Fusarium root rot of canola (*Brassica napus*): Occurrence, pathogenicity and yield losses in Alberta, Canada

by

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Abstract

Root rot is a soilborne disease of canola (*Brassica napus*) caused by a pathogen complex. Canola crops were surveyed in Alberta, Canada, in 2021 and 2022 to assess the composition and diversity of fungi associated with root rot in this region. Isolates were identified to genus and/or species based on colony and morphological characteristics, with *Fusarium* spp. found to be predominant. The identity of *Fusarium* spp. was confirmed by analysis of the translation elongation factor 1-α (TEF-1α) and internal transcribed spacer (ITS) sequences. *Fusarium avenaceum* was recovered most frequently, followed by *F. redolens* and *F. solani*. Most isolates caused moderate to severe disease on canola under greenhouse conditions, with *F. avenaceum* and *F. sporotrichioides* among the most aggressive species. *Fusarium sporotrichioides* and *F. commune* were identified for the first time as canola root rot pathogens. Principal component analysis demonstrated the utility of combining disease severity and growth measurements in assessments of pathogenicity. Analysis of the ITS sequence successfully distinguished various *Fusarium* species, with the exception of closely related phylogenetic species like *F. avenaceum*, *F. acuminatum*, and *Fusarium tricinctum*. The TEF-1α sequence analysis facilitated species differentiation. Concatenating the ITS and TEF-1α sequences provided more diversified grouping information. No geographic or year effects were observed on fungal diversity or aggressiveness. In another study, the impact of *F. avenaceum* and *Fusarium proliferatum* on canola yields was evaluated in field and greenhouse experiments, and the interaction between *F. proliferatum* and *F. oxysporum* was also explored. Inoculation with any of the three species resulted in significant disease severity and reduced seedling emergence compared with non-inoculated controls, leading to yield reductions of up to 35%. Notably,

there was a strong correlation ($r = 0.93$) between root rot severity at the seedling stage and at maturity. Regression analysis indicated a linear decline in emergence with increasing disease severity. Furthermore, disease severity at maturity adversely affected pod number per plant and seed weight per plant, with both parameters ultimately reaching zero at a severity of four on a 0-4 scale. Co-inoculation with *F. oxysporum* and *F. proliferatum* induced more severe root rot than inoculation with each species on its own, suggesting synergistic interactions between these fungi. In a final study, the host range of *F. proliferatum* was investigated through greenhouse inoculation experiments involving wheat, barley, faba bean, pea, lentil, canola, lupine and soybean. All crops were at least partly susceptible, developing mild to severe root rot at the seedling and adult plant stages, and showing significant reductions in growth. In general, barley and wheat demonstrated higher tolerance to infection, followed by faba bean and pea. Soybean, canola, lupine, and lentil were most susceptible. Canola and soybean were particularly vulnerable to *F. proliferatum* at the pre-emergence stage, while infection greatly reduced lentil biomass. Reductions in barley emergence and other growth parameters, however, occurred only under a high inoculum concentration. Understanding the identity, pathogenicity, and relative prevalence of *Fusarium* spp., along with improved knowledge of their impact on yields, will contribute to better management of canola root rot.

Preface

Haitian Yu submitted this dissertation in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Ms. Yu conducted most of the laboratory experiments and statistical analyses in Chapters 2 and 3, all of the experiments and analyses presented in Chapters 4, 5, and 6. She prepared the first draft of all chapters. The chapters were then extensively reviewed and edited by her supervisors, Dr. Stephen Strelkov and Dr. Sheau-Fang Hwang, who made suggestions and editorial changes for each chapter. Ms. Yu then addressed these suggestions as appropriate.

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Haitian Yu conducted all of the experiments, performed the statistical analysis, and wrote the first draft of the manuscript. Haitian Yu, Stephen Strelkov, and Sheau-Fang Hwang contributed to development of the research concept. Haitian Yu, Kan-Fa Chang, and Sheau-Fang Hwang designed the study. Stephen Strelkov and Sheau-Fang Hwang reviewed multiple versions of the manuscript. Stephen E. Strelkov provided project guidance and extensively edited the manuscript. Sheau-Fang Hwang and Stephen Strelkov secured funding support, and supervised and administered the project. All authors contributed to manuscript revision, read and approved the submitted version.

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List of Abbreviations

AG: Antagonistic group

ANOVA: Analysis of variance

Avr: Avirulence

BLAST: Basic local alignment search tool

BLG: Blackleg (*Leptosphaeria maculans*).

WM: White mould (*Sclerotinia sclerotiorum*).

CAD: Canadian dollars

CFU: Colony forming unit

DS: Disease severity

FAO: Food and Agriculture Organization of the United Nations

FOSC: *Fusarium oxysporum* species complex

ITS: Internal transcribed spacer sequence

IGS: Intergenic spacer region

PH: Plant height

PCR: Polymerase chain reaction

PDA: Potato dextrose agar

RPB2: RNA polymerase II gene

RRC: Root rot complex

SE: Seedling emergence

SSR: Sclerotinia stem rot

TEF-1α: Translation elongation factor 1-alpha region

Chapter 1 General introduction and literature review

1.1 Importance of canola (*Brassica napus* L.)

Canola (syn. rapeseed; *Brassica napus* L.) is among the most important oilseed crops worldwide (Kirkegaard et al., 2021) and was grown on 36.8 million ha in 2021 (*FAOSTAT*, 2023). Approximately 24% of this acreage was planted in Canada, which is the world's largest producer of canola; in 2023, 18.3 million t were harvested across the country, mainly on the Prairies, representing 19.3% of global production (*FAOSTAT*, 2023; Statistics Canada, 2023). The canola industry is estimated to contribute $29.9 billion CAD annually to the Canadian economy, an increase of 35% over the past decade (Nichol et al., 2023). In 2014, the Canola Council of Canada established a goal to expand average yield from about 33.7 bu/ac (~2.27 t/ha) to 52 bu/ac (~3.50 t/ha) by 2025, to meet a projected global demand of 26 million t per year (https://www.canolacouncil.org/download/126/about-us/2503/keep-it-coming-2025-the-strategy). However, while significant gains in yields have been realized (with an average of ~40 bu/ac or 2.69 t/ha by 2023), it seems unlikely that this goal can be achieved based on the original timeframe. Multiple abiotic factors, including soil fertility and weather, may limit yields (Kutcher et al., 2010; Morrison et al., 2016), as can biotic factors including insects, weeds and diseases (Cornelsen et al., 2023).

1.2 Major canola diseases

Several diseases pose a threat to canola yield, with blackleg, clubroot, Sclerotinia stem rot, and root rot ranking among the most common and important in Canada (Derbyshire & Denton-Giles, 2016; Strelkov et al., 2021; Wang et al., 2020). Additionally, the emergence of Verticillium stripe in recent years has raised concerns (Zheng et al., 2020).

These canola diseases not only have the potential to reduce yields significantly, but also can also adversely affect the quality of the crop.

Blackleg was one of the first major diseases to constrain the production of canola in Canada (Kharbanda & Tewari, 1996). The causal agent, *Leptosphaeria maculans* (Sowerby) Karst., predominantly affects the lower stem and crown, resulting in the development of dry rot or cankers at the basal stem in severely infected plants (Zhang & Fernando, 2018). The management of blackleg relies heavily on the cultivation of genetically resistant canola cultivars (Dolatabadian et al., 2022); this resistance is mediated by major resistance genes in the host, which recognize specific pathogen Avr genes in the pathogen (Fernando et al., 2007). Knowledge of the pathogen race structure and avirulence gene composition is important for the effective deployment of blackleg resistance and monitoring of the *L. maculans* population is an ongoing process.

Clubroot, a soilborne root disease caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is widespread across much of Alberta and is increasingly common in Saskatchewan and Manitoba (Hollman et al., 2023). The disease first emerged as an issue on the Prairie canola crop in 2003 (Tewari et al., 2005), at which time no management strategies, except for extended rotations out of susceptible hosts, were available (Strelkov & Hwang, 2014). Over the past two decades, multiple methods have been developed for clubroot control, with the most significant being genetic resistance (Peng et al., 2014). The extensive cultivation of clubroot-resistant cultivars has enabled the continued production of canola even in the presence of *P. brassicae*, and has helped to mitigate the impact of the outbreak (Strelkov et al., 2018). Nonetheless, the emergence of ‘resistance-breaking’ strains of the pathogen represents a significant challenge to sustainable clubroot

management, and integrated approaches for disease control need to be applied (Hollman et al., 2023; Strelkov et al., 2018).

Sclerotinia stem rot, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is endemic across the canola production regions of western Canada. Stem rot first manifests as soft, watery lesions on canola stems and branches, developing into bleached, shredded lesions that may cause premature ripening and plant death (Kamal et al., 2015). Fungal sclerotia are prominent signs of infection and are produced inside the affected stems. Effective control measures for stem rot primarily involve timely fungicide application along with disease forecasting and comprehensive risk assessment tools (Derbyshire & Denton-Giles, 2016). In recent years, several stem rot tolerant canola hybrids have become available for growers, although none is fully resistant to *S. sclerotiorium* (Ding et al., 2021).

Verticillium stripe is an emerging disease of canola in Canada, caused by the fungus *Verticillium longisporum* (C. Stark) Karapapa, Bainr. & Heale. The disease was first reported in Manitoba in 2014 (Dolatabadian et al., 2022), and subsequently confirmed (either by testing for the presence of pathogen DNA or, in some cases, isolation of the fungus) across multiple provinces, including British Columbia, Alberta, Saskatchewan, Ontario, and Quebec (http://www.inspection.gc.ca/plants/plant-pests-invasive-species/diseases/verticillium-stripe/eng/1420746212959/1420746213803). The development of Verticillium stripe is associated with wilting, stunting, and chlorosis of the leaves and lateral branches; stems become discolored, and infected plants undergo premature senescence (Fradin & Thomma, 2006). The occurrence of small black microsclerotia on infected tissues is an important sign of the pathogen. The closely related fungus *Verticillium dahliae* Kleb can cause wilt symptoms on canola (Hwang et al., 2017),

although its prevalence on this crop seems rarer. There are limited Verticillium stripe management options in Canada at present, given the recent identification of this disease, although resistance breeding programs are already underway in other countries (Dolatabadian et al., 2022).

Finally, root rot also presents a substantial threat to canola, primarily attributed to three groups of soilborne pathogens: *Rhizoctonia solani* Kühn, *Fusarium* spp., and *Pythium* spp. Despite ongoing efforts, the effective prevention and management of root rot remains a significant challenge. Addressing this disease will demand the implementation of comprehensive strategies, the availability of which is limited at present (Chen et al., 2014; Hwang et al., 2014). Root rot represents the focus of this dissertation, and is discussed more fully in the sections below.

1.3 Root rot of canola

1.3.1 The root rot complex

Root rot is one of the most widespread and damaging diseases affecting many plant species (Bodah, 2017). Common symptoms include root and basal stem decay, often accompanied by seedling stage indications such as pre- and post-emergence damping off (Arora et al., 2021) and seedling blight (Wang et al., 2006). Symptoms, including wilting (Abdel-Monaim & Ismail, 2010), leaf yellowing (Liao et al., 2023) and crown rot (Rebollar-Alviter et al., 2020), can persist throughout all stages of plant growth. A diverse array of microorganisms has been implicated in root rot, such as bacteria (*Pectobacterium* spp., *Dickeya* spp.), fungi (*Heterobasidion* spp., *Armillaria* spp., *Phellinus* spp., *Rosellinia* spp., *R. solani*, *Fusarium* spp.), and oomycetes (*Pythium* spp., *Phytophthora* spp., *Aphanomyces* spp.) (Bodah, 2017). Among these, however, *R. solani*, *Fusarium* spp., and

Pythium spp. stand out as the predominant causal agent agents of the root rot complex (Williamson-Benavides & Dhingra, 2021).

1.3.2 *Rhizoctonia solani*

Rhizoctonia solani is a basidiomycete comprised of genetically distinct anastomosis groups (AGs) defined by their ability to anastomose or fuse with each other. Populations of the fungus classified as AG 2-1 and AG 4 have been reported as most virulent on canola/oilseed rape (Broders et al., 2014; Kataria & Verma, 1992), with AG 2-1 primarily inciting root rot and damping-off in seedlings and adult plants, and AG 4 causing basal stem rot in adult plants (Verma, 1996). Nonetheless, other anastomosis groups of *R. solani*, including AG 8 (Khangura et al., 1999) and AG 10 (Schroeder & Paulitz, 2012), also have been reported to infect canola.

Root rot caused by *R. solani* can have a significant impact on yield. Losses of up to 65% have been observed on individual plants as a result of infection (Klein-Gebbinck & Woods, 2002), with yield reductions of 20-30% reported on a field scale in severely infected crops in western Canada (Reddy et al., 1993). Strategies for the management of this pathogen are limited. Cultural practices, such as the manipulation of seeding date, seeding depth, and rotation out of host crops, have shown some efficacy in reducing root rot (Kharbanda & Tewari, 1996; Teo et al., 1988; Yitbarek et al., 1988). Fungicidal seed treatments with Helix Xtra (thiamethoxam + difenconazole + metalaxyl + fludioxonil) and Prosper FX (clothianidin + carboxin + trifloxystrobin + metalaxyl) significantly enhanced canola emergence and yield, particularly when combined with cultural disease control methods (Hwang et al., 2014). The efficacy of seed treatments, however, declines under high inoculum pressure (Hwang et al., 2015). No fully resistant canola genotypes have

been reported, although some accessions exhibited moderate resistance to *R. solani* AG 8 and AG 10 (Babiker et al., 2013), or AG 2-1 and AG 4 (Lamprecht et al., 2011).

1.3.3 *Pythium* species

The genus *Pythium* was recognized as an important group of root pathogens by the early 1900s, with a wide distribution and host range (Hendrix & Campbell, 1973). As oomycetes, *Pythium* spp. can produce sexual oospores, resilient structures that survive in the soil for many years (Arora et al., 2021). Symptoms of Pythium root rot primarily involve a rapid decomposition of the entire primary root, often extending up to the basal stem (Bodah, 2017). *Pythium ultimum* and *P. aphanidermatum* in particular are reported to be *Brassica* crop pathogens (Williamson-Benavides & Dhingra, 2021), although other species have been implicated (Stanghellini et al., 2014).

Despite their contribution to the development of seedling blight and root rot of canola, *Pythium* spp. are less studied than other pathogens involved in the root rot complex. Nonetheless, Pythium seedling blight caused by *P. ultimum* can significantly reduce canola stand establishment (28%-83%) and yield (Hwang et al., 2015). The use of seed treatments led to reductions in disease severity, while seed size and seeding depth had no effect (Hwang et al., 2015). Soil fumigation is effective, but given its high cost, is applied only for certain high-value horticultural crops (Cook, 2006). Diverse crop rotations can reduce pathogen populations in the soil (Hwang et al., 2009), while no canola cultivars with genetic resistance to *Pythium* are available.

1.3.4 *Fusarium* species

The genus *Fusarium* was first described as *Fusisporium* by Link (1809). Taxonomic studies of *Fusarium* species typically rely on multiple factors, including morphological,

biological, and genetic characteristics (Chandra et al., 2011; Geiser et al., 2004; Leslie & Summerell, 2008; O'Donnell et al., 2012; Summerell et al., 2003). Over time, the genus has undergone extensive taxonomic study and revision. Previously, approximately 1,000 species were described within *Fusarium*; however, through careful integration of morphological and phylogenetic characteristics, this number was later reduced to 70 species (Refai et al., 2015). Genetic studies, particularly with the aid of next-generation sequencing, have contributed significantly to the understanding of *Fusarium* taxonomy (Chandra et al., 2011).

While *R. solani* continues to be a significant concern (Broders et al., 2014; Hwang et al., 2009; Zhou et al., 2014a; Zhou et al., 2014b), *Fusarium* spp. have also emerged as another major cause of this disease (Chen et al., 2014; Hwang et al., 2009; Zhou et al., 2014a). *Fusarium avenaceum* (Fr.) Sacc. was first reported as causing seedling blight in canola (*B. rapa* and *B. napus*) in Alberta in the 1980s (Calman et al., 1986). More recently, *Fusarium acuminatum* Ellis & Everh. and *F. avenaceum* were identified as the predominant and most aggressive species, respectively, associated with root rot in this province (Chen et al., 2014). Crown or root rot caused by *Fusarium* spp. also occurs on canola or rapeseed crops worldwide. For instance, a field survey in Iran indicated that *F. solani* was predominant and *Fusarium equiseti* (Corda) Sacc. most aggressive among *Fusarium* spp. recovered from canola (Larki & Farrokhi Nejad, 2015). In Australia, *F. acuminatum* and *Fusarium semitectum* Berk. & Ravenel were identified as agents causing root disease in canola at both the seedling and adult plants stages (Li et al., 2007).

Infection by *Fusarium* spp. can hamper germination and lead to the death of newly emerged seedlings, resulting in pre- and post-emergence damping-off, respectively. It can

also cause root rot of mature plants, ultimately resulting in decreased yields (Bailey, 2003). Similarly, Fusarium wilt of canola, attributed to *F. oxysporum* and *F. avenaceum*, can also lead to yield losses ranging from 15.9% to 75.2% under field conditions in susceptible cultivars (Benard et al., 2003; Lange et al., 2007). Understanding of how these pathogens affect the yield and growth of canola is, however, currently limited, necessitating further investigation.

As with *R. solani*, the management of root rot caused by *Fusarium* spp. is challenging. Based on the results of greenhouse experiments, diverse crop rotations were suggested to reduce populations of *Fusarium*, *Pythium*, and *Rhizoctonia* in the soil (Hwang et al., 2009). Under field conditions, fungicidal seed treatments reduced disease pressure from *F. avenaceum* and improved canola emergence and yield (Hwang et al., 2015). The availability of genetic resistance appears limited, although winter rapeseed germplasm resistant to *F. oxysporum* and *Fusarium culmorum* (Wm. G. Sm.) Sacc. has been reported (Starzycki et al., 2007). Similarly, the canola cultivar 'Serw 6' displayed resistance to damping-off, root rot, and wilt under field conditions in an Egyptian study (Abdel-Hafez et al., 2021). The management of Fusarium root rot may be hindered by the involvement of multiple species in disease development, given that each possesses its own mycological and virulence characteristics.

1.3.4.1 *Fusarium avenaceum*

Fusarium avenaceum is one of the most common *Fusarium* plant pathogens (Yli-Mattila et al., 2018), with a broad host range and causing various diseases of cereal, legume, and vegetable crops (Calman et al., 1986; Feng et al., 2010; Koike et al., 2015). The fungus produces mycotoxins that have the potential to contaminate grains and other agricultural

products (Munkvold, 2017). Isolates of *Fusarium avenaceum* exhibit distinctive morphological characteristics that aid in their identification. This filamentous fungus typically forms colonies on agar media that are cottony or woolly in texture, with white to pale pink mycelium (Yli-Mattila et al., 2018). Asexual reproduction occurs via the production of microconidia and macroconidia on conidiophores, which contribute to fungal dissemination. Unlike many other *Fusarium* spp., *F. avenaceum* does not appear to produce chlamydospores (thick-walled survival structures) (Leslie & Summerell, 2008; Yli-Mattila et al., 2018). While the presence of the *MAT-1* and *MAT-2* mating types has been confirmed in *F. avenaceum* (Kerényi & Hornok, 2002), a teleomorph stage has not been identified.

The fungus can infect various cereal crops including wheat (*Triticum* spp.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and rye (*Secale cereale* L.), which may lead to mycotoxin contamination of the kernels (Munkvold, 2017; Salas et al., 1999; Yli-Mattila, 2010). It also causes, among others, root rot of lupine (*Lupinus angustifolius* L.) (Holtz et al., 2011), carrot (*Daucus carota* L. subsp. *sativus* (Hoffm.) Thell. (Stanković et al., 2015), potato (*Solanum tuberosum* L.) (García-Núñez et al., 2016), field-grown leek (*Allium ampeloprasum* L.) (Koike et al., 2015), lentil *(Lens culinaris* Medik.), chickpea *Cicer arietinum* L.), pea (*Pisum sativum* L.) (Chatterton et al., 2019; Feng et al., 2010; Gibert et al., 2022; Moparthi et al., 2021; Safarieskandari et al., 2021), and soybean (Chang et al., 2018; Zhou et al., 2018). On canola, *F. avenaceum* causes root rot and seedling blight (Chen et al., 2014; Yu et al., 2023a).

1.3.4.2 *Fusarium oxysporum*

Fusarium oxysporum is a well-studied plant pathogen known for its broad host range

and complex nature, which has led to the identification of numerous *formae speciales* or 'special forms' (Edel-Hermann & Lecomte, 2019). These *formae speciales* can cause a variety of symptoms on different crops, and collectively constitute the *Fusarium oxysporum* species complex (FOSC) (Refai et al., 2015). Since they are morphologically similar, different *formae speciale*s are generally distinguished based on molecular analysis of conserved regions of DNA. These include the internal transcribed spacer (ITS) region, translation elongation factor 1-alpha (TEF-1α) gene, intergenic spacer (IGS) region, and the RNA polymerase II second largest subunit (RPB2) gene (Kistler, 1997; Magdama et al., 2020).

Different *formae speciales* of *F. oxysporum* are adapted to different hosts. Over 120 distinct *formae speciales*, each associated with particular host ranges, have been identified (Michielse & Rep, 2009; Refai et al., 2015). The fungus causes root rot of many crops, such as chickpea (Zhou et al., 2021), pea (Gargouri-Jbir et al., 2023; Safarieskandari et al., 2021), soybean (*Glycine max* L.) (Chang et al., 2018), faba bean (*Vicia faba* L.) (Clarkson, 1978; Yu et al., 2023c), and canola (Gaetán, 2005; Yu et al., 2023a). While infection of the root and crown results in the development of typical root rot symptoms, some *formae speciales* of *F. oxysporum* can invade the host vascular system, causing wilts (Gordon, 2017). For example, *F. oxysporum* f. sp. *lycopersici* causes vascular wilt of tomato (*Solanum lycopersicum* L.) (Srinivas et al., 2019), *F. oxysporum* f. sp. *cubense* (Foc) is responsible for banana (*Musa acuminata* Colla) wilt (Mostert et al., 2017; Ploetz, 2015), and *F. oxysporum* f. sp. *phaseoli* (Fop) induces vascular wilt in common beans (*Phaseolus vulgaris*) (Buruchara & Camacho, 2000; Henrique et al., 2015). Another special form, *F. oxysporum* f. sp. *conglutinans*, is associated with vascular wilt of crucifers, including

cabbage (Liu et al., 2020; Tong et al., 2022) and canola/oilseed rape (Klassen et al., 2007). In canola, the management of vascular wilt relies mainly on planting resistant cultivars (Mehraj et al., 2020). Some *formae speciales* may be further subdivided into races, attacking specific genotypes within a host species, highlighting physiologic specialization of this pathogen at the cultivar level (Edel-Hermann & Lecomte, 2019).

1.3.4.3 *Fusarium proliferatum*

Fusarium proliferatum (Matsush.) Nirenberg is a mycotoxigenic fungus able to infect a diverse variety of crops. From a taxonomic perspective, it is classified within the *Fusarium solani* species complex (FSSC), a genetically heterogeneous group of fungi (Aoki et al., 2014). The precise classification of this species relies primarily on sophisticated molecular techniques, particularly the sequencing of conserved regions such as the internal transcribed spacer (ITS), fumonisin biosynthetic gene (FUM 1), and the translation elongation factor 1-alpha (TEF-1α) gene (Alizadeh et al., 2010; Armengol et al., 2005; Azuddin et al., 2021; Jurado et al., 2010). These specific genetic markers are important for distinguishing *F. proliferatum* from other closely related species within the FSSC. Furthermore, they enable exploration of genetic diversity and genetic relationships within this species complex. Morphological features, including the presence of chains of microconidia, also provide clues to distinguish *F. proliferatum* from other similar *Fusarium* species (Leslie & Summerell, 2008).

The life cycle of *F. proliferatum* shares common features with many other *Fusarium* species, but incorporates both sexual and asexual stages. Asexual conidia that develop on specialized structures called conidiophores act as the primary inoculum (Ichikawa & Aoki, 2000). Upon encountering a susceptible plant, the conidia germinate and give rise to

appressoria, facilitating penetration of the host surface. Following successful penetration, the fungus invades the plant tissues, resulting in a range of disease symptoms. In addition to asexual reproduction, *F. proliferatum* can undergo sexual reproduction when two compatible mating types of the fungus come into contact (Mohd Zainudin et al., 2017). As an ascomycete, sexual reproduction results in the formation of ascospores (sexual spores), which contribute to genetic diversity in fungal populations (Mohd Zainudin et al., 2017).

Like many other *Fusarium* spp., *F. proliferatum* has a wide host range (Abbas et al., 1999; Gálvez & Palmero, 2022; Zhan et al., 2010). Infection by this fungus is of particular concern given its production of fumonisins and other mycotoxins, which can contaminate food and feed (Proctor et al., 2010). *Fusarium proliferatum* also contributes to the development of Fusarium head blight (FHB), a major disease of many cereal crops (Molnár, 2016; Molnár et al., 2023). There are also many reports documenting the involvement of this fungus in many other diseases, particularly root rots and wilts, across diverse plant taxa. For example, *F. proliferatum* has been shown to cause wilt of carnation (*Dianthus caryophyllus* L.) in Spain and wilt of cauliflower (*Brassica oleracea* var. *botrytis* L.) in China (Basallote-Ureba et al., 2016; Yan, 2020). It has been implicated in root and bulb rots of garlic (*Allium sativum* L.) in France, Egypt, and other regions since the mid-2000s (Chrétien et al., 2020; Elshahawy et al., 2017; Galal et al., 2002), and causes crown and stem rot and pith necrosis of cannabis (*Cannabis sativa* L., marijuana) in Canada. In the United States and Canada, it has been identified as a common pathogen on soybean roots (Chang et al., 2015; Díaz Arias et al., 2011; Punja, 2021). In addition, *F. proliferatum* is as a major constraint in the fruit industry, contributing to significant losses in banana and red-fleshed dragon fruit (*Selenicereus undatus* (Haworth) Hunt) (Masratul

Hawa et al., 2013).

1.4 Hypothesis and objectives

Root rot has become a significant concern for canola production in Canada and worldwide (Hwang et al., 2015a; Schroeder & Paulitz, 2012). Despite the importance of this disease, however, canola root rot has not been studied extensively. It is likely that the involvement of multiple *Fusarium* spp. hinders the development of effective control strategies, particularly given that despite their wide host rage, there is limited information regarding which *Fusarium* species are the major pathogens causing this disease on canola. Moreover, the impact of these pathogens on the growth and yield of canola has not been quantified, nor is there recent data regarding the virulence of these fungi on some of the other important crops grown on the Prairies of western Canada. Such knowledge is critical for developing effective strategies for the management of root rot, and determining when and where the application of control measures are warranted.

The primary aim of this project was to provide up-to-date information on root rot disease of canola, and included four specific objectives: (1) to evaluate the incidence of root rot in canola and identify the major pathogens; (2) to characterize the diversity, virulence, and genetic structure of pathogen populations; (3) to assess the impact on the yield and growth of canola; and (4) to clarify the host range of selected *Fusarium* species. We hypothesized that root rot is widespread and that multiple pathogen species contribute to disease development. We also hypothesized that *Fusarium* spp. are the primary causal agents, representing the most prevalent and aggressive pathogens. Finally, we hypothesized, based on previous reports, that *Fusarium* spp. exhibit a broad host range and are capable of inducing severe reductions in the growth and yield of canola.

Chapter 2 Canola disease survey in central-northern Alberta in 2021

2.1 Introduction

Canola (*Brassica napus* L.) is an important cash crop in Alberta, with 2.4 million ha seeded in 2020 (Government of Canada, 2020). Unfortunately, soilborne diseases can negatively affect canola yield and quality. A survey of 35 canola fields was conducted in central and northern Alberta between August and October 2021, with root and stem samples collected to evaluate the incidence of soil-borne pathogens.

2.2 Materials and methods

Thirty-five canola fields were sampled near Edmonton, St. Albert, Namao, Morinville, Gibbons, Josephsburg, Villeneuve, Redwater, Opal and Bruderheim, Alberta. The samples were collected along W-shaped transects in each field, with approximately 50 m between sampling points. All plants within a 1-m^2 quadrat were examined at each of five points along each transect. The incidence of clubroot, blackleg, root rot, and white mould were noted at each sampling point. Lower stem and root samples were also collected at random from low-lying areas in the fields. Samples showing symptoms of root rot and/or yellowing stems were taken to the laboratory, where they were analysed for the presence of fungal pathogens. The outer layer of the root and lower stem surfaces was peeled off, and only those samples with inside surfaces showing discolored tissues were sectioned and transferred to a 96-cell tray which was placed in a lidded, autoclavable box (116 mm x 80 mm x 20.6 mm) (Figures 2.1 and 2.2). This method excluded most saprophytic microorganisms from the isolation process, thereby allowing detection of only endophytic pathogenic fungi. The tray was immersed in a 1% bleach solution for 2 min, rinsed in sterile distilled water, and then the pieces of tissue were incubated on potato dextrose agar for 10-12 days under ambient light at room temperature. The fungal isolates obtained were sub-cultured for

purification and identified visually. The percentage of pathogen-free samples and the mean percent incidence of each pathogen was calculated for the root samples from each location.

2.3 Results and discussion

A total of 926 canola lower stem and root samples were collected at random from the W-shaped transects and low-lying areas in the surveyed fields. Despite dry weather through most of the summer (Alberta Government. 2021), a number of plants with root rot symptoms were observed. The incidence of root rot was highest at Edmonton, with a mean incidence of 4.1%, although 50% of the roots were infected at one sampling point (Table 2.1). At Morinville and Redwater, the overall incidence of root rot was also > 4%. Blackleg incidence was greatest at Morinville, Opal, and Bruderheim. Some canola crops near Edmonton were heavily infected with clubroot, with a mean incidence of 7.2% and one sampling point where 95% of the roots were infected. Most of the other areas showed little to no clubroot infection. A small amount of white mould was observed at Edmonton, St. Albert, Morinville and Gibbons.

Fusarium spp. were recovered most frequently from the root samples, occurring at an incidence of 85% across all locations (Table 2.2). The next most common fungi associated with the roots included *Alternaria* spp. (incidence of 13.9%), *Rhizoctonia* spp. (11.3%) and *Rhizopus* spp. (6.1%). A total of five unknown isolates were detected in canola roots collected at Morinville. During processing, evidence of mechanical damage resulting from insect feeding was observed on some roots. These damaged areas likely served as colonization access points for invasion of the roots by pathogenic fungi. In the lower stems, *Fusarium* spp. also were the predominant pathogenic fungi, occurring at an incidence of 81.7%, followed by *Alternaria* spp. (incidence of 40.6%), *Rhizoctonia* spp. (7.1%) and *Rhizopus* spp. (2.1%) (Table 2.3). The incidence of *Fusarium* spp. was greater than reported in the previous year (Yang et al. 2021). Other fungi including *Penicillium* spp. were detected occasionally in both infected root and stem samples. The high

incidence of *Fusarium*, *Alternaria* and *Rhizoctonia* in both belowground and aboveground tissues suggests an interaction of these fungi during infection of canola. In many cases, *Fusarium* was found in association with *Rhizoctonia* or *Alternaria* spp. Possible synergistic effects among these pathogens should be investigated further.

Table 2.1. Incidence (%) of disease in canola plants collected in central Alberta, 2021.

Location	No. of fields	Root Rot		Blackleg		Clubroot		White mould	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Bruderheim	3	0.4	0-5	4.0	0-9	0.07	0-1	0	0
Edmonton	7	4.1	0-50	2.9	0-16	7.2	0-95	0.2	0-4
Gibbons	3	0.4	0-2	1.3	0-5	0	0	0.07	0-1
Josephburg	2	0.2	0-2	0.6	0-3	0	0	0	0
Morinville	6	4.1	0-30	4.3	0-20	0.2	0-6	0.04	0-1
Namao	1	1.2	0-5	0	0	0	0	0	0
Opal	1	0.2	0-1	4.2	0-11	0.2	0-1	0	0
Redwater	4	4.4	0-25	0.9	0-5	0	0	0	0
St. Albert	5	1.4	0-7	1.7	0-9	0	0	1.1	0-8
Villeneuve	3	0.9	0-8	2.3	0-5	0	0	0	0
Total/Average	35	1.7	0-50	2.2	0-20	0.8	0-95	0.1	0-8

Table 2.2. Incidence of fungi recovered from diseased canola roots collected in central and northern Alberta, 2021.

Location	No. of fields	No. of roots tested	*Fusarium* spp. Mean% [a]	*Alternaria* spp. Mean%	*Rhizoctonia solani* Mean%	BLG[b] Mean%	WM[c] Mean%	*Rhizopus* spp. Mean%	*Penicillium* spp. Mean%
Bruderheim	3	30	98.3	6.7	15.0	0.0	5.0	3.3	0.0
Edmonton	8	128	85.8	25.9	2.8	2.2	2.4	9.3	8.3
Gibbons	2	25	79.0	21.7	23.6	3.0	14.7	5.9	11.7
Josephburg	2	29	87.4	23.2	20.6	0.0	0.0	2.7	0.0
Morinville	6	115	88.6	6.6	3.8	0.0	2.5	9.8	2.4
Namao	1	9	100.0	0.0	0.0	0.0	0.0	11.1	0.0
Opal	1	13	46.2	0.0	7.7	0.0	23.1	0.0	0.0
Redwater	4	82	75.3	20.8	9.7	0.0	7.4	4.3	0.0
St. Albert	4	60	97.8	16.2	9.4	0.0	0.0	14.3	0.9
Villeneuve	3	33	91.6	17.9	20.4	0.0	0.0	0.0	0.0
Total/Average	34	524	85.0	13.9	11.3	0.5	5.5	6.1	2.3

[a] Mean% = mean percent incidence.

[b]BLG = Blackleg (*Leptosphaeria maculans*).

[c]WM = White mould (*Sclerotinia sclerotiorum*).

Table 2.3. Incidence of fungi recovered from diseased canola stems collected in central and northern Alberta, 2021.

Location	No. of fields	No. of stems tested	*Fusarium* spp. Mean% [a]	*Alternaria* spp. Mean%	*Rhizoctonia solani* Mean%	BLG[b] Mean%	WM[c] Mean%	*Rhizopus* spp. Mean%	*Penicillium* spp. Mean%
Bruderheim	3	28	100.0	31.0	5.3	7.3	7.3	0.0	0.0
Edmonton	3	51	86.0	36.4	2.0	7.1	0.0	0.0	2.0
Gibbons	2	25	76.1	21.7	29.8	3.0	14.7	5.9	0.0
Josephburg	2	28	56.1	59.1	0.0	16.4	0.0	0.0	0.0
Morinville	6	143	79.3	33.9	0.0	8.2	0.8	3.2	6.2
Opal	1	20	65.0	30.0	15.0	5.0	5.0	5.0	0.0
Redwater	1	28	96.4	35.7	3.6	0.0	0.0	3.6	3.6
St. Albert	4	36	82.2	77.7	4.2	0.0	0.0	0.0	1.3
Villeneuve	3	43	94.4	39.8	4.0	9.2	0.0	1.3	0.0
Total/Average	25	402	81.7	40.6	7.1	6.2	3.1	2.1	1.5

[a] Mean% = mean percent incidence

[b]BLG = Blackleg (*Leptosphaeria maculans*).

[c]WM = White mould (*Sclerotinia sclerotiorum*).

Figure 2.1. Pathogen isolation tray. Trays contained 96 cells, each of which could hold 3-6 pieces of plant tissue.

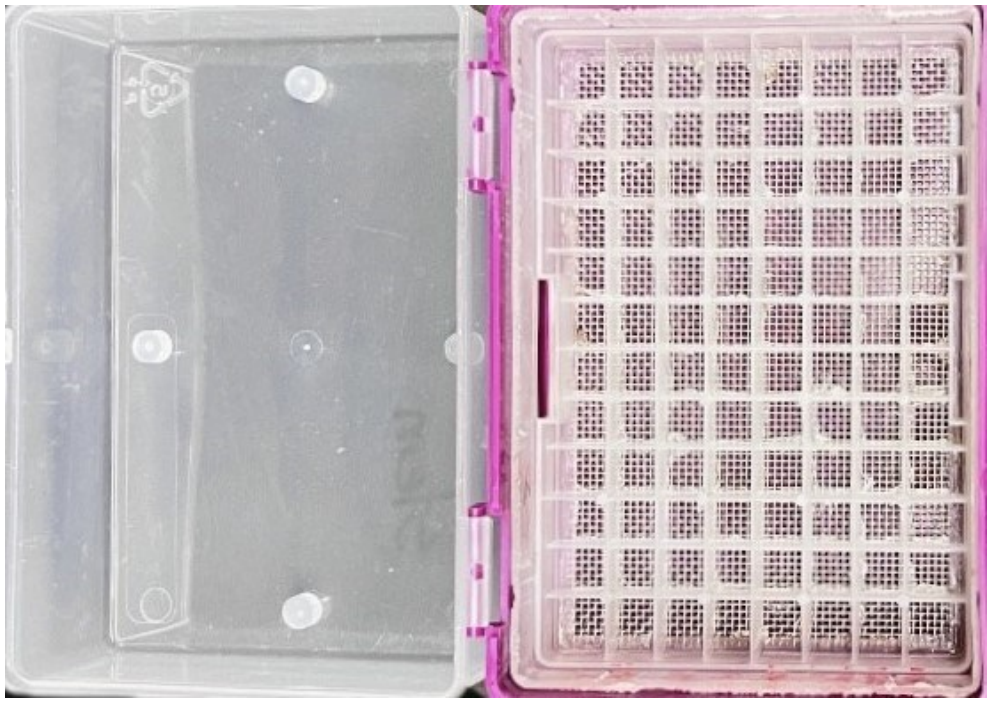


Figure 2.2. An autoclavable box to accommodate the isolation tray with the samples. The bottom of the box (right) can be filled with sodium hypochlorite solution (1%) and followed up with three rinses of sterile distilled water. The lid for the box is shown on the left.

