has included the infection process of the fungus (Burgess *et al.* 1975; Wildermuth and McNamara 1994; Percy *et al.* 2012; Knight and Sutherland 2016), genetic resistance including testing genetic structures, aggressiveness and toxigenicity of *F. pseudograminearum* populations (Bentley *et al.* 2008; Obanor and Chakraborty 2014; Khudhair *et al.* 2019), and yield loss in barley and bread wheat cultivars (Klein *et al.* 1989; Liu *et al.* 2012; Kirkegaard *et al.* 2004).

Barley and wheat cultivars have a wide range of responses to infection with the common root rot pathogen *B. sorokiniana* (Wildermuth *et al.* 1992). In Australia, commercial bread wheat cultivars range from moderately resistant to very susceptible (Lush *et al.* 2018), and significant yield losses have been reported (Wildermuth *et al.* 1992). The yield loss in susceptible cultivars ranged from 13.9% to 23.9%, whereas yield losses in partially resistant cultivars ranged from 6.8% to 13.6% (Wildermuth *et al.* 1992). Wildermuth and McNamara (1991) reported an increase in *B. sorokiniana* populations under wheat, barley and triticale, indicating the potential for these crops to maintain or increase inoculum levels.

The symptoms of crown rot and common root rot disease are similar, and therefore difficult to distinguish without conducting pathogen isolation and identification tests. Crown rot symptoms caused by different *Fusarium* species are also indistinguishable. The symptoms of crown rot start 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 discoloration 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, whereas leaf sheaths and stems have been used for rating crown rot (Wildermuth *et al.* 1992; Burgess *et al.* 2001). A strong association has been observed between sub-crown internode browning and resistance to common root rot in barley and wheat inoculated with *B. sorokiniana* (Wildermuth *et al.* 1992). This has not been demonstrated for

F. pseudograminearum crown rot of wheat (Wildermuth and McNamara 1994; Percy *et al.* 2012).

Although multiple fungal species can be associated with crown rot and common root rot, and barley, bread wheat, durum wheat, oat and triticale have been reported as potential hosts of these fungal species, the comparative ability of these fungi to cause significant crown and common root rot disease across these winter cereal species has not been examined in detail. Knight and Sutherland (2017) and Hollaway *et al.* (2013) compared the visual discoloration caused by a single strain of each of *F. pseudograminearum* and *F. culmorum* across a range of winter cereal species. In these studies, *F. culmorum* caused discoloration on winter cereals the same as or less than *F. pseudograminearum*. Furthermore, *F. graminearum* has not been considered an important pathogen causing crown rot in Australia (Akinsanmi *et al.* 2004). However, with a changing climate, *F. graminearum* could be a significant pathogen causing crown rot on hosts such as barley, bread wheat, durum wheat, oat and triticale. Thus, the present study aimed to examine the visual discoloration caused by four crown rot and common root rot pathogens (*F. pseudograminearum*, *F. culmorum*, *F. graminearum* and *B. sorokiniana*) on leaf sheaths and sub-crown internodes of single, commercially important cultivars of barley, bread wheat, durum wheat, oat and triticale. This study further examined additional host responses to inoculation with the four pathogens through 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 in crop rotations 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 7 days at 25°C for the *Fusarium* species and 22°C for *B. sorokiniana*. Mycelium was scraped off two CZA plates for each strain and mixed into individual bags (1 kg) of a mix of sterilised (autoclaved twice) grains of bread wheat (650 g) and barley (350 g) and incubated at 25°C in the dark. After 7 days, the bags were shaken manually every 2 or 3 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°C for a further 14 days and stirred every 2 days. Individual inocula were then ground for ~10 s in an electric hammer mill (Christy and Norris 8″ Lab Mill; Christy Turner, Ipswich, UK) to pass through a 2-mm sieve. All inoculum bags were sealed and stored at 4°C 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

Table 1. Location and source of each strain of *Fusarium pseudograminearum*, *F. culmorum*, *F. graminearum*, and *Bipolaris sorokiniana*
BRIP, Queensland Plant Pathology Herbarium

Species	Strain	BRIP accession	Collection year	Collection location	Source of strain
F. pseudograminearum	*Fp*1	64949	2009	Emerald, Qld	Crown rot affected stem
	*Fp*2	64952	2012	Irvingdale, Qld	
F. culmorum	*Fc*1	64973	2010	Unknown, NSW	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	

