Fusarium Root Rot of Soybean in Southern Alberta: Pathogen Aggressiveness and Disease Management

by

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Abstract

Fusarium root rot is a major disease of soybean (*Glycine max*) in many regions worldwide. As soybean is a relatively new crop in Alberta, Canada, studies were undertaken to determine the occurrence and severity of root rot in this province, identify and characterize the *Fusarium* species associated with disease development, and evaluate seed treatments and host resistance as disease management tools. Root rot was found in all soybean crops surveyed in 2013 and 2014. A total of seven species of *Fusarium* were identified from infected roots based on their morphological characteristics, with *Fusarium avenaceum*, *F. solani*, *F. oxysporum*, and *F. acuminatum* occurring most frequently. In greenhouse bioassays, isolates of *F. proliferatum* and *F. acuminatum* were the most aggressive, although some isolates of *F. acuminatum* caused little disease. Seed treatment with ipconazole + metalaxyl + fludioxonil, ipconazole + metalaxyl, fludioxonil + metalaxyl, or penflufen + prothioconazole + metalaxyl resulted in relatively high crop emergence rates (≥70%) under field conditions, while in greenhouse experiments, ipconazole + metalaxyl and carbathiin + thiram provided the best results. The resistance of soybean genotypes to *F. avenaceum* varied significantly, with genotype 90M01 having the highest emergence rate (83%) under field conditions. In greenhouse trials, the genotype Tundra was most resistant to root rot, whereas TH29002RR was the most susceptible. The results suggest that while root rot represents a significant challenge to soybean production in Alberta, fungicidal seed treatments and planting of genotypes with improved resistance may help to mitigate the impact of this disease.

Preface

This thesis is an original work by Mr. Ronald Nyandoro. Chapters 2 and 3 have been published as:

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I was responsible for the data collection and analysis in the published articles, as well as in Chapters 4 and 5 (which have not yet been published). Summer students and other staff at Alberta Agriculture and Forestry (Crop Diversification Centre North, Edmonton, and Crop Diversification Centre South, Brooks) provided assistance in establishing and maintaining the field and greenhouse experiments, and in the collection and processing of samples.

I wrote the first drafts of all the thesis chapters, including those published as articles. Each draft was reviewed and edited by Dr. Stephen E. Strelkov, as well as by Dr. Kan-Fa Chang and Dr. Sheau-Fang Hwang. In addition, Dr. Ahmed, Mr. Turnbull, Dr. Howard, and Dr. Harding (Alberta Agriculture and Forestry) provided feedback on the two published articles. My supervisor Dr. Strelkov, in collaboration with Dr. Hwang and Dr. Chang, provided me with guidance and corrections throughout the thesis.

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Dedication

This thesis is dedicated to my wife, Violet, and daughter Ruvimbo for their support and for bearing with me when I had to spend a lot of time away working on field research.

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Contents

List of Tables

List of Figures

Chapter 1: General Introduction and Literature Review

1.1 Introduction

Soybean (*Glycine max* (L). Merr) is widely cultivated for its seed, which has numerous uses (Singh et al. 2006). It is an East Asian legume species, and its wild ancestor (*Glycine soja)* is found in a region stretching from China, Japan, Korea, Taiwan and Russia. It is the only one of 25 known *Glycine* species that has been domesticated (Hymowitz 2008). A study that involved the integration of geographic distribution with microsatellite genotype and phenology between wild and landrace soybeans suggested a single center of origin (Guo et al. 2010), which is believed to be in China where *G. soja* is found in abundance (Li 1994).

Soybeans were first cultivated in east Asia between the 15th and 16th centuries, Europe between the 16th and 17th centuries, and North America in 1765 (Hymowitz 2004). In Canada, soybeans may have been cultivated on a measurable scale as early as 1855 in Ontario, reaching Alberta in 1922 (Shurtleff and Aoyagi 2010). The crop has since become of economic importance in this country, and its acreage in Alberta has increased as well.

Soybeans grow well under a diversity of climatic and soil conditions, and exhibit a wide genetic variability, especially in areas where the wild types grow (Dong et al. 2001). Soybeans grow particularly well in hot summers that offer the optimum growing temperatures, in the range of 20°C to 30°C. Soil salinity is an issue in soybean growth and can be a limiting factor; soils with > 40 nM NaCl are considered to be too saline for ideal growth (Stoddard et al. 2006). Soil nitrogen content is only critical at the crop establishment stage, after which the plants can fix their own nitrogen in a mutually symbiotic relationship with the bacterium *Bradyrhizobium japonicum* Kirchner (1896) (Jordan 1982).

Soybean is consumed directly or indirectly in a variety of forms, including as roasted seeds, soy milk, soy meat, sprouts, as a vegetable, soy sauce, cooking oil, margarine, or in cheeses. It is an economically important crop globally and important for the provision of vegetable oil and protein for both humans and their livestock (Boerma and Spetcht 2004). Its seed has an average composition of 40% protein, 35% carbohydrate, 20% oil, and 5% minerals (Liu 1997). Besides being a source of oil and protein in human diets, soybean has over 200 applications in the food, feed, and industrial sectors (Jenks et al. 2007). Its industrial uses include the manufacture of products such as printing ink, biodiesel, and protein fiber for blending with cotton, wool or other chemical fibers. An agriculturally important by-product in the processing of soybean is the meal or cake, which is rich in protein and is used as a key ingredient in the manufacture of animal feed.

On a global scale, soybean occupies the largest acreage of any legume crop and represents 60% of the world production of oilseeds (Sudaric et al. 2008). In southern Alberta, the area seeded to soybeans is relatively small and was estimated at 2,280 ha in 2010. The acreage of soybean has great potential to increase further in the future, given the continuous efforts to develop new cultivars with early maturity and cold resistance.

The development of the soybean industry in Canada, and in Alberta in particular, has faced numerous agronomic challenges, chief among which are biotic factors. Global losses in soybean production resulting from infection by parasitic bacteria and fungi have been estimated at 11% (Oerke 2006). This magnitude of loss could be even worse where it not for the disease control efforts of soybean growers. In 1998, estimated losses resulting from infection by *Fusarium* species were >7,300 and >86,000 metric tons in Canada and the United States of America, respectively (Wrather et al. 2001). Such high losses tend to discourage current as well

as potential producers of soybeans. Leslie et al. (1993) reported that *F. solani* and *F. oxysporum* were the predominant *Fusarium* species isolated from soybean roots exhibiting symptoms of root rot in North America. There is, therefore, a need to develop strategies to mitigate the impact of root rot if soybean production is to remain lucrative for farmers in areas where conditions are conducive for disease development.

1.2 Soybean diseases

Soybeans can be infected by more than 300 pathogenic microbial species worldwide, with only a few of these pathogens causing economic damage to the crop (Hartman et al. 1999). The amount of damage caused by a particular pathogen depends on the factors that constitute the disease triangle, namely the virulence of the pathogen, the susceptibility of the host, as well as whether or not environmental conditions are conducive for disease development. Diseases of soybean in North America root rots (caused by a complex of pathogens including *Pythium* Pringsheim (1858)*, Rhizoctonia solani* Kühn and *Fusarium oxysporum* Schlecht. emend. Snyder and Hansen)*,* white mold (*Sclerotinia sclerotiorum* (Lib). de Bary), phytophthora root and stem rot (*Phytophthora* de Bary), charcoal root rot (*Macrophomina phaseoli* (Maubl.) S.F. Ashby), powdery mildew (*Microsphaera diffusa* Cooke and Peck) (Risula et al. 2014, Wrather and Koenning 2006), and bacterial blight (*Pseudomonus syringae* pv. *glycenia* Van Hall (1904)). The diseases that affect soybean can be generally divided into seed, root, or foliar diseases.

1.2.1 Seed and Seedling diseases

After planting into moist soil, a soybean seed starts germinating as soon as it imbibes some water. Any pathogens that are present may start to grow as well (Hartman and Hill 2010). Seed and seedling diseases manifest themselves mostly as seed rots, seedling blights, and root rots. The radicle and primary root of emerging seedlings can be attacked immediately by fungi

present in the soil, with infection quickly spreading up the hypocotyl to cause disease on the cotyledons and compromising the subsequent yield (Hartman and Hill 2010). Severe infection of seeds and emerging seedlings will result in pre-emergence damping-off, which reduces crop stand in the field.

Phytophthora sojae Kaufmann and Gerdemann causes the most severe damage at the seedling stage through seed rots as well as root rots (Hartman et al. 1999). Commercial soybeans have high resistance to this pathogen owing to the availability of resistance genes, which have been shown to have durability across soybean producing regions (Dorrance et al. 2003).

1.2.2 Foliar diseases

Foliar diseases of soybean include bean pod mottle, *Septoria* brown spot (*Septoria glycines* Hemm.), powdery mildew (*Microsphaera diffusa*), downy mildew (*Peronospora sojae* Lehm.), brown stem rot [(*Phialophora gregata* Alli.), *Diaporthe phaseolorum, Fusarium* spp.)], white mold (*S. sclerotiorum*), Asian soybean rust (*Phakospora pachyrhizi* Syd.), and bacterial blight (*P. syringae* pv. *glycenia*). Bean pod mottling, caused by the bean pod mottle virus (BPMV), consists of green to yellow leaf mottling, leaf distortion, leaf pluckering, and sometimes terminal necrosis (Hobbs et al. 2003). The virus is seed borne and vector transmitted and therefore good seed hygiene and pest control are effective in the management of the disease. Another viral disease, soybean mosaic virus (SMV), occurs widely across the globe and is common in soybeans, causing light and dark mosaic patterns on leaves, stunted growth, and seed mottling (Hartman et al. 1999). The disease can be managed through seed hygiene and vector control.

White mold is a disease that primarily attacks the stem of the plant, leading to wilting and death of the upper portion of the plant. The disease can be diagnosed by the presence of white

cottony mold in the stem of the plant. *Sclerotinium sclerotiorum* produces phytotoxins, such as sclerin, which complement the activity of endopolygalacturonase in the destruction of plant tissue (Favaron et al. 2004). Stem rot can be controlled effectively through the application of foliar fungicides.

Soybean rust is a disease caused by the pathogen *P. pachyrhizi*, which manifests itself as tan to dark brown or reddish brown pustules (globose uredinia) on the lower side of the leaf (Hartman et al. 1999). The disease attacks plants at any growth stage and may cause early defoliation. Disease development is favored by prolonged leaf wetness. The management of rust is possible with foliar fungicides, but the timing of application is critical (Mueller et al. 2009).