Chapter 3 Canola disease survey in north-central Alberta in 2022

3.1 Introduction

In Alberta, 2.6 million ha were seeded to canola in 2022 (Canola Council of Canada 2022). However, soil-borne diseases reduce canola yield and quality, especially in years with good soil moisture. A survey of 51 canola crops in central and northern Alberta was conducted in September 2022 to collect diseased root and stem samples, which were used to assess the incidence of soil-borne pathogens throughout the region.

3.2 Materials and methods

Fifty-one canola crops in 10 counties across north-central Alberta (Table 3.1) were surveyed for various diseases following a W-shaped transect in each field. The survey was conducted post-harvest for each field. All plants within a 1-m^2 quadrat were examined at each of the five points along the transect. The incidence of clubroot, blackleg, root rot, and sclerotinia stem rot was recorded at each sampling point. A total of 4553 canola plant samples were collected at random from the surveyed fields. Diseased samples showing symptoms of root rot were taken to the laboratory to determine the causal organisms. The superficial layer (epidermis and cortex) of the diseased roots and stems was excised and only the interior vascular tissues that showed discolouration were cut and transferred to a 96-cell tray which was placed in a lidded, autoclavable box (116 mm x 80 mm x 20.6 mm). The tray was immersed in 1% sodium hypochlorite solution for 1-2 min then rinsed in sterile distilled water three times for 5 min each, after which the tissue pieces were

transferred to potato dextrose agar (PDA) plates amended with 0.1 g/L each of chloramphenicol and streptomycin to suppress bacterial growth (Strauss and Dillard 2009) and incubated for 10-12 days under ambient light at room temperature. The fungal isolates recovered were identified visually based on their morphological characteristics. The percent incidence of each pathogen was calculated for the root (Table 3.2) and stem samples (Table 3.3) from each field. The mean isolation rate was obtained by averaging data from 51 fields.

3.3 Results and discussion

The incidence of root rot of canola varied considerably among fields and locations (Table 3.1). The highest incidence occurred in Strathcona County, with a mean of 27.9%. At one sampling point, 51% of the roots were infected. In Lamont and Thorhild, the overall incidence of root rot was >8%. Blackleg incidence was greatest in Beaver County followed by Flagstaff. Some canola crops near Lamont were heavily infected with clubroot, with a mean incidence of 23% and one sampling point where 51% of the roots were infected. Most of the other areas showed little to no clubroot infection. A low amount of sclerotinia stem rot was observed in Westlock, Strathcona, Barrhead and Beaver.

During pathogen isolation, the basal portion of the stem and root was cut and various sizes of blackish tissues appearing in the cross-sections indicated that the blackleg pathogen was well established. Cross-sections of the root also indicated that the pathogen usually extended into the upper stems and downward into the tap roots. A total of 1871

root and 1045 stem diseased samples were assessed (Tables 3.2 and 3.3). The isolation frequencies of the pathogens from the roots varied among fields and locations (Table 3.2). *Fusarium* spp. were recovered from the roots at all locations, occurring at an average incidence of 88.2%, followed by *Leptosphaeria maculans* (15.6%), *Rhizoctonia* spp. (11.1%), and *Rhizopus* spp. (7.6%). During processing, evidence of mechanical damage resulting from insect feeding was observed on many roots. In some cases, these damaged areas were localized and did not result in any further intrusion by pathogenic fungi, likely due to dry soil conditions. However, many damaged roots were heavily infected by *Fusarium* spp., *R. solani* or other fungi causing root tissues to become blackened or rotted under wet soil conditions.

Fusarium spp. were the predominant pathogen recovered from the lower stems also, occurring at an average incidence of 66.8%, followed by *L. maculans* (36.0%), *Alternaria* spp. (14.2%), *Sclerotinia sclerotiorum* (8.8%), and *Rhizoctonia* spp. (6.7%) (Table 3.3). The highest average *S. sclerotiorum* infection rate occurred in Sturgeon (20%), followed by Thorhild (19.5%) and Lamont (16.5%), while the lowest occurred in Flagstaff (1%).

Combinations of *Fusarium* spp. and *L. maculans* (13.1%) or *Fusarium* spp. with *R. solani* (9.7%) were recovered from some canola roots, suggesting a pathogen complex (Table 3.2). Further study is needed to clarify whether synergistic effects occur among these pathogens on canola plants.

The incidence of *Fusarium* spp. was greater than reported in previous years (Yang et

al. 2021; Yu et al. 2022a). This could reflect, at least in part, higher than normal moisture levels during the growing season. Most regions in the surveyed area received over 100 mm of rain in July and August 2022 (Alberta Government 2022).

Table 3.1. Incidence (%) of disease in canola plants collected in north-central Alberta, 2022.

Location (County)	Field #	Root Rot		Blackleg [a]		Clubroot [b]		Sclerotinia stem rot [c]	
		Mean%	Range%	Mean%	Range%	Mean%	Range%	Mean%	Range%
Barrhead	5	12.0	1-22	12.7	0-45	19.1	0-66	3.0	0-10
Beaver	5	22.9	4-34	38.5	15-55	0.2	0-1	3.9	0-9
Flagstaff	6	23.2	9-42	32.0	16-50	0.0	0	5.2	3-7
Lamont	5	8.6	4-11	11.0	8-14	23.0	0-51	6.7	3-12
Leduc	5	19.8	2-31	14.8	9-29	9.3	0-45	9.4	3-19
Parkland	5	13.2	9-19	21.0	8-32	1.6	0-5	11.5	3-16
Strathcona	5	27.9	14-51	3.7	0-19	0.0	0	1.4	0-7
Sturgeon	5	25.5	5-36	11.8	5-22	0.4	0-2	4.5	1-10
Thorhild	5	10.0	2-15	13.8	5-23	0.5	0-1	7.3	2-9
Westlock	5	22.7	18-31	19.2	3-35	4.5	0-12	0.9	0-2
Total/Average	51	19.6	1-51	17.3	0-55	5.2	0-66	5.0	0-19

[a] Blackleg = *Leptosphaeria maculans*

[b] Clubroot = *Plasmodiophora brassicae*

[c] Sclerotinia stem rot = *Sclerotinia sclerotiorum*

Table 3.2. Incidence of fungi recovered from diseased canola roots collected in central and northern Alberta, 2022.

Location (county)	No. of fields	No. of roots tested	*Fusarium* spp. Mean% [a]	BLG [b] Mean%	*F* + BLG[c]	SSR[d] Mean%	*R. solani*[e]	*F* + *Rs*[f]	*Alternaria* spp. Mean%	*Rhizopus* spp. Mean%	Others[g] Mean%
Barrhead	5	163	90.2	9.5	6.2	0	19.5	18.9	3.3	5.8	4.9
Beaver	5	279	97.6	8.3	7.9	0	8.3	8.3	0.2	7.3	7.9
Flagstaff	6	330	97.7	14.1	13.2	0.3	10.0	6.5	0.4	3.3	2.2
Lamont	5	88	93.0	16.1	11.4	3.9	4.0	4.0	5.8	10.8	1.8
Leduc	5	144	92.9	20.0	17.0	6.6	13.7	13.3	0.7	8.9	3.8
Parkland	5	185	88.8	13.8	12.9	2.8	8.9	8.9	3.7	5.4	6.3
Strathcona	5	177	71.0	17.1	16.9	0	11.4	5.7	5.1	7.1	2.1
Sturgeon	5	175	82.4	28.1	21.9	1.4	14.1	11.4	4.9	9.9	4.7
Thorhild	5	90	76.6	8.6	6.8	2.3	13.7	12.7	1.0	6.8	7.7
Westlock	5	240	91.6	19.9	16.4	0.7	7.5	7.2	1.2	10.6	5.0
Total/Average	51	1871	88.2	15.6	13.1	1.8	11.1	9.7	2.6	7.6	4.6

[a] Mean% = mean percent incidence

[b] BLG = Blackleg (*Leptosphaeria maculans*)

[c] *F* + BLG = *Fusarium* spp. + blackleg

[d] SSR = Sclerotinia stem rot (*Sclerotinia sclerotiorum*)

[e] *R. solani* = *Rhizoctonia solani*

[f] *F* + *Rs* = *Fusarium* spp. + *Rhizoctonia solani*

[g] Others including *Penicillium* spp., *Trichoderma* spp., and unknown species

Table 3.3. Incidence of fungi recovered from diseased canola lower stems collected in central and northern Alberta, 2022.

Location (county)	No. of fields	No. of stems tested	*Fusarium* spp. Mean% [a]	BLG [b] Mean%	*F* + BLG[c]	SSR [d] Mean %	*R. solani*[e]	*F* + *Rs*[f]	*Alternaria* spp. Mean%	*Rhizopus* spp. Mean%	Others[g] Mean%
Barrhead	5	89	72.7	35.8	23.4	2.9	5.6	5.7	14.2	1.3	3.2
Beaver	5	126	54.0	23.7	20.1	2.5	3.0	2.3	13.2	0.6	4.0
Flagstaff	6	103	61.6	26.7	13.9	1.0	9.4	8.6	9.8	0	1.1
Lamont	5	89	55.9	24.7	10.5	16.5	0.5	0.5	18.8	0	0.5
Leduc	5	86	83.7	35.3	29.2	7.7	6.4	5.4	18.0	1.0	2.3
Parkland	5	119	82.9	33.5	28.6	7.7	15.9	15.9	4.8	1.6	6.0
Strathcona	5	60	73.0	57.5	34.4	9.4	13.3	13.3	9.7	0	4.3
Sturgeon	5	153	68.6	40.5	23.4	20.6	3.8	2.2	14.4	2.4	4.4
Thorhild	5	95	39.6	51.1	22.0	19.5	2.6	2.6	21.2	0	0.9
Westlock	5	125	76.2	31.2	21.7	0	6.2	4.6	17.8	5.0	13.3
Total/Average	51	1045	66.8	36.0	22.7	8.8	6.7	6.1	14.2	1.2	4.0

[a] Mean% = mean percent incidence

[b] BLG = Blackleg (*Leptosphaeria maculans*)

[c] *F* + BLG = *Fusarium* spp. + blackleg

[d] SSR = Sclerotinia stem rot (*Sclerotinia sclerotiorum*)

[e] *R. solani* = *Rhizoctonia solani*

[f] *F* + *Rs* = *Fusarium* spp. + *Rhizoctonia solani*

[g] Others including *Penicillium* spp., *Trichoderma* spp., and unknown species

Chapter 4 Prevalence, pathogenicity and diversity of canola (*Brassica napus*) root rot fungi in Alberta, Canada

4.1 Introduction

Root rot is a widely distributed disease causing significant yield losses in many agriculturally important crops worldwide (Bodah, 2017). The pathogens responsible for this disease are collectively referred to as the root rot complex (RRC), which consists mostly of fungi and fungal-like microorganisms (oomycetes), and to a lesser extent some bacteria and viruses (Bodah, 2017). The first indicators of root rot include poor germination and reduced emergence (Arora et al., 2021; Wang et al., 2006). Disease development also can be accompanied by aboveground symptoms such as crown rot (Rebollar-Alviter et al., 2020), leaf yellowing (Liao et al., 2023), wilting and stunting (Abdel-Monaim & Ismail, 2010). Plants typically cannot recover once these aboveground symptoms appear (Williamson-Benavides & Dhingra, 2021), and the severity of root rot is exacerbated under wet conditions. In Alberta and other provinces of western Canada, root rot represents an important constraint to canola (syn. oilseed rape; *Brassica napus* L.) production, causing yield losses of up to 35% (Yu et al., 2023a). This is a cause for concern, as canola contributes $29.9 billion CAD annually to the national economy (Canola Concil of Canada, 2023), with the vast majority of production in western Canada.

Root rot first emerged as a threat to Canadian canola production in the late 1980s, approximately 10 years after the start of commercial cultivation of this crop (Acharya et

al., 1984; Gugel et al., 1987; Kataria & Verma, 1992). In studies conducted in the 1980s and 1990s, the primary cause appeared to be *Rhizoctonia solani* J.G. Kühn (Acharya et al., 1984; Gugel et al., 1987; Kataria & Verma, 1992), although *Fusarium avenaceum* R.J. Cook was also reported as one of the causal agents of canola seedling blight during this period (Calman et al., 1986). By the 2000s, *Fusarium* spp. had emerged as a major component of the canola RRC (Chen et al., 2014; Hwang et al., 2009; Zhou et al., 2014a) together with *R. solani* (Broders et al., 2014; Fernandez, 2007; Hwang et al., 2009; Zhou et al., 2014a; Zhou et al., 2014b). Surveys conducted in central Alberta in the early 2000s identified *Fusarium acuminatum* Ellis & Everh as the predominant *Fusarium* species and *F. avenaceum* as the most aggressive species causing seedling blight of canola (Chen et al., 2014). The last major analysis of *Fusarium* spp. on canola in Alberta, however, was conducted on samples collected from 2009 to 2011 (Chen et al., 2014). As such, information on pathogen composition in this region is somewhat outdated, particularly given that numerous other *Fusarium* spp. have been implicated in root rot of canola and other *Brassicas*.

In Australia, *F. acuminatum* and *Fusarium semitectum* (Desm.) Sacc. were identified as the major agents causing root diseases of canola seedlings and adult plants (Li et al., 2007). In the United States, *F. acuminatum*, *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, *Fusarium solani* (Mart.) Sacc, and *Fusarium sporotrichioides* Sherb. were recovered from diseased root samples of *Brassica carinata* L., and the virulence of these

species was confirmed on several cultivars of this crop (Okello et al., 2018). In Poland, foot rot of rapeseed seedlings caused by *F. oxysporum* and *Fusarium culmorum* (Wm.G.Sm.) Sacc. is commonly encountered (Starzycki et al., 2007). A field survey in Iran identified *F. solani* as the predominant species causing root and crown rot in canola, while *Fusarium equiseti* (Corda) Sacc. was the most aggressive (Heydari et al., 2010; Larki & Farrokhi Nejad, 2015); recent studies also isolated *F. acuminatum* and *Fusarium clavum* J.W. Xia, L. Lombard, Sand.-Den., X.G. Zhang & Crous from diseased canola (Nemati Mondanipour et al., 2021). On vegetable *Brassicas*, most studies have concentrated on Fusarium wilt and its control (Mehraj et al., 2020; Mourou et al., 2023; Oksana et al., 2022).

With over 80 species worldwide, a wide host range and rapid growth, *Fusarium* spp. have become important constraints to the production of canola and other crops (Arie, 2019). The identification of individual species of *Fusarium* has traditionally relied on colony and morphological characteristics (Leslie & Summerell, 2008). More recently, species identification and understanding of *Fusarium* taxonomy have been enhanced by the advent of modern genetic, PCR-based and sequencing technologies. Molecular analysis of conserved gene regions, such as the internal transcribed spacer (ITS) region, the translation elongation factor 1-α (TEF-1α) gene, the intergenic spacer (IGS) region, and RNA polymerase II (RPB2) gene enables precise identification of species and an improved knowledge of species diversity and distribution (Chandra et al., 2011; Kistler, 1997;

Magdama et al., 2020).

The management of root rot can be challenging. Only partial resistance, controlled by numerous quantitative trait loci, has been reported in canola (Yang & Verma, 1992). Cultural practices and fungicidal seed treatments can improve canola emergence and yield, but not necessarily under high disease pressure (Hwang et al., 2015). Other strategies, such as diverse crop rotations (Hwang et al., 2009), have also been suggested to reduce root rot severity. However, none of these strategies on its own is sufficient to combat root rot, and an integrated approach is required. Current knowledge of the prevalence, pathogenicity and diversity of root rot pathogens of canola can aid in the development of such an approach. Therefore, the aim of this study was to characterize the *Fusarium* spp. associated with canola root rot in Alberta, to generate the first information on this topic in over a decade.

4.2 Materials and Methods

4.2.1 Disease surveys

Root rot surveys

Canola crops in north-central Alberta were surveyed for the occurrence of root rot in late August to October 2021 (Yu et al., 2022a) and September 2022 (Yu et al., 2023b). Thirty-five and 51 fields were visited in each of 2021 and 2022, respectively, and symptomatic root and lower stem samples were collected for processing in the laboratory. The incidence of root rot was calculated as the percentage of diseased samples relative to

the total collected in each field, with five 1-m^2 quadrats sampled per field along a W-transect (Yu et al., 2023b; Yu et al., 2022a). The surveyed region is illustrated in Figure 4.1.

Fungal isolate recovery

A total of 3,842 symptomatic root and stem samples were cultured for pathogen recovery. This number included 524 root and 402 stem samples collected in 2021, and 1,871 root and 1,045 stem samples collected in 2022. In brief, samples were surface-sterilized in 1% bleach (NaOCl) for 2 min and rinsed 3× in sterile distilled water. The rinsed samples were incubated on potato dextrose agar (PDA) at room temperature under a 12 h cycle of ambient light for 10-12 days. Recovered pathogens were identified to the genus and/or species level by examining the colony and morphological characteristics of each colony, such as the microsclerotia produced by *R. solani* (Nasimi et al., 2024), and the colony color and microscopic structures (macroconidia, microconidia, chlamydospores, and phialides) produced by species of *Fusarium* (Leslie & Summerell, 2008).

4.2.2 Isolation and molecular identification of *Fusarium* species

Hyphal tip purification and species identification

The *Fusarium* isolates tentatively identified based on colony and morphological characteristics as described above were sub-cultured for hyphal tip purification (Li et al., 2019) and their identities confirmed by molecular analysis. Purified isolates were cultured on PDA for 7-10 days as above and stored at 4°C for future use. The DNA of 230 selected

isolates, including 151 isolates recovered from samples collected in 2021 and 79 isolates recovered from samples collected in 2022, was extracted from 7-day-old PDA cultures following the Quick-Start protocol (www.qiagen.com/HB-2522) of the DNeasy Plant Pro Kit (Qiagen, Hilden, Germany). DNA concentration and quality were assessed with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Toronto, Canada) using the default setting for a DNA assay. The samples were then diluted to a final concentration of 20 ng DNA/µL with nuclease-free water (Thermo Fisher Scientific) and stored at -20°C until needed.

The DNA extracted from each of the 230 fungal isolates was subjected to PCR using two sets of primers: EF-2 (5'-GGARGTACCAGTSATCATGTT-3') and EF-3 (5'-GTAAGGAGGASAAGACTCACC-3'), targeting the TEF-1α gene (O'Donnell et al., 1998); and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') (White, 1990), targeting the ITS region. Reactions were conducted in a total volume of 20 µL, which included 1 µL DNA template (20 ng/µL), 1 µL of each forward and reverse primer (10 µM), 10 µL HotStar Taq Master Mix (Qiagen), 0.25 µL bovine serum albumin (10 mg/mL) (Thermo Fisher Scientific), and 6.75 µL nuclease-free water (Thermo Fisher Scientific). Thermocycler conditions consisted of an initial heat activation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 30 sec (marked with 55°C for 90 sec for the EF-2/EF-3 primer pair), and extension at 72°C for 1 min. A final extension step was

conducted at 72℃ for 10 min. Amplicons were purified with a QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions, and sequenced at the Molecular Biology Service Unit (MBSU) of the University of Alberta, Edmonton, Canada.

The TEF-1α and ITS sequences obtained were used to confirm the species classification of each isolate via BLAST searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of the National Center for Biotechnology Information (NCBI) nucleotide databases. Isolates were grouped into species based on a sequence identify threshold of 97% to 100% (Chen et al., 2014). Morphological characterization and molecular identification were combined to determine the final identity of the isolates at the species level.

4.2.3 Pathogenicity test

The pathogenicity of 205 *Fusarium* isolates (130 isolates collected in 2021 and 75 isolates collected in 2022) was assessed on the canola cv. 'Westar' under greenhouse conditions according to Yu et al. (Yu et al., 2022b) with some modifications. Briefly, 473 mL cups (Uline, Toronto, ON) were filled with 400 mL of Promix PGX potting medium (Sun-Gro Canada Inc., Seba Beach, AB, Canada). Fourteen-day old fungal cultures were sectioned into small (3 mm × 3 mm) pieces using a sterilized scalpel, and then spread evenly on the surface of the potting mix within each cup (at a rate of one colony or plate per cup). Ten 'Westar' seeds were planted in each cup, with four cups (replicates) allocated for each isolate (treatment). An equal number of cups was designated for the non-inoculated control, with the same quantity of PDA pieces distributed alongside the seeds.

Two trials were conducted, with treatments arranged in a randomized complete block design. The plants were watered and fertilized (20N: 20P: 20K) as needed.

Following inoculation, the cups were transferred to a greenhouse maintained at 25℃ with a 12-h photoperiod. Seedling emergence data were collected on the 7th day after seeding, while plant height was assessed on the 14th day. On the 21st day after seeding, the plants were uprooted and their roots thoroughly washed with tap water before being assessed for disease severity as described below. Subsequently, the plants were air-dried at 25℃ for 7 days, and the shoots and roots were separated by cutting. Shoot and root dry weights were determined for each experimental unit.

4.2.4 Disease ratings

Root rot severity was assessed on a 0-4 scale (Hwang et al., 1994), where: 0 = healthy roots; 1 = small, light-brown lesions on < 25% of the tap root; 2 = brown lesions on 25-49% of the tap root; 3 = brown lesions on 50-74% of the tap root, tap root constricted; and 4 = tap root severely girdled, brown lesions on > 75% of the tap root with limited lateral roots. The final disease severity per experimental unit was determined by averaging the values of all the individual plants within each cup (replicate).

4.2.5 Emergence, plant height, and shoot and root dry weights

Seedling emergence was determined by counting all surviving plants in each experimental unit 7 days after seeding. Plant height was measured from the soil line to the shoot apex using a ruler at 14 days after seeding. Shoot and root dry weights were assessed

separately for each replicate with a weighing scale (Fisher Science Education SLF 303, Thermo Fisher). Reductions in seedling emergence, plant height, and shoot and root dry weights were calculated relative to non-inoculated controls according to the equation: Reduction = $[(D_{ck} - D_{tr}) / D_{ck}] \times 100\%$, where: D_{ck} represents the control (non-inoculated) treatment and D_{tr} represents the inoculated treatment.

4.2.6 Phylogenetic tree construction

Three independent phylogenetic trees were constructed for 157 isolates based on their (i) ITS sequence, (ii) TEF-1α sequence, and (iii) the concatenated ITS and TEF-1α sequences. These included 112 representative isolates collected in 2021 (110 *Fusarium* isolates and one isolate each of *Trichoderma paraviridescens* and *Neonectria* sp.), 31 representative *Fusarium* isolates collected in 2022, one reference sequence from NCBI, and 13 isolates from the culture collection of the Applied Plant Pathology Lab, University of Alberta. The latter 13 cultures consisted of one isolate of *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg and two isolates each of *Fusarium graminearum* (Schwein.) Petch, *F. solani*, *Fusarium redolens* Wollenw, *F. oxysporum*, *F. avenaceum*, and *F. acuminatum*. Sequence characters were equally weighted in all the phylogenetic analyses, with alignment performed using the MUSCLE algorithm in MEGA 3.0 (Edgar, 2004). Maximum Parsimony (MP) trees were generated with default parameters and bootstrapping using 1000 replicates. All phylogenetic trees were reconstructed using R Studio v. 4.1.2 (RStudio Team, 2020). Species identity was assigned

to isolates in the ITS and TEF-1α sequence trees. In the concatenated sequence tree, each isolate was assigned species identity, along with disease severity and reductions in emergence, root dry weight, and shoot dry weight. Consistency among the single-locus phylogenetic trees and the combined gene phylogeny was evaluated based on the overall topology of each tree (Lin et al., 2023).

4.2.7 Data analysis

The normality of the data was assessed using the Shaprio-Wilk test, while homogeneity was evaluated with Levene's test within ANOVA. Statistical analyses were conducted in R Studio v. 4.1.2 (RStudio Team, 2020). Differences were considered significant when the *p*-value was < 0.05 unless otherwise indicated. In cases where a significant interaction was detected between repetition and treatment, the data were analyzed separately to account for the effects of these factors on the results. In the pathogenicity test, a t-test was employed using R Studio v. 4.1.2 (RStudio Team, 2020) to compare the non-inoculated control and inoculated treatments. Correlation analysis was conducted to assess the relationship among disease severity and plant growth parameters. Principal Component Analysis (PCA) was used to explore the potential utility of parameters for evaluating pathogenicity. Root rot severity and reductions in seedling emergence, plant height, and root and shoot dry weight were used to generate the PCA biplot. Phylogenetic trees were constructed in MEGA 3.0, and then modified in R Studio.

4.3 Results

4.3.1 Root rot incidence and pathogen recovery

In 2021, root rot was identified in 34 of the 35 surveyed fields, with disease incidence ranging from 0% to 50% and averaging 1.7% (Yu et al., 2022a). In the lab, *Fusarium* spp. were recovered from 41.7% to 100% of the diseased root samples collected from each field and an average of 85.0% across all fields (Figure 4.1). While other fungi, including *R. solani*, were also recovered from the root samples, their incidence was relatively low. *Fusarium* was also the predominant genus recovered from diseased lower stem samples collected in 2021.

Root rot was detected in all of the 51 fields surveyed in 2022, with the disease incidence (average of 19.6% and range of 1% to 51%) generally much higher than in 2021 (Yu et al., 2023b). As in 2021, *Fusarium* spp. continued to be the most commonly found fungi, recovered from root samples collected from each field at rates of 25% to 100%. On average, *Fusarium* spp. were recovered from 88.2% of root samples across all fields (Figure 4.1). *Rhizoctonia solani* and *Leptosphaeria maculans* (Sowerby) P. Karst were among the few other pathogens with recovery rates exceeding 10% in the root samples. Among the lower stem samples collected in 2022, *Fusarium* spp. were the most prevalent (recovered from 66.8% of samples), followed by *L. maculans* (present in >30% of samples) (Figure 4.1).

4.3.2 *Fusarium* species identification and prevalence

In 2021, a total of 207 purified isolates were recovered from 35 fields. One hundred fifty-one isolates from 26 fields were selected for species identification based on colony and morphological characteristics combined with analysis of the TEF-1α gene and ITS region. Of these, 149 isolates from 26 fields were identified as *Fusarium*, representing 14 distinct species. These included *F. avenaceum* (74 isolates), *F. redolens* (18 isolates), *F. solani* (14 isolates), *Fusarium torulosum* (Berk. & M.A. Curtis) Nirenberg (11 isolates), *F. culmorum* (5 isolates), *F. equiseti* (4 isolates), *F. acuminatum* (3 isolates), *F. oxysporum* (2 isolates), *Fusarium tricinctum* (Corda) Sacc. (1 isolate), *F. proliferatum* (1 isolate), *F. graminearum* (1 isolate), *Fusarium commune* K. Skovg., O'Donnell & Nirenberg (1 isolate), *F. sporotrichioides* (2 isolates), *Fusarium flocciferum* Corda (1 isolate), and an unidentified *Fusarium* species (Supplementary Table 4.1). *Fusarium avenaceum* was the most prevalent species, found in 20 of the 26 fields and representing 49.7% of all *Fusarium* isolates. The next most common species included *F. redolens* (12.1%), *F. solani* (9.4%), and *F. torulosum* (7.4%).

In 2022, a total of 427 purified isolates were recovered from 51 fields. Seventy-nine isolates from 32 fields were selected for species identification, with 75 of these identified as *Fusarium*, representing nine species. These included *F. avenaceum* (36 isolates), *F. solani* (8 isolates), *F. redolens* (7 isolates), *F. oxysporum* (7 isolates), *F. culmorum* (5 isolates), *F. acuminatum* (4 isolates), *F. torulosum* (2 isolates), *F. tricinctum* (2 isolates), *F.*

flocciferum (1 isolate), and three isolates of an unidentified *Fusarium* species (Supplementary Table 4.1). As in 2021, *F. avenaceum* was the most commonly identified species, confirmed in 22 out of 32 fields and representing 48% of all *Fusarium* isolates. The next most common species found in 2022 was *F. solani* (10.7%), alongside *F. redolens* and *F. oxysporum* (both 9.3%). Three species - *F. commune*, *F. equiseti*, and *F. sporotrichioides* – were identified only in the collections made in 2021.

4.3.3 Pathogenicity test

ANOVA indicated no significant ($p > 0.05$) variation in disease severity or other growth parameters (emergence and root and shoot dry weights) between the two repeats of the greenhouse trial. Therefore, the data were combined for further analysis. In contrast, significant variation ($p < 0.05$) among treatments (isolates) was observed in both years. The results of a t-test comparing the inoculated treatments vs. non-inoculated control for all parameters in each respective year are presented in Supplementary Figures 4.1-4.10. As expected, in both years, all the isolates exhibited a significant increase in disease severity relative to the non-inoculated control (Supplementary Figures 4.1 and 4.6). Ninety-eight of the 130 isolates tested from the 2021 survey caused a reduction in canola seedling emergence (Supplementary Figure 4.2) and shoot dry weight (Supplementary Figure 4.4), while 82 isolates caused a decrease in plant height (Supplementary Figure 4.3) and 126 isolates caused a decrease in root dry weight (Supplementary Figure 4.5). Among the 75 isolates tested from the 2022 survey, 49 reduced seedling emergence

(Supplementary Figure 4.7), 52 reduced plant height (Supplementary Figure 4.8), and 49 and 74 reduced shoot (Supplementary Figure 4.9) and root (Supplementary Figure 4.10) weight, respectively.

Effect of Fusarium spp. on disease severity

The disease severity data aggregated for all isolates collected in both years compared with the aggregated non-inoculated controls are shown in Supplementary Table 4.1. Average disease severity ranged from 0.58 to 4.0 (Table 4.1). Three isolates each of *F. solani* and *F. redolens* induced disease severities of <1.0 (Table 4.1). Eighty-three isolates caused intermediate symptoms, with disease severities ranging from 1.0 to 2.0. These included 21 isolates of *F. redolens*, 18 of *F. avenaceum*, 16 of *F. solani*, and 10 isolates of *F. torulosum*, as well as between 1 to 5 isolates each of *F. culmorum*, *F. oxysporum*, *F. acuminatum*, *F. tricinctum*, *F. flocciferum*, *F. proliferatum*, and *F. equiseti* (Table 4.1). Seventy-six isolates caused disease severities ranging from 2.0 to 3.0. These represented 51 isolates of *F. avenaceum*, 5 of *F. culmorum*, 4 of *F. oxysporum*, and 2-3 isolates each of *F. acuminatum*, *F. equiseti*, *F. torulosum*, and *F. solani*, an unidentified *Fusarium* species, and 1 isolate each of *F. commune*, *F. graminearum*, and *F. redolens* (Table 4.1). Finally, 40 isolates caused disease severities >3.0. Thirty-seven of these isolates were identified as *F. avenaceum*, two as *F. sporotrichioides*, and one could not be identified to the species level (Table 4.1).

Effect of Fusarium spp. on seedling emergence

The reduction in seedling emergence caused by inoculation with *Fusarium* spp. ranged from 1.32% to 100% (Table 4.1). Seventy-four of the isolates representing 11 species caused a <25% reduction in emergence. These consisted of *F. redolens* (19 isolates), *F. solani* (18 isolates), *F. avenaceum* (11 isolates), and *F. torulosum* (10 isolates), *F. acuminatum* (4 isolates), *F. culmorum* (4 isolates), *F. tricinctum* (3 isolates), *F. flocciferum* (2 isolates), *F. oxysporum* (1 isolate), *F. proliferatum* (1 isolate), and *F. commune* (1 isolate). Fifty-eight isolates representing nine *Fusarium* species caused a reduction in seedling emergence of 25% to 50%. These comprised 31 isolates of *F. avenaceum*, 5 isolates each of *F. redolens* and *F. culmorum*, 4 isolates each of *F. equiseti* and *F. oxysporum*, 3 isolates of *F. torulosum*, 2 isolates each of *F. solani* and *F. acuminatum*, and 1 isolate each of *F. graminearum* and an unidentified species. Forty-five of the isolates caused reductions of 50% to 75% in seedling emergence, including 39 isolates of *Fusarium avenaceum* as the predominant species, 3 isolates of *F. oxysporum,* 1 isolate of *F. redolens* and 2 unidentifiable isolates. Finally, 28 isolates reduced seedling emergence by >75%, and consisted of 24 isolates of *F. avenaceum*, 2 isolates of *F. sporotrichioides* and 1 isolate each of *F. culmorum* and *F. solani* (Table 4.1). Among all the isolates, only 58, including 17 isolates of *F. solani*, 14 isolates of *F. redolens*, 4 isolates of *F. culmorum*, 3 isolates each of *F. avenaceum*, *F. acuminatum*, *F. oxysporum*, and *F. tricinctum*, and one isolate each of *F. commune*, *F. proliferatum*, *F. flocciferum*, and unidentified species, did not significantly reduce seedling emergence (Supplementary Figs. 4.2 and 4.7).

Effect of Fusarium spp. on plant height

The effects of *Fusarium* spp. inoculation on plant height were mixed, with most isolates causing reductions in the range from 1.55% to 100% and a few causing small increases (Table 4.1). One-hundred and twenty-seven isolates, representing 12 different *Fusarium* species, were identified as causing plant height reductions of <25%. These included 66 isolates of *F. avenaceum*, 14 isolates of *F. redolens*, and 13 isolates of *F. solani*, as well as *F. torulosum* (8 isolates), *F. oxysporum* (7 isolates), *F. acuminatum* (6 isolates), *F. culmorum*, and *F. tricinctum* (3 isolates each). Additionally, there were 2 isolates each of *F. flocciferum* and an unidentified species, and 1 isolate each of *F. proliferatum*, *F. graminearum*, and *F. commune*, which caused reductions in plant height of <25%. Sixty of the tested isolates, representing seven species, caused intermediate reductions (25% to 50%) in plant height. These included 28 isolates of *F. avenaceum*, 11 isolates of *F. redolens*, 7 isolates of *F. solani,* 6 isolates each of *F. culmorum*, 5 isolates of *F. torulosum*, and 1 isolate each of *F. equiseti*, *F. oxysporum*, and an unidentified species. Sixteen isolates caused severe reductions (50% to 75%) in plant height, consisting of 11 isolates of *F. avenaceum*, 3 isolates of *F. equiseti* and 1 isolate each of *F. solani* and *F. culmorum*. Two isolates of *F. sporotrichioides* caused very severe reductions in plant height of >75%. Fifty-nine isolates, including 31 isolates each of *F. avenaceum*, 7 isolates of *F. solani*, 6 isolates of *F. torulosum*, 5 isolates of *F. redolens*, 2 isolates each of *F. culmorum*, *F. acuminatum*, *F. oxysporum*, and *F. flocciferum*, and one isolate each of *F. tricinctum* and *F. graminearum*,

did not significantly reduce plant height (Supplementary Figs. 4.3 and 4.8). Inoculation with 13 isolates representing seven species resulted in numerical increases in plant height, but these increases were significant (p<0.05) only for only one isolate each of *F. redolens* and *F. tricinctum* (Supplementary Figs. 4.3 and 4.8).

Effect of Fusarium spp. on shoot dry weight

In terms of shoot dry weight, the reductions caused by inoculation with *Fusarium* spp. ranged from 0.06% to 98.6%, while a few isolates increased shoot dry weight by up to 67.9% (Table 4.2). Forty-nine of the isolates caused reductions of <25%. These included *F. avenaceum* (13 isolates), *F. solani* (10 isolates), and *F. redolens* (10 isolates), *F. torulosum* (6 isolates), 2 isolates each of *F. acuminatum*, *F. flocciferum*, *F. tricinctum*, and *F. culmorum*, 1 isolate each of *F. graminearum* and *F. oxysporum*. Sixty-seven isolates caused reductions in shoot dry weight of 25 to 50%. These represented nine species including *F. avenaceum* (37 isolates), *F. redolens* (9 isolates) *F. oxysporum* (4 isolates), 3 isolates each of *F. torulosum*, *F. solani*, and *F. acuminatum*, 3 isolate of *F. culmorum*, 2 isolates of *F. equiseti*, and an unidentified species, and 1 isolate of *F. commune*. Twenty-eight isolates of *F. avenaceum*, three isolates of *F. solani*, two isolates of *F. equiseti*, and one isolate each of *F. redolens* and *F. culmorum*, caused reductions in shoot dry weight of 50% to 75%. Twenty-nine isolates including *F. avenaceum* (25 isolates), *F. sporotrichioides* (2 isolates), *F. culmorum* (1 isolate), and an unidentified isolate induced reductions in shoot dry weight of >75% (Table 4.2). Among all the isolates, 12 isolates of

F. avenaceum, 8 isolates of *F. redolens*, 5 isolates of *F. solani*, 2 isolates each of *F. culmorum* and *F. torulosum*, and 1 isolate each of *F. oxysporum*, *F. graminearum*, *F. tricinctum*, and *F. flocciferum* did not significantly reduce shoot dry weight (Supplementary Figs. 4.4 and 4.9). In contrast, 3 isolates of *F. redolens*, 2 isolates each of *F. avenaceum* and *F. solani*, and 1 isolate each of *F. acuminatum*, *F. culmorum*, and *F. torulosum*, induced significant ($p<0.05$) increases in shoot dry weigh (Supplementary Figs. 4.4 and 4.9).