Fisheries, Toowoomba, Queensland. The plant growth medium consisted of self-mulching black Vertosol of the Irving clay soil association, obtained from the Darling Downs, Queensland (Thompson and Beckmann 1959), mixed with river sand (50% sand:50% soil). This mixture was steam-sterilised at 80°C for 40 min and air-dried for 7 days. No fertiliser was added to the mix. The two seedling tests were planted on 5 April and 5 May 2016 (Australian autumn). Three replicate pots each of barley (cv. Grimmett, moderately susceptible to *F. pseudograminearum*) (Sturgess 2014; Lush *et al*. 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. A non-inoculated control treatment was also included for each cereal species. 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, moist soil (280 g, 38% moisture content) was added to pots (5 cm by 5 cm by 10 cm). 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 (160 g). Inoculum (0.45 g) was applied in an even layer to the soil surface of all pots except the non-inoculated control. The inoculum was covered with dry soil (40 g). All pots were placed in a water bath in a glasshouse at 25°C with natural daylengths. The inoculum was activated after 7 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, and 10 plants from each pot were rated for disease severity and assessed for shoot length and shoot dry weight.

Visual discoloration ratings

The severity of lesions on the sub-crown internode and the first three leaf sheaths was assessed using a 0–100% rating scale based on the visual brown to black discoloration. Rating of each tissue occurred in 5% increments where 0 is no discoloration and 100% is completely discoloured tissue. Following visual discoloration ratings, all roots and the 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, *Schwabach, Germany*) for 48 h, after which dry weights were recorded.

Data analyses

The percentage values of visual discoloration on the first three leaf sheaths were added together and divided by three to give a combined leaf sheath percentage. In order to ensure homogeneity of variance, an arcsine square root transformation was applied to the sub-crown internode and the combined leaf sheath rating data. 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, pots, 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 by using the Taylor series approximation. All analyses were performed with ASReml-R (Butler *et al*. 2009) in the R software environment (The R Foundation, Vienna). Significance of fixed effects was assessed using a Wald test with a significance level of 0.05.

Data for visual discoloration of the leaf sheaths and the sub-crown internode have each been presented graphically in two ways to allow comparison of significant differences detected among cultivars and strains.

Results

Comparison of leaf sheath visual discoloration

The appearance of visual symptoms caused by the four pathogens on the first three leaf sheaths of each symptomatic cultivar was similar (Fig. 1). A significant
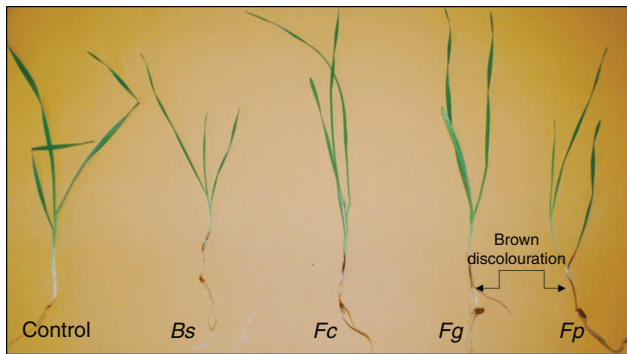
Fig. 1. Example of disease symptoms observed on the first leaf sheaths and sub-crown internode of triticale cv. Endeavour after inoculation. Treatments include non-inoculated control, *Bipolaris sorokiniana* (*Bs*), *Fusarium culmorum* (*Fc*), *F. graminearum* (*Fg*) and *F. pseudograminearum* (*Fp*). This is representative of symptoms observed on barley, bread wheat, durum wheat and oat.

interaction of experiment, pathogen, strain and cultivar ($P = 0.017$) was observed in the leaf sheath ratings (Supplementary Materials, Table S1, available at the journal's website); therefore, the results of the two experiments could not be combined for analysis and are presented individually. *Fusarium pseudograminearum* caused significantly greater visual discoloration than *F. culmorum*, *F. graminearum* and *B. sorokiniana* in barley (cv. Grimmett), triticale (cv. Endeavour) and durum wheat (cv. Hyperno) in both experiments (Fig. 2). The greatest visual discoloration rating was observed for *F. pseudograminearum* in Grimmett (99.6%) in both experiments. Some significant differences were observed between strains of the same pathogen; for example, strains *Fc*2 and *Fg*2 had significantly higher leaf sheath ratings than strain *Fc*1 and *Fg*1, respectively, for Grimmett, Livingston and Endeavour, and strain *Fp*1 had a greater leaf sheath rating than *Fp*2 for Livingston (Fig. 2) in both experiments.