1.2.3 Root rot and sudden death syndrome

Root rot is considered the most prevalent and damaging symptom of infection of soybean by *Fusarium* spp. (Killebrew et al. 1993). This genus of fungi occurs in most soybean growing areas worldwide (Vick et al. 2006). The primary damage to the soybean plant is caused by the destruction of the root system, which results in symptomatic root rot (Leslie et al. 1993). Damage to the root system directly affects water and nutrient uptake by infected plants. A field survey conducted in eastern Canada in 2002, profiling the pathogens causing root rot in soybeans, revealed that *Fusarium* spp. constituted 68% of the fungi isolated from soybean roots (Lévésque 2003, Nelson 1999, Rizvi., Yang 1996, Rupe 1989). Specifically, *F. solani* and *F. oxysporum* have been reported as major causal agents of soybean root rot in North America (Nelson 1999, Zhang et al. 2010).

In greenhouse assays in Ontario, *F. avenaceum* was identified as amongst the most pathogenic root rot causing fungi in soybean (Zhang et al. 2013). Soybeans can develop root and crown rot, and vascular discoloration of the stems (Roy et al. 1989., Rupe 1989). Infection of the

roots by *F. graminearum* initially results in water-soaked lesions that become pinkish-brown in color, spreading up as well as down from the point of initial necrosis (Xue et al. 2007). Some plants may exhibit a rotten, trimmed, and discolored (grey-reddish brown) root system (Hartman et al. 1999). Discolored taproots and basal stems have been described as characteristic symptoms of root rot resulting from Fusarium colonization of the plant phloem and xylem tissue (Navi and Yang 2008). Heavy fungal sporulation can be visible on the root surface under high soil moisture conditions in the late reproductive phase of plant growth (Roy et al. 1997). When sporulation is visible on the root surface, it is diagnostic of root rot (Melgar et al. 1994., Roy 1997).

Soybean seedlings inoculated with *Fusarium* spp. under controlled conditions developed extensive reddish brown to black lesions on the taproots after three weeks (Nelson et al. 1997). The disease can reduce nodulation by the symbiotic *Bradyrhizobium* species and thus compromise nitrogen fixation on infected legumes (Hwang et al. 2003). In some experiments, lesions occurred on taproots of soybean seedlings as early as three days after sowing and Fusarium was successfully isolated from seedling taproots only four days after planting. Lesion incidence was as high as 97% at 10 days after planting (Huang et al. 1998).

Sometimes, root rot symptoms are most visible at the flowering to pod development stages of the soybean crop (Schern and Yang 1999), where they occur as sudden death syndrome. Sudden death syndrome (SDS) is a condition in soybeans that is closely associated with root rot disease, and is one of the most important causes of yield loss in soybean crops in North America (Leandro et al. 2013., Nelson 1999., Wrather et al. 2001). Yield losses associated with SDS result primarily from reduced pod numbers, reduced number of seeds within the pod, as well as empty pods, reflecting a reduction in the capacity for water and nutrient uptake by affected plants as a consequence of root rot (Ameur et al. 2008). In Canada, SDS was first

reported in Ontario in 1996 after a close examination of affected plants that had inter-venal chlorosis, leaf necrotic lesions, discoloration of the roots and vascular systems of the lower stems, poor pod development, and poor pod filling (Anderson and Tenuta 1998). Inconsistences in disease development within and between disease screening experiments have been a major challenge in attempts to fully understand SDS (Hartman et al. 1997).

Under field conditions, yield losses associated with infection by *Fusarium* spp. were reported to be 59% in the case of blight and wilt, 64% in the case of root rot, and as high as 50% as a result of reductions in pod formation (Nelson 1999). Soybean crop losses will likely continue to rise if appropriate disease mitigation measures are not developed, since inoculum levels can build up in the soil. In soybean production, a good crop stand is important for good yields, since it increases pod clearance (height at which lowest pods are set) and crop competitiveness against weeds, both of which have a bearing on crop recovery during harvesting.

Lower soil temperatures at the onset of the growing season create a soil environment that leads to delayed germination and crop emergence. Soil temperatures below 15°C increase root infection and root rot severity by *Fusarium* spp. (Scherm and Yang 1999). Delaying planting until the soil has warmed up to about 15°C or higher will facilitate fast emergence and allow the seedlings to escape pre-emergence infection. On the Canadian prairies, it is recommended that seeding be carried out between May 10 and 25, when the soils have warmed up to a mean of at least 10°C, to enable optimum germination and reduced disease levels (Risula et al. 2014).

1.3 Taxonomy and Classification of Fusarium

Link (1809), cited in Leslie and Summerell (2006), was the first to develop the generic concept of Fusarium based primarily on the presence of the characteristic ‘canoe’-shaped conidia. The shape and size of the conidia are important features in the identification of

Fusarium. A common historical approach to fungal identification was that isolates from individual plant species represented a host-specific species of Fusarium (Leslie and Summerell 2006). Such an approach would eventually result in too many species being named, sometimes even when they represented the same species isolated from different hosts. All modern taxonomic systems for Fusarium are based on the work of Wollenweber and Reinking (1935). These researchers reduced the number of *Fusarium* species from more than 1,000 down to 16 sections, 65 species, and 77 sub-specific varieties and forms (Leslie and Summerell 2006).

At least 18 *Fusarium* species have been recovered from soybean roots in North America and include *F. acuminatum, F. avenaceum, F. chlamydosporum, F. compactum, F. culmorum, F. equiseti, F. graminearum, F. merismoides, F. poae, F. proliferatum, F. pseudograminearum, F. redolens, F. semitectum, F. porotrichioides, F. subglutinans, F. tricintum, F. verticillioide*s, and *F. virguliforme* (Agarwal 1976., Li et al. 2009., Bienapfl 2011., Bienapfl et al. 2010., Broders et al. 2007., Díaz Arias et al. 2008., Díaz Arias et al. 2011a., Díaz Arias et al. 2011b., Díaz Arias et al. 2009., Ellis et al. 2011., French and Kennedy 1963., Killebrew et al. 1993., Leslie and Summerell 2006., Li et al. 2009., McGee et al. 1980., Moretti and Susca 2010., Nelson et al. 1997., Pant and Munkhopadhyvay 2002., Ross 1965., Yang and Feng 2001., Zhang et al. 2010). However, *F. oxysporum* and *F. solani* are the two species that have most commonly been associated with root rot in soybeans (Li et al. 2009., Bienapfl et al. 2010., French and Kennedy 1963., Jasnic et al. 2005., Killebrew et al. 1993., Leslie and Summerell 2006., Zhang et al. 2010).

A number of concepts have been used for the identification of *Fusarium* species. These include generic concepts, morphological species concepts, biological species concepts, and phylogenetic species concepts (Leslie and Summerell 2006). The morphological approach uses both physical and physiological characteristics to distinguish *Fusarium* species with the greatest

weight being placed on the shape of macroconidia, while appearance of microconidia and chlamydospores are also important (Leslie and Summerell 2006). Molecular methods, such as quantitative reverse transcriptase-PCR assays, which allow the quantification of rare transcripts, have been developed and can be used to detect small differences in gene expression in DNA samples (Pfaffl, M. W. 2001). Molecular markers are highly reliable for differentiating strains at an intraspecific level (Hillis and Dixon 1991., Mirete et al. 2003). Species like *F. solani*, for example, can be subdivided into over 50 sub-specific lineages (O'Donnell 2000).

1.4 Root rot disease cycle and epidemiology

When Fusarium infects soybean roots, it produces phytotoxins that are translocated to the leaves, resulting in interveinal scorching (Jin et al. 1996). Foliar symptoms may occur 10-11 days after inoculation of soybeans (Huang et al. 1998). Rapid foliar disease development and dark brown lesions (33-39 mm long) on taproot seedlings were observed on some soybean cultivars 21 days after inoculation with *F. solani* (Huang et al. 1998). By the time foliar symptoms become visible, the root mass of the infected plant has already been reduced, discolored and rotted (Hartman et al. 1999). Root dry mass losses can be as high as 59-74%, while stem discoloration from the point of contact with the soil may range from 11-15 mm (Huang et al. 1998).

Within the same *Fusarium* spp., some isolates may cause yield loss while others may just cause visual symptoms without compromising crop yield (Arias et al. 2013). The strains that cause symptoms without affecting yields could be a useful asset in the development of biological control options for root rot. Determination of the yield reducing effects of *Fusarium* spp. on soybeans can be complicated by other crop-environment interactions, as well as by the timing and method of root sample collection (Arias et al. 2013).

Quantification of the relationship between the severity of SDS on the roots and symptoms on the foliage across soil inoculum densities would help to increase understanding of disease epidemiology, as well as improve methods to screen soybean germplasm for disease resistance (Roy et al. 1997). This knowledge also would help in forecasting disease severity based on the soil inoculum density and in the development of appropriate management strategies. An increase in the density of Fusarium inoculum in the soil is associated with an increase in both root rot and foliar disease symptoms, with this relationship more pronounced in foliar versus root disease (Gongora-Canul 2012).

Fusarium spp. can infect soybeans throughout the growing season (Njiti et al. 1997). In a study where soybean was sown into soil infested with *F. virguliforme*, symptom development occurred faster in the roots (9-18 days) than on the foliage (15-25 days), with the shortest incubation periods associated with higher inoculum densities (Gongora-Canul 2012). The lag in foliar symptom development presumably results from the need for the phytotoxins to be translocated from the roots to the shoots before they can initiate foliar symptom development. In some situations, roots have a high disease severity, compared with mild foliar symptoms on the same individual plant. This reflects that root infection is sometimes limited to the cortical tissue, where toxin translocation up the plant is limited (Navi and Yang 2008). The severity of SDS can also vary year to year in the same field, depending on prevailing weather conditions (Vick et al. 2003, Wrather et al. 1995).

1.5 The role of soil and crop residues

The Fusarium inoculum that initiates disease in soybean crops includes hyphal fragments, macroconidia, microconidia and chlamydospores on crop debris and in the soil (Sutton 1982). When seeded into infested soil, soybean seeds will quickly come into contact with the hyphal

fragments, macrospores, and chlamydospores and infection can ensue (Ellis et al. 2011). In an experiment using a rolled-towel assay where soybean seeds were inoculated with *F. graminearum* spores and rolled in paper towels, mycelial growth was observed on the seeds within 2 to 3 days (Ellis et al. 2011). This speed of infection is fast enough to cause disease on the seeds before they even germinate, especially when planted in cold soils, as is usually the case in southern Alberta. In cold environments, when seed germination and seedling emergence are slow, infection levels can be high.

Sudden death syndrome is favored by wet conditions that may arise from excessive rain, over-irrigation or any factor that results in excessive soil moisture levels (Roy et al. 1989., Scherm and Yang 1996). Disease severity can therefore be reduced by timing seeding so that it does not coincide with periods of elevated soil moisture.

Residue management under reduced tillage systems requires that crop residues be left on the soil surface, therefore resulting in increased inoculum density if those residues are infected (Schaafsma et al. 2005). Destroying the crop residues from the previous crop through burning is an option that may help reduce the inoculum density in the field. However, this practice has numerous drawbacks such as the release of large quantities of smoke into the atmosphere.