Effect of Fusarium spp. on root dry weight

Reductions in root dry weight following inoculation with *Fusarium* spp. ranged from 11.9% to 99.6% (Table 4.2). Four isolates (2 *F. redolens*, 1 *F. culmorum* isolate, and 1 *F. solani*) caused reductions of <25%. Seventeen isolates representing seven species caused reductions in root dry weigh of 25% to 50%, including 6 isolates of *F. avenaceum*, 3 isolates of *F. redolens*, 2 isolates each of *F. oxysporum*, *F. solani*, and *F. torulosum*, and 1 isolate each of *F. proliferatum* and *F. tricinctum*. Seventy-eight isolates, including 11 *Fusarium* species, caused reductions in root dry weight of 50% to 75%. These consisted of *F. avenaceum* (24 isolates), *F. redolens* (15 isolates), *F. solani* (11 isolates), *F. torulosum* (6 isolates), 5 isolates each of *F. acuminatu*, *F. oxysporum*, and *F. culmorum*, 2 isolates each of *F. tricinctum* and an unidentified species, and 1 isolate each of *F. commune*, *F. flocciferum*, and *F. graminearum*. The largest group of 106 isolates caused the most severe reductions (75% to 100%) in root dry weight and included *F. avenaceum* (75 isolates), *F.*

solani (7 isolates), 5 isolates each of *F. redolens* and *F. torulosum*, 4 isolates each of *F. culmorum* and *F. equiseti*, 2 isolates of *F. sporotrichioides*, and 1 isolate each of *F. acuminatum*, *F. flocciferum*, *F. oxysporum*, and an unidentified species (Table 4.2). Among all the isolates, only 1 *F. culmorum* isolate, CS250, did not significantly (p<0.05) reduce the root rot of canola seedlings (Supplementary Fig. 4.10).

Comparison of disease severity between F. avenaceum and other Fusarium species

Most isolates of *F. avenaceum* collected in 2021 caused more severe root rot than did isolates of *F. equiseti*, *F. solani*, *F. redolens*, *F. culmorum* or *F. torulosum* collected in the same year (Figure 4.2). Similar results were observed for the isolate collections made in 2022, with *F. avenaceum* generally causing more severe root rot than *F. acuminatum*, *F. oxysporum*, *F. solani*, *F. culmorum* or *F. redolens*.

Correlations and principal component analysis

Disease severity was negatively correlated with all four plant growth parameters (emergence, plant height, shoot dry weight, root dry weight) examined in the trials conducted with both the 2021 and 2022 isolate collections (Figure 4.3). In contrast, emergence, plant height, shoot dry weight and root dry weight were all positively correlated with one another.

The results of the principal component analysis (PCA) for 2021 and 2022, based on all five parameters for the six most common *Fusarium* spp., are presented in Supplementary Figure 4.11. Overall, the first two principal components (PC1 and PC2)

accounted for more than 85% of the variation among all the isolates in both 2021 (86.2%) and 2022 (91.4%). In PC1, all the parameters, with the exception of plant height, showed relative contributions of approximately -0.40 for both years. Notably, plant height was the primary factor in PC2, with a relative contribution of over 0.70 (data not shown).

4.3.4 Phylogenetic analysis

Phylogenetic tree based on ITS factor sequences

One hundred and forty-three isolates from this study (112 isolates from 2021 and 31 isolates from 2022), 13 isolates from Applied Plant Pathology Lab collection, and one reference sequence downloaded from NCBI were included in the phylogenetic analysis. The ITS sequences obtained from these isolates ranged in size from 469 to 542 bp. Sequence alignment resulted in a sequence matrix containing 577 characters. Within this matrix, there were 372 conserved sites, 160 variable sites, and 113 parsimony-informative sites. The average composition of nucleotides was 23.0% T, 27.7% C, 25.4% A, and 23.9% G. The constructed ITS factor tree had Consistency Index (CI), Retention Index (RI), and Rescaled Consistency (RC) values of 0.748, 0.973, and 0.727, respectively.

The 157 isolates were grouped into five distinct clades as shown in Supplementary Figure 4.12. Clade I comprised 5 isolates collected from multiple fields in 2022, including 1 isolate of *F. tricinctum* and 4 isolates of *F. avenaceum* (Supplementary Figure 4.12). Disease severity caused by isolates in Clade I ranged from 1.89 to 3.38 (Supplementary Table 4.1). Clade II comprised 12 isolates from multiple fields in both years, including 5

isolates of *F. acuminatum*, 6 isolates of *F. avenaceum*, and 1 isolate of *F. torulosum* (Supplementary Figure 4.12). Disease severity caused by isolates in Clade II ranged from 1.50 to 3.50 (Supplementary Table 4.1). Clade III consisted of 19 isolates from multiple fields in both years. This clade comprised 1 isolate of *T. paraviridescens*, 1 isolate of *F. torulosum*, and 17 isolates of *F. solani*, separated into three subgroups. The first subgroup consisted of 11 isolates of *F. solani* and 1 isolate of *F. torulosum*. The second subgroup included 6 isolates of *F. solani*, which were further divided into two sub-subgroups. The third subgroup consisted of the *T. paraviridescens* isolates (Supplementary Figure 4.12). Disease severity caused by isolates in Clade III ranged from 0.61 to 2.54 (Supplementary Table 4.1).

Clade IV consisted of 17 isolates from multiple fields in both years, encompassing a variety of species, including the single isolate of *Neonectria* sp., *F. oxysporum* (4 isolates), *F. equiseti* (3 isolates), *F. sporotrichioides* (2 isolates), *F. culmorum* (4 isolates), and *F. graminearum* (3 isolates). These isolates were organized into six subgroups. The first subgroup comprised 2 isolates of *F. graminearum* and 1 isolate each of *F. culmorum*, and *F. oxysporum*. The second subgroup consisted of 3 isolates of *F. culmorum* and 1 isolate of *F. graminearum*. The third subgroup included 2 isolates of *F. sporotrichioides*. The fourth and fifth subgroups comprised 3 isolates each of *F. equiseti* and *F. oxysporum*, respectively. The last subgroup consisted of the *Neonectria* sp. isolate (Supplementary Figure 4.12). Disease severity caused by isolates in Clade IV ranged from 1.48 to 4.00

(Supplementary Table 4.1). Clade V encompassed 26 isolates from multiple fields in both years, featuring two subgroups. One subgroup contained 2 isolates of *F. proliferatum* and 1 isolate of *F. commune*, while the other subgroup included 23 *F. redolens* isolates, including the reference isolate *F. redolens* 463-5 (FRE_463) from NCBI (Supplementary Figure 4.12). Disease severity caused by isolates in Clade V ranged from 0.64 to 2.04 (Supplementary Table 4.1). An additional 78 isolates from multiple fields in both years, including *F. avenaceum* (65 isolates), *F. torulosum* (9 isolates), *F. tricinctum* (1 isolate), *F. culmorum* (1 isolate), and *F. flocciferum* (2 isolates), were not grouped into any cluster. Disease severity caused by these isolates ranged from 1.22 to 3.88 (Supplementary Table 4.1).

Phylogenetic tree based on TEF-1α sequences

The TEF-1α sequences from the 157 isolates ranged in size from 565 to 664 bp. The aligned sequence matrix contained 839 characters, consisting of 1 conserved site, 660 variable sites, and 665 parsimony-informative sites. The average composition of nucleotides included 22.9% T, 23.1% C, 24.6% A, and 29.4% G. The constructed TEF-1α factor tree yielded CI, RI, and RC values of 0.685, 0.947, and 0.649, respectively.

The isolates were clustered into six distinct groups (Supplementary Figure 4.13). In Clade I, 15 isolates of *F. avenaceum* from multiple fields in both years were grouped together. Two of four isolates collected near Morinville, AB, clustered as a subgroup (Supplementary Figure 4.13). Disease severity caused by isolates in Clade I ranged from

1.60 to 3.54 (Supplementary Table 4.1). In Clade II, 47 isolates of *F. avenaceum* from multiple fields in both years were grouped together. Among these, 32 isolates from multiple fields clustered into one subgroup, while the remaining 15 isolates from multiple fields formed another subgroup (Supplementary Figure 4.13). Disease severity caused by isolates in Clade II ranged from 1.55 to 3.88 (Supplementary Table 4.1). Clade III comprised seven isolates from multiple fields in both years. Within this clade, five isolates of *F. acuminatum*, one isolate of *F. avenaceum*, and one isolate of *F. tricinctum* clustered into three subgroups, respectively (Supplementary Figure 4.13). Disease severity caused by isolates in Clade III ranged from 1.50 to 2.16 (Supplementary Table 4.1). In Clade IV, 13 isolates from multiple fields in both years were grouped together. Within this group, two isolates of *F. flocciferum*, one isolate of *F. tricinctum*, and two isolates of *F. torulosum* were clustered into two subgroups, respectively. The remaining eight *F. torulosum* isolates clustered into three different subgroups (Supplementary Figure 4.13). Disease severity caused by isolates in Clade IV ranged from 1.42 to 2.58 (Supplementary Table 4.1).

In Clade V, 29 isolates from multiple fields in both years, including 22 isolates of *F. redolens*, 4 isolates of *F. oxysporum*, 2 isolates of *F. proliferatum*, and 1 isolate of *F. commune*, were grouped together. Within this group, 22 isolates of *F. redolens* from multiple fields in both years clustered in the first subgroup. The second subgroup comprised 4 isolates of *F. oxysporum*, and the third subgroup included 2 isolates of *F. proliferatum* and 1 isolate of *F. commune* (Supplementary Figure 4.13). Disease severity

caused by isolates in Clade V ranged from 0.64 to 2.45 (Supplementary Table 4.1). Clade VI included 34 isolates from multiple fields in both years, forming nine subgroups. The first subgroup consisted of 4 isolates of *F. culmorum*. The second and third subgroups each included 1 isolate of *F. culmorum* and 3 isolates of *F. graminearum*, respectively. The fourth subgroup comprised 2 isolates of *F. sporotrichioides*, while the fifth subgroup consisted of 3 isolates of *F. equiseti*. The sixth subgroup comprised 1 isolate each of *Neonectria* sp., *T. paraviridescens*, and the *F. redolens* isolate 463-5 (FRE_463) from NCBI. The seventh and eighth subgroups contained 4 and 2 isolates of *F. solani*, respectively, and the ninth subgroup comprised 12 isolates of *F. solani* (Supplementary Figure 4.13). Disease severity caused by isolates in Clade VI ranged from 0.61 to 4.00 (Supplementary Table 4.1). An additional 12 isolates of *F. avenaceum* from multiple fields in both years were not grouped to any clade, and caused disease severities ranging from 1.22 to 3.55 (Supplementary Table 4.1).

Phylogenetic tree based on the concatenated TEF-1α and ITS sequences

To obtain concatenated sequences, the aligned ITS and TEF-1α sequences obtained from the 157 isolates were trimmed with several forward and backward characters deleted and concatenated using MEGA 3.0. The resulting concatenated aligned sequence matrix comprised 1,324 characters, with 548 conserved sites, 597 variable sites, and 383 parsimony-informative sites. The average nucleotide composition was 23.0% T, 25.7% C,

24.7% A, and 26.7% G. The concatenated tree had CI, RI, and RC values of 0.629, 0.940, and 0.591, respectively.

The 157 isolates were grouped into six clades in the concatenated tree (Figure 4.4). Clade I comprised 30 isolates of *F. avenaceum* from multiple fields of both years, in which two subgroups were included. In the first subgroup, 15 isolates were divided further into two subgroups. The second subgroup comprised another 15 isolates from multiple fields (Figure 4.4). Disease severity caused by isolates in Clade I ranged from 1.60 to 3.73 (Figure 4.4 and Supplementary Table 4.1). Clade II consisted of 32 isolates of *F. avenaceum* from multiple fields in both years, divided into two subgroups. The first subgroup included 6 isolates from multiple fields in both years, while the second subgroup comprised 26 isolates from multiple fields in both years (Figure 4.4). Disease severity caused by isolates in Clade II ranged from 1.55 to 3.88 (Figure 4.4 and Supplementary Table 4.1). Clade III included 20 isolates from multiple fields in both years, divided into five subgroups. The first subgroup comprised 2 isolates of *F. flocciferum* and 6 isolates of *F. torulosum*, forming two sub-subgroups, respectively. The second subgroup consisted of 4 isolates of *F. torulosum* and 1 isolate of *F. tricinctum*. The third subgroup included 1 isolate of *F. tricinctum*. The fourth subgroup comprised 5 isolates of *F. acuminatum*, with 2 and 3 isolates, respectively, clustering together. The last subgroup included one isolate of *F. avenaceum* (Figure 4.4). Disease severity caused by isolates in Clade III ranged from 1.42 to 2.58 (Figure 4.4 and Supplementary Table 4.1).

Clade IV consisted of 21 isolates from multiple fields in both years, divided into five subgroups. The first subgroup comprised 11 isolates of *F. solani* and 1 *F. torulosum* isolate. The second subgroup included isolates of *F. solani* divided into two different clusters. One isolate each of *F. culmorum*, *Neonectria* sp., and *T. paraviridescens* were separated into three different subgroups (Figure 4.4). Disease severity caused by isolates in Clade IV ranged from 0.61 to 2.54 (Figure 4.4 and Supplementary Table 4.1). In Clade V, 42 isolates representing eight species from multiple fields were grouped together, with three subgroups. In the first subgroup, 4 isolates of *F. culmorum* were divided into two clusters, 4 isolates of *F. oxysporum* were divided into two clusters, 3 isolates each of *F. equiseti* and *F. graminearum* clustered into two individual clusters, and 2 isolates each of *F. proliferatum* and *F. sporotrichioides* were divided into individual clusters. One isolate of *F. commune* represented the last cluster. In the second subgroup, 22 isolates of *F. redolens* from multiple fields were grouped together. The *F. redolens* reference isolate 463-5 (FRE_463) clustered alone as the third subgroup (Figure 4.4). Disease severity caused by isolates in Clade V ranged from 0.64 to 4.00 (Figure 4.4 and Supplementary Table 4.1). Another 12 isolates of *F. avenaceum* from multiple fields in both years formed an outgroup in the phylogenetic tree (Figure 4.4) with disease severity ranging from 1.22 to 3.55 (Figure 4.4 and Supplementary Table 4.1).

4.3.5 Geographic origins and phylogenetic evolution

No correlation was observed between the geographic origins and phylogenetic

evolution in the phylogenetic tree, nor between the years collected and phylogenetic evolution. Most isolates that induced disease severity > 3.0 clustered in Clades I-II consisting of *F. avenaceum* isolates (Figure 4.4). Additionally, 2 *F. sporotrichioides* isolates from Clade III caused the highest disease severity. Many isolates in Clades I-VI also caused significant reductions in seedling emergence, plant height, and shoot dry weight. However, isolates that induced high reductions in root dry weight were generally distributed across different clades (Figure 4.4).

4.4 Discussion

A proper understanding of the identity, diversity and pathogenicity of common fungi can serve as the foundation for effective plant disease management strategies (Klosterman et al., 2009; Maryani et al., 2019). As an emerging disease, canola root rot has garnered increased attention in recent years (Larki & Farrokhi Nejad, 2015; Nemati Mondanipour et al., 2021). Unfortunately, the last comprehensive etiological study of the fungi associated with the canola RRC in Alberta was carried out with collections made in 2009, 2010 and 2011 (Chen et al., 2014). As such, current information on the identity and prevalence of important root rot pathogens in this province is lacking. The present research indicated that *Fusarium* spp. were the predominant fungi in the RRC during 2021-2022, with an average recovery rate of >80% from roots and >60% from stems across the surveyed fields. Other important canola pathogens recovered in this study included *R. solani* and *L. maculans*.

Over 98% (149 of 151) of the isolates recovered in 2021 and ca. 95% (75 of 79) of the isolates recovered in 2022 were confirmed as *Fusarium* spp. *Fusarium avenaceum* was most common among the species found, representing 50% (75 of 149) and 48% (36 of 75) of the *Fusarium* isolates in 2021 and 2022, respectively. In addition, *F. redolens* (18 isolates in 2021 and 7 isolates in 2022) and *F. solani* (14 isolates in 2021 and 8 isolates in 2022) were also fairly common compared with other species. *Fusarium torulosum* (12 isolates) was frequently isolated in 2021, but *F. oxysporum* (7 isolates) was more frequent in 2022. Three species, *F. commune*, *F. equiseti*, and *F. sporotrichioides*, were identified only in 2021. To our knowledge, this is the first report of *F. sporotrichioides* and *F. commune* causing root rot of canola in anywhere in the world, although the former has been identified from *Brassica carinata* (Okello et al., 2018). Collectively, these findings emphasize considerable species-level diversity among *Fusarium* populations extracted from diseased canola tissues, with *F. avenaceum* emerging as both the most abundant and widely distributed species. Additional species, including *F. solani*, *F. redolens*, *F. oxysporum*, and *F. torulosum*, also exhibited a wide distribution. The identification of some species only in certain years suggests that less common components of the pathogen population may sometimes go undetected. Overall, there was no correlation between field location and species.

Fusarium roseum (Schwein.) Petch was identified as the causal agent of canola root rot in the early 1970s (Berkenkamp & Vaartnou, 1972), while *F. avenaceum* was first

reported as causing canola seedling blight in the early 1980s (Calman et al., 1986). In a more recent study, *F. avenaceum* was also identified as a highly aggressive pathogen causing seedling blight of canola, although the predominant species observed was *F. acuminatum* along with *F. culmorum*, *F. equiseti*, *F. redolens*, and *F. torulosam* (Chen et al., 2014). In reference to other field crops, *Fusarium* spp. have been documented extensively as a cause of root rot in soybean (Nyandoro, 2017; Zhou et al., 2018), chickpea (Zhou et al., 2021), lupine (Chang et al., 2014b; Holtz et al., 2011, 2013), wheat (Moya-Elizondo et al., 2011), barley (Fernandez, 2007; Fernandez et al., 2009; Gentosh et al., 2020), as well as pea and lentil (Chatterton et al., 2019). These crops are commonly suggested as rotation options with canola to achieve sustainable production and mitigate root rot disease (Gill, 2018; Soon et al., 2005). However, the significant species-level diversity of *Fusarium* recovered from canola, coupled with the wide host range of many of these pathogens, highlights the potential limitations of crop rotation for the management of canola root rot. For instance, the predominant pathogen identified in this study, *F. avenaceum*, was also reported to be one of the most aggressive and common species causing root rot of pea (Feng et al., 2010; Safarieskandari et al., 2021) and a cause of significant disease in chickpea, dry bean, faba bean, and lentil (Feng et al., 2010; Hwang et al., 1994; Safarieskandari et al., 2021; Zhou et al., 2021).

Bioassays to evaluate fungal pathogenicity offer a direct means of estimating the visual damage inflicted on the host (Bock et al., 2022), while monitoring of plant growth

parameters aids in understanding the pathogen impact on host development (Chang et al., 2018). In this study, the pathogenicity testing included assessments of disease severity, seedling emergence, plant height, and shoot and root dry weights after inoculation of canola with isolates of *Fusarium*. The results showed a wide range of root rot severities, spanning from 0.58 to 4.0. The majority of isolates (159 out of 205), however, caused moderate to severe root rot, with disease severities ranging from 1.0 to 3.0. Another 40 isolates, consisting of *F. avenaceum* and *F. sporotrichioides*, caused very severe disease (>3.0). Earlier studies have reported *F. avenaceum*, *F. solani*, and *F. equiseti* as causing the most severe symptoms on canola (Chen et al., 2014; Larki & Farrokhi Nejad, 2015).

Similarly, the majority of isolates had notable effects on the growth of the canola plants. Isolates of *F. avenaceum*, along with a few isolates of *F. solani*, *F. redolens*, *F. culmorum*, *F. equiseti*, and *F. sporotrichioides*, stood out as among the most aggressive in reducing root and shoot biomass. Seedling emergence were also reduced by more than 70% of the isolates, with *F. avenaceum*, *F. solani*, *F. sporotrichioides*, and *F. culmorum* identified as the most aggressive species. In contrast, plant height was less affected, with a third of the isolates not having a significant effect on this parameter. Indeed, one isolate each of *F. solani* and *F. tricinctum* significantly increased plant height, and they did not reduce shoot dry weight or seedling emergence. This might indicate that these isolates have some beneficial functions in promoting plant growth. Both *F. solani* and *F. tricinctum* have been reported as potentially beneficial endophytic fungi in plants in responding to biotic

stress (Ma et al., 2023; Pappas et al., 2018).

Correlation analysis indicated a robust negative association between disease severity and all plant growth parameters evaluated. In a previous study, a similar negative correlation was observed between disease severity and canola seedling emergence after co-inoculation with *F. proliferatum* and *F. oxysporum* (Yu et al., 2023a). In contrast, all four growth parameters exhibited positive correlations with each other. Madden et al. recommended principal component analysis (PCA) for quantitatively assessing plant disease epidemics (Madden & Pennypacker, 1979). In this study, PC1 and PC2 accounted for over 85% of the overall variation among all isolates. Additionally, disease severity and the four growth parameters all emerged as major contributors to PC1, while root dry weight and plant height were important in PC2. The use of correlation analysis and PCA of disease severity and growth parameters allows for a more comprehensive evaluation of pathogenicity, disease development and host growth characteristics.

With the advancement of molecular biology techniques, sequencing-based analyses have emerged as valuable tools in taxonomic studies, supplementing traditional phenotypic and morphological methods (Chehri et al., 2015; Yli-Mattila et al., 2002). In the present study, three distinct phylogenetic trees were constructed based on the fungal ITS sequence, TEF-1α sequence, and the concatenated ITS and TEF-1α sequences. The broad topological structures of these phylogenetic trees exhibited similarity and effectively depicted the phylogenetic relationships within the *Fusarium* genus. Notably, the phylogenetic tree

generated from the ITS sequences highlighted distinct clustering for most species, with the exception of genetically related species such as *F. avenaceum*, *F. tricinctum*, *F. torulosum*, and *F. acuminatum*, which grouped together. This was anticipated, as these four species constitute part of the *Fusarium tricinctum* species complex (FTSC), which has emerged as a disease issue in small grain cereals and other crops (Laraba et al., 2022; Munkvold et al., 2021). Similar findings were reported in previous phylogenetic investigations of *Fusarium* species associated with canola seedling blight (Chen et al., 2014), as well as across species originating from diverse geographical locations (Yli-Mattila et al., 2002).

The TEF-1α sequence analysis resulted in six distinct clades, effectively distinguishing all the *Fusarium* species. Additionally, the construction of the phylogenetic tree based on the concatenated sequences of the ITS and TEF-1α regions effectively organized the *Fusarium* species into distinct clades. Across all three phylogenetic trees, the data consistently indicated a relatively low diversity within *F. avenaceum*, aligning with findings from previous studies (Chen et al., 2014; Feng et al., 2010). While a close phylogenetic relationship was observed among *F. avenaceum*, *F. tricinctum*, *F. torulosum*, and *F. acuminatum*, highlighting their genetic similarity, *F. redolens* and *F. oxysporum* exhibited relatively greater genetic distance from *F. avenaceum*. No correlations were between the phylogenetic grouping and geographic origin, year of isolation or pathogenicity characteristics of the isolates. This may reflect the relatively restricted geographic scale of the survey, which was limited to northcentral Alberta, and the fact that

samples were collected over two consecutive years. The phylogenetic tree based on concatenated sequences not only facilitated clear differentiation between the various species, but also allowed further classification within species, as exemplified by *F. avenaceum*. This suggests that employing concatenated sequences in phylogenetic tree construction is a superior strategy, particularly for species with limited genetic diversity. Similarly, concatenated analyses of multi-locus DNA sequence data are widely recognized as a powerful and commonly used method for exploring evolutionary and phylogenetic relationships between isolates, for example with the *Fusarium oxysporum* species complex (Achari et al., 2020). In an earlier report, the enhanced discriminatory capacity of the combined phylogenetic tree was particularly pronounced for species like *F. avenaceum*, *Fusarium arthrosporioides* Sherb., and *F. tricinctum*, which exhibit morphological similarities (Yli-Mattila et al., 2002).

In conclusion, this study addressed the emerging challenge of canola root rot via a comprehensive investigation of its causal agents and their impact on plant growth. *Fusarium* spp. represent an important component of the canola RRC, with this study suggesting that *F. avenaceum* is the predominant and most aggressive species. Principal component analysis confirmed the necessity of a comprehensive assessment of pathogenicity. Phylogenetic analysis, based on the ITS and TEF-1α sequences, offered insights into the genetic relationships among *Fusarium* species, with concatenated sequences proving particularly effective in discerning closely related populations. This

study also highlighted the need for integrated strategies for root rot management, and emphasizing the role of both disease assessment and growth parameter evaluation in understanding pathogenicity. The findings provide an update on the status of the canola RRC in Alberta and pave the way for more targeted control measures to mitigate its impact on crop production.

Table 4.1. Root rot severity and reductions in emergence and plant height following inoculation of the canola cv. 'Westar' with isolates representing different *Fusarium* species under greenhouse conditions.

Species[a]	Disease Severity[b]					Reduction in Emergence[c] (%)					Reduction in Height[d] (%)				
	Range (0-4)	0-1.0	1.01-2.0	2.01-3.0	3.01-4.0	Range	<25	25.1-50	50.1-75	>75	Range	<25	25.1-50	50.1-75	>75
FAC	1.50-2.41	0	3	3	0	19.7-37.7	4	2	0	0	-1.9-17.7	6	0	0	0
FAV	1.22-3.88	0	17	51	37	10.5-93.4	11	31	39	24	-8.6-71.6	66	28	11	0
FCO	2.04	0	0	1	0	10.5	1	0	0	0	14.70	1	0	0	0
FCU	1.46-2.37	0	5	5	0	13.1-80.3	4	5	0	1	10.1-60.0	3	6	1	0
FEQ	1.86-2.52	0	1	3	0	38.2-50.0	0	4	0	0	47.9-57.5	0	1	3	0
FFL	1.42-1.72	0	2	0	0	16.4-17.1	2	0	0	0	6.9-8.9	2	0	0	0
FGR	2.19	0	0	1	0	44.70	0	1	0	0	2.40	1	0	0	0
FOX	1.48-2.45	0	4	4	0	14.8-59.2	1	4	3	0	2.5-33.1	7	1	0	0
FPR	1.71	0	1	0	0	14.5	1	0	0	0	-9.7	1	0	0	0
FRE	0.58-2.04	3	21	1	0	1.3-52.5	19	5	1	0	-18.6-47.2	14	11	0	0
FSO	0.61-2.54	3	16	2	0	2.6-77.0	18	2	0	1	-5.3-54.9	13	7	1	0
FSP	4	0	0	0	2	100	0	0	0	2	91.2-100	0	0	0	2
FTO	1.19-2.58	0	10	3	0	6.6-44.7	10	3	0	0	-4.3-40.7	8	5	0	0
FTR	1.78-1.96	0	3	0	0	11.5-23.0	3	0	0	0	-8.6-17.1	3	0	0	0
Fsp	2.41-3.48	0	0	2	1	29.5-73.8	0	1	2	0	17.6-35.0	2	1	0	0
Total	0.58-4.0	6	83	76	40	1.3-100	74	58	45	28	-18.6-100	127	60	16	2

[a]FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCO, *Fusarium commune*; FCU, *Fusarium culmorum*; FEQ, *Fusarium equiseti*; FFL, *Fusarium flocciferum*; FGR, *Fusarium graminearum*; FOX, *Fusarium oxysporum*; FPR, *Fusarium proliferatum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; FSP, *Fusarium sporotrichioides*; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; and

Fsp, unidentified species

[b]Root rot disease severity (0-4 scale) at 21 days after seeding; the number of isolates of each species under each set of values is indicated

[c]Reduction in seedling emergence in the inoculated treatment relative to the non-inoculated control; the number of isolates of each species under each set of values is indicated

[d]Reduction in plant height at 14 days after seeding in the inoculated treatment relative to the non-inoculated control; the number of isolates of each species under each set of values is indicated

Table 4.2. Reductions in shoot and root dry weights of canola cv. 'Westar' following inoculation with isolates representing different *Fusarium* species under greenhouse conditions.

Species[a]	Shoot Dry Weight Reduction[b] (%)					Root Dry Weight Reduction[c] (%)				
	Range	<25	25.1-50	50.1-75	>75	Range	<25	25.1-50	50.1-75	>75
FAC	-26.5-37.3	3	3	0	0	55.8-77.7	0	0	5	1
FAV	-38.9-94.9	15	37	28	25	30.6-98.2	0	6	24	75
FCO	30.5	0	1	0	0	63.5	0	0	1	0
FCU	-19.6-75.2	5	3	1	1	18.8-84.9	1	0	5	4
FEQ	42.5-54.2	0	2	2	0	78.0-84.1	0	0	0	4
FFL	5.3-24.9	2	0	0	0	54.5-75.1	0	0	1	1
FGR	24.7	1	0	0	0	64.7	0	0	1	0
FOX	-13.7-47.2	4	4	0	0	45.5-79.7	0	2	5	1
FPR	-6.7	1	0	0	0	41.2	0	1	0	0
FRE	-67.9-52.2	15	9	1	0	11.9-82.2	2	3	15	5
FSO	-24.3-58.8	15	3	3	0	23.3-87.7	1	2	11	7
FSP	96.4-98.6	0	0	0	2	96.6-99.6	0	0	0	2
FTO	-33.7-43.9	10	3	0	0	46.9-79.1	0	2	6	5
FTR	-12.8-20.3	3	0	0	0	43.9-62.6	0	1	2	0
Fsp	27.3-82.5	0	2	0	1	61.2-93.9	0	0	2	1
Total	-67.9-98.6	74	67	35	29	11.9-99.6	4	17	78	106

[a]FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCO, *Fusarium commune*; FCU, *Fusarium culmorum*; FEQ, *Fusarium equiseti*; FFL, *Fusarium flocciferum*; FGR, *Fusarium graminearum*; FOX, *Fusarium oxysporum*; FPR, *Fusarium proliferatum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; FSP, *Fusarium sporotrichioides*; FTO, *Fusarium torulosum*, FTR, *Fusarium tricinctum*; and

Fsp, unidentified species

[b]Reduction in shoot dry weight in the inoculated treatment relative to the non-inoculated control; the number of isolates of each species under each set of values is indicated

[c]Reduction in root dry weight in the inoculated treatment relative to the non-inoculated control; the number of isolates of each species under each set of values is indicated

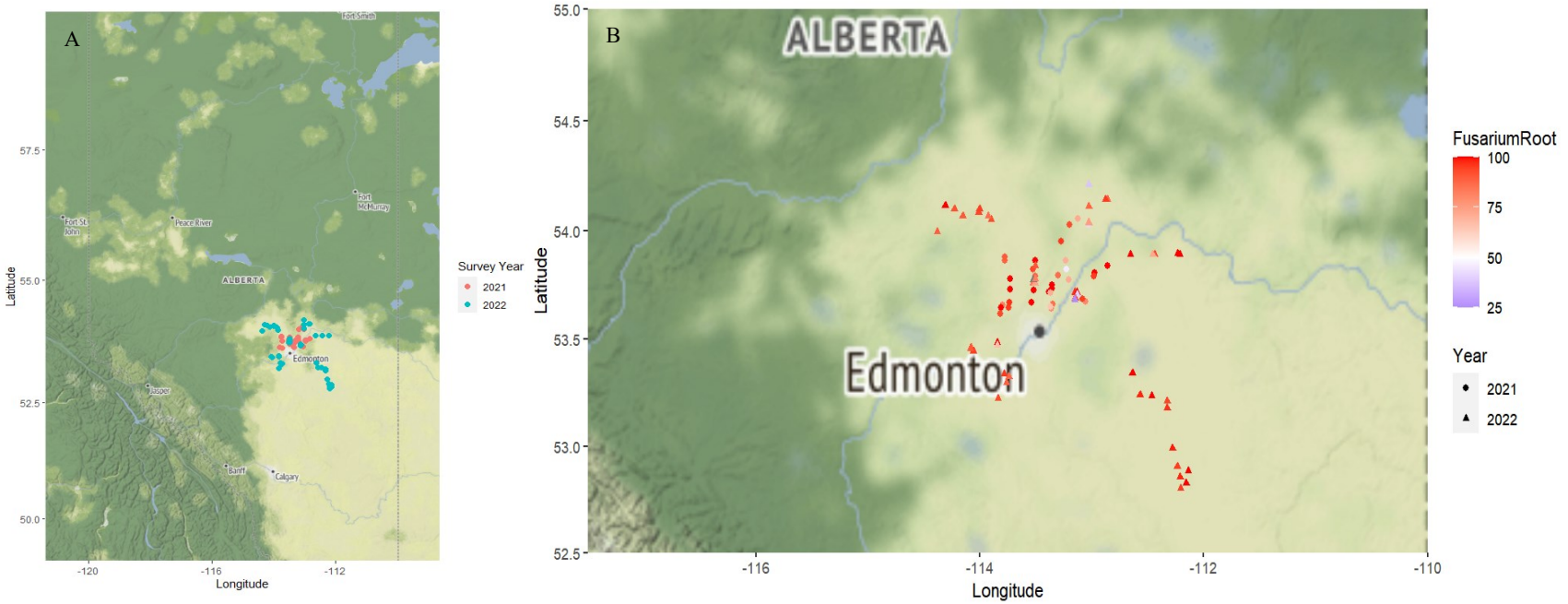


Figure 4.1. Map of Alberta (A) and the incidence of *Fusarium* recovered from diseased canola root samples (B) collected across 86 field sites in 2021 and 2022. Longitude and latitude indicate the position of surveyed fields on the maps. Fields visited in 2021 and 2022 are indicated in red and blue, respectively, in panel (A), and as circles and triangles in panel (B). FusariumRoot represents the incidence (%) of *Fusarium* spp. recovered from root samples within each surveyed field, as reflected on the color scale.

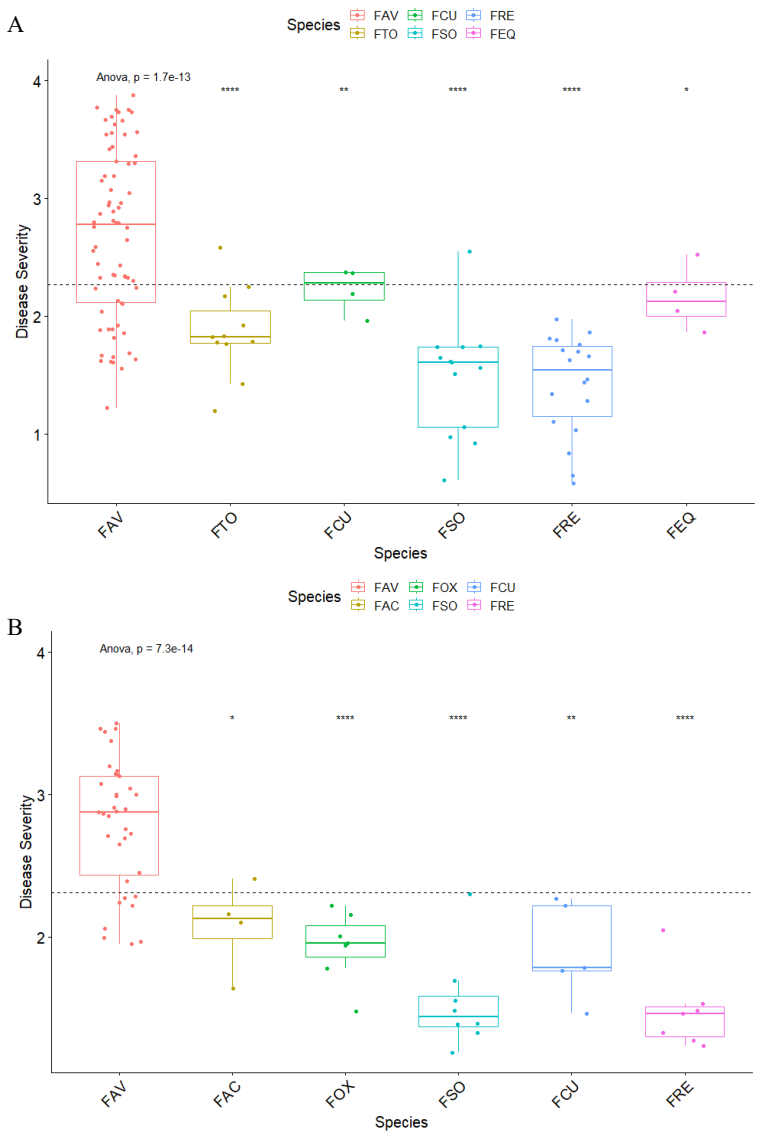


Figure 4.2. Root rot disease severity on the canola cv. 'Westar' caused by isolates representing different *Fusarium* spp. recovered in 2021 (A) and 2022 (B). FAV, *Fusarium avenaceum*; FCU, *Fusarium culmorum*; FRE, *Fusarium redolens*; FTO, *Fusarium torulosum*; FSO, *Fusarium solani*; FEQ, *Fusarium equiseti*; FOX, *Fusarium oxysporum*; and FAC, *Fusarium acuminatum*. Inoculations were conducted under greenhouse conditions and rated on a 0 to 4 scale (Hwang et al., 1994), where: 0 = healthy roots and 4 = tap root severely girdled, brown lesions on >75% of the tap root with limited lateral roots. ns, no significant difference in disease severity between *Fusarium avenaceum* and each of the other *Fusarium* species on a t-test; *, significant difference at $p < 0.05$; **, significant difference at $p < 0.01$; ***, significant difference at $p < 0.001$; and ****, significant difference at $p < 0.0001$. The dashed lines represent the overall mean disease severity across species.