For most of the *Fusarium* spp. strains, the visual discoloration on Grimmett leaf sheath tissue was significantly greater than on all other cereals (Fig. S1). Hyperno (0.09–30%) and Endeavour (0.002–30%) had less visual discoloration than Grimmett (0.33 to 99.6%) and Livingston (1.14–51.53%) when infected by all pathogens, whereas oat cv. Genie exhibited significantly ($P < 0.05$) less visual discoloration across all pathogens (0.03–2.5%) (Fig. S1). The *B. sorokiniana* strains caused low levels of visual discoloration (<20%) across the cultivars, with Livingston exhibiting significantly greater visual discoloration than the other cultivars.

Comparison of sub-crown internode visual discoloration

A significant ($P < 0.001$) pathogen × strain × cultivar interaction for the sub-crown internode rating was observed (Table S2). The greatest visual discoloration ratings were observed in Grimmett inoculated with *F. pseudograminearum* strains *Fp*1 and *Fp*2 (100%), *F. culmorum* strain *Fc*2 (97.6%), and *F. graminearum* strain *Fg*2 (59.8%) (Fig. 3). Sub-crown

internode visual discoloration in Livingston inoculated with *F. pseudograminearum* strains ranged from 32.3% to 64.7%, with *B. sorokiniana* strains from 16.5% to 58.2%, and a 31% visual discoloration rating was observed with the *F. culmorum* strains (Fig. 3). Significant variation between strains was observed in sub-crown internode visual discoloration of Grimmett inoculated with *F. culmorum* and *F. graminearum*, Livingston inoculated with *F. pseudograminearum* and *B. sorokiniana*, and Genie inoculated with *F. culmorum* (Fig. 3).

Differences among cultivars varied depending on the pathogen and the strain. Grimmett (0.5–100%) and Livingston (0.9–64.7%) exhibited the greatest visual discoloration on the sub-crown internode, whereas Genie (0–12%) and Endeavour and Hyperno (0–13.7%) had low levels of sub-crown internode visual discoloration (Fig. S2).

Shoot length

The shoot length of cultivars varied significantly ($P < 0.001$; Table S3) in response to pathogen inoculation (Fig. 4). In most instances, the inoculated treatments had a reduced shoot length compared with 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 with the control (Fig. 4). Oat cultivar Genie had the lowest levels of reduction in shoot length across all pathogens (6–12%) (Fig. 4).

Shoot dry weight

A significant interaction of cultivars and strains within the pathogens was observed for shoot dry weight ($P = 0.035$; Table S4). The reduction in dry shoot weight between control and inoculated plants was significant for Grimmett inoculated with both *F. pseudograminearum* strains (45.1–57%), *Fc*2 (11.6%) and *Fg*2 (10.7%) (Fig. 5). Genie seedlings inoculated with strains *Fg*1 and *Fp*2 also had a significant decreases of 11% and 9%, respectively, in dry shoot weight compared with the control. The dry shoot weight of Livingston was significantly lower than the control when inoculated with *Fp*1 (17.2%).

Discussion

Our study identified significant variation among visual discoloration ratings caused by crown rot and common root rot pathogens on leaf sheath and sub-crown internode tissues of five winter cereals. To the best of our knowledge, this is the first direct comparison of disease caused by these four pathogens across major winter cereal species. This information will be important for predicting the disease risk across these cereal species, and the potential implications of crop rotations in the presence of these pathogens.