1.6 Host-pathogen interactions

Root rot of soybean (sudden death syndrome or SDS) has been attributed largely to infection by a complex of *F. oxysporum* and *F. solani*, with other pathogenic organisms playing a peripheral role (Farias and Griffin 1989., Ferrant and Carrol 1981., Nelson 1999). Some studies on the occurrence of *Fusarium* spp., pathogenicity on soybeans, and genetic resistance of soybean cultivars, however, have not been conclusive (Arias et al. 2013). In some reports, *F.*

oxysporum has been noted as a saprophytic fungus associated with soybean, colonizing the plant roots without causing any symptoms (Farias and Griffin 1990).

Damping-off and SDS have well documented economic impacts on soybean production, but there has not yet been a thorough documentation of the losses associated with wilts and root rots (Arias et al. 2013). Despite its widespread occurrence in soybean growing areas, the impact of Fusarium root rot in particular has not been properly quantified, largely as a consequence of the similarity of symptoms with those associated with other diseases.

Fusarium solani has been linked to root rot symptoms on soybean seedlings, causing significant reductions in crop emergence and yield potential under both open field and greenhouse conditions (Killebrew et al. 1988). This pathogen has been linked to the root rot complex, causing a range of symptoms that include wilting, damping-off, rots, vascular discoloration and root cortex decay (Farias and Griffin 1989., Leslie and Summerell 2006., Nelson 1999).

Fusarium oxysporum caused yield reductions of 47.6% and 55.6%, respectively, on two susceptible cultivars under field conditions (Leath et al. 1985). In infected soybean plants, reductions in total root length and total root surface area were reported to be more reliable indicators of fungal pathogenicity than is root rot severity (Arias et al. 2013). Root rot severity was strongly negatively correlated to shoot and root mass, while root morphological characteristics were not strongly correlated to the same growth parameters (Arias et al. 2013). Some studies have suggested that yield losses associated with Fusarium infection are incurred only if the pathogen colonizes the root vascular tissue and not just the root surface (Navi and Yang 2008).

Fusarium oxysporum can cause severe damping-off, with up to 75 % of inoculated plants succumbing to the disease under greenhouse conditions (Arias et al. 2013). If such high levels of pathogenicity occur in the field, the yield reduction is likely to be significant. Under field and greenhouse conditions, nine species of *Fusarium* (*F. acuminatum, F. equiseti, F. graminearum, F. oxysporum, F. proliferatum, F. semitectum, F. solani, F. sporotrichioides, and F. virguliforme*) all caused symptoms at the vegetative and reproductive phases of soybean growth. The symptoms included root rots, alterations in root morphology, as well as yield reductions (Arias et al. 2013).

Plants infected by *F. graminearum* and *F. virguliforme* developed light brown to black root discoloration and had low root and shoot mass, root length and volume. Infection with *Fusarium* also resulted in poor plant development as a result of reductions in nodulation and the translocation of water and nutrients (Aiken et al. 1996., Cichy et al. 2007., Grant et al. 1981). Many types of *F. oxysporum*-plant interactions (pathogenic, saprophytic, mutualistic, and antagonistic) have been reported, with some isolates being more aggressive in greenhouse than field experiments (Arias et al. 2013).

Fusarium solani f. sp. *glycines* produces toxins in liquid medium, and fungal culture filtrates induced toxicity on soybean callus tissue, cotyledons, germinating seeds, as well as on plants (Lim et al. 1990). Fusarium phytotoxins have been linked to the development of foliar symptoms, which has enabled their widespread use in the evaluation of resistance of soybean to infection (Hartman et al. 1997). A detailed understanding of host resistance mechanisms would, however, require additional knowledge of the other factors that favor phytotoxin production as well as the role of environmental factors in the disease triangle (Huang et al. 1998). The yield response to stand reduction as a result of root rot disease has sometimes been found to be

insignificant, probably owing to the ability of the remaining plants to take advantage of the niches created in the thinner plant stands and exhibit compensatory growth (Stivers et al. 1980).

1.7 Root rot disease management strategies

Approaches that include cultural practices like crop rotation with non-host crops, sowing of certified high quality seed, selection of cultivars with partial resistance, removal of infected crop residues, application of fungicide treatments, and selection of soils with high fertility have all been identified as helpful for managing root rot (Nelson 1999., Zhang et al. 2012). An integrated approach to the management of SDS is the most sustainable way of achieving healthy, vigorously growing crops. An increase in plant vigor increases the ability of the crop to resist pathogen attack and achieve maximum productivity.

1.7.1 Cultural methods

Current disease management options for SDS include the use of resistant soybean germplasm and a variety of cultural practices to mitigate the impact of the disease. These practices include delayed seeding, which allows the soil to warm up enabling faster germination and emergence (Grau et al. 2004), sub-soiling, which improves soil aeration and also speeds up seedling germination and emergence, and chisel plowing and disc plowing of the soil, which bury crop residues and lower pathogen inoculum levels (Vick et al. 2006). In areas where there is a high risk of SDS, late planting after the soil has warmed up has been recommended as a management option (Yang and Navi 2006).

Root colonization and the concomitant development of foliar symptoms depend on environmental conditions (Scherm and Yang 1996), soil physical conditions (Scherm and Yang 1996, Vick et al. 2003), soil chemistry (Rupe et al. 1996), as well as soil biological factors (McLean and Lawrence 1995, Melgar et al. 1994). Elevated soil moisture levels coupled with

depressed soil temperatures at the crop establishment stage increase SDS severity (Hirrel 1987., Vick et al. 2003). Planting soybeans in warm, moderately moist soil will therefore play a significant role in reducing the impact of root rot in a crop planted in an infested field.

Activities that compact the soil also increase the occurrence and severity of SDS (Roy et al. 1997, Scherm and Yang 1996., Scherm et al. 1998., Vick et al. 2003). Soil compaction hinders drainage and extends the periods during which the soil is saturated (Chong et al. 2005). Conversely, practices that improve soil drainage and aeration become useful in situations where the threat of SDS is high. Sub-soiling (ripper-tilling) the soil to as deep as 40-45 cm reduces the soil bulk density and increases porosity, thereby helping to ameliorate the problem of root rot (Vick et al. 2003).

Short rotations and continuous cropping of soybeans have been linked to increases in the incidence and severity of root rot (Bradley 2008). Such practices encourage inoculum build-up by ensuring continuous host availability for the pathogen. Rotating with non-host crops represents one of the most effective root disease management options available (Bradley 2008).

1.7.2 Biological methods

The continuous use of large quantities of agro-chemicals in the management of fungal diseases poses health and environmental hazards over and above the risk of development of fungicide resistance in the targeted species (Haas et al. 2000). Bio-fungicides offer an environmentally friendly alternative to the chemical fungicides. When biological control agents are isolated from the same environments in which they are to be used, the chances are greater that they will colonize the rhizosphere effectively, increase in concentration and offer long-term crop protection (Chao et al. 1986). The isolation of biocontrol agents from the rhizosphere of the

targeted crop species has yielded more effective bio-fungicides compared with the isolation of the same biocontrol agent from the rhizosphere of a different plant species (Cook 1993).

Some *F. oxysporum* strains are antagonistic to pathogenic strains and thus could become useful biocontrol agents in the management of *F. oxysporum* and other rhizosphere pathogens (Fravel et al. 2003., Menjivar et al. 2011). The non-pathogenic *F. oxysporum* competes with the pathogenic strains for infection sites, thus providing control through competitive displacement and also through induction of host systemic resistance (Fravel et al. 2003). Isolating and culturing these non-pathogenic strains may result in the identification of biocontrol agents that can be used to inoculate the soil at seeding and reduce infection by virulent strains of the fungus. *Clonostachys rosea*, a fungus that occurs naturally in most soils, also is a potential bio-fungicide (Schroers et al. 1999).

The bacterium *Bacillus subtilis*, which occurs commonly in a range of ecological niches (Pang et al. 1998), is an important *Fusarium* antagonist (Zhang et al. 2009). Species of *Bacillus*, *Serratia*, and *Pseudomonas fluorescens* have demonstrated potential as bio-control agents against pea plant pathogens, in addition to having plant growth stimulation properties (Wang et al. 2003., Ryder et al. 1999). Strains of *B. subtilis* inhibited the germination of macroconidia of *F. graminearum* by 14-32% and of macroconidia of *F. oxysporum* by 20-48%. Mycelial growth of these species was also inhibited (Zhang et al. 2009).

Not all root rot reducing strains of *B. subtilis* are effective in protecting germinating seeds (Zhang et al. 2009). The placement of a biocontrol agent close to the target site of action is critical for effective colonization and suppression of infection by the pathogen (Weller 1988). The soil environment also plays a critical role in the effectiveness of biocontrol agents, since most of these are sensitive to factors such as soil type and pH (Schmidt et al. 2004).

1.7.3 The use of genetic resistance

The management of Fusarium in soybean can be a challenging task given the general absence of highly resistant varieties, as well as the presence of a multitude of alternative hosts for the pathogen (Bradley 2008). The cropping of resistant varieties is the most practical and economic disease management strategy, and there is a need to investigate the resistance in commercial soybean varieties (Wrather and Koenning 2006). A disease management system that centers on inherent resistance is most desirable because it costs less for producers and reduces the need for the application of pesticides. However, soybean cultivars with high levels of resistance are not yet available on the market (Nelson 1999., Wang et al. 2003).

Partial resistance has been observed in some soybean lines (Hartman et al. 1997). Mueller et al. (2002), working with 6,037 soybean accessions, noted high resistance to *F. solani* in some of these lines. Lines that exhibit resistance can be incorporated into soybean breeding programs to introduce Fusarium resistance in commercial cultivars. A cultivar may, however, have significant resistance to one species of Fusarium but still be susceptible to others, and this represents a challenge to breeders trying to develop varieties with resistance to the entire root rot complex (Zhang et al. 2010).

Soybean cultivars tested in greenhouses, growth chambers and open fields exhibited varying degrees of tolerance to *F. solani* f. sp. *glycines* (Hartman et al. 1997., Melgar et al. 1994., Rupe et al. 1991., Stephens et al. 1993). A dominant gene, for example, is linked to *F. solani* resistance in some soybean varieties, while others exhibit quantitative resistance (Stephens et al. 1993).

Great emphasis has been placed on *F. solani* in screening soybean varieties for resistance, since this fungus historically has caused very large yield losses (Nelson 1999., Wang et al.

2003). Very little work has been carried out on understanding the genetics of resistance to most other *Fusarium* species that infect soybeans (Huang et al. 1998). There is a need to focus on all species of Fusarium when screening soybean varieties for resistance, as this would ensure broad spectrum protection.