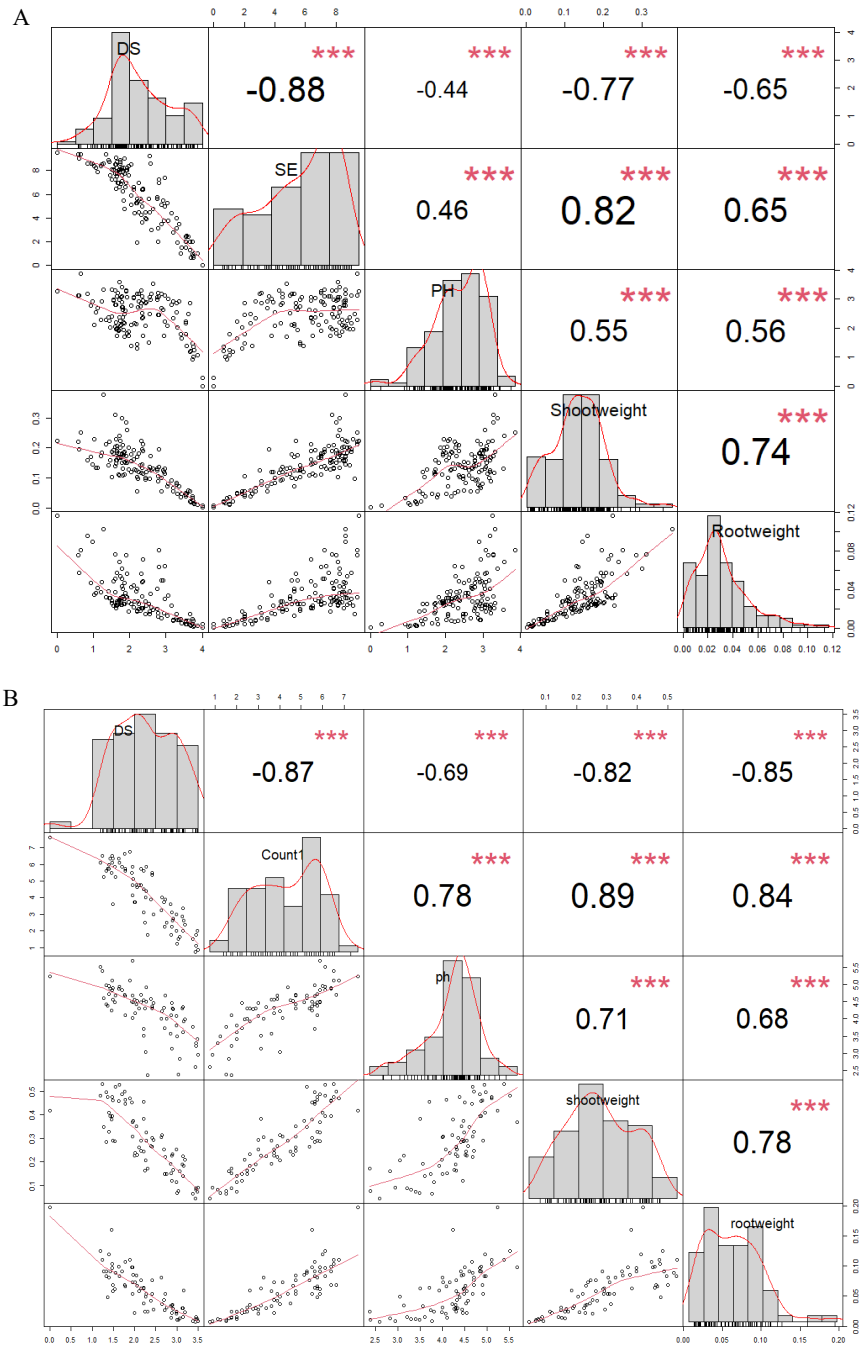


Figure 4.3. Correlation analysis between disease severity (DS), seedling emergence (SE or Count1), plant height (PH or ph), shoot dry weight (Shootweight or shootweight) and root dry weight (Rootweight or rootweight) after inoculation of the canola cv. 'Westar' with isolates of *Fusarium* collected in 2021 (A) and 2022 (B). Inoculations were conducted under greenhouse conditions. *, significant correlation at $p < 0.05$; **, significant correlation at $p < 0.01$; ***, significant correlation at $p < 0.001$.

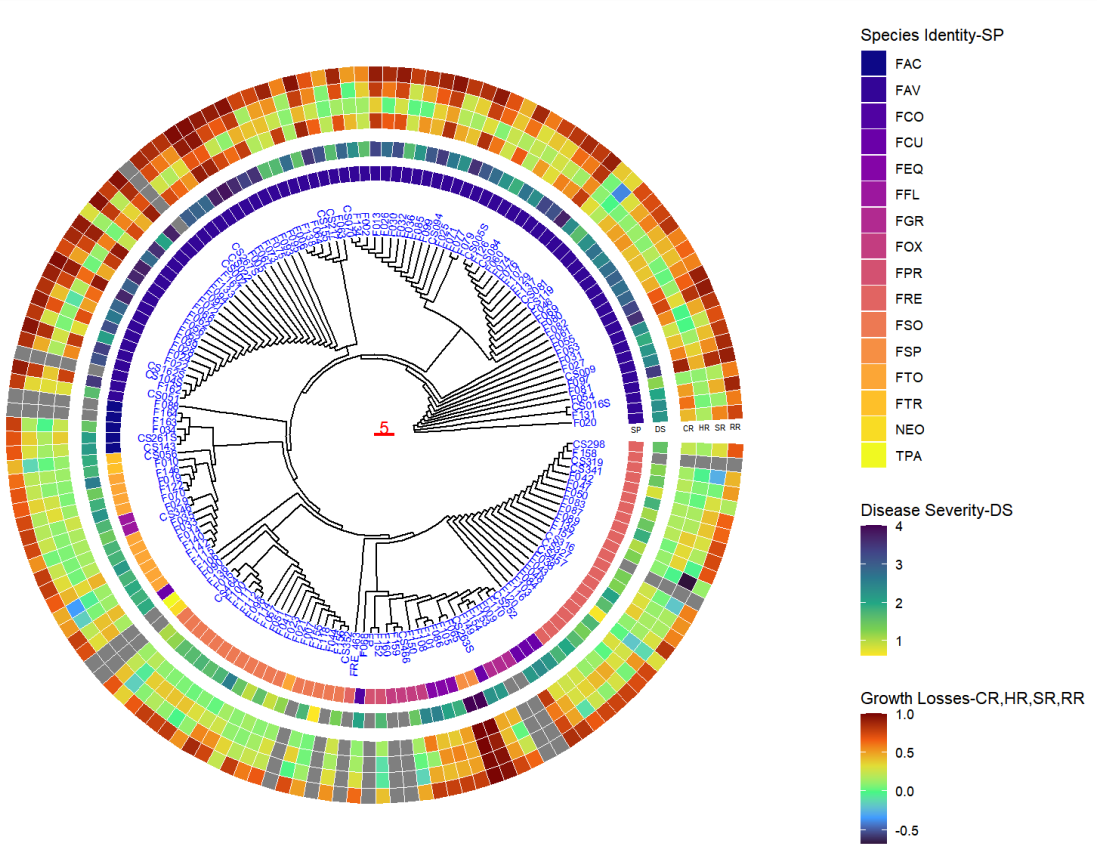


Figure 4.4. Maximum parsimony tree based on the concatenated internal transcribed spacer (ITS) and elongation factor (EF1-α) sequences from 157 fungal isolates, including 112 isolates recovered from canola in 2021 (F002-F146), 31 isolates recovered from canola in 2022 (CS002-CS466), 13 reference isolates from a laboratory culture collection (FP, F153-F164), and sequences from one *Fusarium redolens* isolate FRE_463 retrieved from GenBank, National Center for Biotechnology Information (NCBI). SP, species identity; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCO, *Fusarium commune*; FCU, *Fusarium culmorum*; FEQ, *Fusarium equiseti*; FFL, *Fusarium flocciferum*; FGR, *Fusarium graminearum*; FOX, *Fusarium oxysporum*; FPR, *Fusarium proliferatum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; FSP, *Fusarium sporotrichioides*; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; NEO, *Neonectria* sp.; and TPA, *Trichoderma paraviridescens*. DS, disease severity on rated on a 0 to 4 scale (Hwang et al., 1994), where: 0 = healthy roots and 4 = tap root severely girdled, brown lesions on >75% of the tap root with limited lateral roots. Growth Losses, reductions in seedling emergence (CR), plant height (HR), shoot dry weight (SR), and root dry weight (RR) following inoculation with each fungal isolate and relative to the corresponding non-inoculated control.

Chapter 5 Characterization of the virulence and yield impact of *Fusarium* species on canola (*Brassica napus*)

5.1 Introduction

Canola (*Brassica napus* L.) is an important crop on the Canadian prairies, contributing an average of $10.0 billion annually in farm cash receipts between 2017 and 2021 (Mattson et al., 2007). The production of this crop, however, can be constrained by root rot, a soilborne disease caused by a complex of pathogens, including *Fusarium* spp., *Rhizoctonia solani*, and *Pythium* spp.(Zhou et al., 2014a). Root rot is commonly associated with seed decay, damping off, and the decomposition of infected root tissues, resulting in substantial yield losses, particularly under conditions favorable for disease development (Hwang et al., 2015a; Larki & Farrokhi Nejad, 2015). Partial or nearly complete yield losses have been reported because of root rot in canola (Calman et al., 1986; Hwang et al., 1986; Kataria & Verma, 1992; Verma, 1996). In western Canada, *R. solani* is one of the primary pathogens responsible for root rot and damping off (Kataria & Verma, 1992). However, *Fusarium* species, including *F. oxysporum* and *F. avenaceum*, also play crucial roles in the root rot complex (Chen et al., 2014; Hwang et al., 1994; Yu et al., 2022a; Zhou et al., 2014a). In recent years, *F. proliferatum* has been reported with increasing frequency as a cause of root rot, crown rot, yellowing, and wilting in various vegetable, fruit, and field crops (Amby et al., 2015; Li et al., 2017; Palacios et al., 2015; Proctor et al., 2010). Several *Fusarium* spp., including *F. proliferatum*, were the most frequently isolated fungi

in diseased canola root and stem samples collected from central and northern Alberta, Canada, in 2021 (Yu et al., 2022a). Inoculating canola with these *F. proliferatum* isolates resulted in significant reductions in seedling emergence and plant height, confirming the pathogenicity of this fungus on canola (Yu et al., 2022a). The wide range of hosts susceptible to *Fusarium* spp. (Li et al., 2017; Safarieskandari et al., 2021), coupled with their ability to survive as saprophytes and produce long-lived resting structures (chlamydospores), makes these fungi challenging to manage (Nelson et al., 1994). Furthermore, a recent study (Delgado-Baquerizo et al., 2020) suggests that the projected increase in temperatures due to climate change may increase the occurrence and severity of root rot and related diseases in the coming decades.

While the cultivation of resistant crops is widely recognized as a cost-effective and environmentally friendly strategy for disease management (Dolatabadian et al., 2022), the availability of resistance to Fusarium root rot appears to be restricted (Okello et al., 2018). Additionally, the effectiveness of crop rotation and other cultural management strategies can be limited for this disease (Marburger et al., 2015), primarily due to the broad host range of *Fusarium* spp., which limits options for non-susceptible crops to include in the rotations. Fungicide seed treatments have been widely applied to manage soilborne pathogens (Broders et al., 2007; Esker & Conley, 2012), but their efficacy against different *Fusarium* spp. varies (Ellis et al., 2011; Munkvold & O'Mara, 2002). While one study investigated the impacts of seed size, fungicidal seed treatment, seeding depth, and seeding

date on canola seedling blight caused by *F. avenaceum* (Hwang et al., 2015), the capacities of different *Fusarium* spp. to cause root rot in canola, and their effects on yields, have not been evaluated. Moreover, both *F. proliferatum* and *F. oxysporum* have been identified as capable of invading the xylem, causing similar symptoms including wilting and xylem discoloration (Klassen et al., 2007; Punja, 2021). These shared patterns in disease progression imply similarities in the interactions between these *Fusarium* species and the host plant. This study aimed to enhance understanding of the potential impacts of *F. proliferatum*, *F. avenaceum*, and *F. oxysporum* on canola production, and had three specific objectives: (1) assessing the abilities of different *Fusarium* spp. to cause root rot; (2) evaluating the effects of *Fusarium* spp. on seedling emergence and yield; and (3) investigating the interrelationship between *F. proliferatum* and *F. oxysporum* in disease development and growth reduction in canola.

5.2 Materials and Methods

5.2.1 Isolates of *Fusarium* spp. and inoculum production

One isolate each of *F. proliferatum* (P002), *F. avenaceum* (F4A), and *F. oxysporum* (FOC4), originally collected from canola tissues showing symptoms of root rot (Yu et al., 2022a), were included in this study. All isolates were stored at 4 °C on potato dextrose agar (PDA) until use.

An inoculum of each isolate was generated separately on a wheat grain medium (Hwang, 1988). Briefly, grains of wheat (1 L) were soaked overnight in tap water, and then

placed in a Hi Patch Mushroom Spawn Bag (Western Biologicals, Aldergrove, BC, Canada). The bag was sealed with a foam insert, secured with a collar, and autoclaved for 90 min. After it cooled to room temperature, the grain was inoculated with 0.5 cm diam. agar plugs excised from 14-day-old cultures of *F. proliferatum*, *F. avenaceum*, or *F. oxysporum* produced on PDA. The inoculated grains were mixed thoroughly by shaking and placed in an incubation chamber under darkness at room temperature for 5 weeks, allowing for complete colonization of the kernels. Subsequently, the inoculum was allowed to air-dry at 25 °C for 3 days, ground, and passed through a 2.0 mm mesh sieve. The grain inoculum was stored at 4 °C for a maximum of 2 months until further use.

5.2.2 Effects of *F. proliferatum* and *F. avenaceum* under greenhouse conditions

The abilities of *F. proliferatum* and *F. avenaceum* to cause root rot on canola, as well their impact on plant growth parameters, were evaluated under greenhouse conditions. The experiments were arranged in a randomized complete block design with five replicates, utilizing the canola hybrids '45CS40', 'L234', 'CS2000', and '45H31'. In trials with *F. avenaceum*, the open-pollinated canola cultivar 'Westar' was included as an additional host. Each canola genotype was planted at a density of 10 seeds per cup in 473 mL cups (Uline, Toronto, ON, Canada) filled with 400 mL of Promix PGX potting medium (Sun-Gro Canada Inc., Seba Beach, AB, Canada). The grain inoculum of each fungal species was blended with the potting medium at specific ratios, determined based on a preliminary assessment of isolate aggressiveness. For *F. proliferatum*, the grain inoculum was mixed

with the potting medium in the following ratios (*v*/*v*): 1:67, 1:100, 1:133, 1:200, 1:400, 1:1000, and 1:2000. These ratios resulted in inoculum densities ranging from 9×10^4 to 3×10^3 colony-forming units (cfu) per gram of potting mix. The grain inoculum of *F. avenaceum* was mixed with the potting mix (*v*/*v*) in the following ratios: 3:17, 3:22, 2:23, 3:47, 1:24, and 1:49. These ratios resulted in inoculum densities ranging from 9×10^5 to 1.2×10^5 cfu per gram of soil mixture. Controls did not receive grain inoculum from either species (i.e., 0:1 (*v*/*v*), 0%).

After inoculation, the cups were carefully transferred to a greenhouse and maintained at approximately 25 °C under a 12 h photoperiod. Seedling emergence was recorded on the 7th day after seeding. On the 21st day after seeding, the plants were gently uprooted and thoroughly washed with tap water to assess root rot severity, as described below. All experiments were repeated.

5.2.3 Effects of *F. proliferatum* and *F. avenaceum* under field conditions

The effects of *F. proliferatum* and *F. avenaceum* inoculation on the growth and yield of canola were assessed under field conditions. Two field experiments were conducted at the St. Albert Research Station (53°42′ N, 113°38′ W), St. Albert, Alberta, over two years (seeded on 4 June 2021 and 24 May 2022), with one site in 2021 and two sites in 2022. No occurrences of root rot disease were previously observed at the designated field site. Two widely cultivated canola hybrids, ‘45H31’ and ‘CS2000’, were sown in plots treated with grain inoculum, as previously described by Hwang et al. (Hwang et al., 2015). Each

plot consisted of four 6 m rows with 30 cm spacing between rows, seeded at a rate of 0.7 g seeds per row. Trials were arranged in a randomized split-plot design with four replicates, with varieties as the main plot and different inoculum levels as subplots. For *F. avenaceum*, the grain inoculum was added with the canola seed at rates of 0 (control), 50, 100, and 150 mL per 6 m row. For *F. proliferatum*, grain inoculum was added with the canola seed at rates of 0 (control), 5, 10, and 15 mL per 6 m row. The relative rates of inoculum added for each species were based on a preliminary assessment of aggressiveness, as described above. Emergence at the seedling stage, disease severity at the flowering stage, and yield per plot at maturity were recorded for both experiments.

5.2.4 Effects of *F. proliferatum* and *F. oxysporum* alone or in combination under greenhouse conditions

To assess the impacts of *F. proliferatum* and *F. oxysporum,* both individually and in combination, on root rot development and canola growth and yield, two repeated greenhouse experiments were conducted using the canola hybrids '45H31' and 'CS2000'. Canola seeds were sown in rectangular boxes (45 cm length × 30 cm width × 20 cm depth, 16 Qt.; Sterilite, Townsend, MA, USA), filled with 5 L of Promix PGX potting medium (Sun-Gro Canada Inc., Seba Beach, AB, Canada). The planting density was maintained at 12 seeds per row, with 3 rows per box.

Grain inocula of *F. proliferatum* and *F. oxysporum* were applied by mixing with the soilless mix in various ratios, determined based on a preliminary assessment. These ratios

(*v*/*v*/*v*) included: 0 mL *F. proliferatum* (*Fp*): 0 mL *F. oxysporum* (*Fo*): 5000 mL soilless mix (control treatment), 75 mL *Fp*: 0 mL *Fo*: 5000 mL soilless mix, 0 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix, 22.5 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix, 75 mL *Fp*: 22.5 mL *Fo*: 5000 mL soilless mix, 75 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix, 37.5 mL *Fp*: 37.5 mL *Fo*: 5000 mL soilless mix, and 22.5 mL *Fp*: 22.5 mL *Fo*: 5000 mL soilless mix. Each treatment was replicated four times in separate boxes. The boxes were placed in a greenhouse at a temperature of approximately 25 °C under a 12 h photoperiod. The experiment was arranged in a randomized complete block design.

Seedling emergence was assessed on the 7th day after seeding. On the 21st day after seeding, plants from the second row were carefully uprooted and washed under tap water to assess root rot severity, as described below. The remaining two rows were allowed to grow until maturity, and disease severity, seed weight, and the number of pods were recorded for each plant at maturity. Seed weight and the number of pods per experimental unit (box) were calculated by adding the values obtained from all individual plants within the corresponding box.

5.2.5 Effects of *F. proliferatum* and *F. oxysporum* alone or in combination under field conditions

To evaluate the effects of *F. proliferatum* and *F. oxysporum* alone or in combination on canola growth and yield, another field experiment was conducted in 2022 at two sites at the St. Albert Research Station, where no prior instances of root rot disease had been

identified. The canola hybrids '45H31' and 'CS2000' were sown in plots treated with grain inoculum, as previously described by Hwang et al. (Hwang et al., 2015). Plots consisted of four 6 m rows with 30 cm spacing between rows, sown at a rate of 0.7 g canola seeds per row, as described above. Trials were arranged in a randomized split-plot design, with varieties as the main plot and inoculum levels as the subplots, and four replicates per treatment. The experiment was carried out with grain inocula of *F. proliferatum* and *F. oxysporum* mixed with sand at different ratios, which were then applied at a final rate of 100 mL per row. These ratios included: 0 mL *Fp*: 0 mL *Fo*: 100 mL sand (control treatment), 50 mL *Fp*: 0 mL *Fo*: 50 mL sand, 0 mL *Fp*: 50 mL *Fo*: 50 mL sand, 15 mL *Fp*: 50 mL *Fo*: 35 mL sand, 50 mL *Fp*: 15 mL *Fo*: 35 mL sand, 50 mL *Fp*: 50 mL *Fo*: 0 mL sand, 25 mL *Fp*: 25 mL *Fo*: 50 mL sand, and 15 mL *Fp*: 15 mL *Fo*: 70 mL sand. Seedling emergence, disease severity at flowering, and yield per plot at the maturity stage were recorded.

5.2.6 Disease ratings

Root rot severity was assessed on a 0–4 scale (Hwang et al., 1994), for which: 0 = healthy roots; 1 = small, light-brown lesions on <25% of the tap root; 2 = brown lesions on 25–49% of the tap root; 3 = brown lesions on 50–74% of the tap root, tap root constricted; and 4 = tap root severely girdled, brown lesions on >75% of the tap root with limited lateral roots. In greenhouse studies, the final disease severity per experimental unit (cup or box) was calculated by averaging the values of all of the individual plants within

each cup or box. In field experiments, the final root rot severity per plot was determined by averaging the values of 20 representative plants randomly selected from each plot.

5.2.7 Seedling emergence and seed weight

Seedling emergence was determined by counting all surviving plants per experimental unit (cup or box) in the greenhouse or per plot in the field. The seed weight was calculated by adding the yield of all individual plants within each experimental unit in the greenhouse or per plot in the field.

5.2.8 Weather data acquisition

Weather data (precipitation and temperature) for the St. Albert Research Station were collected using the Current and Historical Alberta Weather Station Data Viewer (https://acis.alberta.ca/acis/weather-data-viewer.jsp; accessed on 13 November 2022), Government of Alberta.

5.2.9 Data analysis

Normality was assessed using the Shapiro-Wilk test, while the homogeneity of variance was evaluated using Levene's test within ANOVA. All analyses were carried out in R Studio v. 4.1.2 (RStudio Team, 2020). Differences were considered significant at $p \leq 0.05$ unless otherwise noted. If there was a significant interaction suggested between repetition (site or year) and treatment, the data were analyzed separately. Linear regression analysis was performed to evaluate the relationships between root rot severity and plant yield parameters under greenhouse conditions.

5.3 Results

5.3.1 Temperature and rainfall

Precipitation and temperature at the St. Albert Research Station differed between 2021 and 2022, particularly in June, July, and September (Supplementary Table 5.1 and Supplementary Figure 5.1). The precipitation totals in June and July of 2022 surpassed those of 2021, with values of 129.3 mm and 43.3 mm, respectively, compared with 41.6 mm and 28.7 mm in the previous year.

Monthly mean temperatures, as well as the maximum and minimum temperatures, were higher in August and September of 2022 in comparison with those of 2021. Nonetheless, very high temperatures (>30 °C) occurred earlier in 2021, specifically during the final week of June. The daily maximum temperatures during that period ranged from 30.7 °C to 35.9 °C. In contrast, a maximum temperature of >30 °C did not occur until July 28 in 2022, while the highest recorded temperature (33.7 °C) was in early September. No spell of very high temperatures lasted more than three consecutive days during the 2022 growing season.

5.3.2 Effects of *F. proliferatum* and *F. avenaceum* under greenhouse conditions

In nearly all cases, canola seedling emergence was reduced significantly following inoculation with *F. proliferatum* or *F. avenaceum* (Figure 5.1). Particularly large reductions in emergence were observed under moderate to high inoculum concentrations. The only

exception was for ‘L234’ after inoculation with *F. avenaceum*, where a significant reduction in emergence was not detected.

As expected, non-inoculated treatments did not develop any symptoms of root rot (disease severity = 0). In contrast, treatment with any concentration of *F. proliferatum* (Figure 5.1, A1 and A2) or *F. avenaceum* (Figure 5.1, B1 and B2) inoculum resulted in a significant increase in disease. Root rot generally became more severe with increasing inoculum concentration, with average ratings of 2.3 and 2.1 in ‘45CS40’, 2.3 and 2.0 in ‘L234’, 2.4 and 2.4 in ‘CS2000’, and 2.1 and 2.5 in ‘45H31’, following inoculation with the highest rates of *F. proliferatum* and *F. avenaceum*, respectively. ‘Westar’, which was treated only with *F. avenaceum*, developed a root rot severity rating of 2.9 at the highest inoculum concentration.

5.3.3 Effects of *F. proliferatum* and *F. avenaceum* under field conditions

In the two field experiments, both the year and the interactions between year and treatment had a significant effect on most parameters tested, including yield and emergence. Additionally, notable site effects were observed in 2022 for certain parameters (emergence after inoculation with *F. avenaceum*), although the interactions between site and other factors were largely non-significant. Consequently, for subsequent analysis, the data sets from the two years were examined separately, without considering the site effect.

In 2021, inoculation with *F. proliferatum* resulted in a reduction in yield, while simultaneously increasing root rot severity compared with the non-inoculated control

(Table 5.1). Both cultivars exhibited a significant increase in disease severity with increasing inoculum concentration, while emergence decreased from the control to the moderate inoculum concentration. The yield of cultivar 'CS2000' experienced a significant reduction (as high as 29.80%) under high inoculum concentration. Cultivar '45H31', on the other hand, incurred yield losses ranging from 6.96% to 10.00%. Likewise, in 2022, inoculation with *F. proliferatum* had detrimental effects on yield, emergence, and disease severity. The root rot severities in both cultivars were significantly higher than those of their respective controls under all inoculum levels, and increased with escalating inoculum concentration. The seedling emergence of '45H31' declined across all inoculum levels, while the emergence of 'CS2000' was reduced at moderate to high inoculum concentrations. Inoculation had a significant effect on the yield of '45H31', with yield losses of 12.76%, 13.01%, and 25.77% under low, moderate, and high inoculum concentrations, respectively. Likewise, for 'CS2000', all treatments also led to a significant decrease in yield, ranging from 18.16% to 31.37%.

In 2021, both cultivars exhibited reduced yield and emergence, as well as increased root rot severity, following inoculation with *F. avenaceum* (Table 5.2). Yield losses ranged from 9.04% to 19.68% for '45H31' and from 7.21% to 17.79% for 'CS2000'. Similarly, in 2022, there were consistent decreases in both yield and emergence for both cultivars, while disease severities exhibited significant increases with increasing *F. avenaceum*

inoculum level (Table 5.2). In that year, yield losses ranged from 21.07% to 33.66% for '45H31' and from 22.53% to 30.38% for 'CS2000'

In general, for both *Fusarium* species, the performance outcomes of the two cultivars with respect to emergence, root rot severity, and yield were not significantly different over the two years of the experiment.

5.3.4 Co-inoculation with *F. proliferatum* and *F. oxysporum* under greenhouse conditions

The ANOVA conducted on the two repeats of this experiment confirmed that variances in emergence, disease severity at the seedling stage, and disease severity at maturity were homogeneous. As a result, the data from the two repeats were combined. However, for yield parameters (pod number and seed weight per experimental unit) with significantly distinct variances between the two repeats ($p < 0.01$), separate analyses were conducted. Linear regression analysis indicated a highly significant correlation ($p < 0.001$) between root rot severity at the seedling stage and at maturity. This correlation was strong for both the combined data for the two cultivars and for the data for each individual cultivar ($r = 0.93$). Additionally, disease severity at the seedling stage had a significant effect ($p < 0.001$) on emergence, with correlation coefficients of approximately −0.80 (Figure 5.2). The cultivar 'CS2000′ showed greater seedling emergence and lower disease severity across all treatments relative to '45H31'.

Nearly all of the inoculated treatments had significantly lower emergence and more severe disease relative to the controls. Root rot severity varied between 2.99 and 3.60 for cultivar '45H31', and between 2.56 and 3.39 for 'CS2000′ (Table 5.3). The 75 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix treatment showed the lowest emergence, but the highest disease severity, for both cultivars. No significant difference was found between the 22.5 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix treatment and the 75 mL *Fp*: 22.5 mL *Fo*: 5000 mL soilless mix treatment, which had equivalent total levels of grain inoculum applied. Treatment with 37.5 mL *Fp*: 37.5 mL *Fo*: 5000 mL soilless mix resulted in reductions in nearly all parameters, including seedling emergence, seed weight, and pod number. Additionally, significant differences were observed in terms of emergence and root rot severity at maturity when comparing this treatment to inoculation with 75 mL *Fp*: 0 mL *Fo*: 5000 mL soilless mix or 0 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix. In comparison to treatments with 75 mL *Fp*: 0 mL *Fo*: 5000 mL soilless mix or 0 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix, the treatment with 22.5 mL *Fp*: 22.5 mL *Fo*: 5000 mL soilless mix (which combined both *Fusarium* spp.) had a nearly identical impact on most parameters, despite containing less grain inoculum overall. Seed weight and pod number per box decreased significantly in the two repeated trials under all inoculated treatments, with the decline becoming greater as the amount of grain inoculum increased (Table 5.4); reductions exceeding 50% were observed at the higher inoculation rates.

Significant negative correlations ($p < 0.05$) were found between disease severity at maturity and both seed weight and pod number. These correlations held true for each cultivar, as well as when analyzing the combined data of the two cultivars. Similarly, a linear decrease in seed weight and pod number per plant was observed with increasing disease severity at maturity. As disease severity approached 4.0, the reduction in these parameters reached as high as 100% (Figure 5.3). These findings indicate a strong association between disease severity at maturity and adverse effects on seed weight and pod number.

5.3.5 Effects of co-inoculation with *F. proliferatum* and *F. oxysporum* under field conditions

In the co-inoculation field experiments, variance was homogeneous for yield, disease severity, and emergence between the two sites ($p > 0.05$). For the other parameters, the *p*-values ranged from 0.01 to 0.05, suggesting no significant interaction between sites, treatments, or cultivars. As a result, the data from both sites were combined for the subsequent analysis.

All of the inoculated treatments experienced a detrimental effect on disease severity, emergence, and yield for both cultivars when compared with their respective non-inoculated controls (Table 5.5). The disease severity in all of the inoculated treatments was significantly greater than in the non-inoculated controls. No significant differences were observed, however, among the various inoculated treatments. Disease severity in the

controls remained consistently low (<0.40). In contrast, for cultivar ‘45H31′, disease severity ranged from 1.58 to 1.89 in the inoculated treatments, while for ‘CS2000′, it ranged from 1.60 to 1.86. Similarly, the seedling emergence of both cultivars was reduced by all of the inoculations. Treatment with a mixture of 50 mL *Fp*: 50 mL *Fo*: 0 mL sand resulted in the lowest emergence rate. In addition, treatment with a combination of 25 mL *Fp*: 25 mL *Fo*: 50 mL sand showed significantly lower emergence compared to the treatment with 50 mL *Fp*: 0 mL *Fo*: 50 mL sand or treatment with 0 mL *Fp*: 50 mL *Fo*: 50 mL sand. However, no significant differences were observed between treatment with 50 mL *Fp*: 0 mL *Fo*: 50 mL sand and treatment with 0 mL *Fp*: 50 mL *Fo*: 50 mL sand, or between treatment with 15 mL *Fp*: 50 mL *Fo*: 35 mL sand and treatment with 50 mL *Fp*: 15 mL *Fo*: 35 mL sand.

Almost all of the treatments significantly reduced the yields of both cultivars. Yield losses for ‘45H31′ ranged from 10.30% in the treatment with 15 mL *Fp*: 15 mL *Fo*: 70 mL sand to 34.67% in the treatment with 50 mL *Fp*: 50 mL *Fo*: 0 mL sand. No significant differences in yield were observed among treatments receiving the same amount of total grain inoculum, irrespective of whether they were applied individually or in combination. In a similar fashion, for cultivar ‘CS2000′, yield losses ranged from 12.24% (in the treatment with 50 mL *Fp*: 0 mL *Fo*: 50 mL sand) to 30.16% (in the treatment with 50 mL *Fp*: 50 mL *Fo*: 0 mL sand). However, a significant difference was observed when comparing the treatment with 25 mL *Fp*: 25 mL *Fo*: 50 mL sand to the treatment with 50

mL *Fp*: 0 mL *Fo*: 50 mL sand, with the former showing a notably lower yield. In contrast, no significant differences were found between the treatment with 50 mL *Fp*: 0 mL *Fo*: 50 mL sand and the treatment with 0 mL *Fp*: 50 mL *Fo*: 50 mL sand, nor between the treatment with 15 mL *Fp*: 50 mL *Fo*: 35 mL sand and the treatment with 50 mL *Fp*: 15 mL *Fo*: 35 mL sand. No significant differences were detected among the canola cultivars for yield, seedling emergence, or disease severity for any of the treatments.

5.4 Discussion

The management of root rot in canola can be difficult, particularly given the involvement of multiple *Fusarium* species in disease development. The minimal inoculum densities required to induce disease, whether under natural conditions or in vitro, varied depending on the virulence of the strains (DeVay et al., 1997; Elmer, 2002; Netzer, 1976; Saremi et al., 2011; Zhou et al., 2014a). In our study, we employed pre-identified densities as distinct treatments to accurately simulate the disease-conducive inoculum conditions in nature. In this study, the application of *F. avenaceum*, *F. proliferatum,* or *F. oxysporum* at the seedling stage resulted in severe disease and reduced emergence. These findings are consistent with previous research by Chen et al. (Chen et al., 2014), who observed that *Fusarium* spp. collected from soil and diseased canola plants in central Alberta caused significant seedling blight and root rot, with *F. avenaceum* in particular found to be highly aggressive on canola. Moreover, in the current study, disease severity increased with higher inoculum concentrations of both *F. proliferatum* and *F. avenaceum*, leading to

decreases in emergence. These results align with similar studies on canola, in which *F. avenaceum* was found to reduce seedling emergence (Zhou et al., 2014a), as well as studies on bean, in which *Fusarium solani* f. sp. *phaseoli* caused severe root rot (Sippell & Hall, 1982), and on lentil, in which *F. avenaceum* reduced seedling survival (Hwang et al., 1994).

Under field conditions, inoculation with *F. avenaceum*, *F. proliferatum*, and *F. oxysporum* individually, or with a combination of *F. proliferatum* and *F. oxysporum*, resulted in significant yield reductions for both of the cultivars examined, highlighting the virulence of all three *Fusarium* spp. on canola. Similarly, previous studies also reported severe yield losses from Fusarium seedling blight and Fusarium wilt of canola (Lange et al., 2007). These findings underscore the importance of managing Fusarium root rot to safeguard canola crops and ensure optimal yields. Nonetheless, the yield losses resulting from inoculation with *F. avenaceum* or *F. proliferatum* were more pronounced in 2022 than in 2021. In both years, seedling emergence was reduced when cultivars were inoculated with *F. proliferatum*, while the reductions caused by *F. avenaceum* were particularly prominent in 2022. These findings align with a study by Chang et al. (Chang et al., 2011), who reported similar losses in the stand establishment (22–31%) and seed yield (19%) of lupine caused by *F. avenaceum*. The greater yield losses observed in 2022 likely reflected more severe disease that year, even for similar treatments. While most *Fusarium* spp. are known to prefer warm temperatures (Li et al., 2017; Safarieskandari et al., 2021), the very hot and dry conditions experienced in 2021 may not have been

conducive to disease development. Moreover, the hot and dry weather in 2021 per se may have contributed to reduced seedling emergence, as suggested by the significant differences observed between the non-inoculated controls in 2021 and 2022; around 400 seedlings per plot were recorded in 2022, compared with fewer than 100 seedlings per plot in 2021. Previous studies have documented the influences of temperature and soil moisture on the severity of root rot caused by *Fusarium* spp. For example, Yan et al. (Yan & Nelson, 2022) reported that *F. solani* and *F. tricinctum* caused the greatest root rot in soybean at 20 °C in sandy loam soil and at 15 °C in a silt loam. Additionally, they found that, when the temperature reached 28 °C, most infections occurred at soil moisture levels of 40% to 80% water holding capacity. Similarly, severe root rot symptoms were observed on lentil at temperatures ranging from 20 °C to 27.5 °C under controlled conditions, with fewer symptoms observed in warmer or cooler soils (Hwang et al., 2000).

In the field experiments, co-inoculation with *F. proliferatum* and *F. oxysporum* had similar effects on the emergence and yield relative to inoculation with a single *Fusarium* species. In general, all inoculated treatments showed a reduction in these parameters with increasing disease severity. These findings align with previous studies investigating the effects of *F. avenaceum* on canola and faba bean (Chang et al., 2014a; Zhou et al., 2014a), in which changes in growth parameters became more pronounced with increasing inoculum levels. Furthermore, in the current study, no significant differences were observed among treatments combining a high level of *F. proliferatum* (50 mL *Fp*: 0 mL

Fo: 50 mL sand) and a high level of *F. oxysporum* (0 mL *Fp*: 50 mL *Fo*: 50 mL sand), a high level of *F. proliferatum* and a low level of *F. oxysporum* (50 mL *Fp*: 15 mL *Fo*: 35 mL sand), or a low level of *F. proliferatum* and a high level of *F. oxysporum* (15 mL *Fp*: 50 mL *Fo*: 35 mL sand). It is worth noting, however, that the treatment combining a moderate level of *F. proliferatum* and a moderate level of *F. oxysporum* (25 mL *Fp*: 25 mL *Fo*: 50 mL sand) resulted in a greater reduction in seedling emergence and yield than when a high level of a single *Fusarium* species was applied. Similar results were observed with respect to seedling emergence and root rot severity under greenhouse conditions. These findings suggest that there may be synergistic interactions between the two *Fusarium* species under certain combinations or inoculum concentrations. Further studies involving multiple strains of each species are needed to confirm the widespread occurrence of the synergistic phenomenon among different *Fusarium* species in canola root rot.

Synergistic interactions between pathogens can occur when they benefit mutually from biochemical signals essential for pathogenesis, or when they exchange resources necessary for survival, leading to functional complementation (Abdullah et al., 2017; Monod, 1949). For instance, an additive interaction was reported between *F. oxysporum* and *R. solani* in causing root rot in soybean (Datnoff & Sinclair, 1988). Likewise, it was observed that, when maize ears were inoculated with a spore mixture of *Fusarium graminearum* and *Fusarium verticillioides*, or when *F. graminearum* was sequentially followed by *F. verticillioides*, the competitiveness of the latter species improved (Picot et

al., 2012). Additionally, prior infection by *F. graminearum* was found to benefit subsequent infections by *F. verticillioides* (Picot et al., 2012). Similar interactions have been observed in bacteria, where resources may be shared, benefiting all species while reducing mutual competition (Platt et al., 2012; Platt & Bever, 2009). In contrast, competitive interactions were detected among four *Fusarium* spp. in causing Fusarium head blight of wheat (Beccari et al., 2017). These studies underscore the complexity of relations among pathogens and emphasize the need for further research to improve understanding of the mechanisms behind such interactions.