Fusarium pseudograminearum strains caused greater visual discoloration than any of the other pathogens on Grimmett, Livingston, Hyperno and Endeavour. Similar results 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 each of
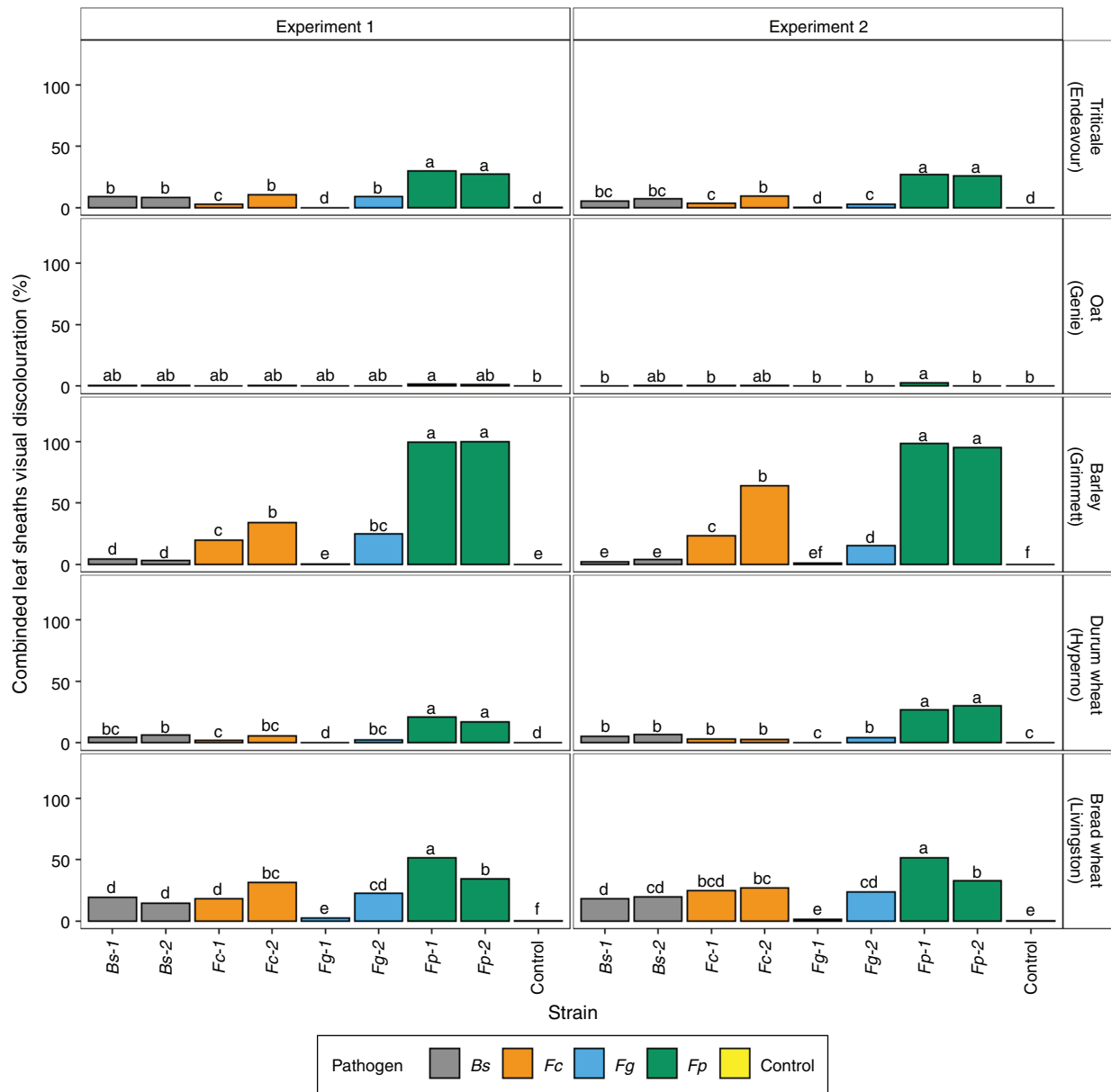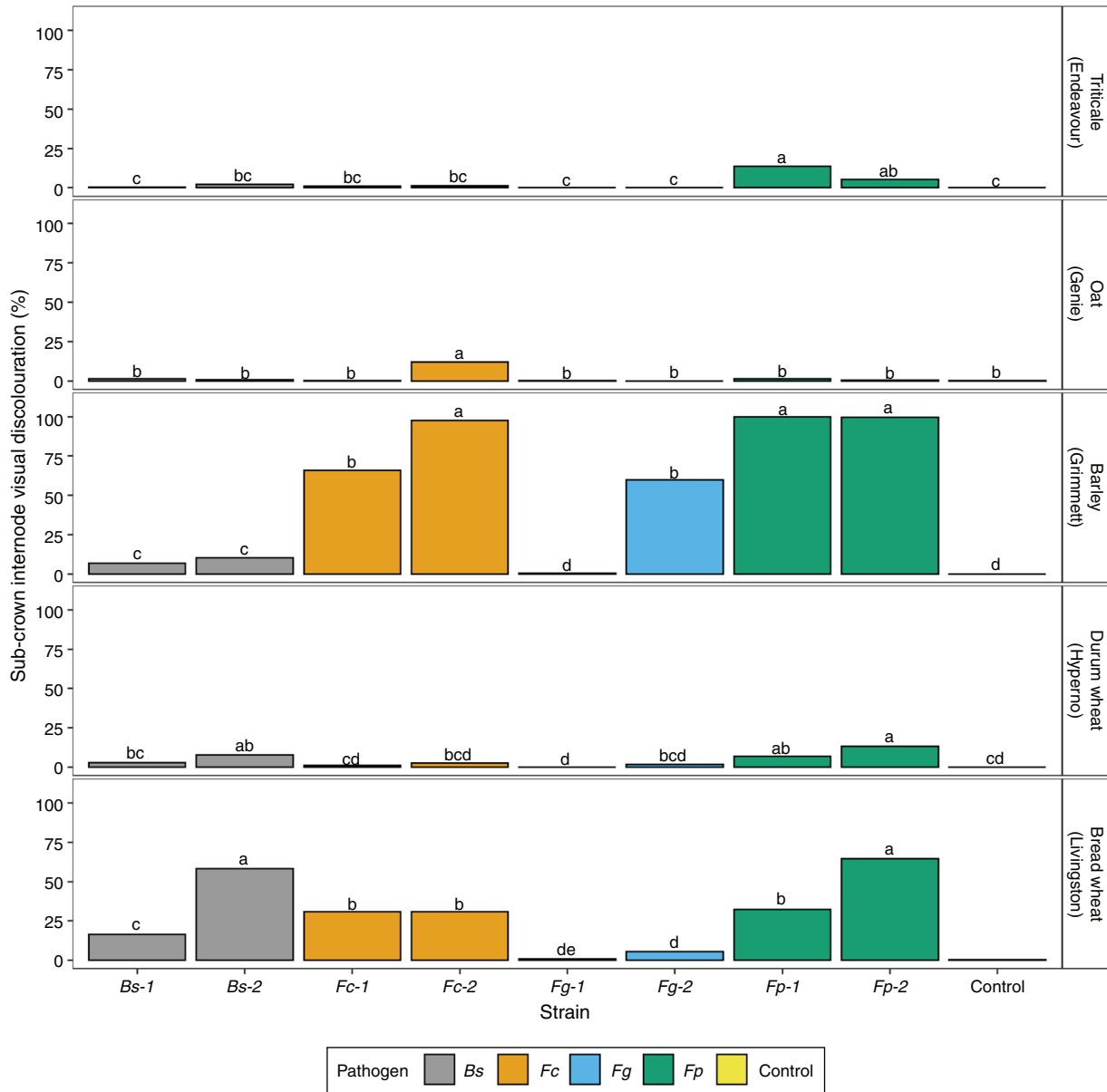
Fig. 2. Mean combined leaf sheath visual discoloration ratings for each experiment × strain × cultivar interaction. Treatments include *Fusarium pseudograminearum* (strains *Fp*1 and *Fp*2), *F. culmorum* (strains *Fc*1 and *Fc*2), *F. graminearum* (strains *Fg*1 and *Fg*2), *Bipolaris sorokiniana* (strains *Bs*1 and *Bs*2), and non-inoculated control for each host. Means with the same letter are not significantly different within a cultivar and experiment at α = 0.05.