1.7.4 Seed treatment with fungicides

Seedling diseases that attack the roots of soybean are a common problem where soil temperatures are low and soil moisture levels are high (Bradley 2008). These conditions extend the germination period, when the seedling is most vulnerable to pathogen attack and make fungicidal seed treatments necessary (Bradley 2008). Environmental conditions play a significant role in determining the effectiveness of seed treatments, and when conditions are not favorable for disease, yield differences decline between fungicide-treated and untreated seeds (Bradley 2008).

Soybean seed treatments can be effective in protecting crops against soil-borne pathogenic organisms and helping to ensure good yields. Soybean seed treatment with metalaxyl resulted in a positive yield response in the presence of *Phytophthora megasperma* Drechs, f. sp. *glycinea* Kaun Erwin and environmental conditions conducive for disease development (Guy et al. 1989). *Fusarium graminearum* responded inconsistently to metalaxyl and fludioxonil when these products were used individually, but control was better when the two chemicals were mixed. This suggests that mixing fungicides for seed treatment can be an effective strategy in widening their spectrum of action (Broders et al. 2007). In some experiments, captan or fludioxonil provided superior root rot protection when used as seed treatments, while azoxystrobin offered very low protection and fungicide concentration did not alter efficacy (Ellis et al. 2011).

1.8 Research hypotheses and objectives

The aim of the research presented in this thesis was to evaluate the occurrence of root rot of soybean in Alberta, Canada, and identify the causal agents of the disease in this region. Emphasis was also placed on management of the disease, focusing on an examination of the effectiveness of fungicide seed treatments and the use of varietal genetic resistance under both greenhouse and field conditions. Specifically, the research objectives were to: (1) determine root rot incidence and severity in southern Alberta (where most soybeans are grown in the province), (2) characterize the *Fusarium* species associated with root rot, and (3) evaluate fungicidal seed treatments and varietal resistance as possible root rot management strategies. I hypothesized that root rot is a widespread disease on soybean crops in Alberta, and that *Fusarium* species are the main causal agent of the disease in this province.

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Chapter 2: The Occurrence of Soybean Root Rot in Southern Alberta, Canada, in 2013

2.1 Introduction

Soybean (*Glycine max* (L.) Merr) has great potential as an alternative cash crop to canola in southern Alberta farming systems. The potential profitability of soybean has been a driving force in its growth as an important crop in Canadian agriculture (Dorff 2007). A number of crop production issues, however, need to be addressed, including root rot caused by fungi belonging to the genus *Fusarium.* A comprehensive survey of soybean crops was conducted in August 2013 across the southern Alberta region to assess root rot occurrence in soybean fields.

2.2 Methods

The survey was conducted in August 2013 when the soybean crop was at the pod set-early pod filling stages of growth. Root samples were collected from 28 fields across 7 different locations in southern Alberta (Fig. 2.1). The samples were collected along W-transects in each field. Twenty plants were dug out at each sampling point for a total of 100 root samples per field. Plants were also collected in low lying areas of the field where they were observed to be severely stunted or dead. The roots were gently shaken to rid them of excess soil, sealed in plastic bags, and placed on ice in cooler boxes to avoid spoilage. At the end of each day, the root samples were stored at 4°C to maintain freshness until they could be taken back to the laboratory for further processing. In the laboratory, the roots were gently washed under running water to rid them of soil. They were then rated visually for root rot on a 0-4 scale as described by Chang et al. (2007), where: 0 = normal root color, 1 = 1-25% root discoloration, 2 = 26-50% root discoloration, 3 = 51-75% root discoloration, and 4 = 76-100% root discoloration. The roots

were also rated for nodulation on a scale of 0-4, where: 0 = no nodules, 1 =1-5 nodules, 2 = 6-10 nodules, 3 = 11-15 nodules, and 4 = >15 nodules per root system. The root samples were partially dried by opening the bags and placing them in the greenhouse overnight to reduce moisture levels and enable storage at 4°C for future pathogen isolation work.

2.3 Results and Discussion

Root rot was observed in all of the 28 fields surveyed, although the levels of disease severity varied. These results are similar to what was observed in a survey conducted in 2012 (Chang et al. 2013). Low lying areas in the field where water gathers during storms tended to be associated with patches in which stunted and dead plants were often observed. Diseased plants in most cases could be pulled easily out of the ground (in cases where the soil was at field capacity) due to severe damage on the root system. Some plants showed stunting and yellowing of the bottom leaves, which were sacrificed as the root system became inadequate to sustain the entire plant. Some plants had completely dried up after losing the entire root system to root rot, and these were particularly evident in low lying areas.

The lowest incidence of root rot (45%) was found in samples collected near Brooks, while the highest incidence (100%) was recorded near Duchess (Table 2.1). Dry conditions at Brooks may have been less favorable for disease development. Generally, root rot severity increased from the south to the north across the sampled region. It was lowest in Taber (average disease severity = 1.2) and highest in Lacombe (average disease severity = 1.8). This is likely a result of generally lower temperatures further north, which may have delayed crop emergence and provided the soil borne pathogens with more time to infect the plants and cause disease. Sites at which a high incidence of disease was recorded also had a high disease severity. Stunted or completely dead plants had severe root damage and depressed nodulation.

It should be noted that *Sclerotinia sclerotiorum*, the causal agent of white mold, also was identified in 20 out of the 28 surveyed fields. White mold was very severe in some crops, with notable wilting of the foliage. The high prevalence of white mold may reflect short canola (*Brassica napus*)-soybean rotations that allow pathogen inoculum build up, since canola also is a host for *S. sclerotiorum*. Another disease, bacterial blight (*Pseudomonas syringae*), was noted in experimental field plots at the Crop Diversification Centre – South, Alberta Agriculture and Forestry, Brooks, but not in any of the commercial crops inspected.

2.4 References

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Table 1.1 Root rot incidence in soybean crops in southern Alberta in 2013.

Location	No. of surveyed fields	Root rot incidence (%)		Root rot severity (0-4)		Root nodulation (0-4)	
		Range	Mean	Range	Mean	Range	Mean
Brooks	9	45-85	71.4	0.7-1.8	1.4	0.8-2.8	1.8
Duchess	3	47-100	67	0.99-3.4	2	1.7-2.9	2.3
Lacombe	2	73-77	75	1.72-1.9	1.8	2.1-2.2	2.1
Medicine Hat	6	51-92	69	0.52-3	1.6	1.1-2.7	1.7
Taber	2	66-69	67.7	1.1-1.3	1.2	2.3-2.4	2.4
Tilley	4	58-87	71.4	0.9-2.3	1.5	0.5-1.3	0.9
Vauxhall	2	69-71	70.3	1.2-1.6	1.4	1.6-1.7	1.7

Note: Root rot severity was rated on a scale of 0-4, based on the percentage of the roots on an individual plant showing discoloration, where: 0 = normal root color, 1 = 1-25% root discoloration, 2 = 26-50% root discoloration, 3 = 51-75% root discoloration, and 4 = 76-100% root discoloration. Root nodulation was rated on a scale of 0-4, based on the number of nodules on the root system, where: 0 = no nodules, 1 =1-5 nodules, 2 = 6-10 nodules, 3 = 11-15 nodules, and 4 = >15 nodules per root system.

Figure 2.1 Location of fields surveyed for the occurrence and severity of soybean root rot in southern Alberta in August, 2013.

Figure 2.2 A low lying spot in a field near Vauxhall, Alberta (August 2013), with severe soybean root rot.

Chapter 3: The Occurrence of Soybean Root Rot in Southern Alberta, Canada, in 2014

3.1 Introduction

Development of short-season cultivars has greatly increased the potential of soybean (*Glycine max* (L.) Merr.) as a profitable cash crop in southern Alberta farms. As a result, soybean is rapidly becoming an important crop in Canadian agriculture (Dorff 2007). However, production issues have developed, including root rot which has been reported to be a major challenge in Canada (Chang et al. 2013). A survey was conducted in August 2014 across the southern Alberta region to assess the occurrence of root rot and its impact on soybean crops.

3.2 Methods

The survey was conducted over the period of August 17-23, 2014, when the soybean crops were at the pod set to early pod filling stages of growth. Root samples were collected from 28 fields in 9 different locations (Bow Island, Brooks, Duchess, Jenner, Medicine Hat, Seven Persons, Taber, Tilley, and Vauxhall) in southern Alberta (Fig. 3.2). Samples were collected from 5 points in each field along W-shaped transects. Twenty plants were dug out of the soil at each sampling point, for a total of 100 root samples collected per field. Plants also were collected outside the sampling points (primarily, low lying areas of the field), where they were observed to be severely stunted or dead. The roots were gently shaken to rid them of excess soil, sealed in plastic bags, and placed on ice in cooler boxes to avoid spoilage.

Once transported back to the laboratory, the roots were washed gently under running water and then rated visually for root rot on a 0-4 scale as described by Chang et al. (2007), in which: 0 = normal root color, 1 = 1-25% root discoloration, 2 = 26-50% root discoloration, 3 =

51-75% root discoloration, and 4 = 76-100% root discoloration or a dead plant. The root samples also were rated for nodulation on a scale of 0-4, where: 0 = no nodules, 1 = 1-5 nodules, 2 = 6-10 nodules, 3 = 11-15 nodules, and 4 = >15 nodules per root system.

3.3 Results and Discussion

Root rot was observed in all 28 fields surveyed, but disease severity varied. The disease was most severe (1.7) in Tilley and least (0.3) in Brooks (Table 3.1). Plants in low lying areas of the fields where water and salts accumulate during storms tended to be severely stunted or dead. In most cases, the diseased plants could be pulled out of the ground easily, especially when the soil was at field capacity, as a result of severe constriction of the root system. Some plants showed stunting and yellowing of the lower leaves, likely because the root system was inadequate to sustain the plant. Other plants, particularly in the low lying areas, had completely dried up after losing the entire root system to the disease.

The lowest incidence of root rot (18%) was found in samples collected at Brooks, while the highest incidence (73%) was recorded at Taber (Table 3.1). The lower incidence of root rot in Brooks and Duchess may have been a reflection of dry field conditions in that region of the province during much of the summer (Table 3.2). Dry conditions are unfavorable for disease development. Root nodulation was highest (3.4) at Jenner and lowest (1.1) in Vauxhall. Overall, root rot disease incidence and severity in the surveyed locations were lower in 2014 than in 2013 (Nyandoro et al. 2015), and nodulation was superior. This suggests that lower disease pressure enables the plants to form more nodules from symbiotic associations with *Bradyrhizobium japonicum*.

3.4 References

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Table 3.1 Root rot incidence, severity and nodulation in soybean crops surveyed in southern Alberta in 2014.