Co-inoculation with *F. proliferatum* and *F. oxysporum* under greenhouse conditions yielded results similar to those from the field experiments. In general, all inoculations led to a significant reduction in seedling emergence, seed weight per plant, and the number of pods per plant. Notably, disease severity at maturity was strongly and negatively correlated with both seed weight and pod number per plant, with regression analysis indicating a linear decline in these parameters as disease severity increased. At a maximum disease severity of 4.0, both yield parameters approached zero. These findings are similar to those reported by Wang et al. (Wang et al., 2020), who observed a decrease in seed yield and pod number per plant caused by blackleg disease in canola. By establishing a clear relationship between disease severity and yield parameters such as seed weight and pod production, the regression models developed in this study provide a quantitative understanding of the impact of Fusarium root rot on canola.

In conclusion, all three *Fusarium* species were shown as capable of causing severe root rot in canola, reducing yields, as well as emergence and growth. Furthermore, co-inoculation with *F. proliferatum* and *F. oxysporum* resulted in greater disease severity than inoculation with each species on its own, suggesting possible synergistic interactions between these pathogens. The identification of significant negative linear correlations of seedling emergence, pod number per plant, and seed weight per plant with disease severity also underscores the need to implement more effective root rot management strategies. The knowledge from this study could be used to inform such strategies, and to more fully document the potential economic impact of Fusarium root rot in canola.

Table 5.1. Comparison among different treatments on canola seedling emergence, root rot severity, and yield following inoculation with *Fusarium proliferatum* under field conditions in Edmonton, Alberta, in 2021 and 2022.

Cultivar	Treatment [a]	Emergence [b]		Disease Severity [c]		Yield (kg/ha) [d]		Yield losses (%) [e]	
		2021	2022	2021	2022	2021	2022	2021	2022
'45H31'	Control	96.8 A	426 a	0.25 A	0.323 a	2.30 A	3.92 a		
	Low	53.4 B	321 b	0.94 B	1.579 b	2.14 A	3.42 ab	6.96%	12.76%
	Moderate	29.9 C	337 b	1.41 C	1.979 c	2.13 A	3.41 ab	7.39%	13.01%
	High	18.0 C	286 b	1.72 D	2.078 c	2.07 A	2.91 b	10.00%	25.77%
'CS2000'	Control	77.9 A	396 a	0.20 A	0.329 a	2.45 A	4.24 a		
	Low	51.2 B	331 ab	1.01 B	1.699 b	2.02 AB	3.47 b	17.55%	18.16%
	Moderate	25.3 C	311 bc	1.45 C	1.881 bc	2.12 AB	2.99 bc	13.47%	29.48%
	High	12.7 C	260 c	1.74 D	2.132 c	1.72 B	2.91 c	29.80%	31.37%

[a] Control refers to non-inoculated check plots; Low, Moderate, and High indicate treatments receiving low, moderate, and high inoculum levels, respectively. With regards to result columns, superscript letters indicate the following: [b] seedling emergence per plot; [c] average root rot disease severity (0–4 scale) per plot; [d] yield per hectare, calculated from the plot yield of each treatment; [e] yield losses, expressed as the percentage reduction for each inoculated treatment in relation to its corresponding control. Means in a column and category followed by the same uppercase or lowercase letter do not differ based on the LSD at $p \leq 0.05$.

Table 5.2. Comparison among different treatments on canola seedling emergence, disease severity, and yield following inoculation with *Fusarium avenaceum* under field conditions in Edmonton, Alberta, in 2021 and 2022.

Cultivar	Treatment [a]	Emergence [b]		Disease Severity [c]		Yield (kg/ha) [d]		Yield losses (%) [e]	
		2021	2022	2021	2022	2021	2022	2021	2022
'45H31'	Control	64.9 A	406 a	0.17 A	0.328 a	1.88 A	4.13 a		
	Low	59.8 AB	222 b	1.14 B	1.451 b	1.71 A	3.26 b	9.04%	21.07%
	Moderate	58.8 B	147 c	1.21 B	1.788 c	1.56 A	3.21 bc	17.02%	22.28%
	High	57.6 B	123 c	1.29 B	2.095 d	1.51 A	2.74 c	19.68%	33.66%
'CS2000'	Control	60.4 A	390 a	0.40 A	0.501 a	2.08 A	3.95 a		
	Low	59.7 A	240 b	1.07 B	1.580 b	1.93 A	3.06 b	7.21%	22.53%
	Moderate	56.2 A	180 bc	1.21 B	1.735 c	1.71 A	2.96 b	17.79%	25.06%
	High	55.9 A	131 c	0.93 B	2.015 d	1.71 A	2.75 b	17.79%	30.38%

[a] Control refers to non-inoculated check plots; Low, Moderate, and High indicate treatments receiving low, moderate, and high inoculum levels, respectively. With regards to result columns, superscript letters indicate the following: [b] seedling emergence per plot; [c] average root rot disease severity (0–4 scale) per plot; [d] yield per hectare, calculated from the plot yield of each treatment; [e] yield losses, expressed as the percentage reduction for each inoculated treatment in relation to its corresponding control. Means in a column and category followed by the same uppercase or lowercase letter do not differ based on the LSD at $p \leq 0.05$.

Table 5.3. Comparison among different treatments on emergence and root rot severity in the canola cultivars '45H31′ and 'CS2000′ following inoculation with *Fusarium proliferatum* and *Fusarium oxysporum*, alone or in combination, under greenhouse conditions.

Treatment [a]	'45H31'			'CS2000'		
	Emergence [b]	Disease Severity (Seedling Stage) [c]	Disease Severity (Maturity) [d]	Emergence [b]	Disease Severity (21 Days) [c]	Disease Severity (Maturity) [d]
Control	31.38 A	0.00 A	0.00 A	32.50 a	0.00 a	0.00 a
75 *Fp*: 0 *Fo*: 5000 mix	18.62 B	3.31 B	3.00 B	24.62 bc	2.83 bc	2.94 b
0 *Fp*: 75 *Fo*: 5000 mix	16.38 BC	3.01 B	3.17 BCD	27.25 ab	2.56 b	2.81 b
22.5 *Fp*: 75 *Fo*: 5000 mix	12.50 BC	3.15 B	3.29 BCDE	23.00 bc	2.86 bc	2.97 bc
75 *Fp*: 22.5 *Fo*: 5000 mix	9.25 CD	3.41 B	3.49 DE	20.88 bc	3.06 bc	2.98 bc
75 *Fp*: 75 *Fo*: 5000 mix	4.88 D	3.59 B	3.60 E	11.88 d	3.34 c	3.39 d
37.5 *Fp*: 37.5 *Fo*: 5000 mix	9.75 CD	3.47 B	3.35 CDE	17.38 cd	2.79 bc	3.30 cd
22.5 *Fp*: 22.5 *Fo*: 5000 mix	17.38 B	2.99 B	3.09 BC	25.62 ab	2.60 b	2.98 bc

[a] Control, no inoculum (0 mL *F. proliferatum* (*Fp*): 0 mL *F. oxysporum* (*Fo*): 5000 mL soilless mix); 75 *Fp*: 0 *Fo*: 5000 mix = 75 mL *Fp*: 0 mL *Fo*: 5000 mL soilless mix; 0 *Fp*: 75 *Fo*: 5000 mix = 0 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix; 22.5 *Fp*: 75 *Fo*: 5000 mix = 22.5 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix; 75 *Fp*: 22.5 *Fo*: 5000 mix = 75 mL *Fp*: 22.5 mL *Fo*: 5000 mL soilless mix; 75 *Fp*: 75 *Fo*: 5000 mix = 75 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix; 37.5 *Fp*: 37.5 *Fo*: 5000 mix = 37.5 mL *Fp*: 37.5 mL *Fo*: 5000 mL soilless mix; and 22.5 *Fp*: 22.5 *Fo*: 5000 mix = 27.5 mL *Fp*: 27.5 mL *Fo*: 5000 mL soilless mix. With regards to result columns, superscript letters indicate the following: [b] seedling emergence (number of plants) per box; [c] root rot disease severity (0–4 scale) at 21 days after seeding;

[d] root rot disease severity (0–4 scale) at maturity. Means in a column and category followed by the same uppercase or lowercase letter do not differ based on the LSD at $p \leq 0.05$.

Table 5.4. Comparison among different treatments on seed weight and pod number of the canola cultivars '45H31′ and 'CS2000′ following inoculation with *Fusarium proliferatum* and *Fusarium oxysporum*, alone or in combination, at maturity under greenhouse conditions.

Treatment [a]	'45H31'				'CS2000'			
	Seed Weight (g) [b]		Pod Number [c]		Seed Weight (g) [b]		Pod Number [c]	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	17.70 A	68.90 a	975 A	1855 a	12.71 A	64.00 a	645 A	2009 a
75 *Fp*: 0 *Fo*: 5000 mix	4.58 B	15.50 b	208 B	462 b	4.03 B	18.90 b	162 B	540 b
0 *Fp*: 75 *Fo*: 5000 mix	3.14 B	16.30 b	170 B	448 b	4.41 B	23.30 b	184 B	504 b
22.5 *Fp*: 75 *Fo*: 5000 mix	3.75 B	13.40 b	171 B	385 b	4.91 B	16.50 b	178 B	380 b
75 *Fp*: 22.5 *Fo*: 5000 mix	3.71 B	13.90 b	153 B	388 b	4.38 B	23.50 b	169 B	494 b
75 *Fp*: 75 *Fo*: 5000 mix	3.88 B	13.70 b	132 B	410 b	3.96 B	18.40 b	130 B	388 b
37.5 *Fp*: 37.5 *Fo*: 5000 mix	4.13 B	21.60 b	149 B	541 b	3.85 B	18.30 b	127 B	405 b
22.5 *Fp*: 22.5 *Fo*: 5000 mix	4.64 B	22.60 b	182 B	551 b	4.61 B	23.20 b	162 B	560 b

[a] Control, no inoculum (0 mL *F. proliferatum* (*Fp*): 0 mL *F. oxysporum* (*Fo*): 5000 mL soilless mix); 75 *Fp*: 0 *Fo*: 5000 mix = 75 mL *Fp*: 0 mL *Fo*: 5000 mL soilless mix; 0 *Fp*: 75 *Fo*: 5000 mix = 0 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix; 22.5 *Fp*: 75 *Fo*: 5000 mix = 22.5 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix; 75 *Fp*: 22.5 *Fo*: 5000 mix = 75 mL *Fp*: 22.5 mL *Fo*: 5000 mL soilless mix; 75 *Fp*: 75 *Fo*: 5000 mix = 75 mL *Fp*: 75 mL *Fo*: 5000 mL soilless mix; 37.5 *Fp*: 37.5 *Fo*: 5000 mix = 37.5 mL *Fp*: 37.5 mL *Fo*: 5000 mL soilless mix; and 22.5 *Fp*: 22.5 *Fo*: 5000 mix = 27.5 mL *Fp*: 27.5 mL *Fo*: 5000 mL soilless mix. With regards to result columns, superscript letters indicate the following: [b] total seed weight per box; [c] total pod number per box. 1st, first repeat; 2nd, second repeat of experiment. Means in a column and category followed by the same uppercase or lowercase letter do not differ based on the LSD at $p \leq 0.05$.

Table 5.5. Comparison among different treatments on canola seedling emergence, disease severity, and yield of '45H31′ and 'CS2000′ following inoculation with *Fusarium proliferatum* and *Fusarium oxysporum*, alone or in combination, under field conditions in Edmonton, Alberta, in 2022.

Treatment [a]	'45H31'				'CS2000'			
	Emergence [b]	Disease Severity (Flowering Stage) [c]	Yield (kg/ha) [d]	Yield losses (%) [e]	Emergence [b]	Disease Severity (Flowering Stage) [c]	Yield (kg/ha) [d]	Yield losses (%) [e]
Control	276.8 A	0.399 A	3.98 A		278.1 a	0.398 a	3.92 a	
50 *Fp*: 0 *Fo*: 50 sand	182.2 BC	1.751 B	3.28 B	17.59%	189.4 b	1.604 b	3.44 ab	12.24%
0 *Fp*: 50 *Fo*: 50 sand	162.0 BCD	1.728 B	3.22 B	19.10%	151.8 b	1.795 b	3.09 bcd	21.17%
15 *Fp*: 50 *Fo*: 35 sand	146.1 CD	1.748 B	3.25 B	18.34%	148.0 b	1.859 b	3.00 bcd	23.47%
50 *Fp*: 15 *Fo*: 35 sand	158.9 BCD	1.741 B	3.25 B	18.34%	160.0 b	1.741 b	3.34 bc	14.80%
50 *Fp*: 50 *Fo*: 0 sand	81.8 E	1.889 B	2.60 C	34.67%	67.9 c	1.764 b	2.72 e	30.61%
25 *Fp*: 25 *Fo*: 50 sand	128.6 D	1.581 B	3.21 B	19.35%	102.0 c	1.741 b	2.85 cd	27.30%
15 *Fp*: 15 *Fo*: 70 sand	193.6 B	1.684 B	3.57 AB	10.30%	164.0 b	1.718 b	3.23 bcd	17.60%

[a] Control, no inoculum (0 mL *F. proliferatum* (*Fp*): 0 mL *F. oxysporum* (*Fo*): 100 mL sand); 50 *Fp*: 0 *Fo*: 50 sand = 50 mL *Fp*: 0 mL *Fo*: 50 mL sand; 0 *Fp*: 50 *Fo*: 50 sand = 0 mL *Fp*: 50 mL *Fo*: 50 mL sand; 15 *Fp*: 50 *Fo*: 35 sand = 15 mL *Fp*: 50 mL *Fo*: 35 mL sand; 50 *Fp*: 15 *Fo*: 35 sand = 50 mL *Fp*: 15 mL *Fo*: 35 mL sand; 50 *Fp*: 50 *Fo*: 0 sand = 50 mL *Fp*: 50 mL *Fo*: 0 mL sand; 25 *Fp*: 25 *Fo*: 50 sand = 25 mL *Fp*: 25 mL *Fo*: 50 mL sand; and 15 *Fp*: 15 *Fo*: 70 sand = 15 mL *Fp*: 15 mL *Fo*: 70 mL sand. With regards to result columns, superscript letters indicate the following: [b] seedling emergence (number of plants) per plot; [c] root rot disease severity (0–4 scale) at the flowering stage; [d] yield per hectare, calculated from the plot yield for each treatment; [e] yield losses are the percentage reduction for each inoculated treatments in relation to corresponding control. Means in a column and category followed by the same uppercase or lowercase letter do not differ based on the LSD at $p \leq 0.05$.

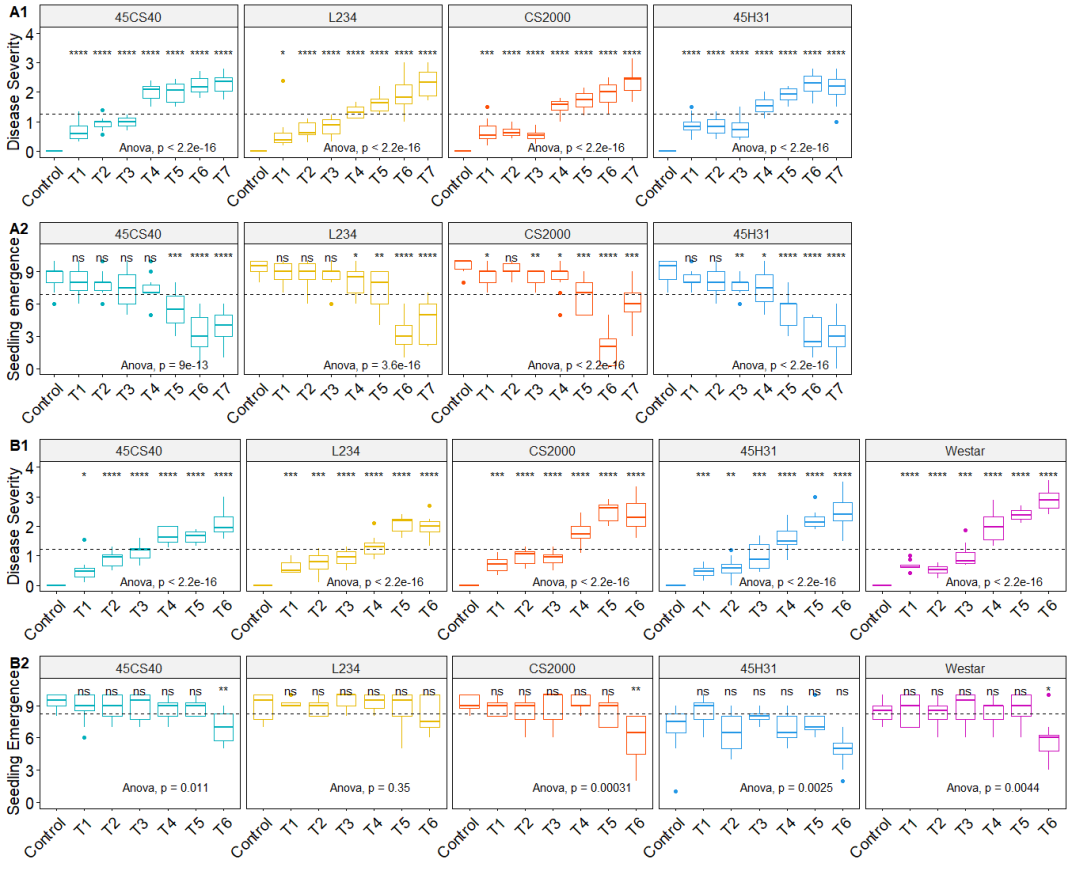


Figure 5.1. Comparison of the effect of inoculation with *Fusarium proliferatum* on root rot severity (**A1**) and seedling emergence (**A2**) in the canola cultivars '45CS40', 'L234', 'CS2000', and '45H31', and *Fusarium avenaceum* on root rot severity (**B1**) and seedling emergence (**B2**) in the canola cultivars '45CS40', 'L234', 'CS2000', '45H31' and 'Westar', under greenhouse conditions. (**A1**, **A2**): Control, non-inoculated control of each cultivar; T1, 1:2000 (*v*/*v*) ratio of *Fusarium proliferatum* (*Fp*) to potting mix; T2, 1:1000 (*v*/*v*) ratio of *Fp* to potting mix; T3, 1:400 (*v*/*v*) ratio of *Fp* to potting mix (*v*/*v*); T4, 1:200 (*v*/*v*) ratio of *Fp* to potting mix; T5, 1:133 (*v*/*v*) ratio of *Fp* to potting mix (*v*/*v*); T6, 1:100 (*v*/*v*) ratio of *Fp* to potting mix; T7, 1:67 (*v*/*v*) ratio of *Fp* to potting mix. (**B1**, **B2**): Control, non-inoculated control of each cultivar; T1, 1:49 (*v*/*v*) ratio of *Fusarium avenaceum* (*Fa*) to potting mix (*v*/*v*); T2, 1:24 (*v*/*v*) ratio of *Fa* to potting mix (*v*/*v*); T3, 3:47 (*v*/*v*) ratio of *Fa* to potting mix; T4, 2:23 (*v*/*v*) ratio of *Fa* to potting mix; T5, 3:22 (*v*/*v*) ratio of *Fa* to potting mix; T6, 3:17 (*v*/*v*) ratio of *Fa* to potting mix. ns, no significant difference between the treatment and corresponding control based on a *t*-test; *, significant difference at $p \leq 0.05$; **, significant difference at $p \leq 0.01$; ***, significant difference at $p \leq 0.001$; and ****, significant difference at $p \leq 0.0001$. The dashed lines represent the overall mean for each parameter.

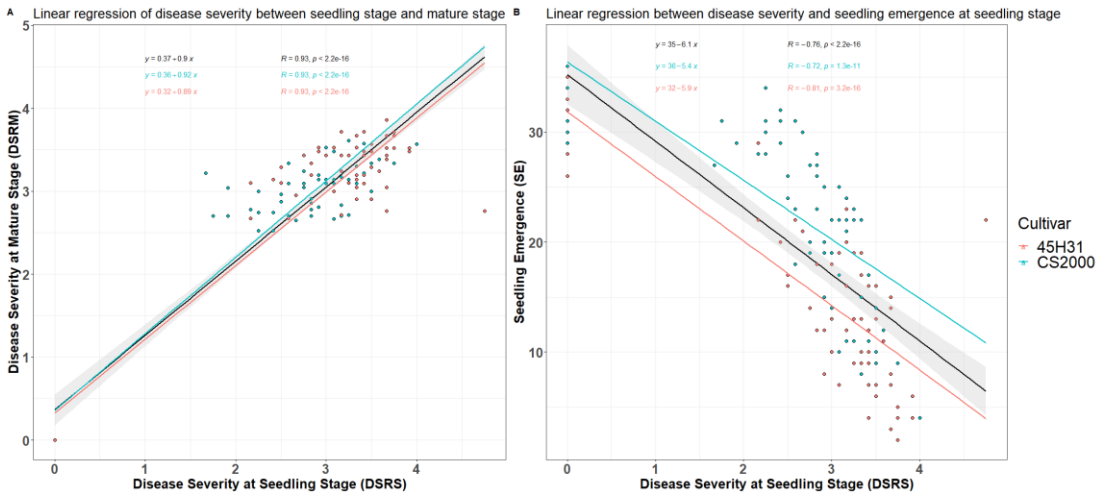


Figure 5.2. Linear regression between average canola root rot disease severity at the seedling stage and at maturity (**A**), and between average disease severity at the seedling stage and seedling emergence (**B**) for all treatments under greenhouse conditions. Black line, linear regression based on the combined data for the cultivars '45H31' and 'CS2000'; pink line, linear regression for '45H31'; blue line, linear regression for 'CS2000′. DSRS, root rot disease severity (0–4 scale) at the seedling (21 days after seeding) stage; DSRM, root rot disease severity (0–4 scale) at maturity; SE, seedling emergence (number of plants) per box.

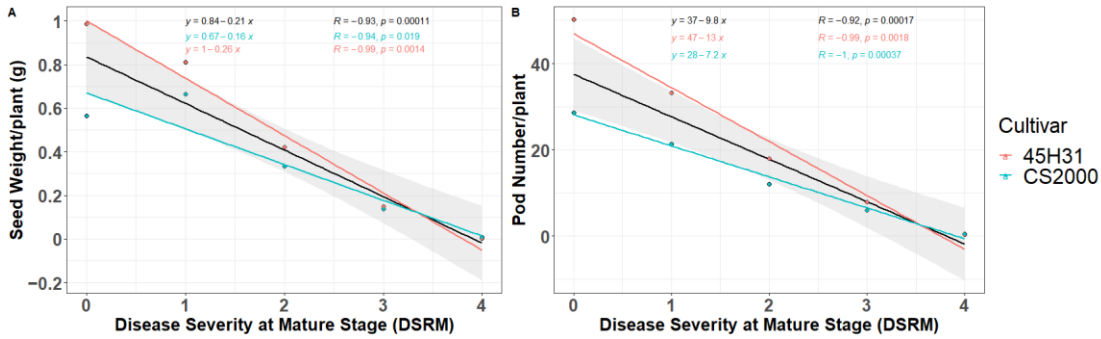


Figure 5.3. Linear regression between canola yield parameters (seed weight per plant (**A**) and pod number per plant (**B**)) and root rot disease severity at maturity. Black line, linear regression based on the combined data for the cultivars '45H31' and 'CS2000'; pink line, linear regression for '45H31'; blue line, linear regression for 'CS2000'. DSRM, root rot disease severity (0–4 scale) at maturity; Seed Weight/plant, the average seed weight for all plants at each DSRM level (0–4); Pod Number/plant, the average pod number for all plants at each DSRM level (0–4).

Chapter 6 Host range of *Fusarium proliferatum* in western Canada

6.1 Introduction

Root rot is a destructive disease affecting many crops worldwide. It is caused by a variety of pathogens collectively known as the root rot complex, which includes, among others, *Rhizoctonia* spp., *Fusarium* spp., *Pythium* spp., and *Aphanomyces* spp. (Williamson-Benavides & Dhingra, 2021). Infection by these pathogens typically results in reduced plant growth and impaired biological function of the affected organs, leading to symptoms such as rotting, wilting, yellowing, and discoloration of plant tissues (Rampersad, 2020).

Among these causal agents, the genus *Fusarium* includes widely distributed pathogenic fungi capable of infecting a broad range of plants (Bodah, 2017). *Fusarium proliferatum* in particular has been reported to infect various hosts, including field, fruit and vegetable crops (Abbas et al., 1999; Gálvez & Palmero, 2022; Zhan et al., 2010). The fungus has been increasingly reported as a causal agent of tissue rot and wilt diseases in many crops worldwide, including (among others) carnation (*Dianthus caryophyllus* L.) (Basallote-Ureba et al., 2016), cauliflower (*Brassica oleracea* var. *botrytis* L.) (Yan, 2020), garlic (*Allium sativum* L.) (Chrétien et al., 2020; Elshahawy et al., 2017; Galal et al., 2002), onion (*Allium cepa* L.), maize (*Zea mays* subsp. *mays*), rice (*Oryza sativa* L.), and sugarcane (*Saccharum officinarum* L.) (Alizadeh et al., 2010). *Fusarium proliferatum* is also regarded as an important pathogen in the fruit industry, causing significant economic

losses in banana (Li et al., 2017) and red-fleshed dragon fruit (Masratul Hawa et al., 2013). In Canada, *F. proliferatum* has been reported to cause crown and stem rot and pith necrosis in greenhouse-grown cannabis (*Cannabis sativa* L.) (Punja, 2021), as well as root rot of soybean (*Glycine max* L.) (Chang et al., 2015), with similar reports from the United States (Díaz Arias et al., 2011). The fungus is also recognized as a threat to food and feed quality due to the production of mycotoxins such as fumonisins, which can adversely affect sphingolipid metabolism, leading to chronic and acute diseases in humans and other animals (Proctor et al., 2010).

An understanding of the host range of plant pathogens is critical for effective disease management (Morris & Moury, 2019). In the context of soilborne pathogens, host range often guides the choice of crops included in a rotation for disease mitigation. The inclusion of non-host crops can decrease the risk of disease development (Krupinsky et al., 2002), and implementing diverse crop rotations may contribute to reducing pathogen populations in the soil (Hwang et al., 2009). On the other hand, the selection of susceptible or inappropriate hosts can result in an increase in inoculum levels and more severe disease development. Moreover, anticipated increases in root-related diseases, attributed to climate change (Delgado-Baquerizo et al., 2020), coupled with the widespread occurrence of pathogenic soilborne agents in agricultural systems (Dixon & Tilston, 2010), underscores the need for improved knowledge of the virulence of microbial pathogens and their capacity to cause disease on different hosts. Such knowledge is essential for reducing

losses from root rot and other diseases.

In the Prairies region of western Canada, *Fusarium* spp. have been identified as the predominant pathogens responsible for root rot in multiple crops (Chen et al., 2014; Hwang et al., 1994, Yue et al., 2022a). Among the most prevalent species of *Fusarium*, *Fusarium avenaceum* has demonstrated aggressiveness on legumes, cereals, and canola (Chen et al., 2014; Fernandez, 2007; Safarieskandari et al., 2021). To our knowledge, however, there are no reports on the host range of *F. proliferatum* or its impact on plant germination or growth. The aim of this study was to investigate the reaction of eight important field crops grown in western Canada, including wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.), lentil (*Lens culinaris* L. ssp. *culinaris*), canola (*Brassica napus* L.), lupine (*Lupinus angustifolius* L.), and soybean (*Glycine max* L.), to inoculation with *F. proliferatum*. The results of this research may offer insights for formulating effective strategies to control root rot.

6.2 Materials and Methods

6.2.1 Pathogen material

An aggressive isolate of *F. proliferatum*, designated P002 and originally collected from canola with symptoms of root rot (Yu et al., 2022a), was selected for this study. Fungal inoculum was generated on a wheat grain medium as described previously (Hwang, 1988). Briefly, 1 L of grain was soaked in tap water overnight at room temperature, and then transferred to a Hi-Patch mushroom spawn bag (Western Biologicals, Aldergrove,

BC). The opening on the bag was closed with a foam insert and secured with a collar, and the grain was autoclaved for 90 min. After it cooled to room temperature, ~250 plugs (0.5-diam.) from a 14-day-old *F. proliferatum* culture grown on potato dextrose agar (PDA) were added, and the grain was thoroughly mixed. It was then incubated for 5 weeks in darkness at room temperature, allowing for complete fungal colonization of the grain. The inoculated grain was air-dried for 3 days at 25℃, ground to a powder (particle size ranging from 1-2 mm) and passed through a 2.0-mm-mesh. The ground grain inoculum was then stored in a cooler at 4℃ for a maximum of 2 months until use.

6.2.2 Host reaction at the seedling stage

The response of eight crop species to *F. proliferatum* inoculation was assessed under greenhouse conditions at the seedling stage. The crops evaluated included wheat ('Katepwa', 'AC Crystal', and 'Lillian'), barley ('AB Tofield' and 'Canmore'), faba bean ('Malik' and 'Fabelle'), pea ('CDC Greenwater', 'AAC Carver', 'CDC Amarillo', and 'AAC Barrhead'), lentil ('CDC Nimble' and 'CDC Lima CL'), canola ('Westar' and 'L255PC'), lupine ('Arabella' and 'Mirabor'), and soybean ('AAC Mandor', 'OT15-02', and 'AKRAS R2'). Briefly, grain inoculum was mixed with Promix PGX potting medium (Sun Gro Canada Inc., Seba Beach, AB, Canada) at ratios of 1:300 (v:v) (0.33%, ‘low inoculum’ concentration) or 1:150 (v:v) (0.67%, ‘high inoculum’ concentration) grain inoculum to potting medium, corresponding to 3×10^4 or 6×10^4 colony forming units (cfu) per g potting medium, respectively. Each host variety was sown in 473-mL cups (Uline, Toronto,

ON) filled with 400 mL of the inoculated potting medium at a density of 10 seeds per cup and maintained under greenhouse conditions at ~25℃ with a 12-h photoperiod. The plants were watered as needed and fertilized (15N-15P-15K) weekly. Control treatments consisted of seeds grown in non-inoculated potting medium. The experiment was arranged in a randomized complete block design (RCBD) with five replicates (cups) per treatment, and the entire experiment was repeated.

At 21 days after seeding, the plants were removed from the potting medium, and the roots washed thoroughly with tap water to assess root rot severity as described below. After completion of the disease assessment, the plants were dried for 7 days at ca. 25℃, the shoots and roots were separated by cutting, and the respective dry weights measured for each replicate (cup).

6.2.3 Root rot development at maturity

To evaluate host reactions to *F. proliferatum* at the adult plant stage, greenhouse trials were conducted with one cultivar each of canola (‘Westar’), faba bean (‘Fabelle’), soybean (‘AKRAS R2’), lupine (‘Arabella’), barley (‘Canmore’) and wheat (‘AC Crystal’), and three cultivars of pea (‘CDC Greenwater’, ‘AAC Carver’, ‘CDC Amarillo’). The plants were sown at a density of 10 seeds per cup and grown in 400 mL Promix PGX potting medium (Sun Gro Canada Inc.) inoculated with grain inoculum at a ratio of 1:300 (v:v) (corresponding to 3×10^4 cfu per g potting medium) as above. Control treatments did not receive any grain inoculum, and the experiment was arranged in a RCBD with three

replicates (cups) per treatment.

After flowering, the plants were gently removed from the potting medium and the roots washed with tap water to assess disease severity as described below. Diseased root tissue samples of the faba bean 'Fabelle', soybean 'AKRAS R2', lupine 'Arabella', wheat 'AC Crystal' and pea cultivars 'CDC Greenwater' and 'AAC Carver', were collected, flash-frozen in liquid nitrogen, and stored at -20℃ prior to DNA extraction.

6.2.4 Disease ratings

Root rot severity was assessed on a 0-4 scale (Hwang et al., 1994), illustrated for each crop in Supplementary Figure 6.1, where: 0 = healthy roots; 1 = small, light-brown lesions on < 25% of the tap root; 2 = brown lesions on 25-49% of the tap root; 3 = brown lesions on 50-74% of the tap root, tap root constricted; and 4 = tap root severely girdled, brown lesions on > 75% of the tap root with limited lateral roots. The final disease severity per experimental unit (cup) was calculated by averaging the values of all the individual plants within each cup.

6.2.5 Emergence, plant height, shoot and root dry weights

Emergence was determined by counting all surviving plants in each experimental unit 7 days after seeding. Plant height was measured from the soil line to the shoot apex with a ruler at 14 days after seeding. Shoot and root dry weights per experimental unit were determined separately on a weighing scale (Fisher Science Education SLF 303, Thermo Fisher Scientific, Mississauga, ON). Reductions in seedling emergence, plant height, and

shoot and root dry weights were calculated relative to non-inoculated controls according to the equation:

$$\text{Reduction} = [(D_{ck} - D_{tr}) / D_{ck}] \times 100\% \quad (1)$$

Where: D_{ck} represents the control (non-inoculated) treatment and D_{tr} represents the inoculated treatment.

6.2.6 PCR detection of *F. proliferatum*

The presence of fungal DNA in host tissues was determined by PCR analysis with the *F. proliferatum*-specific primers TH5-F and TH6-R (Waalwijk et al., 2003). The specificity of the primers was confirmed by testing on *F. proliferatum* isolate P002 and isolates of *F. avenaceum*, *Fusarium graminearum*, *Fusarium solani*, *Fusarium redolens* and *Fusarium oxysporum* obtained from the culture collection of the Applied Plant Pathology Lab, University of Alberta (Yu et al., 2022a). Fungal DNA was extracted from pure PDA-grown cultures using a DNeasy Plant Pro Kit (Qiagen, Hilden, Germany) according to the Quick-Start protocol (www.qiagen.com/HB-2522). Extractions of total genomic DNA from inoculated and control plant roots were conducted using the same protocol and kit from 0.1 g of tissue. The quantity and quality of the DNA samples was assessed in a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific), and the final concentration of the DNA was adjusted to 20 ng/μL with nuclease-free water (Thermo Fisher Scientific). The samples were stored at -20℃ until needed.

PCR was conducted in a 20 μL reaction volume, which included 1 μL of DNA

template (20 ng/μL), 1 μL of each of the forward and reverse primers TH5-F and TH6-R (10 μM), 10 μL of HotStarTaq master mix (Qiagen), 0.25 μL of bovine serum albumin (10 mg/mL) (Thermo Fisher Scientific), and 6.75 μL of nuclease-free water (Thermo Fisher Scientific). Amplification reactions consisted of an initial heat activation at 95℃ for 10 min, followed by 40 cycles of denaturation at 94℃ for 1 min, annealing at 60℃ for 30 s, and extension at 72℃ for 1 min. A final extension step was conducted at 72℃ for 5 min. Positive and negative controls (*F. proliferatum* DNA or equivalent volume of nuclease-free water, respectively) were included in all PCR assays. Amplicons were resolved by agarose gel electrophoresis and the presence of a single band near the predicted amplicon size (~330 bp) was regarded as a positive result for *F. proliferatum* in the sample tested.

6.2.7 Data analysis and Principal Component Analysis

All statistical analyses were conducted in R Studio v. 4.1.2 (RStudio Team, 2020). The Shapiro-Wilk test was used to assessing normality, while the homogeneity of variance was evaluated using Levene's test within ANOVA. Unless otherwise noted, differences were considered significant at $p < 0.05$. If a significant interaction was detected between repetition and treatment, the data were analyzed separately to account for the effects of these factors on the results. Principal Component Analysis (PCA) was utilized to group the cultivars/crops into clusters based on their responses to *F. proliferatum* infection at the seedling stage, and to identify the primary parameters for evaluating host susceptibility. Root rot severity and reductions in seedling emergence, plant height, root and shoot dry

weight were used to generate the PCA biplot.

6.3 Results

6.3.1 Root rot development at the seedling stage

The variance in emergence, plant height, and root dry weight was not significant ($p > 0.05$) in the two repeats of the experiment conducted at the seedling stage, while significant differences in root rot severity and shoot dry weight were observed. Interactions between repeat and cultivar or crop and inoculum concentration were not found to be significant, indicating consistent trends in the results. Due to their overall similarity and absence of significant interactions, the datasets from the two repeated experiments were combined for further statistical analysis.

All the crop species developed symptoms of root rot. Between 2-4 cultivars were examined per crop, and while some variability in disease severity was observed among cultivars of the same crop, none was completely resistant (Supplementary Table 6.1). When averaged across cultivars, disease severity ranged from 1.29 to 3.03 for each of the crops at the low inoculum concentration, and from 1.98 to 3.42 at the high inoculum concentration (Table 6.1). Root rot was significantly more severe at the high vs. low inoculum concentration for all the species. When comparing disease severity across the eight crops, wheat and barley generally developed the mildest symptoms at both inoculum concentrations, followed by faba bean, lentil and pea (Table 6.1). At the lower inoculum concentration, there were no significant differences in root rot severity among the latter

three crops, while at the higher concentration, disease was more severe in lentil vs. faba bean or pea. Root rot development tended to be most severe in canola, lupine and soybean, although at the high inoculum concentration, disease severity in these crops was similar to lentil (Table 6.1). As expected, no disease symptoms were observed in any of the control (non-inoculated) treatments.

6.3.2 Emergence, plant height, shoot and root dry weights at the seedling stage

Inoculation with the low concentration of *F. proliferatum* did not affect plant height, emergence, or shoot and root dry weights in barley; in this crop, only the high inoculum treatment significantly reduced these parameters relative to the control (Table 6.2). Similarly, no reductions in plant height and shoot dry weight were observed for wheat at the low inoculum concentration, although significant reductions were found in emergence and root dry weight in this treatment. At the high inoculum concentration, plant height was significantly reduced in wheat relative to the control, while shoot dry weight declined compared with the low inoculum concentration. Wheat emergence and root dry weight in the high inoculum treatment declined further compared with the low inoculum treatment (Table 6.2). In faba bean, seedling emergence declined relative to the control under the high but not low inoculum concentrations, while treatment with either concentration resulted in significant declines in height and shoot and root dry weights. In the case of all the other crop species, treatment with either the low or high *F. proliferatum* inoculum concentrations resulted in significant decreases in height, emergence, and shoot and root

dry weights relative to their respective controls, with these declines usually more pronounced in the high inoculum treatment (Table 6.2). The reductions in plant height, seedling emergence, and shoot and root dry weights for each of the 2-4 cultivars evaluated per crop species are included in Supplementary Figure 6.2.