F. pseudograminearum and *F. culmorum* in seedlings of six winter cereals (barley, durum wheat, oat, rye (*Secale cereale*), spring wheat and triticale) and three summer cereals (maize (*Zea mays*), rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*)). *Fusarium pseudograminearum* caused greater discoloration than *F. culmorum* in all of the cereals except oat, rye, maize and rice, for which only minimal disease was reported.

In our study, *F. graminearum* caused brown discoloration on the sub-crown internode and leaf sheaths of Grimmett and Livingston at levels similar to *F. culmorum* but less than

F. pseudograminearum. *Fusarium graminearum* has not historically been considered an important crown rot pathogen in Australia (Obanor and Chakraborty 2014). However, Dyer *et al*. (2009) and Obanor and 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 isolated from the crowns in areas where head blight occurred in northern New South Wales and in warm to subtropical areas with moderate to high rainfall in Queensland (Backhouse and Burgess 2002; Akinsanmi *et al*. 2004). The
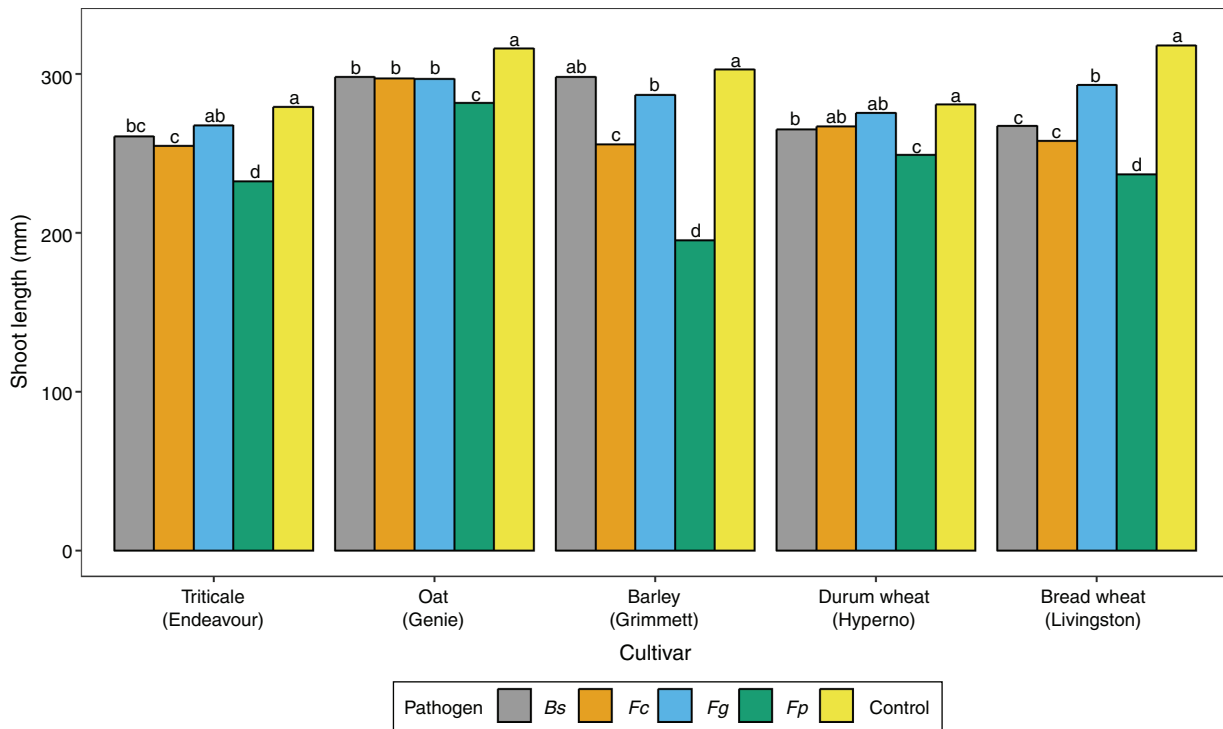
Fig. 3. Mean values of the sub-crown internode visual discoloration rating for the pathogen × strain × cultivar interaction. Treatments include *Fusarium pseudograminearum* (strains *Fp*1 and *Fp*2), *F. culmorum* (strains *Fc*1 and *Fc*2), *F. graminearum* (strains *Fg*1 and *Fg*2), *Bipolaris sorokiniana* (strains *Bs*1 and *Bs*2), and non-inoculated control for each host. Means with the same letter are not significantly different among pathogens within a cultivar at α = 0.05.

results support the potential for *F. graminearum* to contribute to crown rot disease in Australia under favourable conditions. However, only *F. graminearum* strains isolated from Fusarium head blight were available in the present study, and along with the difference in visual discoloration caused by each isolate, it suggests that further assessment with strains from crown rot infections should be investigated.