Location	No. of fields surveyed	Root rot incidence (%)		Root rot severity (0-4)		Root nodulation (0-4)	
		Range	Mean	Range	Mean	Range	Mean
Bow Island	2	36-37	37	0.5-0.7	0.6	2.9-3.5	3.2
Brooks	3	9-24	18	0.2-0.4	0.3	2.2-3.8	3.3
Duchess	2	22-25	24	0.4-0.4	0.4	1.4-2.3	1.9
Jenner	2	52-70	61	0.8-1.4	1.1	3.0-3.5	3.4
Medicine Hat	6	10-50	34	0.2-1.1	0.7	0.4-2.7	2.2
Seven Persons	4	14-46	28	0.4-0.7	0.5	0.2-2.6	1.7
Taber	2	61-85	73	1.0-1.8	1.4	2.8-3.0	2.9
Tilley	5	38-62	50	0.7-1.8	1.7	1.1-3.2	2.2
Vauxhall	2	29-72	51	0.5-1.9	1.2	0.9-1.3	1.1
TOTALS	**28**	**9-85**	**40**	**0.2-1.9**	**0.9**	**0.2-3.8**	**2.3**

Note: Root rot severity was rated on a scale of 0-4, based on the percentage of the roots on an individual plant showing discoloration, where: 0 = normal root color, 1 = 1-25% root discoloration, 2 = 26-50% root discoloration, 3 = 51-75% root discoloration, and 4 = 76-100% root discoloration. Root nodulation was rated on a scale of 0-4, based on the number of nodules on the root system, where: 0 = no nodules, 1 =1-5 nodules, 2 = 6-10 nodules, 3 = 11-15 nodules, and 4 = >15 nodules per root system.

Table 3.2 Precipitation (mm) and atmospheric temperature (°C) among surveyed sites in 2013.

	Brooks			M. Hat			Lacombe			Taber			Vauxhall		
Date	**Jun**	**Jul**	**Aug**	**Jun**	**Jul**	**Aug**	**Jun**	**Jul**	**Aug**	**Jun**	**Jul**	**Aug**	**Jun**	**Jul**	**Aug**
1	15.9	20.9	16.9	16.1	22.9	18.2	13.1	14			11	18.3	15.3	21.5	17.8
2	15.9	25.2	16.2	16.7	16.5	16.6	6.3	17	16.6		23.8		15	25	15.4
3	11.9		13.1	10.1	19.5	15.8	9	14	14.9	12	23		11.3		14.5
4	11.2	20.6	16.4	11.1	15.9	17.9	3.9	10	15.1	12.3	23		11.4	22.5	16.3
5	14.9	16.8	16.7	14.3	15.4	19.4	6.8	10	16.5	16.8			16	18.1	17.1
6	15.4	15.6	16.6	17.3		18.2	2.4	7.9	15.5	17.5		17	15.9	17.3	17.1
7	18.8	14.6	18	20.6	9.4	18.5	10.5	6.2	14.1			18.5	19.5	15.5	19.1
8	15.9	14.4	15.4	16.8	11.6	17.1	8.4	5.8			15.3	16.5	16.1	14.9	16.6
9	12.9	17.1	16.4	14.3	11.6	17.1	5.4	7.4	15.9		18.5		12.8	17.8	16.3
10	12.3	20.6	20.3	13.6	13.4	21	6.1	9.7	17.6	14	20.5		12.9	21.1	19.9
11	12.5	20.7	20.6	12.1	15.9	22.6	1.1	7	18.6	11.8			11.4	20.7	21.9
12	13.5	14.8	20.4	16	9	21.8	9	4	17.4	14.3		20	13.2	16.4	20
13	11.8	14.3	20.1	13.8	10.8	21	5.3	5.1	18.2			18.5	12.8	15.1	18.5
14	13.4	16.4	20.9	14.3	6.3	23.3	7.8	8.8	19.4			21.8	12.9	17.2	21.2
15	15.6	14.8	22.7	16.5	10.7	25.6	10.2	7.2	18.4		15.5		15.8	14.7	22.4
16	14.2	14.1	22.2	13.8	6.3	24.6	8.1	4.2	19.1				14.6	14.5	21.7
17	14.8	17.9	21.8	16.8	11.8	24	3.8	8	18.4	16.8			16.2	18.8	22.1
18	18.3	20.3	21.1	22.3	15	22	6.6	14	16.9	20			18.7	20	20.6
19	17.9	19.6	20.3	21.6	12.2	22.5	13.1	13	15.6	19.8		22.5	18.9	20	22.6
20	15.7	20.5	17.5	15	15.7	20	11.9	12	13.6	15		18.5	14.3	21.1	17.1
21	11.6	18.8	14.4	14.6	15.9	16.8	10.1	13	11.9			16.5	12.3	19.7	14.8
22	15.4	19.6	18.5	16.4	15.1	21.5	6	14	15.7		20.5		15.1	20.3	19
23	13.3	19.8	18.6	16.7	15.3	23.2	8.4	12	15.3		20.5		15	21.1	21.7
24	14.9	19.6	20.4	18.1	16.2	21.9	6.8	12	16.8	16	21.3		15.6	21.4	20.6
25	18	17.4	19.6	18.9	12.6	22.6	10.8	11	16.8	17	19		17.1	18.8	20.2
26	17.9	18.7	21.8	18.7	11.3	24.7	7.4	11	17.1	18.3		21.8	18.9	18.8	22.1
27	18.1	16.1	19.3	18.3	14.2	22.5	7.8	9.8	16.2	20		21.5	19.4	17.6	20.8
28	20.1	13.6	18.7	21.6	10.1	22	9	8	16			18.8	20	14.8	18.9
29	20	13.2	20.9	21.8	8.6	23.4	11.6	5.9	17.8		13.3	22.8	19.7	12.2	21.1
30	21	13.8	20.6	23.9	7.3	22	12.5	2.3	18.3		14.5		22.3	13.7	20
31		16.2	16.8		7.4	17.4			16.7		19			17.2	15.9
Mean	**15.4**	**17.5**	**18.8**	**16.7**	**12.8**	**20.8**	**7.97**	**9**	**16.6**	**16.1**	**18.6**	**19.5**	**15.7**	**18.3**	**19.1**

Figure 3.1 A low lying area of a field near Tilley, AB (August 2014), with the soybean crop exhibiting symptoms of severe root rot**.**

Figure 3.2 Map showing the approximate locations of the soybean crops in surveyed southern Alberta in 2014.

Chapter 4: Aggressiveness of *Fusarium* Species Recovered From Soybean Crops in Southern Alberta

4.1 Introduction

The genus *Fusarium* includes several fungi that are pathogenic to soybeans, causing diseases that include wilts, sudden death syndrome (SDS), root rot, and seed and seedling diseases (Armstrong and Armstrong 1950., Broders et al. 2007., El-Kazzaz et al. 2008., McGee et al. 1980., Nelson 1999., Rizvi and Yang 1996., Schlub et al. 1981., Warren and Kommedahl 1973., Yang and Feng 2001). This genus has been described as one of the most abundant and aggressive groups of plant pathogens (Nelson et al. 1981). Soybean crop losses in Canada as a direct result of Fusarium root rot were estimated to exceed 7,300 metric tons in 1998 (Wrather et al. 2001). Disease symptoms on infected soybeans may include depressed crop emergence, dark brown root lesions, and decay of the entire taproot (Nelson 1999). The significant amount of heterogeneity in the morphology of the microscopic structures exhibited by the genus poses a challenge when it comes to delimitation of its species through a morphological approach.

Fusarium species are largely soil-borne pathogens that infect a wide range of host plants, causing primary or secondary infections (El-Kazzaz et al. 2008., Nelson et al. 1993). Apart from the soil, they also inhabit plant roots, shoots, plant debris and decaying plant material (Aoki et al. 2003., Nelson et al. 1983., Newson and Martin 1953). A large number of *Fusarium* species have been recovered from soybean roots, including: *Fusarium acuminatum, F. avenaceum, F. chlamydosporum, F. compactum, F. culmorum, F. equiseti, F. graminearum, F. merismoides, F. poae, F. proliferatum, F. pseudograminearum, F. redolens, F. semitectum, F. porotrichioides, F. subglutinans, F. tricintum, F. verticillioide*s, and *F. virguliforme* (Agarwal 1976., Li et al. 2009.,

Bienapfl 2011., Bienapfl et al. 2010., Broders et al. 2007., Díaz Arias et al. 2008., Díaz Arias et al. 2011a., Díaz Arias et al. 2011b., Díaz Arias et al. 2009., Ellis et al. 2011., French and Kennedy 1963., Killebrew et al. 1993., Leslie and Summerell 2006., McGee et al. 1980., Moretti and Susca 2010., Nelson et al. 1997., Pant and Munkhopadhyvay 2002., Ross 1965., Yang and Feng 2001., Zhang et al. 2010). However, *F. oxysporum* and *F. solani* are the two species that have been associated most commonly with root rot in the United States and other soybean growing regions (Li et al. 2009., Bienapfl et al. 2010., French and Kennedy 1963., Jasnic et al. 2005., Killebrew et al. 1993., Leslie and Summerell 2006., Zhang et al. 2010).

The production of soybeans has been increasing in Alberta, Canada. In 2014, approximately 4 900 ha were seeded to the crop (Gabruch and Gietz 2014), and in 2015 about 5 300 ha were grown, mainly in southern regions of the province. While the production of soybean is still limited relative to other crops in Alberta, soybean could become an attractive choice for farmers, given the development of early maturing varieties well-suited to the short growing season in this province. Unfortunately, root rot has become an important constraint to soybean production, with surveys carried out in southern Alberta showing that the disease is prevalent in most fields (Chang et al. 2013., Nyandoro et al. 2014., Nyandoro et al. 2015). *Fusarium* species were the most common fungi isolated from soybean roots collected in the 2012 and 2013 surveys.

The current system of *Fusarium* species delimitation is based primarily on distinct morphological and physiological characters, with even subtle differences in a characteristic sometimes being used to delineate a species (Llorens et al. 2006). Distinctive characters, including the size and shape of macroconidia, microconidia and chlamydospores, colony aerial appearance and pigmentation, as well as growth rate on agar media are used to identify species

(Leslie and Summerell 2006). The morphological species concept is generally applicable to any fungal group and has been long used in species delimitation (Taylor et al. 2000). The challenge, however, is that there is a wider spectrum of species than there are readily distinguishable characters for those species (Leslie and Summerell 2006). This makes the process of morphological species delimitation a difficult task. Amongst morphological characteristics, the shape of the macroconidia represents the primary basis upon which species of *Fusarium* are delimited. Nonetheless, there can be confusion, since the shape of the macroconidium may vary depending on the environment in which the fungus is cultured (Leslie and Summerell 2006). Molecular markers also can be highly reliable for differentiating strains at an intraspecific level (Hillis and Dixon 1991., Mirete et al. 2003). Nonetheless, despite some of the challenges associated with the morphological identification of species, this approach is useful for classification of biodiversity, since it forms the groundwork for delimitation of species in the absence of complete DNA sequences, or in the absence of resources or equipment to carry out DNA analysis.