6.3.3 Host reaction at maturity

Based on the ANOVA, variance in root rot severity between the two repeats of the experiment conducted at the adult plant stage was not significant, while significant differences were observed among hosts. Therefore, the disease severity data from the two repeats was combined to conduct a comparison among crops/cultivars.

At maturity, all crops presented symptoms of root rot, with disease severities ranging from 1.08 to 3.57 (Table 6.3). As was observed at the seedling stage, the lowest disease severity (1.08-1.15) at maturity was found on barley and wheat, followed by faba bean (2.00), one of the pea cultivars ('ACC Carver', 2.62), and canola, soybean and the other two pea cultivars (3.00-3.26). The most severe root rot developed on lupine (disease severity = 3.57). PCR analysis with *F. proliferatum*-specific primers confirmed the presence of a single band of 300-400-bp (corresponding to the expected ~330 bp amplicon), which was obtained only from symptomatic plant tissues (Supplementary Figure 6.3).

6.3.4 Principal Component Analysis

Principal Component Analysis (PCA) was conducted on root rot severity and reductions in plant height, seedling emergence, and shoot and root dry weights of all

individual cultivars of each species at the seedling stage under the low and high *F. proliferatum* inoculum concentrations. The first principal component (PC1) explained 84.4% of the variance among cultivars at the low inoculum concentration and 87.1% of the variance at the high inoculum concentration. The second principal component (PC2) explained 7.7% and 8.9% of the variance at the low and high inoculum concentrations, respectively (Figure 6.1). The five parameters (root rot severity and reductions in plant height, seedling emergence, and shoot and root dry weights) collectively contributed to the most significant variation among all cultivars in PC1, with relative contributions ranging from -0.471 to -0.393 (Supplementary Table 6.2). The negative values indicated that these parameters were inversely related to the variation observed in PC1, implying that as disease severity increased or reductions in plant growth parameters became more pronounced, the values along PC1 decreased. Root rot severity and reduction in root dry weight represented the most significant components in the second principal component (PC2), with relative contributions over 0.8 and < -0.5, respectively (Supplementary Table 6.2). The broad distribution of the cultivars along the PC1 and PC2 axes of the biplots indicated significant variation among cultivars of some crops, including faba bean and pea at both the high and low inoculum concentrations, canola and soybean under low inoculum, and lupine and lentil under high inoculum (Figure 6.1).

6.4 Discussion

The host range of a pathogen can play an important role in designing effective disease

management strategies (Morris & Moury, 2019). In this study, *F. proliferatum* caused root rot on all evaluated crops, although based on symptom severity and its impact on emergence, plant height and root and shoot dry weights, soybean, lupine, canola, and lentil appeared to be most susceptible. Conversely, based on these parameters, barley and wheat, followed by faba bean, showed greater tolerance to infection. Similar trends in susceptibility were observed at both the seedling and adult plant stages across the crops, although the significant reductions in emergence at the seedling stage may highlight the risk posed to stand establishment by early infection. Other studies have reported similar phenomena, including reports with pea root rot caused by *Aphanomyces euteiches* (Bodah, 2017), dry root rot of chickpea (*Cicer arietinum*) caused by *Rhizoctonia bataticola* (Bodah, 2017; Pande et al., 2004), and Fusarium root rot of soybean (Arias et al., 2013) at various growth stages. These observations suggest that tolerance to infection can persist in a host genotype, suggesting the potential to mitigate disease pressure as plants mature. Conversely, susceptibility also continues into the later stages of growth.

Crop rotation is often regarded as a primary method for reducing pathogen inoculum, although its effectiveness depends on the availability of non-host crops (Gálvez & Palmero, 2022; Hwang et al., 2009; Marburger et al., 2015). The fairly wide host range of *F. proliferatum* found in this study was not necessarily unexpected, given the reports of this pathogen on many host species (Alizadeh et al., 2010; Díaz Arias et al., 2011; Punja, 2021; Stepien et al., 2011; Yan, 2020). However, the virulence observed on soybean, lupine,

canola, lentil, and pea, and to a lesser extent on barley, wheat and faba bean, is concerning within a western Canadian context, since these represent most of the crops that can be grown in this region (Chang et al., 2015; O'Donovan et al., 2007; St. Luce et al., 2015). This suggests that rotation may have limited efficacy as a management strategy for root caused by *F. proliferatum*, particularly since a rotation length of at least four years is recommended for most crops (Cramer, 2000; Hwang et al., 2009). In addition to rotation and other cultural approaches, chemical treatments and biological control may contribute to the management of root rot (Bodah, 2017; Hwang et al., 2003; Nyandoro et al., 2019; Williamson-Benavides & Dhingra, 2021). Genetic resistance, however, can be one of the most effective approaches for plant disease control.

Recent studies have indicated a high level of resistance in *Allium fistulosum* and *Allium schoenoprasum* accessions to isolates of *F. oxysporum* and *F. proliferatum* (Galván et al., 2008). Resistance to Fusarium crown rot also has been widely reported in wheat and barley, in most cases associated with nonpathogen-specific quantitative trait loci (QTL) (Liu & Ogbonnaya, 2015). Similarly, QTLs associated with resistance to Fusarium root rot have also been identified in pea (Coyne et al., 2019; Rubiales et al., 2023), as has partial resistance or tolerance to root rot in soybean (Nyandoro et al., 2019). There are limited reports, however, of resistance or enhanced tolerance in faba bean (Rubiales & Khazaei, 2022), canola (Yu et al., 2023a), lentil (Zitnick-Anderson et al., 2021), or lupine (Hwang et al., 2014b). Nonetheless, the milder severity of root rot caused by *F. proliferatum*

observed on barley and wheat in this study suggests that cereal crops may be less favorable hosts. In an earlier study, barley demonstrated yield tolerance to Fusarium crown rot caused by *Fusarium pseudograminearum* (Liu et al., 2012), while wheat genotypes with tolerance to Pythium root rot have also been reported (Higginbotham et al., 2004). While no oat genotypes were included in the present study, the response of this crop to *F. proliferatum* may be worth evaluating, as another report indicated that oats had greater tolerance than other cereal crops to root and crown rot caused by *Fusarium* spp. (Collins et al., 2018; Saad et al., 2022). The significant differences in root severity observed among cultivars of some species (faba bean, canola, and lentil) suggested diversity in the reaction to *F. proliferatum* within some crops, which may prove helpful in the development of root rot management plans.

Molecular identification and phylogenetic analysis based on the translation elongation factor 1-alpha (TEF-1α) sequences, along with mating studies on multiple isolates from diverse hosts and locations, have suggested that there is no relation between *F. proliferatum* isolates and their hosts or geographic origins (Azuddin et al., 2021; Jurado et al., 2010; Proctor et al., 2010; Stepien et al., 2011). Nevertheless, an evaluation of vegetative compatibility among isolates of *F. proliferatum* indicated that isolates recovered from maize, onion, sugarcane, and rice could be classified into different vegetative compatibility groups (VCGs), indicating a correlation between VCG and host preference (Alizadeh et al., 2010). The fungal isolate used in the current study was obtained from

canola (Yu et al., 2022a), and assessments of the host responses to additional isolates collected from different species may be warranted to confirm the reactions (relative tolerance or degree of susceptibility) observed here.

Principal Component Analysis has been recommended for the study of plant diseases given its ability to evaluate the relative importance of different variables in a quantitative manner (Madden & Pennypacker, 1979). For example, PCA has been used to assess leaf features, including color, shape, and texture, which could be used for disease identification (Wang et al., 2012), and for evaluation of drought tolerance in canola based on morphological and agronomic traits (Zhu et al., 2011). In this study, the two principal components (PC1 and PC2) explained over 90% of the original variation among cultivars, indicating the capacity of these components to capture most of the diversity in responses to *F. proliferatum*. All five parameters used in the PCA were identified as major factors contributing to PC1. The PCA analysis highlighted the importance of considering multiple factors, including disease severity and reductions in growth parameters, for an improved assessment of host responses to the fungus. When only root rot severity was evaluated, all crops appeared susceptible to *F. proliferatum*, despite some variation in the disease ratings. However, evaluation of the effect of the fungus on other parameters, such as emergence and biomass, highlighted the greater tolerance observed in some crops, particularly barley and wheat, relative to others. While disease severity often is used as the primary measure of fungal pathogenicity (Arabi & Jawhar, 2013; Naseri & Mousavi, 2013) or host range

(Safarieskandari et al., 2021), the effects of a pathogen on plant biomass, plant height, and seedling emergence have also been explored in several studies (Arias et al., 2013; Chang et al., 2020; Hwang et al., 2003). Notable reductions in dry weight (> 50%) were documented for *Allium* spp. inoculated with *F. proliferatum*, reflecting severe root rot (Palmero et al., 2012). To the best of our knowledge, this study is the first to apply PCA for evaluation of the utility of different plant-related parameters for host range identification in *F. proliferatum*.

This study indicated that barley, wheat, and, to a lesser extent, faba bean showed a degree of tolerance to *F. proliferatum*, whereas canola and other legume crops exhibited greater susceptibility. Additionally, although there was variability in susceptibility levels among cultivars of these species, none proved to be fully resistant. Considering the significance of these crops in western Canadian agriculture, rotations might not entirely succeed in minimizing Fusarium root rot.

Table 6.1. Root rot severity on eight crop species at 21 days after seeding in potting medium treated with different concentrations of *Fusarium proliferatum* inoculum.

Treatment[a]	Root Rot Severity[b]							
	Wheat	Barley	Faba bean	Pea	Lentil	Canola	Lupine	Soybean
Control	0.00 a, A	0.00 a, A	0.00 a, A	0.00 a, A	0.00 a, A	0.00 a, A	0.00 a, A	0.00 a, A
Low	1.29 b, A	1.47 b, AB	1.77 b, BC	1.97 b, C	1.96 b, C	2.66 b, D	2.87 b, DE	3.03 b, E
High	2.12 c, A	1.98 c, A	2.51 c, B	2.73 c, B	3.35 c, C	3.38 c, C	3.33 c, C	3.42 c, C

[a] Control, non-inoculated; Low, treated with a low concentration (3×10^4 colony forming units (cfu)/g potting medium) of *F. proliferatum* inoculum; High, treated with a high concentration (6×10^4 cfu/g potting medium) of *F. proliferatum* inoculum. The results for each plant species represent the averages from 2-4 cultivars of each crop (see Supplementary Table 6.1 for the data for the individual cultivars).

[b] Root rot disease severity as assessed on a 0-4 scale (Hwang et al., 1994), where: 0 = healthy roots; 1 = small, light-brown lesions on < 25% of the tap root; 2 = brown lesions on 25-49% of the tap root; 3 = brown lesions on 50-74% of the tap root, tap root constricted; and 4 = tap root severely girdled, brown lesions on > 75% of the tap root with limited lateral roots.

Note: Different lowercase letters indicate significant differences ($p < 0.05$) within columns, while different uppercase letters indicate significant differences within rows.

Table 6.2. Average plant height, seedling emergence, and shoot and root weights of eight crop species at various times after seeding in potting medium treated with different concentrations of *Fusarium proliferatum* inoculum.

Treatment[a]	Plant Height (cm)[b]							
	Wheat	Barley	Faba bean	Pea	Lentil	Canola	Lupine	Soybean
Control	18.31 a	17.19 a	10.14 a	15.66 a	11.33 a	3.73 a	12.52 a	6.76 a
Low	17.27 a	17.21 a	7.79 b	8.13 b	4.34 b	1.83 b	5.62 b	2.66 b
High	12.01 b	10.87 b	6.86 b	5.59 c	2.16 c	1.34 b	3.60 c	1.81 b
	Seedling Emergence[c]							
	Wheat	Barley	Faba bean	Pea	Lentil	Canola	Lupine	Soybean
Control	9.57 a	9.80 a	6.55 a	9.38 a	9.35 a	9.40 a	9.90 a	8.70 a
Low	8.80 b	9.15 a	6.70 a	5.85 b	3.80 b	2.55 b	4.75 b	1.53 b
High	6.53 c	7.10 b	4.75 b	2.78 c	0.65 c	0.45 c	1.85 c	0.53 c
	Shoot Dry Weight (g)[d]							
	Wheat	Barley	Faba bean	Pea	Lentil	Canola	Lupine	Soybean
Control	0.42 ab	0.45 a	1.95 a	1.14 a	0.56 a	0.35 a	1.49 a	1.24 a
Low	0.45 b	0.50 a	1.68 b	0.67 b	0.12 b	0.18 ab	0.61 b	0.39 b
High	0.29 a	0.36 a	1.42 c	0.27 c	0.02 b	0.04 b	0.18 c	0.25 b
	Root Dry Weight (g)[e]							
	Wheat	Barley	Faba bean	Pea	Lentil	Canola	Lupine	Soybean
Control	0.55 a	0.54 a	0.87 a	0.69 a	0.30 a	0.07 a	0.33 a	0.32 a
Low	0.37 b	0.46 a	0.74 b	0.53 b	0.07 b	0.05 a	0.11 b	0.08 b
High	0.19 c	0.22 b	0.71 b	0.19 c	0.02 b	0.01 a	0.04 b	0.06 b

[a] Control, non-inoculated; Low, treated with a low concentration (3×10^4 colony forming units (cfu)/g potting medium) of *F. proliferatum* inoculum; High, treated with a high concentration (6×10^4 cfu/g potting medium) of *F. proliferatum* inoculum. The results for each plant species represent the averages from 2-4 cultivars of each crop (see Supplementary Table 6.1 for the data for the individual cultivars).

[b] Plant height measured at 14 days after seeding.

[c] Seedling emergence (average number of plants per experimental unit (cup)) measured at 7 days after seeding.

[d] Shoot dry weight measured at 21 days after seeding.

[e] Root dry weight measured at 21 days after seeding.

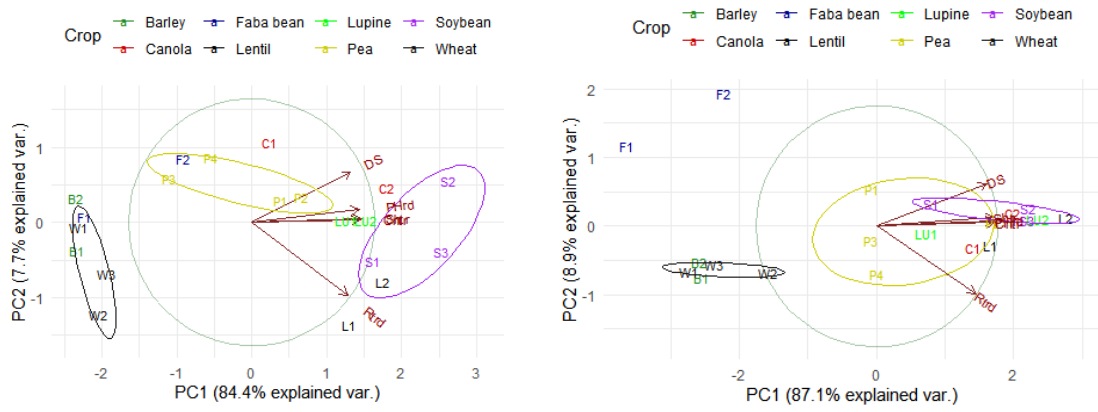
Note: Different lowercase letters indicate significant differences ($p < 0.05$) within columns.

Table 6.3. Comparison of root rot severity at maturity (after flowering) on nine cultivars representing seven different crop species grown in potting medium inoculated with *Fusarium proliferatum*.

Crop	Cultivar	Disease Severity[a]
Barley	Canmore	1.08 a
Wheat	AC Crystal	1.15 a
Faba bean	Fabelle	2.00 b
Pea	AAC Carver	2.62 c
Canola	Westar	3.00 d
Soybean	AKRAS R2	3.06 d
Pea	CDC Amarillo	3.20 d
Pea	CDC Greenwater	3.26 d
Lupine	Arabella	3.57 e

[a] Root rot disease severity as assessed on a 0-4 scale (Hwang et al., 1994), where: 0 = healthy roots; 1 = small, light-brown lesions on < 25% of the tap root; 2 = brown lesions on 25-49% of the tap root; 3 = brown lesions on 50-74% of the tap root, tap root constricted; and 4 = tap root severely girdled, brown lesions on > 75% of the tap root with limited lateral roots. Disease assessments were conducted following the completion of flowering for each cultivar/crop.

Note: Different lowercase letters indicate significant differences ($p < 0.05$) within columns.



(**a**) (**b**)

Figure 6.1. Principal Component Analysis based on root rot disease severity (DS) and reductions in seedling emergence (Ctrd), plant height (PHrd), shoot dry weight (Shrd), and root dry weight (Rtrd) of 20 cultivars representing eight crop species grown in potting medium treated with a low (a) or high (b) concentration of *Fusarium proliferatum* (3×10^4 and 6×10^4 colony forming units/g potting medium, respectively). Disease severity and shoot and root dry weights were assessed at 21 days after seeding, while emergence was measured at 7 days and plant height was measured at 14 days after seeding. B1, barley cultivar 'AB Tofield'; B2, barley 'Canmore'; W1, wheat 'Katepwa'; W2, wheat 'AC Crystal'; W3, wheat 'Lillian'; P1, pea 'CDC Greenwater'; P2, pea 'AAC Carver'; P3, pea 'CDC Amarillo'; P4, pea 'AAC Barrhead'; S1, soybean 'AAC Mandor'; S2, soybean 'OT15-02'; S3, soybean 'AKRAS R2'; LU1, lupine 'Arabella'; LU2, lupine 'Mirabor'; L1, lentil 'CDC Nimble'; L2, lentil 'CDC Lima CL'; F1, faba bean 'Malik'; F2, faba bean 'Fabelle'; C1, canola 'Westar'; C2, canola 'L255PC'.

Chapter 7 Conclusions

7.1 Introduction

Root rot is a significant threat to canola (*Brassica napus* L.) production in western Canada (Chen et al., 2014; Hwang et al., 2014). The disease results from infection by various soilborne pathogens, including *Rhizoctonia solani* J.G. Kühn, *Fusarium* spp., and *Pythium* spp., which collectively constitute the root rot complex (RRC). Unfortunately, current strategies for the control of this disease are limited (Chen et al., 2014; Hwang et al., 2014). An improved understanding of the identity, diversity, and aggressiveness of the primary pathogens responsible for root rot of canola, as well as knowledge of their impact on this and other crops grown in the region, will aid in the development of improved disease management strategies (Klosterman et al., 2009; Maryani et al., 2019). As such, the generation of this information was a major focus of this dissertation.

Chapter 1 of the thesis served as an introduction and literature review, setting the context for the work conducted. Chapters 2 and 3 described canola root rot surveys in northcentral Alberta carried out in 2021 and 2022; these surveys not only provided an overview of the disease situation in the region, but also enabled the collection of large amounts of root and lower stem samples that served as the basis for most of the subsequent research. The prevalence, pathogenicity and diversity of canola root rot fungi was evaluated in Chapter 4, while the virulence of selected *Fusarium* species and their yield impact on canola was examined in Chapter 5. In the final data chapter (Chapter 6), the host

range of one of these fungi, *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, was determined. The following sections address the conclusions and recommendations stemming from these studies.

7.2 The occurrence, pathogenicity, and identity of pathogens causing root rot of canola

Root rot first emerged as a threat to Canadian canola production in the late 1980s, approximately 10 years after the start of commercial cultivation of this crop, with *R. solani* identified as the primary cause (Acharya et al., 1984; Gugel et al., 1987; Kataria & Verma, 1992). Later, *Fusarium* species were also identified as major contributors to the development of root rot in canola cropping systems (Chen et al., 2014; Hwang et al., 2009; Zhou, Chen, et al., 2014). In Alberta, however, the last major analysis of root rot and its *Fusarium* causal agents was completed a decade ago (Chen et al., 2014). As a result, there is need for updated information on the etiology and epidemiology of this disease.

Analysis of canola tissue samples collected in 2021 and 2022 indicated that *Fusarium* spp. were the most common root rot pathogens, with an average recovery rate exceeding 80% from roots and over 60% from stems. In contrast, *R. solani* was recovered only from approximately 10% of the samples. The identification of the *Fusarium* isolates to species level relied on colony and morphological characteristics, complemented by sequence analysis of the internal transcribed spacer (ITS) and translation elongation factor-1α (TEF-1α) sequences. This analysis indicated that *Fusarium avenaceum* R.J. Cook was most

prevalent, followed by *Fusarium redolens* Wollenw., *Fusarium solani* (Mart.) Sacc., and *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen. The results suggested a shift from earlier studies, when *Fusarium acuminatum* Ellis & Everh. was most common (Chen et al., 2014). In total, 14 different *Fusarium* spp. were detected in 2021, with nine species identified in 2022, highlighting the diversity of *Fusarium* populations in northcentral Alberta. Notably, *Fusarium sporotrichioides* Sherb. and *Fusarium commune* K. Skovg. were identified as causative agents of canola root rot for the first time.

While aggressiveness varied across species and isolates, the vast majority of fungi caused moderate to severe root rot. *Fusarium avenaceum* and *F. sporotrichioides* in particular were highly aggressive, causing not only severe disease symptoms but also significant reductions in emergence, plant height, and root and shoot weights. All of these parameters were included in principal component analysis, which enabled a comprehensive assessment of pathogenicity among species and isolates. A negative correlation was found between disease severity and both emergence and seedling growth, which underscored the detrimental effects of *Fusarium* infection on early plant development. Phylogenetic analysis based on the ITS and TEF-1α sequences indicated no correlations between aggressiveness, geographic origin or year collected among the species, suggesting consistent distribution and pathogenicity in the study.

7.3 The influence of *Fusarium* spp. on canola growth and yield

Field and greenhouse experiments were conducted to assess the effects of *F. avenaceum*, *F. proliferatum*, and *F. oxysporum* on the growth and yield of canola. Additionally, the interaction between *F. proliferatum* and *F. oxysporum* was also examined. All three *Fusarium* species demonstrated the ability to induce severe root rot, resulting in yield reductions of up to 35%, along with compromised emergence and growth. Additionally, two yield loss models were developed: one correlating seed weight per plant with disease severity, and the other linking pod number per plant with disease severity. In these models, yield parameters decreased linearly as disease severity increased; on a scale of 0 to 4.0, yield approached zero as disease severity reached 4.0, suggesting a 25% decline in yield for each unit increase in disease severity.

Co-inoculation with *F. oxysporum* and *F. proliferatum* resulted in more severe root rot compared with inoculation with either species individually. This suggested synergistic interactions between these fungi, resulting in more severe disease development. The identification of significant negative linear correlations between seedling emergence, yield parameters, and root rot severity emphasizes the need to adopt more efficient root rot management tactics, particularly in the context of simultaneous infection by multiple pathogens.

7.4 Host range of *Fusarium proliferatum*

While crop rotation is crucial for controlling soilborne diseases, its effectiveness is contingent upon the inclusion of non-host plants in the cropping sequence (Gálvez &

Palmero, 2022; Hwang et al., 2009; Stepien et al., 2011). As such, the development of useful rotations depends on an understanding of the host range of plant pathogens (Morris and Moury, 2019). Although the host ranges of *F. avenaceum*, *F. oxysporum*, *F. solani*, and *F. redolens* have been studied extensively, limited information has been reported for *F. proliferatum*, and no reports are available from western Canada. Considering the significance of canola in Canada, it is important to explore the host range of newly emerging pathogens for improved disease management.

The host range of *F. proliferatum* was assessed by inoculating various crop species, including legumes (faba bean, soybean, lupine, lentil, and pea), cereals (wheat and barley), and canola, all of which are widely cultivated in western Canada. Generally, *F. proliferatum* caused root rot on all evaluated crops. However, based on symptom severity and its impact on emergence, plant height, and root and shoot dry weights, soybean, lupine, canola, and lentil appeared to be the most susceptible. Conversely, barley and wheat, followed by faba bean, showed greater tolerance to infection based on these parameters. Similar trends were observed across the crops at both the seedling and adult plant stages. The detection of crops and some cultivars that exhibited greater tolerance to *F. proliferatum* may prove helpful in the development of improved rotations for root rot management. Moreover, the evaluation of multiple factors beyond disease severity, such as seedling emergence, in principal component analysis provided a more complete understanding of the host responses to infection. The significant reductions in emergence

at the seedling stage may indicate particular risk to stand establishment from early infection.

7.5 Future directions

The results presented in this thesis mark a first step toward generating the information necessary for improved root rot management in canola. Further investigations should concentrate on the biology of *Fusarium* spp. within the canola root rot pathosystem. These studies could include experiments on host-pathogen interactions, the reproductive and survival traits of the pathogen, as well as the response of these fungi to environmental factors within canola cropping systems. A refined understanding of the genetic regulation of root rot tolerance in canola, for example through the identification of quantitative trait loci, could also be beneficial. Additionally, pinpointing biocontrol agents exhibiting broad efficacy against the primary components of the root rot complex might serve as an eco-friendly strategy for disease management. Given the resilience of *Fusarium* pathogens in the soil, investigations into exploiting the chemical and physical properties of soil to diminish inoculum longevity may also be justified. Given the dynamic nature of the pathogen population, field surveys should be conducted regularly to keep information updated. It is clear that an integrated approach will be required for the enhanced and sustainable management of canola root rot.

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Appendix A: Supplementary materials for Chapter 4

Supplementary Table 4.1. Pathogenicity of *Fusarium* isolates causing root rot and their impact on growth of canola seedlings.

Isolate	Species[a]	Disease Severity[b]	Countrd[c]	Phrd[d]	Shootrd[e]	Rootrd[f]	Field No.[g]	Tree[h]
CS139	FAC	2.41	27.9%	16.7%	27.8%	72.6%	2022-21	
CS143	FAC	2.16	24.6%	14.3%	30.5%	68.4%	2022-25	1
CS178	FAC	1.64	19.7%	5.7%	-26.5%	57.8%	2022-18	
CS261S	FAC	2.10	37.7%	17.7%	37.3%	70.3%	2022-26	1
F034	FAC	1.50	23.7%	-1.9%	17.0%	77.7%	2021-16	1
F125	FAC	1.79	25.0%	11.3%	19.0%	55.8%	2021-32	
CS002	FAV	2.99	73.8%	27.3%	73.2%	87.8%	2022-11	1
CS005S	FAV	2.24	67.2%	17.7%	61.4%	87.9%	2022-23	1
CS009	FAV	2.22	41.0%	13.4%	41.7%	80.8%	2022-26	1
CS016S	FAV	2.00	50.8%	13.0%	45.5%	88.7%	2022-20	1
CS018S	FAV	2.39	49.2%	12.5%	35.9%	78.2%	2022-18	
CS020	FAV	3.13	62.3%	17.9%	55.1%	84.8%	2022-21	
CS022	FAV	2.91	63.9%	18.4%	60.2%	85.6%	2022-11	1
CS034	FAV	2.76	67.2%	23.4%	64.6%	84.3%	2022-48	
CS048	FAV	2.90	65.6%	17.9%	44.5%	81.7%	2022-31	
CS051	FAV	3.46	77.0%	38.8%	79.4%	92.1%	2022-23	1
CS057	FAV	1.95	37.7%	10.7%	26.4%	72.9%	2022-21	
CS070	FAV	2.88	70.5%	14.9%	71.5%	88.6%	2022-21	1
CS081	FAV	2.28	42.6%	6.1%	30.8%	72.6%	2022-23	1
CS084	FAV	2.69	50.8%	17.3%	39.6%	77.8%	2022-26	1
CS085S	FAV	1.97	32.8%	14.4%	10.2%	56.5%	2022-2	
CS089	FAV	2.71	70.5%	28.6%	64.4%	86.8%	2022-15	
CS092S	FAV	3.00	83.6%	33.3%	84.3%	94.7%	2022-43	
CS123	Fsp	3.48	73.8%	35.0%	82.5%	93.9%	2022-15	
CS094	FAV	2.65	47.5%	19.4%	25.1%	81.4%	2022-37	1
CS138S	Fsp	2.41	29.5%	17.6%	37.1%	61.2%	2022-22	
CS098S	FAV	3.46	85.2%	37.1%	80.6%	93.9%	2022-17	
CS103	FAV	3.04	80.3%	54.5%	82.0%	94.6%	2022-23	
CS104S	FAV	3.14	55.7%	17.0%	46.6%	84.8%	2022-19	1
CS119	FAV	2.73	49.2%	10.7%	47.5%	72.7%	2022-20	1
CS128S	FAV	3.44	90.2%	50.5%	89.6%	96.3%	2022-6	
CS145S	FAV	2.45	60.7%	15.0%	29.7%	86.0%	2022-26	
CS150	FAV	3.00	60.7%	22.1%	39.4%	89.2%	2022-26	

CS155	FAV	3.50	88.5%	43.7%	78.4%	95.8%	2022-47	1
CS162S	FAV	3.38	80.3%	28.0%	84.6%	95.4%	2022-36	1
CS163	FAV	3.14	73.8%	31.2%	66.6%	92.4%	2022-51	
CS185S	FAV	3.07	78.7%	46.0%	61.6%	93.2%	2022-7	
CS202	FAV	2.86	77.0%	21.6%	63.8%	92.7%	2022-38	
CS214	FAV	3.20	68.9%	23.7%	49.8%	88.1%	2022-7	
CS217	FAV	3.17	65.6%	24.9%	56.1%	70.0%	2022-48	1
CS267S	FAV	2.85	52.5%	32.0%	54.1%	83.1%	2022-22	1
CS219	Fsp	2.64	57.4%	23.6%	27.3%	58.7%	2022-46	
CS269S	FAV	2.88	59.0%	40.1%	45.5%	82.5%	2022-19	
CS284	FAV	2.27	23.0%	14.2%	22.4%	72.4%	2022-23	
CS355	FAV	2.06	39.3%	13.6%	44.8%	65.9%	2022-5	
F002	FAV	1.60	23.7%	14.0%	37.5%	56.0%	2021-2	1
F005	FAV	3.75	92.1%	69.1%	92.9%	98.2%	2021-2	1
F006	FAV	1.68	26.3%	41.3%	50.2%	75.9%	2021-3	1
F013	FAV	3.31	80.3%	37.8%	79.5%	92.2%	2021-10	1
F016	FAV	3.66	86.8%	63.7%	90.7%	97.1%	2021-12	
F017	FAV	3.77	89.5%	62.2%	93.6%	97.7%	2021-12	
F020	FAV	2.23	43.4%	16.9%	56.7%	76.1%	2021-13	1
F022	FAV	2.11	32.9%	6.4%	27.9%	81.5%	2021-14	1
F024	FAV	3.19	71.1%	10.1%	70.0%	92.2%	2021-16	1
F025	FAV	2.30	40.8%	15.4%	36.7%	84.3%	2021-16	1
F026	FAV	2.79	67.1%	1.7%	60.4%	86.4%	2021-16	1
F027	FAV	1.89	26.3%	-0.5%	23.0%	71.6%	2021-16	1
F028	FAV	2.65	50.0%	-8.6%	41.6%	83.6%	2021-16	1
F030	FAV	3.19	71.1%	28.1%	75.1%	93.2%	2021-16	1
F031	FAV	2.33	55.3%	-1.5%	47.3%	81.9%	2021-16	1
F032	FAV	1.66	26.3%	1.5%	25.5%	79.1%	2021-16	1
F036	FAV	2.24	52.6%	2.4%	47.1%	82.8%	2021-16	1
F039	FAV	2.88	63.2%	18.0%	75.5%	90.9%	2021-19	1
F046	FAV	2.44	52.6%	12.6%	58.2%	76.2%	2021-21	1
F049	FAV	1.82	34.2%	19.6%	51.6%	72.0%	2021-23	
F052	FAV	3.69	93.4%	63.0%	92.1%	97.6%	2021-23	1
F053	FAV	3.55	75.0%	33.7%	82.6%	93.0%	2021-23	1
F054	FAV	1.22	10.5%	10.2%	17.3%	58.7%	2021-23	1
F055	FAV	3.29	67.1%	12.1%	65.6%	91.2%	2021-24	1
F056	FAV	2.96	47.4%	4.4%	49.5%	73.8%	2021-24	1
F060	FAV	2.34	32.9%	28.3%	35.7%	81.8%	2021-24	1
F061	FAV	2.87	46.1%	2.8%	55.3%	57.6%	2021-24	1
F062	FAV	3.67	78.9%	12.3%	75.7%	87.3%	2021-24	1
F063	FAV	1.65	21.1%	5.2%	7.7%	50.8%	2021-24	1

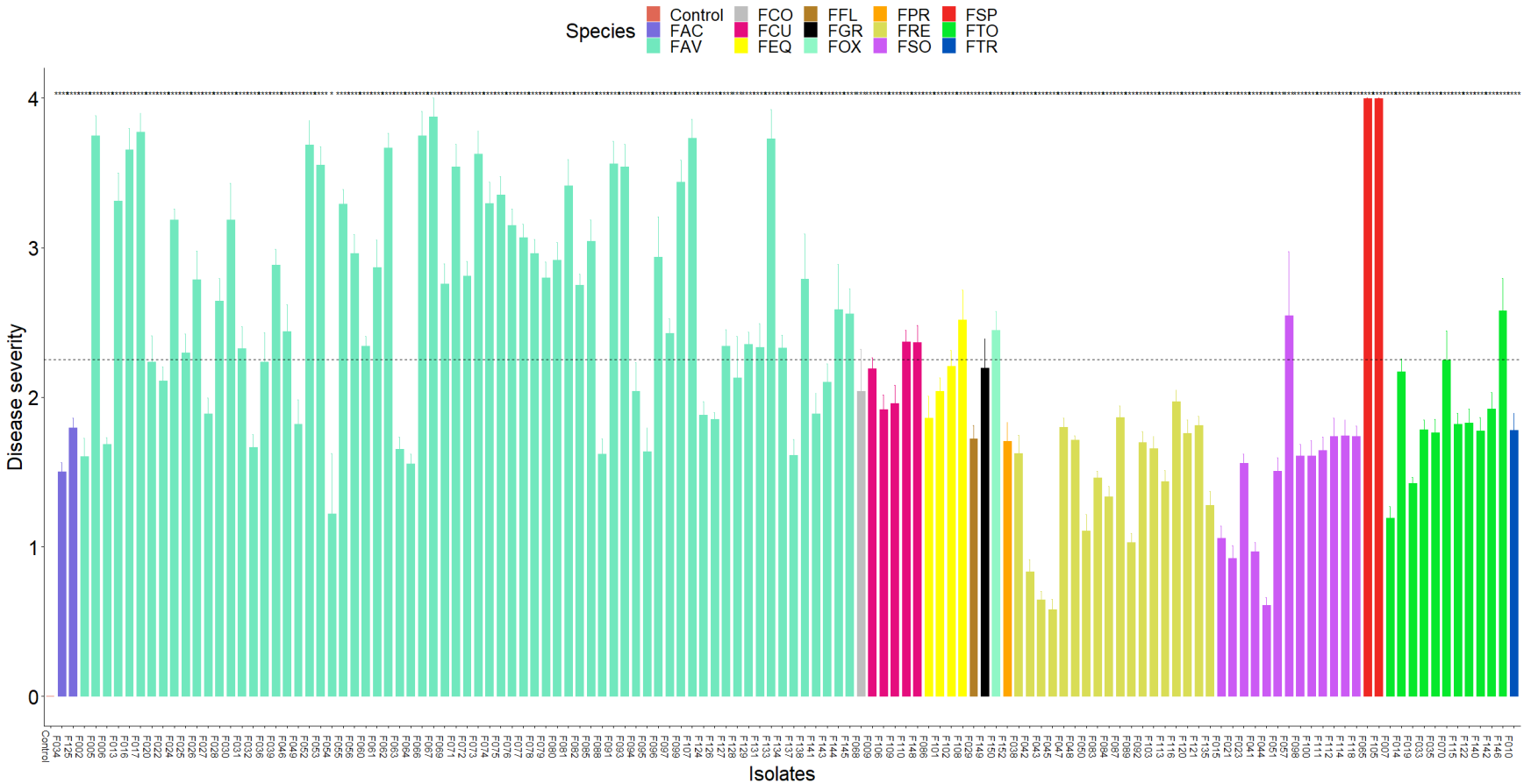
F064	FAV	1.55	15.8%	9.4%	13.7%	57.8%	2021-24	1
F066	FAV	3.75	93.4%	58.6%	90.6%	92.9%	2021-25	1
F067	FAV	3.88	89.5%	67.4%	94.9%	97.9%	2021-25	1
F069	FAV	2.76	40.8%	9.0%	44.6%	82.6%	2021-25	1
F071	FAV	3.54	84.2%	31.4%	77.9%	93.9%	2021-25	1
F072	FAV	2.81	23.7%	5.6%	13.1%	30.6%	2021-25	1
F073	FAV	3.63	75.0%	23.4%	74.4%	93.1%	2021-25	1
F074	FAV	3.30	57.9%	22.0%	63.0%	87.3%	2021-25	1
F075	FAV	3.35	63.2%	10.3%	67.4%	92.2%	2021-25	1
F076	FAV	3.15	53.9%	13.9%	59.5%	80.2%	2021-25	1
F077	FAV	3.07	60.5%	8.2%	63.9%	70.8%	2021-25	1
F078	FAV	2.96	22.4%	31.4%	41.9%	44.7%	2021-25	
F079	FAV	2.79	26.3%	24.8%	39.7%	45.3%	2021-25	1
F080	FAV	2.92	42.1%	19.3%	44.1%	57.4%	2021-25	1
F081	FAV	3.42	89.5%	45.6%	84.7%	91.5%	2021-25	1
F082	FAV	2.75	39.5%	6.1%	41.6%	64.6%	2021-25	1
F085	FAV	3.05	44.7%	36.4%	67.3%	89.1%	2021-26	1
F088	FAV	1.62	31.6%	32.5%	34.6%	79.8%	2021-26	1
F091	FAV	3.56	73.7%	57.3%	79.6%	96.5%	2021-27	1
F093	FAV	3.54	78.9%	45.0%	80.2%	96.4%	2021-27	1
F094	FAV	2.04	46.1%	35.5%	20.5%	78.5%	2021-27	1
F095	FAV	1.63	18.4%	40.1%	15.1%	76.1%	2021-28	1
F096	FAV	2.94	60.5%	47.4%	47.0%	86.8%	2021-28	
F097	FAV	2.43	57.9%	36.7%	54.9%	87.0%	2021-28	1
F099	FAV	3.44	84.2%	49.6%	79.2%	95.3%	2021-29	1
F106	FCU	1.92	15.8%	44.3%	32.4%	72.3%	2021-30	1
F107	FAV	3.73	78.9%	55.3%	82.6%	91.5%	2021-30	1
F124	FAV	1.88	30.3%	20.2%	32.6%	68.8%	2021-32	
F126	FAV	1.85	18.4%	23.4%	31.5%	59.3%	2021-32	1
F127	FAV	2.34	47.4%	22.9%	40.3%	71.5%	2021-32	
F128	FAV	2.13	53.9%	33.7%	43.4%	76.4%	2021-32	
F129	FAV	2.35	32.9%	9.5%	5.1%	38.8%	2021-32	1
F131	FAV	2.33	61.8%	5.4%	12.5%	59.1%	2021-32	1
F133	FAV	3.73	90.8%	71.6%	85.5%	96.5%	2021-32	1
F134	FAV	2.33	43.4%	12.2%	1.1%	51.2%	2021-33	1
F137	FAV	1.61	18.4%	-0.9%	-38.9%	34.3%	2021-33	1
F138	FAV	2.79	78.9%	24.4%	53.5%	89.5%	2021-33	1
F141	FAV	1.89	15.8%	-0.8%	-27.3%	34.7%	2021-33	
F143	FAV	2.10	30.3%	6.8%	13.7%	62.1%	2021-33	
F144	FAV	2.59	68.4%	2.4%	44.0%	81.5%	2021-33	
F145	FAV	2.56	52.6%	5.7%	38.6%	84.7%	2021-33	