Low visual discoloration ratings were observed on the leaf sheaths of most hosts inoculated with *B. sorokiniana* strains, except for Livingston, which also had the greatest visual discoloration on the sub-crown internode. This finding

confirms previous studies suggesting 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 a pathogen species was observed for combined leaf sheath and sub-crown internode ratings in some of the cultivars. Each of the *F. culmorum* and *F. graminearum* strains caused different levels of visual discoloration across the cultivars. *Fusarium pseudograminearum* has been described to vary in aggressiveness between strains, depending on several factors including farming system, geographical factors, and

Fig. 4. Mean values of shoot length for the pathogen × cultivar interaction. Treatments include *Fusarium pseudograminearum* (*Fp*), *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *Bipolaris sorokiniana* (*Bs*), and non-inoculated control. Means with the same letter are not significantly different within a cultivar at α = 0.05.

the genetic diversity of each strain (Akinsanmi *et al.* 2004). A similar scenario is suggested to occur in the other *Fusarium* species assessed.

Low levels of visual discoloration were observed in durum wheat cv. Hyperno on the leaf sheaths and the sub-crown internode after inoculation with each of the pathogens. These results contrast with the findings of Knight and Sutherland (2017), where durum wheat cvv. EGA Bellaroi and Jandaroi had visual discoloration ratings similar to the most diseased cultivars of barley. The low level of disease visual discoloration on durum wheat in our study 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. Liu *et al.* (2012) estimated the fungal biomass in barley, bread wheat and durum wheat cultivars at various times (3, 7, 14, 21, 28 and 35 days) post inoculation with *F. pseudograminearum*. The fungal quantities measured at the six time points of infection were characterised by three distinct phases in each of the cereals. During all stages, the durum wheat cultivar (Wollaroi, highly susceptible) exhibited late and slow fungal accumulation compared with barley cultivars, indicating in the early stages of infection, less disease may also develop. Lush *et al.* (2018), in '2018 wheat varieties, Queensland'

reported that durum wheat cultivars, including Hyperno, are considered susceptible to very susceptible to *F. pseudograminearum*. Data in this guide are from disease assessed at maturity (Lush *et al.* 2018). Although differences in the age of plants were minimal between the present study and that of Knight and Sutherland (2017), the growth conditions and cultivars did vary. The inoculation techniques could also have had an impact on disease progression. Knight and Sutherland (2017) applied 6 µL of an inoculum of 10^6 conidia/mL to the coleoptile at 14 days after planting, whereas we applied the inoculum as a layer above the seeds, which required activation by moisture 7 days after planting. Thus, in the present study, the coleoptile grew through the soil and the inoculum, similar to a natural field infection, compared with direct application of a conidial suspension.

Triticale cv. Endeavour had low levels of visual symptoms on the leaf sheaths and sub-crown internode compared with Grimmett and Livingston. Knight and Sutherland (2017) reported that triticale cvv. Hawkeye and Berkshire had a high level of visual discoloration, with responses to a single strain of each of *F. pseudograminearum* and *F. culmorum* similar to barley, durum wheat and spring wheat cultivars. This difference may be due to the different inoculation methods used or genetic variation among cultivars.