The main objectives of this study were to: (1) determine the *Fusarium* species associated with soybean root rot in Alberta, and (2) investigate the aggressiveness of these species on soybean. An understanding of the composition and aggressiveness of the *Fusarium* species causing root rot of soybean is important for the development of knowledge-based strategies to manage this disease in Alberta.

4.2 Materials and methods

4.2.1 Fusarium isolation from soybean root tissues

Soybean roots were collected from seven areas across southern Alberta (Brooks, Duchess, Lacombe, Medicine Hat, Taber, Tilley, and Vauxhall), representing a total of 28 fields (Fig. 2.2 and Fig. 3.2) in 2013 and 2014. The roots were washed and assessed for the severity of root rot symptoms, and then used for the isolation of fungi belonging to the genus *Fusarium* as described by Leslie and Summerell (2006). Root pieces (5 mm) were excised from both symptomatic and asymptomatic, randomly sampled roots, with five pieces excised per root. The root pieces were surface-disinfected in 1% sodium hypochlorite for one minute, rinsed three times in sterilized distilled water, and blot dried on sterilized paper towels under a laminar flow hood. After blot drying, the five pieces from each root were placed on 100 mm-diameter Petri dishes filled with Potato Dextrose Agar (PDA; Difco) and incubated at room temperature (RT) under continuous light to allow for growth of pathogens infecting the root tissues.

Hyphal tips were then cut from the radiating hyphae under a dissecting microscope. For those fungal colonies that had grown to the point where it was difficult to cut single hyphal tips, isolation was carried out through the single-spore approach. Single-spores were isolated using the sterile needle technique. Briefly, a sterile needle was used to gently touch the aerial portion of the fungal colony, enabling spore attachment to the needle, with the spores immediately plated out on water agar in 100 mm-diameter Petri dishes. The Petri dishes were incubated at RT overnight, and the germinating single-spores were collected under a dissecting microscope and transferred to PDA on 50 mm-diameter Petri dishes to maintain the cultures for future examination under a microscope and virulence assessments.

4.2.2 *Fusarium* species identification

The purified fungal isolates were first placed into 35 groups based on general colony morphology and pigmentation on PDA medium. General groupings included isolates with red aerial mycelia, red pigmentation of the culture medium, orange mycelium, yellow mycelium, white mycelium, slimy mycelium, lack of pigmentation, brown aerial mycelia, brown pigmentation of the media, and purple aerial mycelia. One isolate was arbitrarily selected from each group for species determination. The selected isolates were cultured on Carnation Leaf Agar (CLA) to study their microscopic structures (Pérez-Sierra et al. 2007). The CLA medium was prepared by pouring sterilized water agar into 50 mm-diameter Petri dishes which contained pieces of sterilized carnation leaves as described by Leslie and Summerell (2006). Discs of mycelium were excised from the pure cultures of the isolates maintained on PDA, and were transferred to the CLA medium. The Petri dishes were incubated at 25°C with a 12 h photoperiod.

Identification of the Fusarium isolates was carried out on the basis of the color of the aerial mycelium and pigmentation of the fungal colonies on PDA, and the appearance of the macroconidia, microconidia and chlamydospores on CLA medium (Leslie and Summerell 2006., Nelson et al. 1983). The isolates were examined *in situ* for the presence or absence of sporodochia and spore chains, and on microscope slides for the size and shape of the macroconidia (length, curvature, shape and appearance of the apical and foot cells, abundance as well as the number of septa), microconidia (presence or absence, size, shape, number of septa, and appearance of the conidiogenous cell bearing them), and chlamydospores (presence or absence, whether single or double or cluster or chain formation). The characteristics observed

(Figures 4.1- 4.7) were compared with the species descriptions provided by Leslie and Summerell (2006).

4.2.3 Assessments of aggressiveness

Discs (2 mm^2) from the original single-spore or hyphal tip-derived cultures of 26 identified isolates were excised under the dissecting microscope using a minutien micro-knife and placed on 100 mm-diameter Petri dishes filled with PDA medium, with four fresh Petri dishes prepared per species of *Fusarium*. The inoculated Petri dishes were then placed at RT under continuous light. After 14 days, the cultures were cut into small fragments (~4 mm^2) with a sterile scalpel, 30 mL of sterile distilled water was added per Petri dish, and the resulting suspension was stirred and poured over the potting mix (see below) in which the soybeans were to be planted. A suspension generated from PDA medium that had not been inoculated with any fungal species was prepared in the same manner for use as a control.

The soybean genotype TH29002RR (Pioneer Hi-Bred, Chatham, ON) was used as a test host. The seeds were planted in 500 mL cups filled with Sunshine Aggregate Plus Professional Growing Mix (Sun Gro Horticulture Canada Ltd., Seba Beach, AB) at a density of 7 seeds per cup. The planting cups were filled level to ensure uniform soil volume in all cups and then pressed down with the flat base of a glass flask to create an even surface. The cups were watered to field capacity before inoculation with the Fusarium culture suspensions. Inoculation was carried out by pouring 30 ml of autoclaved water onto the Fusarium culture in each Petri dish, stirring with a glass rod and then evenly pouring the homogenate over the soil. The cups were maintained in a greenhouse maintained at 26^{o}C with a 12 h photoperiod, and watered daily in the morning and evening to ensure high moisture levels around the roots.

The treatments were arranged in a Randomized Complete Block Design (RCBD) and replicated five times. The cups were maintained at field capacity for 14 days after planting. The number of emerged seedlings was counted 2 weeks after inoculation. Shoot length also was measured at 14 days, using a calibrated measuring stick, while the seedlings were still standing in the cups. After emergence and shoot length had been recorded, each seedling was carefully dug out of the potting mix and washed in a tub of standing water. The washed roots were evaluated for root rot severity on a scale of 0-4 as described by Chang et al. (2007), based on the proportion of the root surface showing discoloration, where: 0 = normal root color, 1 = 1-25% root discoloration, 2 = 26-50% root discoloration, 3 = 51-75% root discoloration, and 4 = 76-100% root discoloration. After evaluation of symptom severity, seedlings from the same experimental unit were cut to separate the roots from the shoots and placed in a drier at 35°C for 2 days. Dry weights were recorded separately for the roots and shoots. The entire experiment was repeated for all isolates.

4.2.4 Data analysis

The relative frequency of individual *Fusarium* species across the surveyed sites was calculated using the formula: frequency = (n/N) ×100, where n = number of isolates of an individual species at a specific site, and N = number of isolates of the particular species recovered across the survey area (Roy 1997). Analysis of variance (ANOVA) was carried out on germination, seedling height, root rot severity, and root and shoot dry mass data using the Mixed Model and the General Linear Model (PROC GLM) procedures of SAS 9.3 (SAS Institute Inc., Cary, NC). Means were compared using t-Grouping and Duncan's Least Significant Difference (LSD) at $P \leq 0.05$.

4.3 Results

4.3.1 The recovered *Fusarium* species

A total of 971 *Fusarium* isolates were recovered from the soybean root samples. These were placed into 35 relatively homogeneous groups based on the visual appearance of the colonies on PDA. From these groups, 26 isolates were determined to species according to Leslie and Summerell (2006). Seven species were identified, including *F. culmorum* (2 isolates), *F. avenaceum* (6 isolates), *F. proliferatum* (2 isolates), *F. oxysporum* (2 isolates), *F. acuminatum* (5 isolates), *F. solani* (5 isolates), and *F. redolens* (4 isolates).

Fusarium culmorum produced an olive-brown raised mycelium with a deep red pigmentation on PDA medium (Fig. 4.1). The cultures produced a dark-brown central spore mass (~20 mm) on PDA. The macroconidia (30 µm long) on CLA were short and stout and found in abundance in the aerial parts of the mycelium (some formed on monophialides on hyphae), with blunt apical cells and poorly developed foot cells. The macroconidia had 3-4 septa.

Isolates of *F. avenaceum* produced a white mycelium on PDA, with a brown pigmentation (Fig. 4.2) and abundant bright orange sporodochia. They produced long slender macroconidia (65 µm long) with a slight curvature and thin walls. The macroconidia had 5 septa and were borne on both monophialides and polyphialides.

Fusarium acuminatum developed slow growing colonies of abundant white mycelium with a light brown pigmentation and dark brown spots on PDA (Fig. 4.3). The macroconidia (50 µm) that formed on CLA were slender with a distinct curvature and 3-4 septa. They had distinct foot-shaped basal cells and snout-shaped apical cells. Short cell intercalary chlamydospores were

formed on CLA and no microconidia were present. Microconidia were fusiform and intercalary chlamydospores were present.

Isolates of *F. solani* developed a dense white mycelium with a central spore mass and brown pigmentation that became lighter and creamy at the periphery on PDA (Fig. 4.4). Bright orange sporodochia formed on CLA and the macroconidia (50 µm) were slightly curved to almost straight with 5 septa. Microconidia formed abundantly on false heads and long monophialides. Smooth oval intercalary chlamydospores formed in the hyphae.

Isolates identified as *F. oxysporum* developed a dense rose-burgundy mycelium with a central spore mass and a burgundy-greyish pigmentation that was creamy at the periphery on PDA (Fig. 4.5) and numerous bright orange sporodochia formed on CLA. The macroconidia were slender with thin walls. Oval microconidia formed in abundance and hyphal intercalary chlamydospores were present.

Fusarium redolens formed a white mycelium that sometimes radiated outwards in a feather-like pattern with a patchy dark brown rhizomatous pigmentation on PDA (Fig. 4.6). Colonies developed abundant bright orange sporodochia on CLA. The macroconidia (50µm long) were long, slender and slightly curved with thin walls and distinct foot cells, borne on both monophialides and polyphialides. Oval microconidia were formed and were borne on false heads that occurred abundantly in the aerial mycelium.

Finally, in the case of *F. proliferatum*, isolates developed a white mycelium and light brown pigmentation on PDA (Fig. 4.7) and the macroconidia were approximately 50µm long, slender and slightly curved with thin walls. Microconidia were borne on both monophialides and polyphialides.

4.3.2 Aggressiveness of *Fusarium* species on soybean

4.3.2.1 Root rot

Significant interspecific, and sometimes intraspecific, variation was observed in the severity of root rot caused by the different *Fusarium* species identified in this study (Table 4.1). *Fusarium acuminatum* caused the most severe root rot on the soybeans seedlings, although some isolates of this species were less aggressive. The two *F. proliferatum* isolates tested induced severe root rot on the seedlings, with disease severities of 2.3 and 3.1, respectively, while the six isolates of *F. avenaceum* induced varying levels of root rot severity, ranging between 0.8-2.3. The two *F. oxysporum* isolates caused mild to moderate disease, with root rot severities between 0.5-1.6. The five isolates of *F. solani* evaluated induced mild symptoms of root rot, with disease severities ranging between 0.8-1.5. Isolates of *F. redolens* induced mild symptoms (0.6-1.5) relative to all species tested. As expected, soybean plants grown in non-inoculated potting mix did not develop any root rot.