F068	FCO	2.04	10.5%	14.7%	30.5%	63.5%	2021-25	1
CS246S	FCU	1.76	13.1%	10.1%	-19.6%	65.0%	2022-34	
CS248S	FCU	1.78	24.6%	21.7%	-11.4%	66.6%	2022-32	
CS250	FCU	1.46	27.9%	18.9%	9.3%	18.8%	2022-37	
CS252	FCU	2.22	26.2%	29.1%	-9.4%	67.7%	2022-12	1
CS263S	FCU	2.27	47.5%	41.9%	50.9%	75.1%	2022-48	1
F009	FCU	2.19	80.3%	60.0%	75.2%	84.9%	2021-3	1
F109	FCU	1.96	19.7%	46.8%	42.7%	84.9%	2021-30	
F110	FCU	2.37	31.6%	46.7%	48.9%	82.2%	2021-30	1
F148	FCU	2.37	32.9%	27.8%	15.0%	58.5%	2021-	
F086	FEQ	1.86	50.0%	47.9%	50.1%	78.3%	2021-26	1
F101	FEQ	2.04	38.2%	57.5%	46.3%	78.5%	2021-30	1
F102	FEQ	2.21	40.8%	56.1%	54.2%	84.1%	2021-30	
F108	FEQ	2.52	40.8%	54.1%	42.5%	78.0%	2021-30	1
CS346	FFL	1.42	16.4%	6.9%	5.3%	54.0%	2022-4	1
F029	FFL	1.72	17.1%	8.9%	24.9%	75.1%	2021-16	1
F149	FGR	2.19	44.7%	2.4%	24.7%	64.7%	2021-	1
CS171	FOX	1.94	27.9%	13.9%	-8.0%	50.1%	2022-44	
CS174	FOX	2.22	59.0%	33.1%	8.6%	68.1%	2022-46	
CS218	FOX	1.78	26.2%	10.8%	-6.9%	45.5%	2022-26	
CS358	FOX	2.01	50.8%	20.5%	43.2%	63.2%	2022-44	
CS359	FOX	2.16	37.7%	10.1%	41.2%	61.0%	2022-44	
CS459	FOX	1.95	45.9%	23.3%	26.3%	62.7%	2022-26	
CS466	FOX	1.48	14.8%	2.5%	-13.7%	47.7%	2022-26	1
F150	FOX	2.45	59.2%	11.1%	47.2%	79.7%	2021-	1
F152	FPR	1.71	14.5%	-9.7%	-6.7%	41.2%	2021-	1
CS293	FRE	1.49	52.5%	13.1%	30.4%	70.4%	2022-18	
CS298	FRE	1.53	23.0%	14.6%	31.3%	68.6%	2022-32	1
CS316	FRE	1.33	26.2%	4.9%	-18.6%	57.8%	2022-48	1
CS319	FRE	1.23	14.8%	-2.9%	-26.9%	44.2%	2022-9	1
CS327	FRE	1.27	31.1%	12.0%	16.4%	53.5%	2022-26	1
CS341	FRE	1.46	19.7%	7.5%	5.3%	51.1%	2022-44	1
CS457	FRE	2.04	37.7%	15.4%	15.7%	57.4%	2022-44	1
F038	FRE	1.62	10.5%	14.3%	29.4%	82.2%	2021-18	1
F042	FRE	0.83	9.2%	2.5%	29.5%	17.5%	2021-19	1
F043	FRE	0.64	1.3%	-18.6%	-11.6%	30.8%	2021-19	1
F045	FRE	0.58	1.3%	3.8%	2.2%	34.7%	2021-19	
F047	FRE	1.80	17.1%	16.4%	41.8%	65.1%	2021-21	1
F048	FRE	1.71	30.3%	27.4%	52.2%	74.8%	2021-23	1
F050	FRE	1.10	17.1%	17.6%	39.8%	64.4%	2021-23	1
F083	FRE	1.46	19.7%	37.7%	29.8%	73.4%	2021-26	1

F084	FRE	1.34	1.3%	30.0%	5.6%	72.6%	2021-26	1
F087	FRE	1.86	5.3%	33.8%	17.2%	74.0%	2021-26	1
F089	FRE	1.03	11.8%	35.4%	-2.6%	69.7%	2021-26	1
F092	FRE	1.70	15.8%	41.0%	19.5%	75.8%	2021-27	
F103	FRE	1.65	13.2%	47.2%	28.7%	77.4%	2021-30	1
F113	FRE	1.43	7.9%	31.3%	1.2%	73.1%	2021-30	
F116	FRE	1.97	11.8%	42.6%	9.9%	76.5%	2021-30	1
F120	FRE	1.76	27.6%	35.7%	26.0%	76.0%	2021-30	1
F121	FRE	1.81	17.1%	27.4%	6.6%	71.3%	2021-30	
F135	FRE	1.28	9.2%	-2.1%	-67.9%	11.9%	2021-33	1
CS226	FSO	1.55	31.1%	10.6%	-24.3%	55.1%	2022-16	
CS227	FSO	1.69	23.0%	7.6%	-16.5%	53.7%	2022-20	
CS233S	FSO	1.48	16.4%	6.3%	-10.2%	65.1%	2022-26	
CS258	FSO	2.30	77.0%	54.9%	58.8%	87.7%	2022-9	
CS344	FSO	1.39	21.3%	5.3%	1.7%	39.4%	2022-32	
CS349	FSO	1.39	24.6%	9.9%	21.8%	59.0%	2022-9	
CS352	FSO	1.33	26.2%	15.5%	31.8%	53.7%	2022-12	1
CS464	FSO	1.19	19.7%	-5.3%	-15.5%	36.3%	2022-21	1
F015	FSO	1.06	13.2%	22.3%	40.4%	68.9%	2021-11	1
F021	FSO	0.92	9.2%	14.6%	24.6%	59.4%	2021-13	1
F023	FSO	1.56	14.5%	8.6%	19.4%	79.5%	2021-15	1
F041	FSO	0.97	9.2%	10.8%	35.2%	23.3%	2021-19	1
F044	FSO	0.61	5.3%	4.7%	13.4%	50.4%	2021-19	1
F051	FSO	1.50	14.5%	26.8%	54.2%	75.6%	2021-23	1
F057	FSO	2.54	2.6%	24.2%	56.9%	87.0%	2021-24	1
F098	FSO	1.61	3.9%	39.9%	-1.6%	80.2%	2021-29	1
F100	FSO	1.61	10.5%	37.0%	8.6%	67.8%	2021-29	1
F111	FSO	1.65	7.9%	38.8%	18.4%	79.1%	2021-30	1
F112	FSO	1.74	6.6%	39.9%	10.5%	77.4%	2021-30	1
F114	FSO	1.74	7.9%	30.7%	10.8%	53.7%	2021-30	1
F118	FSO	1.74	9.2%	36.6%	0.1%	72.7%	2021-30	1
F065	FSP	4.00	100.0%	91.2%	96.4%	96.6%	2021-25	1
F105	FSP	4.00	100.0%	100.0%	98.6%	99.6%	2021-30	1
CS255	FTO	1.36	24.6%	16.2%	-14.0%	75.5%	2022-13	
CS289	FTO	1.63	16.4%	6.9%	-9.6%	49.7%	2022-21	
F007	FTO	1.19	11.8%	29.9%	23.0%	67.6%	2021-3	1
F014	FTO	2.17	44.7%	34.7%	43.9%	67.0%	2021-11	1
F019	FTO	1.42	6.6%	27.2%	36.1%	67.2%	2021-12	1
F033	FTO	1.78	19.7%	5.0%	32.6%	79.1%	2021-16	1
F035	FTO	1.76	14.5%	6.4%	24.4%	79.0%	2021-16	1
F070	FTO	2.25	26.3%	10.3%	19.2%	78.5%	2021-25	1

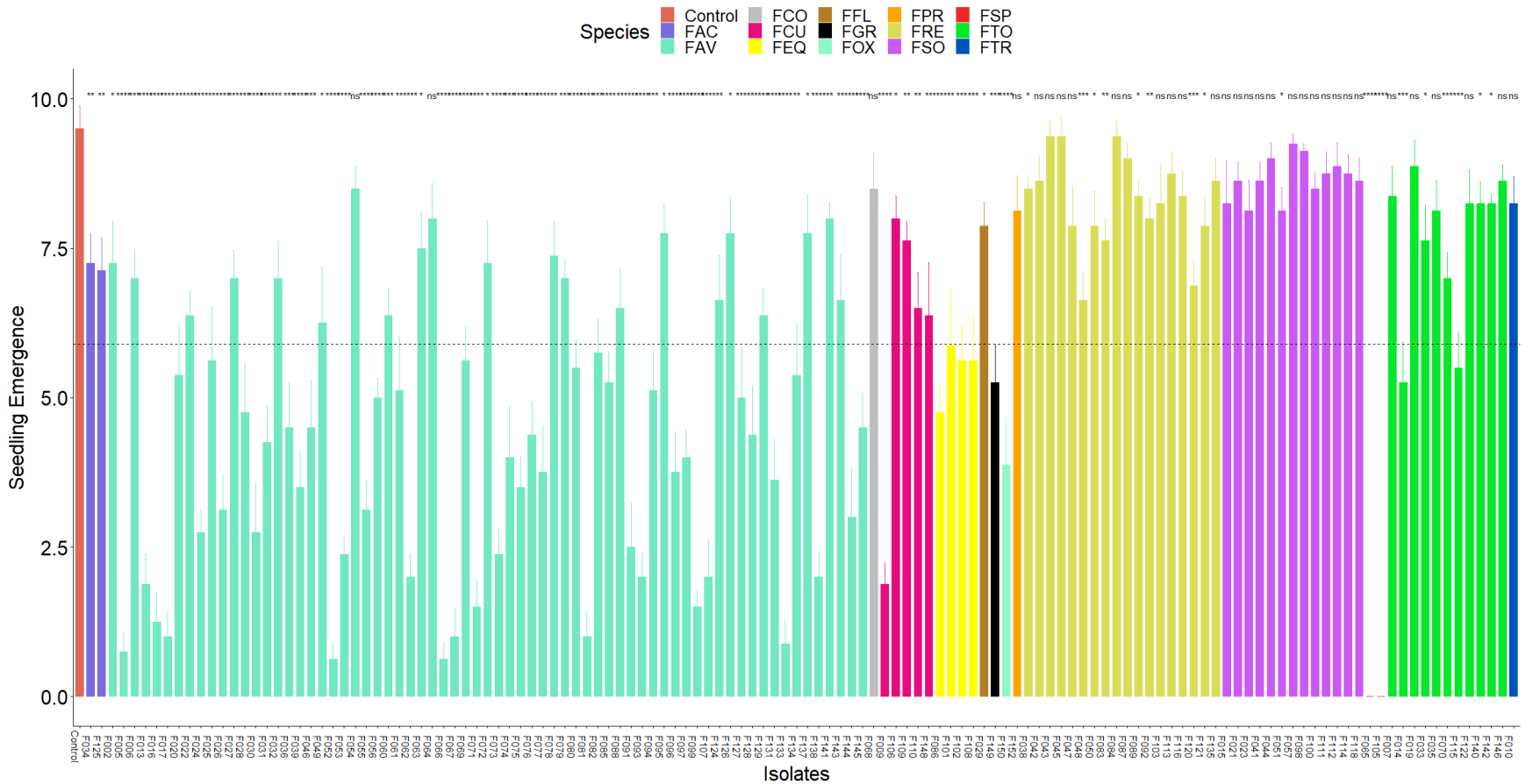
F115	FTO	1.82	42.1%	40.7%	21.5%	78.3%	2021-30	1
F122	FTO	1.83	13.2%	32.9%	17.9%	73.9%	2021-30	1
F140	FTO	1.77	13.2%	-4.3%	-33.7%	46.9%	2021-33	1
F142	FTO	1.92	13.2%	6.4%	-15.8%	51.7%	2021-33	1
F146	FTO	2.58	9.2%	9.0%	22.7%	69.4%	2021-33	1
CS056	FTR	1.89	11.5%	2.4%	-12.8%	43.9%	2022-20	1
CS362	FTR	1.96	23.0%	-8.6%	12.0%	50.3%	2022-6	
F010	FTR	1.78	13.2%	17.1%	20.3%	62.6%	2021-4	1

[a]FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCO, *Fusarium commune*; FCU, *Fusarium culmorum*; FEQ, *Fusarium equiseti*; FFL, *Fusarium flocciferum*; FGR, *Fusarium graminearum*; FOX, *Fusarium oxysporum*; FPR, *Fusarium proliferatum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; FSP, *Fusarium sporotrichioides*; Fsp, unidentified species; FTO, *Fusarium torulosum*, FTR, *Fusarium tricinctum*; [b]Root rot disease severity (0-4 scale) at 21 days after seeding (Hwang et al., 1994); [c]Reduction on seedling emergence caused by corresponding isolate compared with non-inoculated control; [d]Reduction on plant height caused by corresponding isolate compared with non-inoculated control; [e]Reduction on shoot dry weight caused by corresponding isolate compared with non-inoculated control; [f]Reduction on root dry weight caused by corresponding isolate compared with non-inoculated control; [g]Isolated year and field number; [h]Isolates involved in phylogenetic tree construction: 1 represents involved in tree construction.



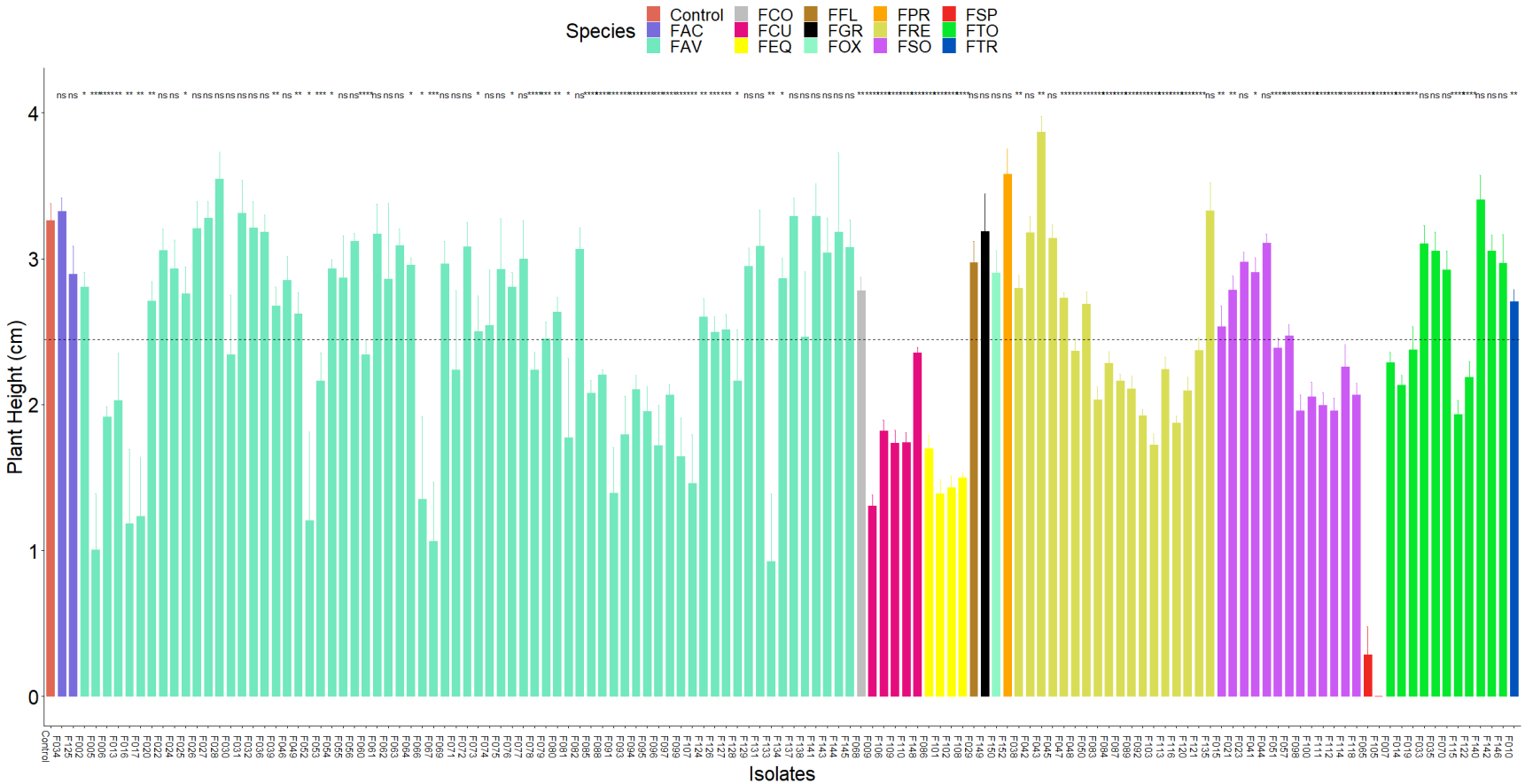
Supplementary Figure 4.1. Impact of *Fusarium* isolates, identified from 2021, on root rot disease severity of canola cv. 'Westar' under greenhouse conditions. Species, species identity; Control, non-inoculated control; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCO, *Fusarium commune*; FCU, *Fusarium culmorum*; FEQ, *Fusarium equiseti*; FFL, *Fusarium flocciferum*; FGR, *Fusarium graminearum*; FOX, *Fusarium oxysporum*; FPR, *Fusarium proliferatum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; FSP, *Fusarium sporotrichioides*; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; ns, no significant difference between the

treatment and corresponding non-inoculated control based on a t-test; *, significant difference at $p < 0.05$; **, significant difference at $p < 0.01$; ***, significant difference at $p < 0.001$; and ****, significant difference at $p < 0.0001$. The dashed lines represent the overall mean for corresponding parameter.



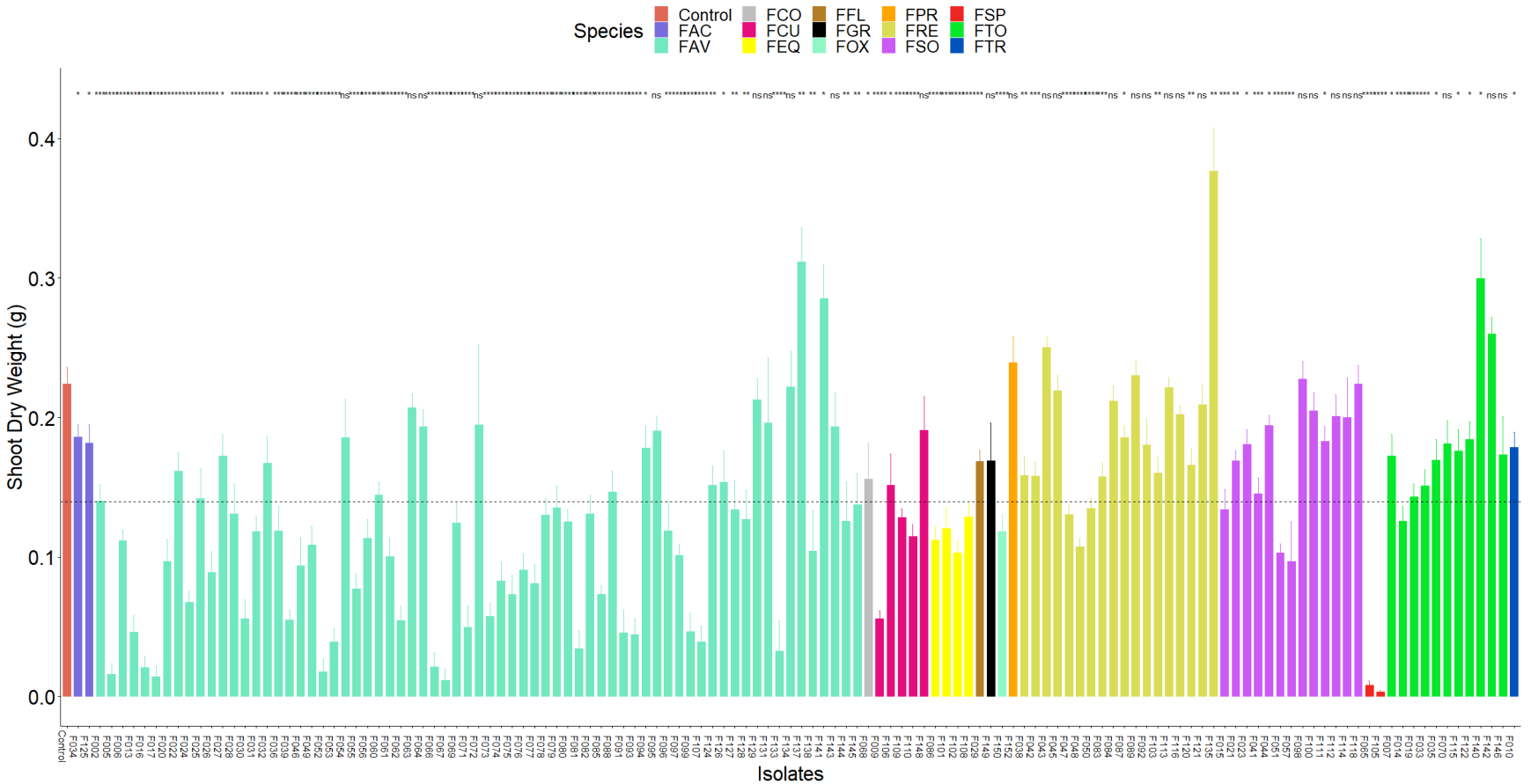
Supplementary Figure 4.2. Impact of *Fusarium* isolates, identified from 2021, on seedling emergence of canola cv. 'Westar' under greenhouse conditions. Species, species identity; Control, non-inoculated control; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCO, *Fusarium commune*; FCU, *Fusarium culmorum*; FEQ, *Fusarium equiseti*; FFL, *Fusarium flocciferum*; FGR, *Fusarium graminearum*; FOX, *Fusarium oxysporum*; FPR, *Fusarium proliferatum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; FSP, *Fusarium sporotrichioides*; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; ns, no significant difference between the

treatment and corresponding non-inoculated control based on a t-test; *, significant difference at $p < 0.05$; **, significant difference at $p < 0.01$; ***, significant difference at $p < 0.001$; and ****, significant difference at $p < 0.0001$. The dashed lines represent the overall mean for corresponding parameter.



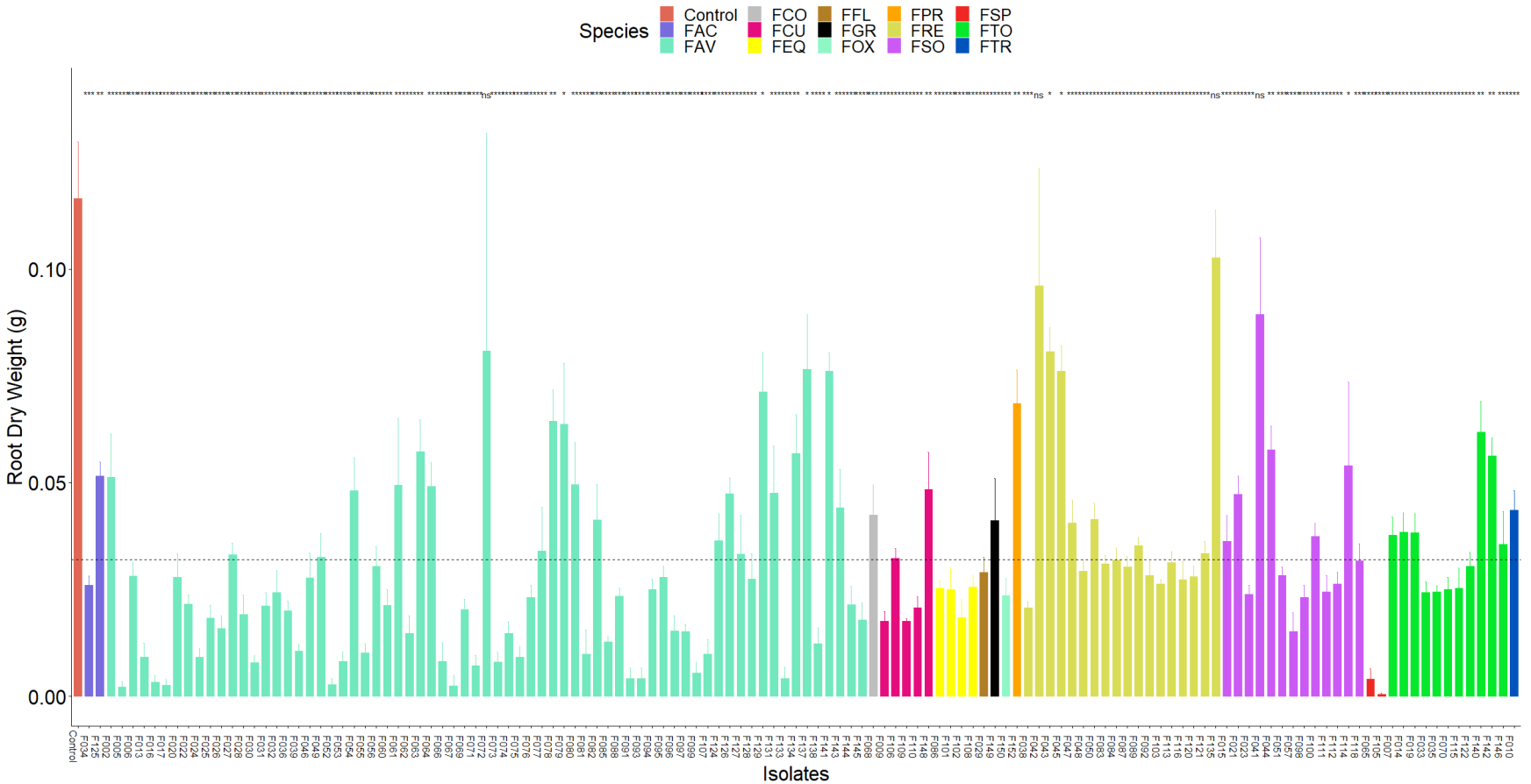
Supplementary Figure 4.3. Impact of *Fusarium* isolates, identified from 2021, on plant height of canola cv. 'Westar' under greenhouse conditions. Species, species identity; Control, non-inoculated control; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCO, *Fusarium commune*; FCU, *Fusarium culmorum*; FEQ, *Fusarium equiseti*; FFL, *Fusarium flocciferum*; FGR, *Fusarium graminearum*; FOX, *Fusarium oxysporum*; FPR, *Fusarium proliferatum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; FSP, *Fusarium sporotrichioides*; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; ns, no significant difference between the treatment and

corresponding non-inoculated control based on a t-test; *, significant difference at $p < 0.05$; **, significant difference at $p < 0.01$; ***, significant difference at $p < 0.001$; and ****, significant difference at $p < 0.0001$. The dashed lines represent the overall mean for corresponding parameter.



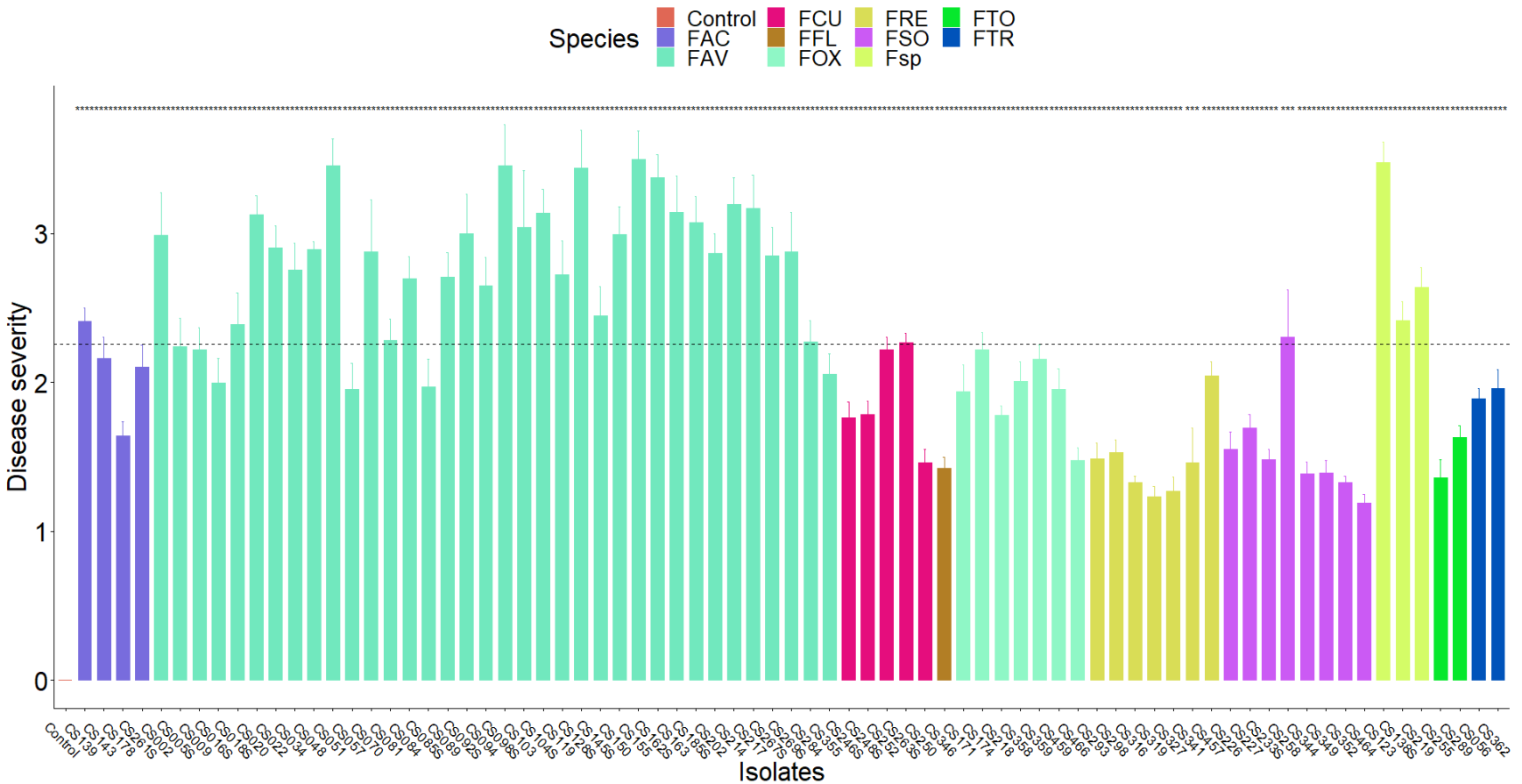
Supplementary Figure 4.4. Impact of *Fusarium* isolates, identified from 2021, on shoot dry weight of canola cv. 'Westar' under greenhouse conditions. Species, species identity; Control, non-inoculated control; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCO, *Fusarium commune*; FCU, *Fusarium culmorum*; FEQ, *Fusarium equiseti*; FFL, *Fusarium flocciferum*; FGR, *Fusarium graminearum*; FOX, *Fusarium oxysporum*; FPR, *Fusarium proliferatum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; FSP, *Fusarium sporotrichioides*; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; ns, no significant difference between the

treatment and corresponding non-inoculated control based on a t-test; *, significant difference at $p < 0.05$; **, significant difference at $p < 0.01$; ***, significant difference at $p < 0.001$; and ****, significant difference at $p < 0.0001$. The dashed lines represent the overall mean for corresponding parameter.



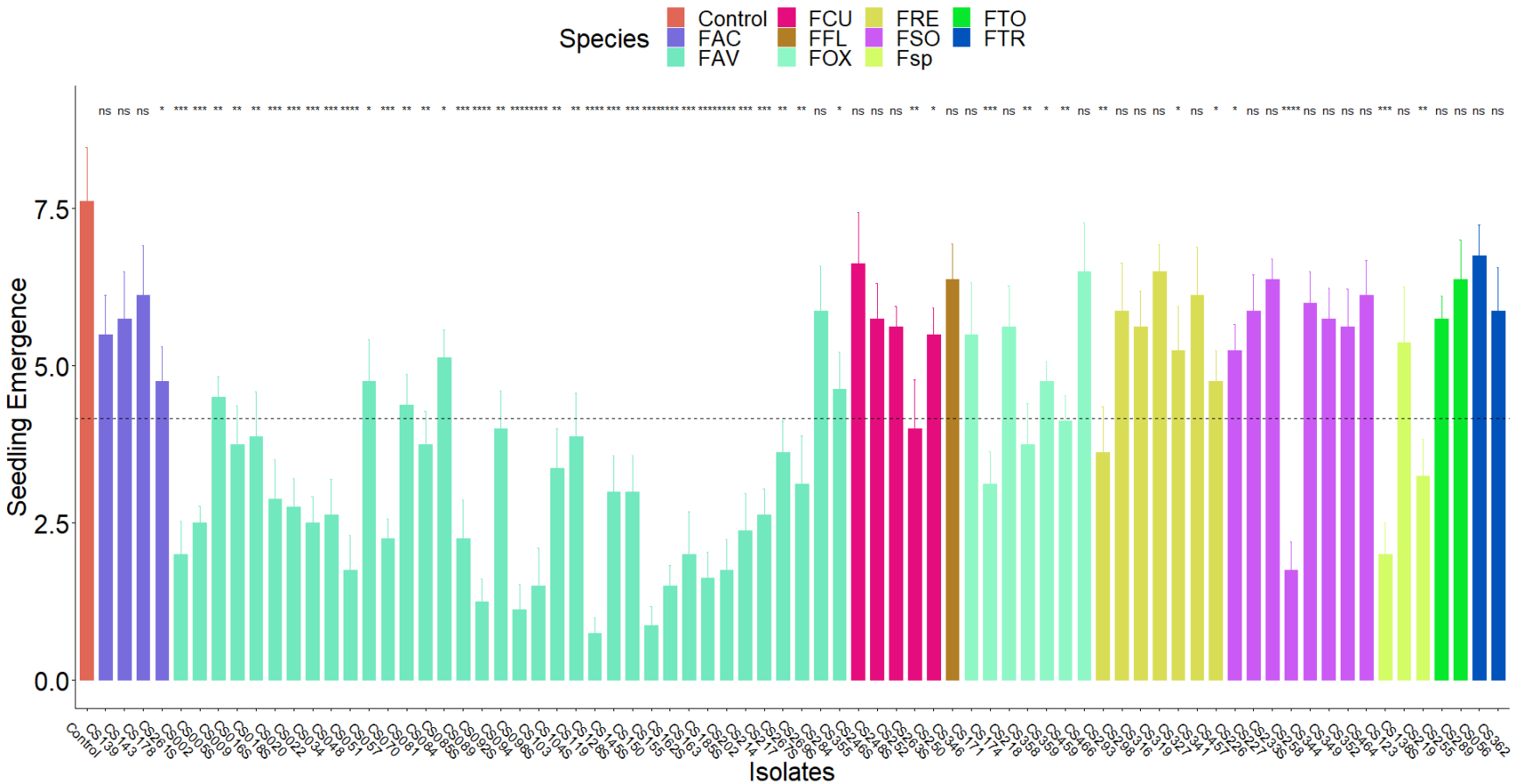
Supplementary Figure 4.5. Impact of *Fusarium* isolates, identified from 2021, on root dry weight of canola cv. 'Westar' under greenhouse conditions. Species, species identity; Control, non-inoculated control; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCO, *Fusarium commune*; FCU, *Fusarium culmorum*; FEQ, *Fusarium equiseti*; FFL, *Fusarium flocciferum*; FGR, *Fusarium graminearum*; FOX, *Fusarium oxysporum*; FPR, *Fusarium proliferatum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; FSP, *Fusarium sporotrichioides*; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; ns, no significant difference between the

treatment and corresponding non-inoculated control based on a t-test; *, significant difference at $p < 0.05$; **, significant difference at $p < 0.01$; ***, significant difference at $p < 0.001$; and ****, significant difference at $p < 0.0001$. The dashed lines represent the overall mean for corresponding parameter.