Oat is considered a resistant or an asymptomatic host of *F. pseudograminearum* (Percy *et al.* 2012; Knight and Sutherland 2017). Low levels of visual discoloration, still
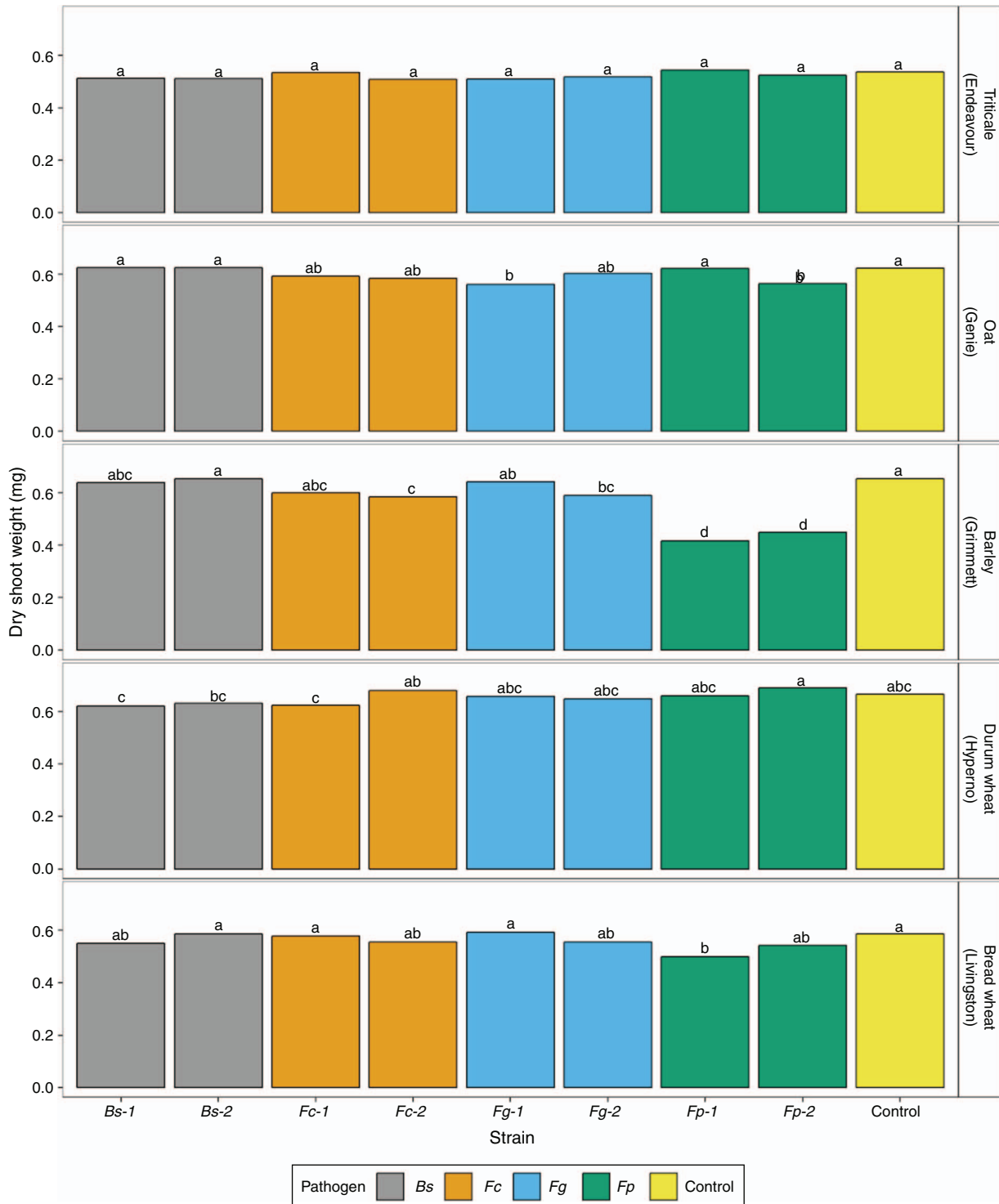
Fig. 5. Mean values of cultivar dry shoot weight for the pathogen × strain × cultivar interaction. Treatments include *Fusarium pseudograminearum* (strains *Fp*1 and *Fp*2), *F. culmorum* (strains *Fc*1 and *Fc*2), *F. graminearum* (strains *Fg*1 and *Fg*2), *Bipolaris sorokiniana* (strains *Bs*1 and *Bs*2), and non-inoculated control for each host. Means with the same letter are not significantly different within a cultivar α = 0.05.

significantly greater than the controls, were observed on the sub-crown internode of Genie when inoculated with one strain of *F. culmorum* and on the leaf sheaths when inoculated with

one strain of *F. pseudograminearum*. Although disease levels were low, the capacity of oats to exhibit discoloration after inoculation with *F. pseudograminearum* and *F. culmorum*

supports recommendations that oat should not be used as a rotational crop for crown rot caused by *F. pseudograminearum* (Nelson and Burgess 1994) or for 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 been extensively 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 in the case of *B. sorokiniana* with Grimmett. Oat cv. Genie had low or no symptoms; however, there was a significant reduction in the shoot length of Genie across all pathogens ($P < 0.05$). The reduction in shoot length indicates that each pathogen had a negative effect on the development of the host. Similarly, Smiley *et al.* (2005) indicated that visual discoloration 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 $Fp1$ significantly reduced dry shoot weight in Grimmett and Livingston, whereas $Fg1$ significantly decreased dry shoot weight in Genie ($P < 0.05$). This outcome contrasts with the findings of 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 with 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 visual discoloration caused by infection with *F. pseudograminearum*, *F. culmorum*, *F. graminearum* and *B. sorokiniana* in five winter cereals species. *Fusarium pseudograminearum* caused the greatest visual discoloration 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, whereas 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 shoot length being more significant than the reduction in 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.

Conflicts of interest

The authors declare no conflict of interest.

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