4.3.2.2 Soybean seedling emergence

All of the Fusarium isolates tested in this study significantly suppressed seedling emergence ($P \leq 0.05$) to varying degrees compared with the pathogen-free control. Seedling emergence varied significantly among soybean plants in response to inoculation with different *Fusarium* species, and sometimes in response to inoculation with different isolates of the same species (Table 4.1). In potting mix inoculated with *F. acuminatum*, germination rates ranged from 40-95%. The lowest emergence rate (40%) was caused by an isolate of this species. Both isolates of *F. proliferatum* strongly suppressed soybean emergence, which ranged from 45-65% following inoculation of the potting mix. The impact of *F. avenaceum* on seedling emergence varied widely, with emergence rates from 60-94%. Inoculation with isolates of *F. redolens* had a

low to moderate impact on emergence (82-96%). Similarly, seedling emergence following inoculation with *F. solani* was 87-97%, and following inoculation with *F. oxysporum*, it was 88-95%. There was a significant ($P \leq 0.05$) and strong negative correlation (-0.8827) between seedling emergence and root rot severity.

4.3.2.3 Seedlings shoot length and dry matter accumulation

There was significant variation ($P \leq 0.05$) in seedling height among the soybean plants inoculated with the various species of *Fusarium*. Inoculation with *F. solani* (average plant height = 47.0 mm), *F. culmorum* (41.5 mm), *F. redolens* (46.0 mm) or *F. oxysporum* (43.2 mm) did not significantly reduce seedling plant height relative to the disease-free control plants (48.3 mm). In contrast, there were significant reductions in height associated with inoculation with *F. avenaceum* (36.8 mm), *F. acuminatum* (37.6 mm), and *F. proliferatum* (29.3 mm). The biggest impact on soybean seedling height was observed following inoculation with *F. acuminatum* isolate FPT062. Seedlings in this treatment had an average height of only 20 mm (Table 4.1).

Dry mass of the roots and shoots of the inoculated soybean seedlings also varied significantly ($P \leq 0.05$) (Table 4.1). All of the *Fusarium* species, with the exception of *F. redolens*, reduced root dry mass relative to the non-inoculated control (0.48 g). The lowest mean root dry matter was observed in seedlings inoculated with *F. avenaceum* (0.16 g). Similarly, inoculation with any of the *Fusarium* species significantly ($P \leq 0.05$) reduced shoot dry mass relative to the disease-free control treatment (1.43 g). The most aggressive *Fusarium* species, based on its impact on shoot dry matter accumulation, was *F. acuminatum*. Plants inoculated with this species had a mean root dry mass of 0.59 g at 14 days after planting (Table 4.1).

4.4 Discussion

The identity and aggressiveness of *Fusarium* species associated with soybean root rot in southern Alberta was examined. Most of the species found have been recovered previously from soybean roots elsewhere in Canada (Zhang et al. 2013., Chang et al. 2015). In Ontario, there have been reports of *F. avenaceum*, *F. oxysporum*, *F. solani*, *F. graminearum*, *F. tricintum*, *F. sporotrichioides*, *F. equiseti* and *F. poae* on soybean (Zhang et al. 2013), and most recently, *F. proliferatum* and *F. culmorum* were identified in Alberta and Manitoba (Chang et al. 2015). The relatively high frequency of recovery of *F. solani* in the current study confirms earlier reports that this species, along with *F. oxysporum*, is one of the most common *Fusaria* on soybean roots in North America (Leslie et al. 1990., Killebrew et al. 1993). In contrast, *F. oxysporum* was not recovered as frequently as might have been expected based on these earlier reports. It is important to note, however, that only a limited number of isolates (26) was classified to species in the current study, and evaluation of additional isolates may be necessary to confirm these observations. *Fusarium proliferatum*, which is regarded largely as a corn pathogen, was also recovered from soybean roots in this study. This species also has been associated with root rot of soybeans in other studies from North America (Díaz Arias et al. 2011a., Chang et al. 2015); it is a cosmopolitan fungus, which infects numerous host plants and occurs commonly as a saprophyte (Leslie and Summerell 2006., Summerell et al. 2003). Therefore, the use of crop rotation as a strategy to manage *F. proliferatum* might be ineffective in fields where the rotation involves crops like corn that serve as alternative hosts.

Species within the genus *Fusarium* are generally recognized as causing disease on soybean via infection of the seeds and roots prior to and after emergence. At least 19 *Fusarium* species have been recovered to date from soybean roots (Bienapfl 2011., Díaz 2011, Farias and

Griffin 1989., Klag et al. 1978., Leslie et al. 1990., Nelson 1999., Nyvall 1976., Pioli et al. 2004., Zhang et al. 2010). The economic impact of some of the associated diseases, including sudden death syndrome and damping-off, is well documented, while the impact of wilts and root rots is less well understood (Díaz et al. 2011). Pre-emergence damping-off, seedling root rot and the impact of disease on growth parameters were assessed for representative isolates of the seven *Fusarium* species identified in this study. All species and isolates induced varying levels of pre-emergence damping-off and root rot under greenhouse conditions, confirming earlier reports that *Fusarium* species are important root-infecting pathogens in soybean crops (Armstrong and Armstrong 1950., Grant et al. 1981., Nelson 1999., Rizvi and Yang 1996., Rupe 1989). As expected, the infection of roots resulted in poor plant development, most likely as a result of reduced nutrient and water translocation capacity (Aiken and Smucker 1996., Atkinson 2000., Cichy and Snap 2007., Himmelbauer et al. 2004).

Fusarium acuminatum has been reported to be highly aggressive on soybean under both greenhouse and field conditions (Díaz et al. 2011). The results of this study show that it consists of both aggressive and less aggressive strains. While some isolates greatly reduced seedling emergence (40%) and caused severe root rot symptoms (3.1 on a scale of 0-4), others were only mildly pathogenic, with soybean emergence rates as high as 95% and mild symptoms of root rot (0.96). These results indicate significant variation in the aggressiveness of different isolates or strains of *F. acuminatum*.

Complexes of *F. oxysporum* and *F. solani* have been strongly associated with root rot in soybean (Farias and Griffin 1989., Farias and Griffin 1990., Ferrant and Carroll 1981., French 1963., Grant et al. 1981., Kikic and Griffin 1998., Killebrew 1993., Nelson 1999., Nyvall 1976), although there is controversy with respect to their relative importance in disease development. In

one study, *F. solani* was described as nonpathogenic on soybeans (French and Kennedy 1963), while another study found that this fungus caused root rot and reduced seedling emergence and yields under both greenhouse and field conditions (Killebrew et al. 1988). In the current study, *F. solani* and *F. oxysporum* caused very mild to moderate disease and had relatively small impact on seedling emergence rates. Significant intraspecific variation in some *Fusarium* species has been associated with the occurrence of various strains within the *F. oxysporum* and *F. solani* complex (Farias and Griffin 1989., French and Kennedy 1963., Leslie and Summerell 2006., Nelson 1999). The mild symptoms of root rot induced by *F. oxysporum* in this study are consistent with previous reports that some strains of *F. oxysporum* have saprophytic tendencies on soybean (Nyvall 1976), and in many cases induce no disease symptoms (Farias and Griffin 1990). Similarly, while some studies have identified isolates of *F. avenaceum* as being highly aggressive on soybean (Zhang et al. 2010), in this study some isolates were only weakly pathogenic, suggesting intraspecific variation in the aggressiveness of this species.

As noted above, *F. proliferatum* traditionally has been associated with seedling, stalk, and ear rots of corn (Munkvold 2003., Munkvold and O'Mara 2002), but was reported recently as an important soybean root rot-inducing pathogen in Iowa (Díaz et al. 2011a). In this study, *F. proliferatum* was very aggressive on inoculated soybeans (50-70% crop emergence and 2.3-3.1 root rot disease severity). The aggressiveness of *F. proliferatum* isolates from Alberta is consistent with the high aggressiveness of isolates collected from Iowa (Díaz et al. 2011b). In contrast, *F. redolens* caused relatively mild disease on soybeans grown in the inoculated potting mix, with crop emergence rates ranging between 82-95% and symptom severity between 0.6-1.5. In the experiments in which *F. redolens* was first identified as a root rot inducing pathogen on

soybeans, the fungus induced significant disease through the development of root necrotic lesions (Bienapfl et al. 2010).

This study provided insights into the spectrum of *Fusarium* species infecting soybean roots in southern Alberta, as well as their aggressiveness. Nonetheless, there is need for further studies that involve isolation of Fusarium directly from soil samples to determine the inoculum density of each species in the soil under typical field conditions. Since *Fusarium* species occur together with other root rot pathogens that include, among others, *Rhizoctonia solani* and *Pythium* species, it would also be worthwhile to investigate how soybean root rot develops after inoculation with a complex of pathogens.

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Table 4.1 Effects of *Fusarium* species on emergence, root rot and growth of the soybean genotype TH29002RR under greenhouse conditions.

Species	Isolate	Emergence (%)	Root rot severity (0-4)	Height (cm)	Root wt. (g)	Shoot wt. (g)
Non-inoculated control						
	n/a	100^{a}	0.0^{j}	48.3^{ab}	0.48^{a}	1.43^{a}
F. avenaceum						
	FPT004	57^{ghi}	2.5^{ab}	30.4^{fg}	0.30^{bcd}	0.81^{c-g}
	FPT026	80^{b-f}	1.5^{c-f}	39.9^{b-f}	0.31^{bcd}	0.98^{bcd}
	FPT027	89^{abc}	0.8^{f-i}	46.4^{abc}	0.38^{ab}	1.00^{bcd}
	FPT030	64^{fgh}	2.2^{bcd}	32.6^{efg}	0.16^{e}	0.54^{g}
	FPT032	77^{c-f}	2.0^{b-e}	36.4^{c-f}	0.33^{bc}	0.94^{b-e}
	FPT073	68^{efg}	2.2^{bc}	35.3^{d-g}	0.19^{de}	0.61^{efg}
F. acuminatum						
	FPT013	93^{abc}	1.1^{f-i}	45.1^{a-d}	0.30^{bcd}	0.85^{c-g}
	FPT016	91^{abc}	1.0^{f-i}	43.6^{a-d}	0.36^{ab}	0.95^{b-e}
	FPT025	86^{a-e}	1.3^{e-i}	43.3^{a-e}	0.34^{bc}	0.90^{b-f}
	FPT044	68^{efg}	2.1^{bcd}	36.3^{c-f}	0.29^{b-e}	0.74^{d-g}
	FPT062	41^{i}	3.1^{a}	19.9^{h}	0.20^{cde}	0.59^{fg}