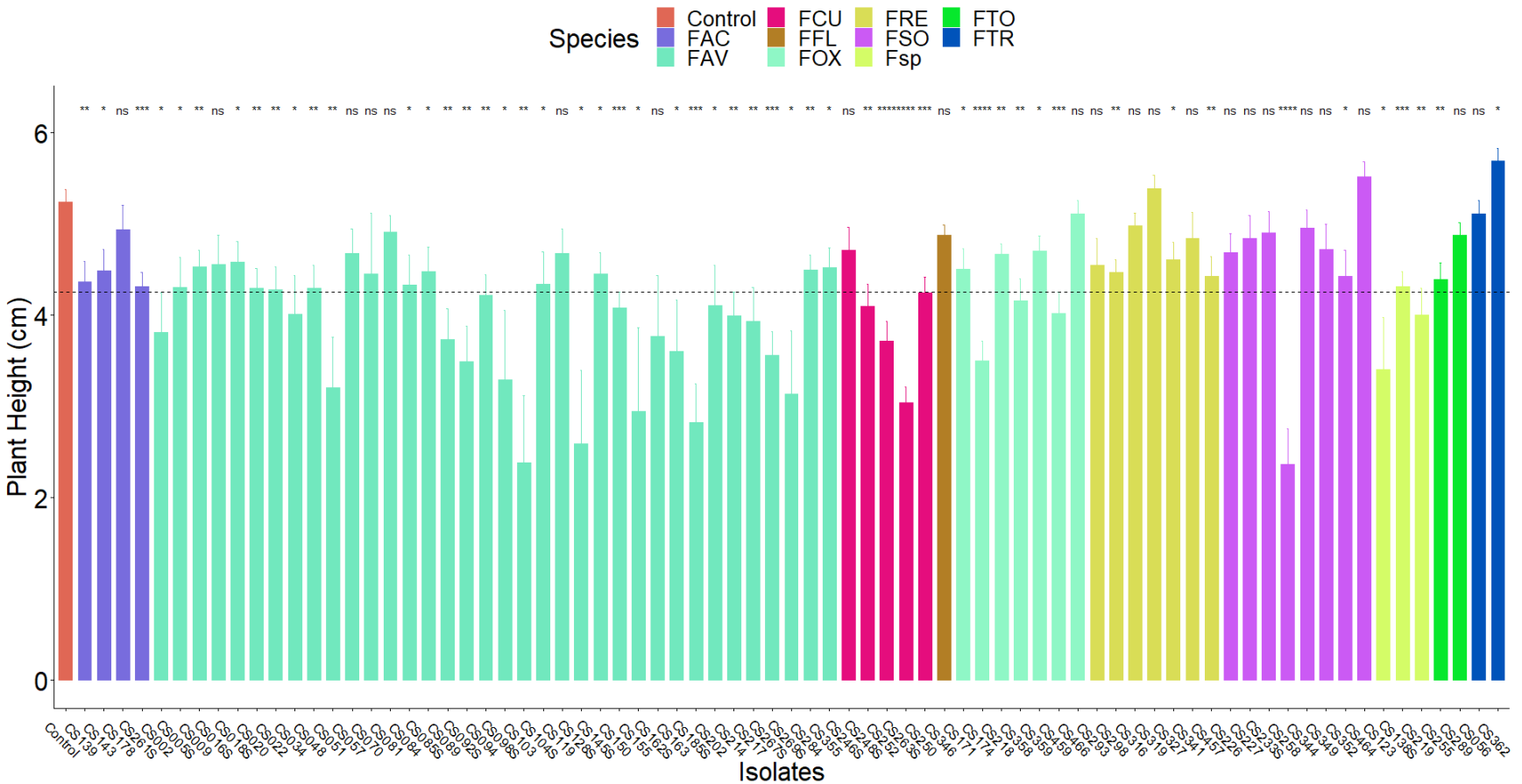
Supplementary Figure 4.6. Impact of *Fusarium* isolates, identified from 2022, on root rot disease severity of canola cv. 'Westar' under greenhouse conditions. Species, species identity; Control, non-inoculated control; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCU, *Fusarium culmorum*; FFL, *Fusarium flocciferum*; FOX, *Fusarium oxysporum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; Fsp, unidentified *Fusarium* sp.; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; ns, no significant difference between the treatment and corresponding non-inoculated control based on a t-test; *, significant difference at $p < 0.05$; **, significant

difference at $p < 0.01$; ***, significant difference at $p < 0.001$; and ****, significant difference at $p < 0.0001$. The dashed lines represent the overall mean for corresponding parameter.



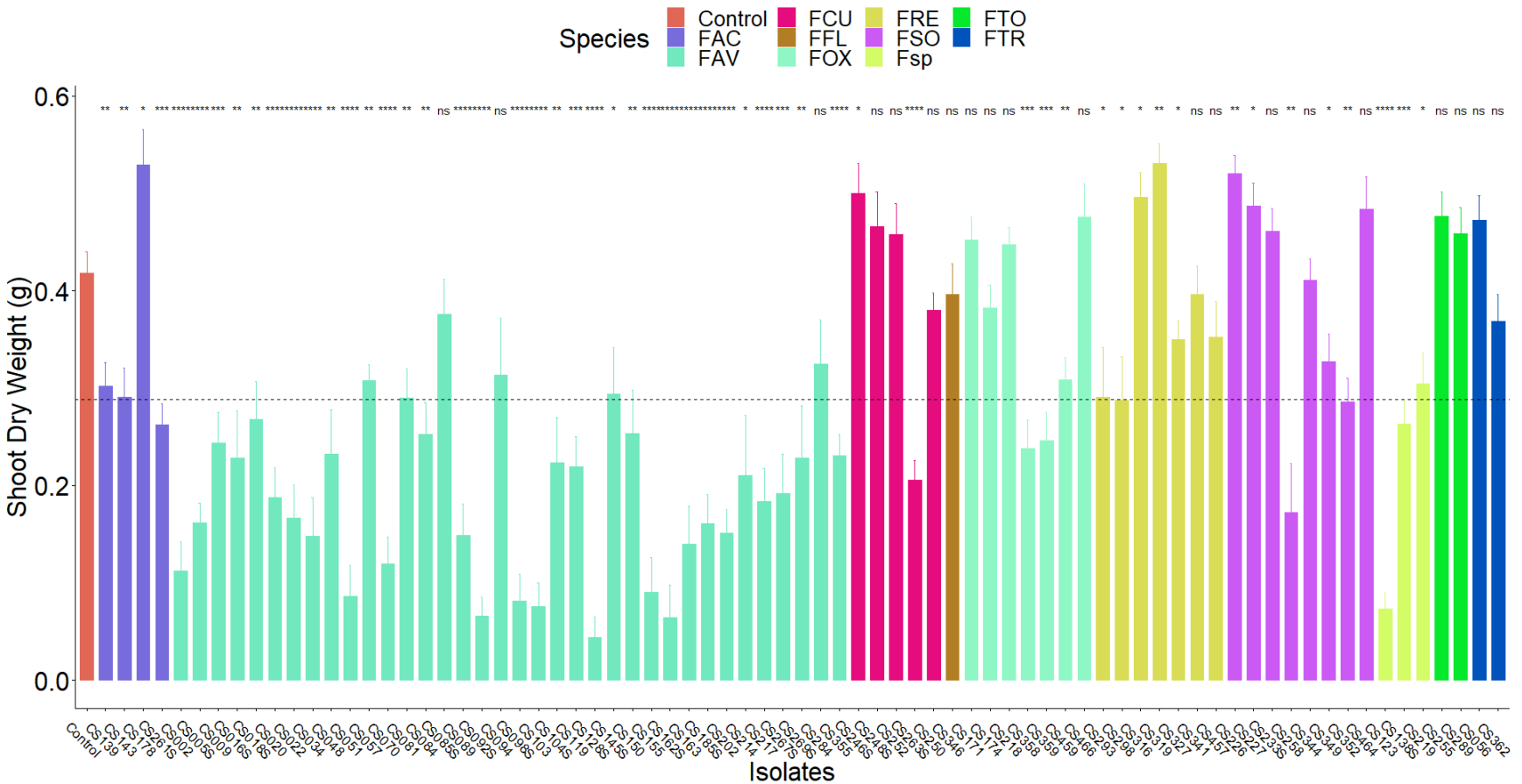
Supplementary Figure 4.7. Impact of *Fusarium* isolates, identified from 2022, on seedling emergence of canola cv. 'Westar' under greenhouse conditions. Species, species identity; Control, non-inoculated control; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCU, *Fusarium culmorum*; FFL, *Fusarium flocciferum*; FOX, *Fusarium oxysporum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; Fsp, unidentified *Fusarium* sp.; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; ns, no significant difference between the treatment and corresponding non-inoculated control based on a t-test; *, significant difference at $p < 0.05$; **, significant

difference at $p < 0.01$; ***, significant difference at $p < 0.001$; and ****, significant difference at $p < 0.0001$. The dashed lines represent the overall mean for corresponding parameter.



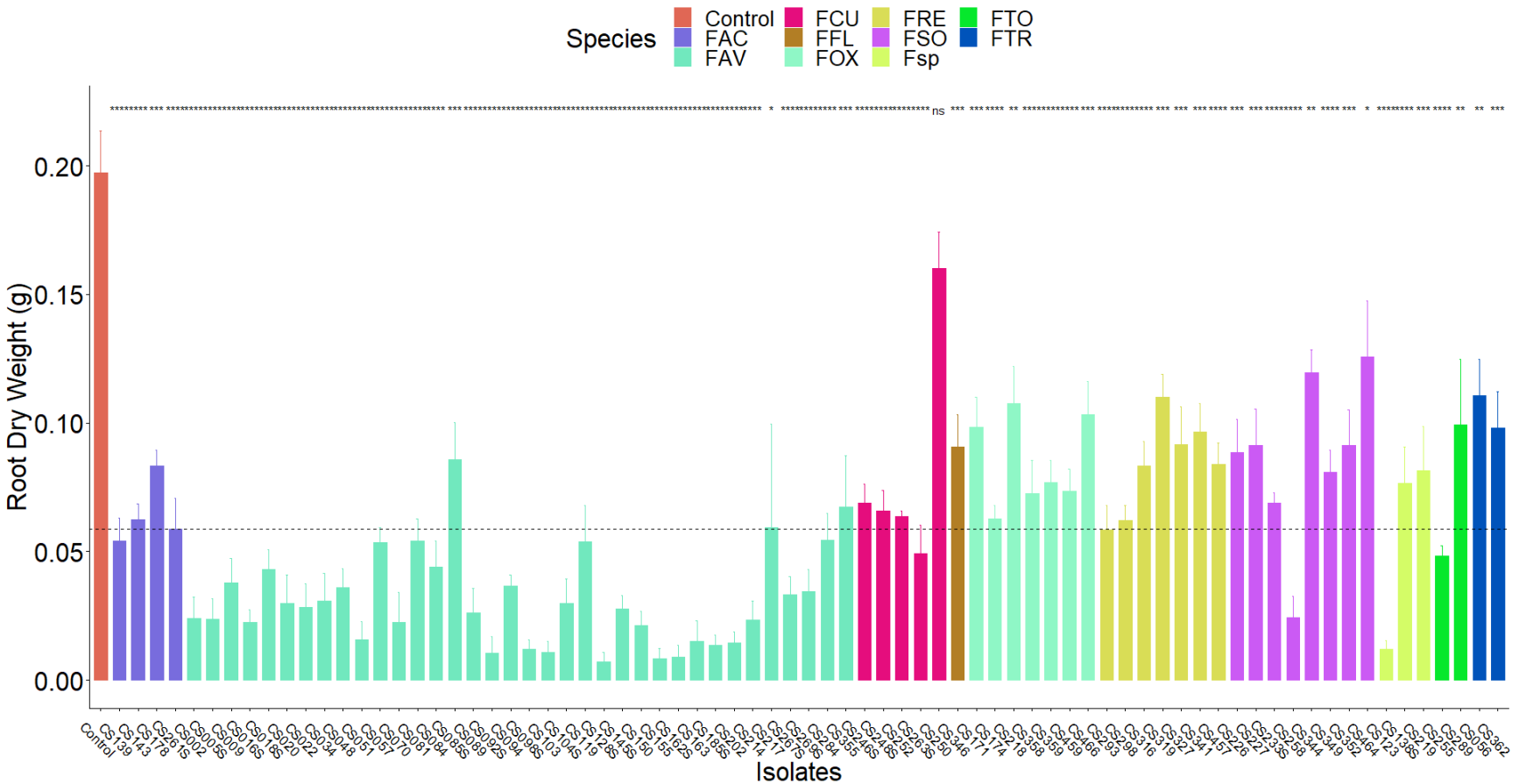
Supplementary Figure 4.8. Impact of *Fusarium* isolates, identified from 2022, on plant height of canola cv. 'Westar' under greenhouse conditions. Species, species identity; Control, non-inoculated control; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCU, *Fusarium culmorum*; FFL, *Fusarium flocciferum*; FOX, *Fusarium oxysporum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; Fsp, unidentified *Fusarium* sp.; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; ns, no significant difference between the treatment and corresponding non-inoculated control based on a t-test; *, significant difference at $p < 0.05$; **, significant difference at $p < 0.01$;

, significant difference at $p < 0.001$; and *, significant difference at $p < 0.0001$. The dashed lines represent the overall mean for corresponding parameter.



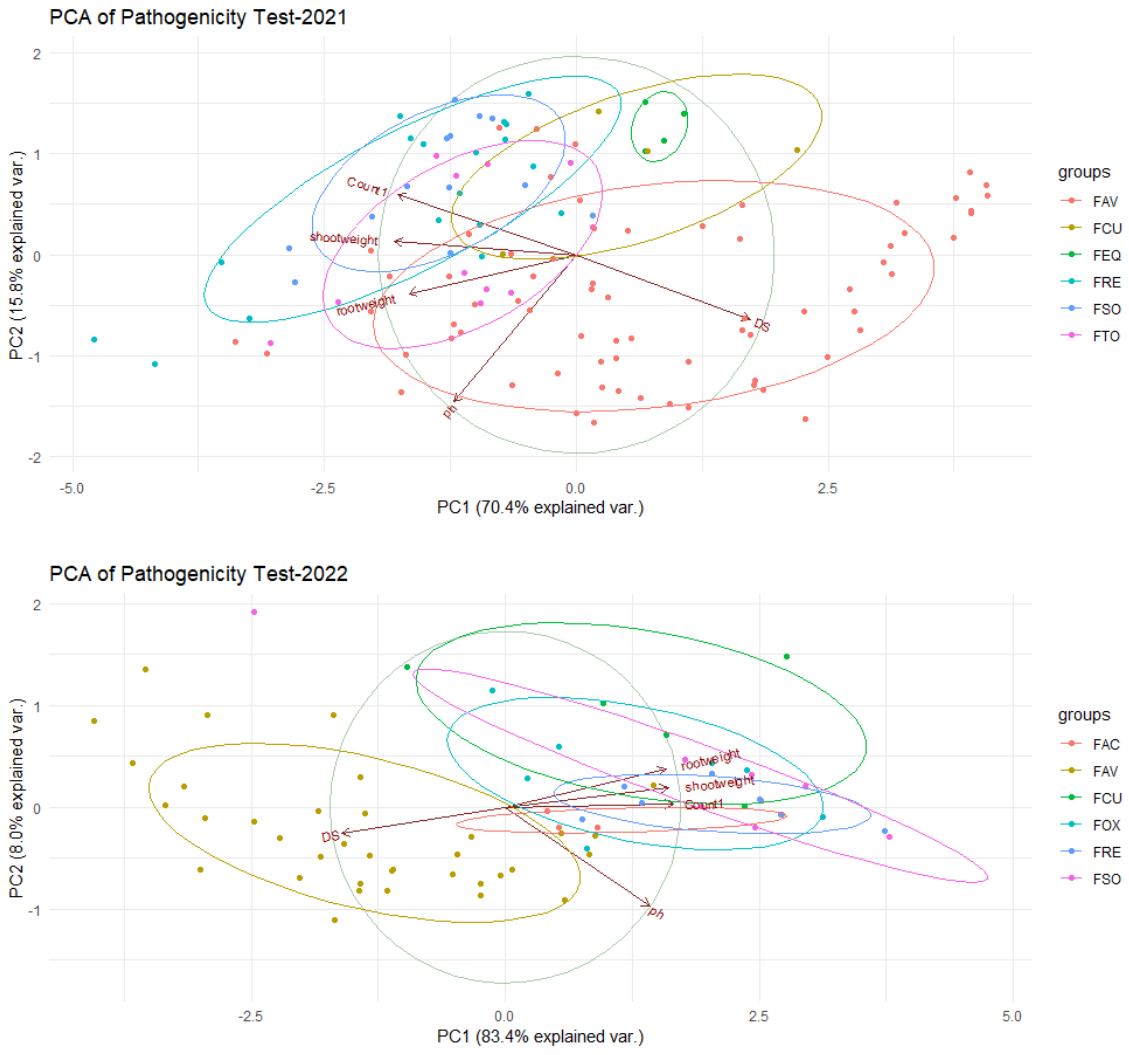
Supplementary Figure 4.9. Impact of *Fusarium* isolates, identified from 2022, on shoot dry weight of canola cv. 'Westar' under greenhouse conditions. Species, species identity; Control, non-inoculated control; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCU, *Fusarium culmorum*; FFL, *Fusarium flocciferum*; FOX, *Fusarium oxysporum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; Fsp, unidentified *Fusarium* sp.; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; ns, no significant difference between the treatment and corresponding non-inoculated control based on a t-test; *, significant difference at $p < 0.05$; **, significant

difference at $p < 0.01$; ***, significant difference at $p < 0.001$; and ****, significant difference at $p < 0.0001$. The dashed lines represent the overall mean for corresponding parameter.

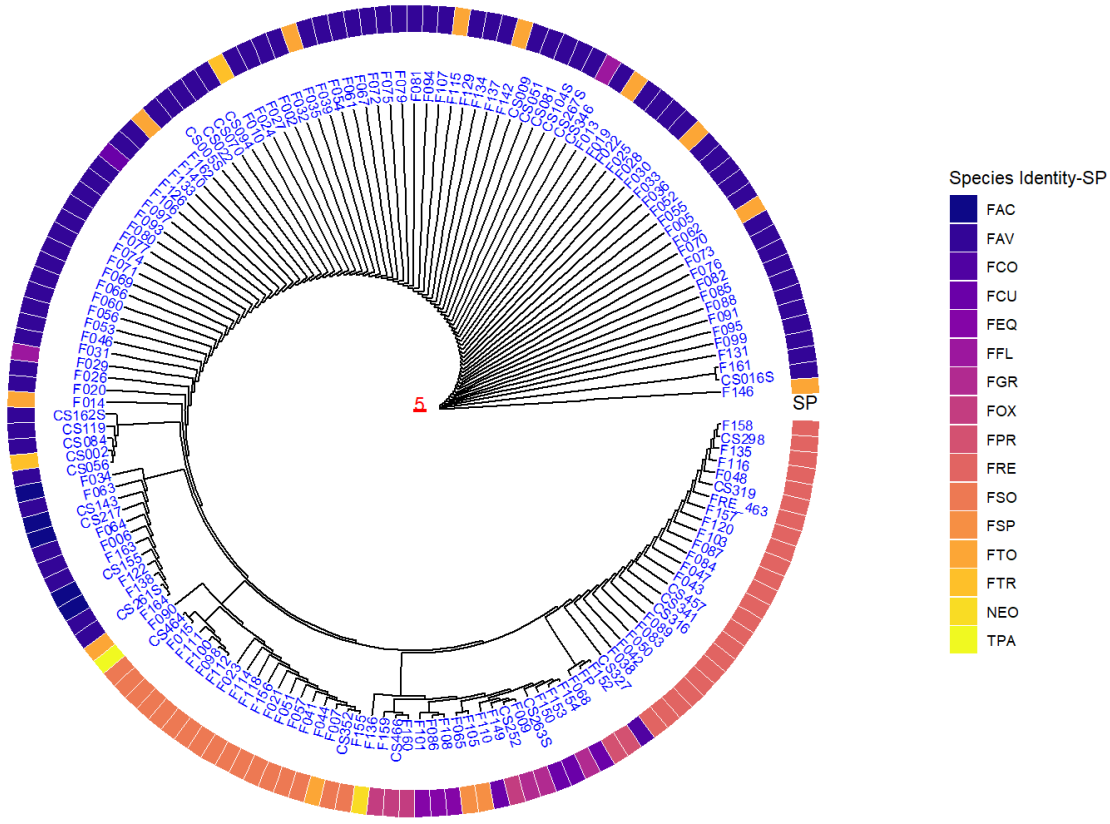


Supplementary Figure 4.10. Impact of *Fusarium* isolates, identified from 2022, on root dry weight of canola cv. 'Westar' under greenhouse conditions. Species, species identity; Control, non-inoculated control; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCU, *Fusarium culmorum*; FFL, *Fusarium flocciferum*; FOX, *Fusarium oxysporum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; Fsp, unidentified *Fusarium* sp.; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; ns, no significant difference between the treatment and corresponding non-inoculated control based on a t-test; *, significant difference at $p < 0.05$; **, significant

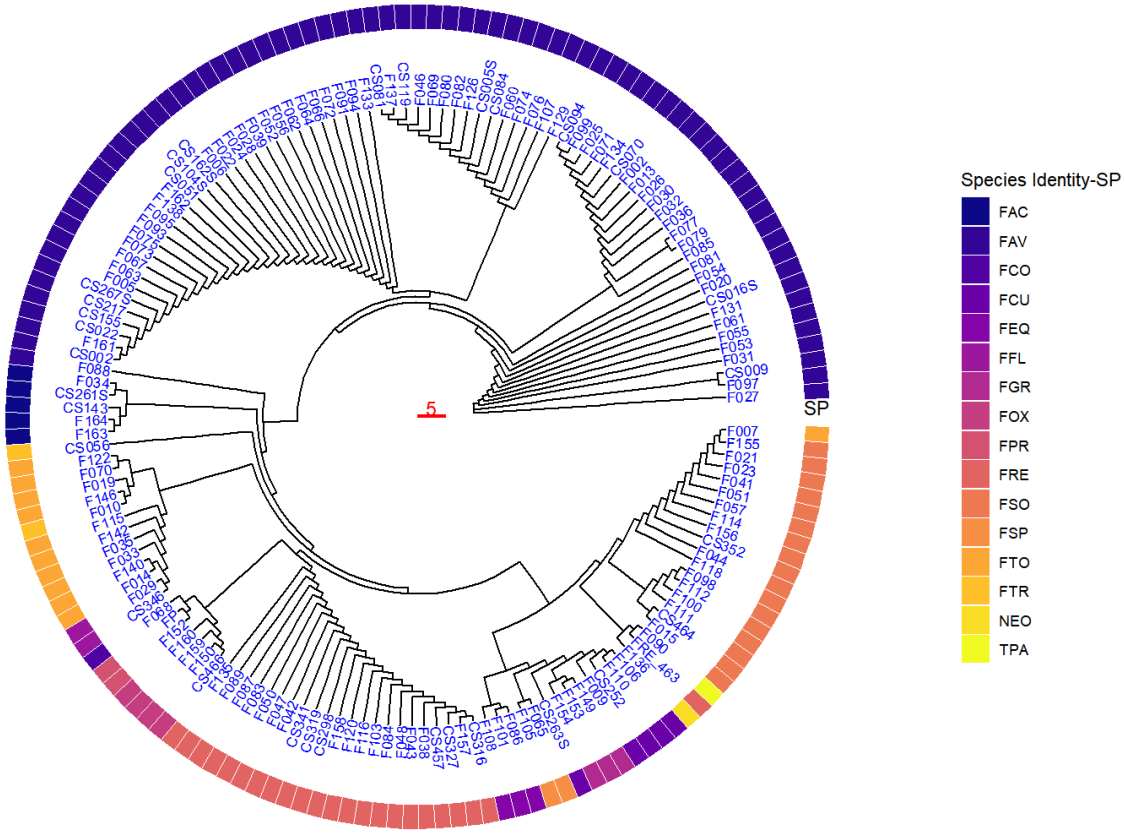
difference at $p < 0.01$; ***, significant difference at $p < 0.001$; and ****, significant difference at $p < 0.0001$. The dashed lines represent the overall mean for corresponding parameter.



Supplementary Figure 4.11. Principal component analysis based on root rot disease severity (DS) and seedling emergence (Count1), plant height (ph), shoot dry weight (shootweight), and root dry weight (rootweight) of canola seedlings after inoculation with the isolates from the common identified six *Fusarium* species in 2021 (top) and 2021 (bottom). groups, species identity; FAV, *Fusarium avenaceum*; FCU, *Fusarium culmorum*; FRE, *Fusarium redolens*; FTO, *Fusarium torulosum*; FSO, *Fusarium solani*; FEQ, *Fusarium equiseti*; FOX, *Fusarium oxysporum*; FAC, *Fusarium acuminatum*.



Supplementary Figure 4.12. Maximum parsimony tree based on the internal transcribed spacer (ITS) sequence of 157 fungal isolates, including 112 isolates recovered from canola in 2021 (F002-F146), 31 isolates recovered from canola in 2022 (CS002-CS466), 13 reference isolates from a laboratory culture collection (FP, F153-F164), and sequences from one *Fusarium redolens* isolate FRE_463 retrieved from GenBank, National Center for Biotechnology Information (NCBI). SP, species identity; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCO, *Fusarium commune*; FCU, *Fusarium culmorum*; FEQ, *Fusarium equiseti*; FFL, *Fusarium flocciferum*; FGR, *Fusarium graminearum*; FOX, *Fusarium oxysporum*; FPR, *Fusarium proliferatum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; FSP, *Fusarium sporotrichioides*; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; NEO, *Neonectria* sp.; and TPA, *Trichoderma paraviridescens*. DS, disease severity on rated on a 0 to 4 scale (Hwang et al., 1994), where: 0 = healthy roots and 4 = tap root severely girdled, brown lesions on >75% of the tap root with limited lateral roots. Growth Losses, reductions in seedling emergence (CR), plant height (HR), shoot dry weight (SR), and root dry weight (RR) following inoculation with each fungal isolate and relative to the corresponding non-inoculated control.

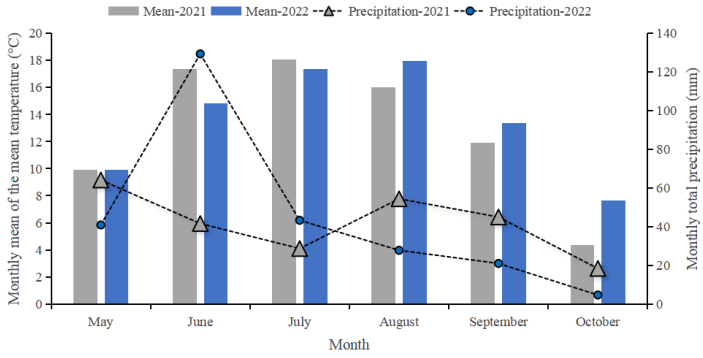


Supplementary Figure 4.13. Maximum parsimony tree based on the elongation factor (EF1-α) sequence of 157 fungal isolates, including 112 isolates recovered from canola in 2021 (F002-F146), 31 isolates recovered from canola in 2022 (CS002-CS466), 13 reference isolates from a laboratory culture collection (FP, F153-F164), and sequences from one *Fusarium redolens* isolate FRE_463 retrieved from GenBank, National Center for Biotechnology Information (NCBI). SP, species identity; FAC, *Fusarium acuminatum*; FAV, *Fusarium avenaceum*; FCO, *Fusarium commune*; FCU, *Fusarium culmorum*; FEQ, *Fusarium equiseti*; FFL, *Fusarium flocciferum*; FGR, *Fusarium graminearum*; FOX, *Fusarium oxysporum*; FPR, *Fusarium proliferatum*; FRE, *Fusarium redolens*; FSO, *Fusarium solani*; FSP, *Fusarium sporotrichioides*; FTO, *Fusarium torulosum*; FTR, *Fusarium tricinctum*; NEO, *Neonectria* sp.; and TPA, *Trichoderma paraviridescens*. DS, disease severity on rated on a 0 to 4 scale (Hwang et al., 1994), where: 0 = healthy roots and 4 = tap root severely girdled, brown lesions on >75% of the tap root with limited lateral roots. Growth Losses, reductions in seedling emergence (CR), plant height (HR), shoot dry weight (SR), and root dry weight (RR) following inoculation with each fungal isolate and relative to the corresponding non-inoculated control.

Appendix B: Supplementary materials for Chapter 5

Supplementary Table 5.1. Extreme high temperature (>30°C) at the St. Albert research station in field trials, 2021-2022

Date	Max Temp (°C)	Date	Max Temp (°C)
26-06-2021	30.8	28-07-2022	31.7
27-06-2021	30.7	13-08-2022	30.7
28-06-2021	35.2	19-08-2022	32.5
29-06-2021	35.2	20-08-2022	31
30-06-2021	35.9	26-08-2022	30.1
01-07-2021	34.3	30-08-2022	30.9
08-07-2021	31.4	31-08-2022	31.8
09-07-2021	32	02-09-2022	31.4
10-07-2021	32.5	03-09-2022	33.7
14-07-2021	30.8	04-09-2022	31.6
31-07-2021	30.1		
05-08-2021	31.1		
13-08-2021	32.2		
14-08-2021	32.3		



Supplementary Figure 5.1. Precipitations and mean temperatures at the site used in field trials, St. Albert, AB, 2021-2022.

Appendix C: Supplementary materials for Chapter 6

Supplementary Table 6.1. Comparison of root rot severity on cultivars representing eight different crop species at 21 days after seeding in potting medium treated with different concentrations of *Fusarium proliferatum* inoculum.

Crop	Cultivar	Disease Severity[a]	
		Low Inoculum[b]	High Inoculum[c]
Wheat	AC Crystal	1.06 a	2.29 AB
	Katepwa	1.46 ab	2.00 A
	Lillian	1.35 ab	2.06 AB
Barley	AB Tofield	1.23 ab	1.86 A
	Canmore	1.72 bcd	2.10 AB
Faba bean	Malik	1.31 ab	2.13 AB
	Fabelle	2.24 de	2.89 CDE
Pea	CDC Amarillo	1.57 abc	2.56 BCD
	AAC Barrhead	2.01 cde	2.37 ABC
	CDC Greenwater	2.03 cde	2.90 CDE
	AAC Carver	2.26 e	3.10 EFG
Lentil	CDC Nimble	1.62 bc	3.01 DEF
	CDC Lima CL	2.30 ef	3.70 H
Canola	Westar	2.33 ef	3.06 DEF
	L255PC	2.99 g	3.68 H
Lupine	Arabella	2.83 fg	2.97 DE
	Mirabor	2.90 g	3.68 H
Soybean	AAC Mandor	2.51 efg	3.13 EFG
	AKRAS R2	3.03 gh	3.53 FGH
	OT15-02	3.55 h	3.61 GH

[a] Root rot disease severity as assessed on a 0-4 scale (Hwang et al., 1994), where: 0 = healthy roots; 1 = small, light-brown lesions on < 25% of the tap root; 2 = brown lesions on 25-49% of the tap root; 3 = brown lesions on 50-74% of the tap root, tap root constricted; and 4 = tap root severely girdled, brown lesions on > 75% of the tap root with limited lateral roots.

[b] Treated with a low concentration (3×10^4 colony forming units (cfu)/g potting medium) of *F. proliferatum* inoculum.

[c] Treated with a high concentration (6×10^4 cfu/g potting medium) of *F. proliferatum* inoculum.

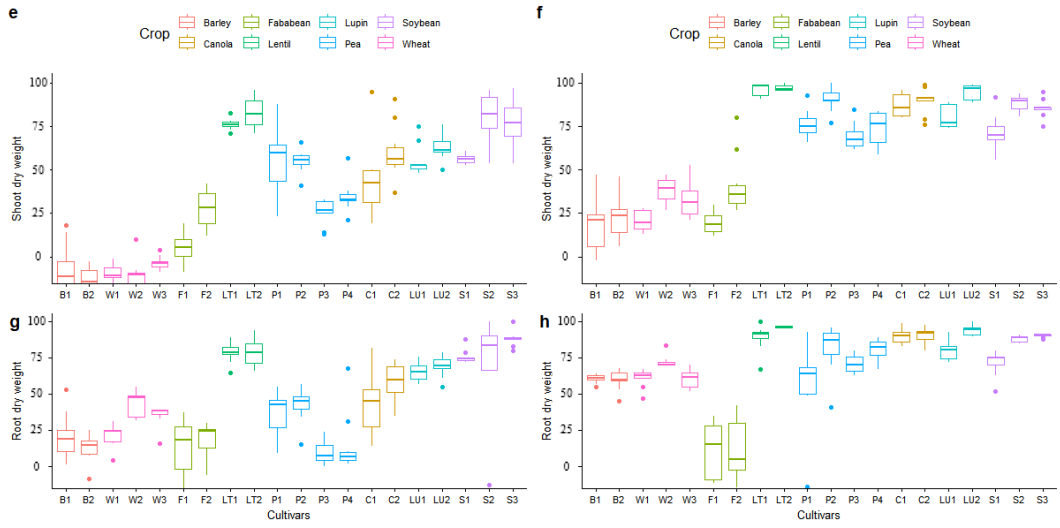
Note: Different lowercase letters indicate significant differences ($p < 0.05$) within the 'Low Inoculum' column, while different uppercase letters indicate significant differences within the 'High Inoculum' column.

Supplementary Table 6.2. Principal Component Analysis of root rot disease severity and reductions in emergence, plant height, shoot and root dry weights of 20 cultivars representing 8 crop species grown in potting medium treated with different concentrations of *Fusarium proliferatum* inoculum.

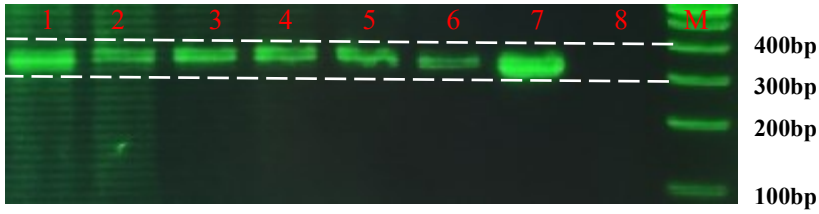
Parameter	Low Inoculum Concentration[a]					High Inoculum Concentration[b]				
	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5
Disease Severity	-0.4254	-0.5600	0.6152	-0.3559	-0.0247	-0.4364	-0.5245	-0.7019	0.0610	-0.1954
Emergence Reduction	-0.4645	-0.0237	0.1613	0.8704	0.0129	-0.4695	-0.0444	0.1828	0.5337	0.6778
Plant Height Reduction	-0.4613	-0.1467	-0.5582	-0.1369	-0.6598	-0.4657	-0.0640	0.1921	-0.8192	0.2664
Shoot Dry Weight Reduction	-0.4707	-0.0379	-0.4358	-0.1826	0.7442	-0.4665	-0.1095	0.5672	0.1987	-0.6397
Root Dry Weight Reduction	-0.4110	0.8143	0.3067	-0.2525	-0.1008	-0.3932	0.8408	-0.3397	0.0297	-0.1491

[a] Low, treated with a low concentration (3×10^4 colony forming units (cfu)/g potting medium) of *F. proliferatum* inoculum; [b]High, treated with a high concentration (6×10^4 cfu/g potting medium) of *F. proliferatum* inoculum.

Supplementary Figure 6.1. Root rot disease rating scale for eight crop species, where: 0 = healthy roots; 1 = small, light-brown lesions on < 25% of the tap root; 2 = brown lesions on 25-49% of the tap root; 3 = brown lesions on 50-74% of the tap root, tap root constricted; and 4 = tap root severely girdled, brown lesions on > 75% of the tap root with limited lateral roots. The scale was adapted from Hwang et al. (Hwang et al., 1994).



Supplementary Figure 6.2. Reductions (%), relative to non-inoculated controls, in plant height, seedling emergence, and shoot and root weights of 20 cultivars representing eight crop species grown in potting medium treated with low (a, c, e, g) or high (b, d, f, h) concentrations of *Fusarium proliferatum* (3×10^4 and 6×10^4 colony forming units/g potting medium, respectively). Plant height (a,b) was measured at 14 days after seeding, seedling emergence (c,d) was measured at 7 days after seeding, shoot dry weight (e,f) was measured at 21 days after seeding, and root dry weight (g,h) was measured at 21 days after seeding. B1, barley cultivar 'AB Tofield'; B2, barley 'Canmore'; W1, wheat 'Katepwa'; W2, wheat 'AC Crystal'; W3, wheat 'Lillian'; P1, pea 'CDC Greenwater'; P2, pea 'AAC Carver'; P3, pea 'CDC Amarillo'; P4, pea 'AAC Barrhead'; S1, soybean 'AAC Mandor'; S2, soybean 'OT15-02'; S3, soybean 'AKRAS R2'; LU1, lupine 'Arabella'; LU2, lupine 'Mirabor'; L1, lentil 'CDC Nimble'; L2, lentil 'CDC Lima CL'; F1, faba bean 'Malik'; F2, faba bean 'Fabelle'; C1, canola 'Westar'; C2, canola 'L255PC'.



Supplementary Figure 6.3. Detection of *Fusarium proliferatum* in plant root tissues by PCR analysis with the *F. proliferatum*-specific primers TH5-F/TH6-R (Waalwijk et al., 2003). Plants were grown in potting medium inoculated with the fungus (3×10^4 cfu/g potting medium) and root tissues were collected shortly after flowering. Total genomic DNA was extracted and subjected to PCR. Lane 1, pea cultivar 'CDC Greenwater'; lane 2, pea 'AAC Carver'; lane 3, faba bean 'Fabelle'; lane 4, soybean 'AKRAS R2'; lane 5, lupine 'Arabella; lane 6, wheat 'AC Crystal'; lane 7, *F. proliferatum* isolate P002 (positive control); lane 8, nuclease-free water (negative control); lane M, 100 bp DNA ladder (Thermo Fisher Scientific, Mississauga, ON).