F. culmorum						
	FPT001	80^{b-f}	1.6^{c-f}	41.1^{b-e}	0.31^{bcd}	1.10^{bc}
	FPT058	86^{a-e}	1.3^{e-i}	41.9^{b-e}	0.34^{bc}	0.96^{bcd}
F. solani						
	FPT035	88^{a-d}	1.0^{f-i}	45.0^{a-d}	0.39^{ab}	1.04^{bcd}
	FPT052	93^{abc}	1.3^{e-i}	48.4^{ab}	0.39^{ab}	1.03^{bcd}
	FPT066	93^{abc}	1.4^{d-h}	45.4^{a-d}	0.30^{bcd}	0.84^{c-g}
	FPT070	88^{a-d}	1.1^{f-i}	43.3^{a-e}	0.31^{bcd}	0.99^{bcd}
	FPT089	98^{ab}	0.7^{g-j}	52.9^{a}	0.43^{ab}	1.20^{ab}
F. proliferatum						
	FPT039	70^{d-g}	2.3^{b}	32.9^{efg}	0.21^{cde}	0.74^{d-g}
	FPT072	48^{hi}	3.1^{a}	25.6^{gh}	0.31^{bcd}	0.74^{d-g}
F. redolens						
	FPT037	96^{ab}	0.6^{ij}	49.0^{ab}	0.38^{ab}	1.11^{bc}
	FPT055	82^{a-f}	1.5^{d-g}	40.3^{b-f}	0.40^{ab}	1.05^{bcd}
	FPT076	96^{ab}	0.8^{f-i}	50.3^{ab}	0.38^{ab}	1.05^{bcd}
	FPT080	93^{abc}	0.7^{g-j}	44.5^{a-d}	0.43^{ab}	1.08^{bcd}
F. oxysporum						

	FPT006	91[abc]	0.7[g-j]	41.8[b-e]	0.30[bcd]	0.85[c-g]
	FPT095	88[abc]	1.6[c-f]	44.5[a-d]	0.41[ab]	0.86[b-g]

Data are the means of four replicates, pooled over two experiments. Data within a column followed by the same letter are not significantly different according to Duncan's New Multiple Range Test ($P \leq 0.05$).

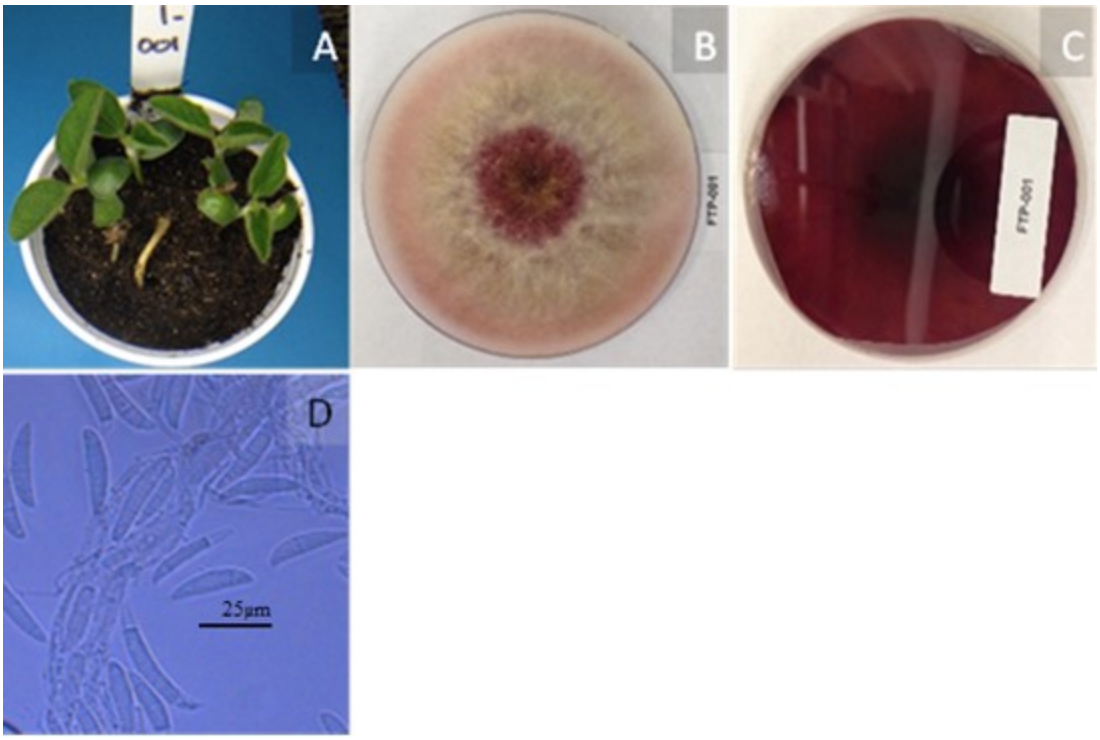

Figure 4.1 Aggressiveness and morphological characteristics of *Fusarium culmorum*. Soybean plant 14 days after planting in soil inoculated with isolate FPT001 (A). Dark brown central spore mass with a diameter of 20 mm and olive brown raised mycelium (B) and deep red pigmentation on PDA (C). Macroconidia (30µm) on CLA short and stout, occurring in abundance in the aerial parts of the mycelium (some formed on monophialides on hyphae) with blunt apical cells and poorly developed foot cells. The macroconidia had 3-4 septa (D).

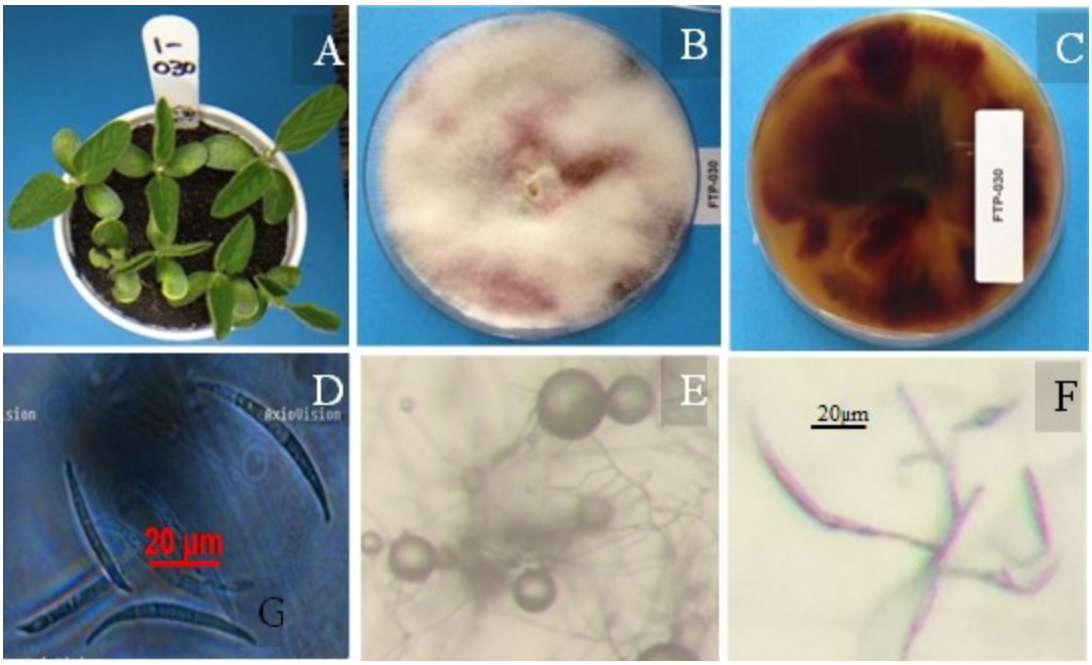

Figure 4.2 Aggressiveness and morphological characteristics of *Fusarium avenaceum*. Soybean plants 14 days after planting in soil inoculated with isolate 1-030 (A). White aerial mycelium on PDA (B), patchy dark brown pigmentation (C), macroconidia (50µm) on CLA (D), abundant bright orange sporodochia (E) and macroconidia (50µm) on CLA, slender and slightly curved with thin walls with distinct foot cells and borne on mono- and polyphialides (F).

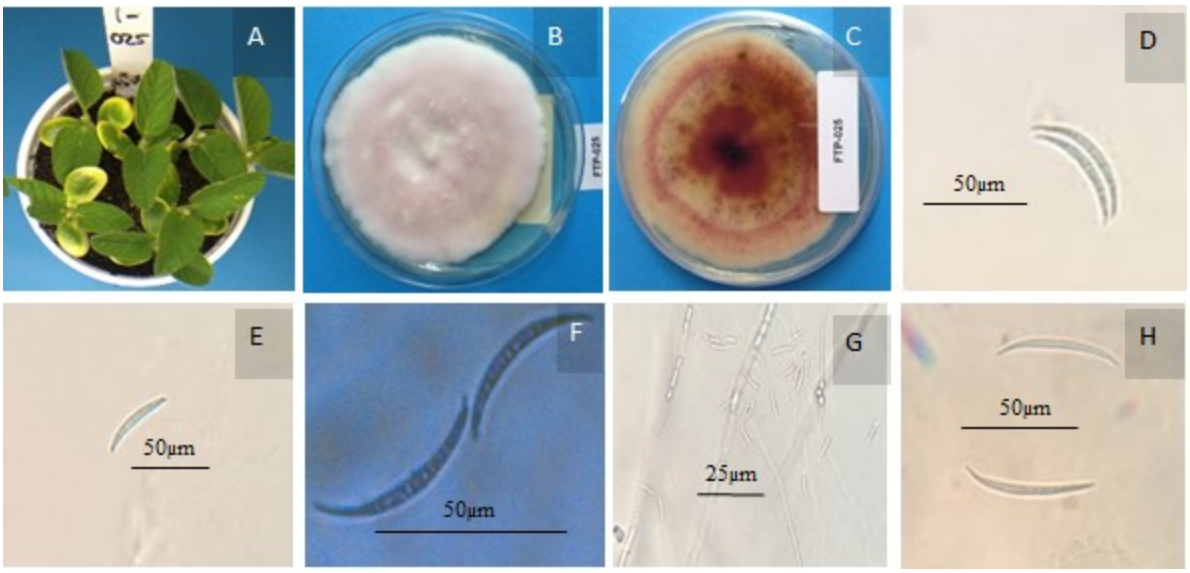

Figure 4.3 Aggressiveness and morphological characteristics of *Fusarium acuminatum*. Soybean plants 14 days after seeding grown on soil inoculated with isolate 1-025 (A). Slow growing abundant white mycelium (B) and a light reddish brown pigmentation with dark brown spots on PDA (C). Macroconidia on CLA slender with a distinct curvature and 3 septa, distinct foot- shaped basal cells and snout-shaped apical cells (D, E, F, H). Macroconidia with short intercalary cell.

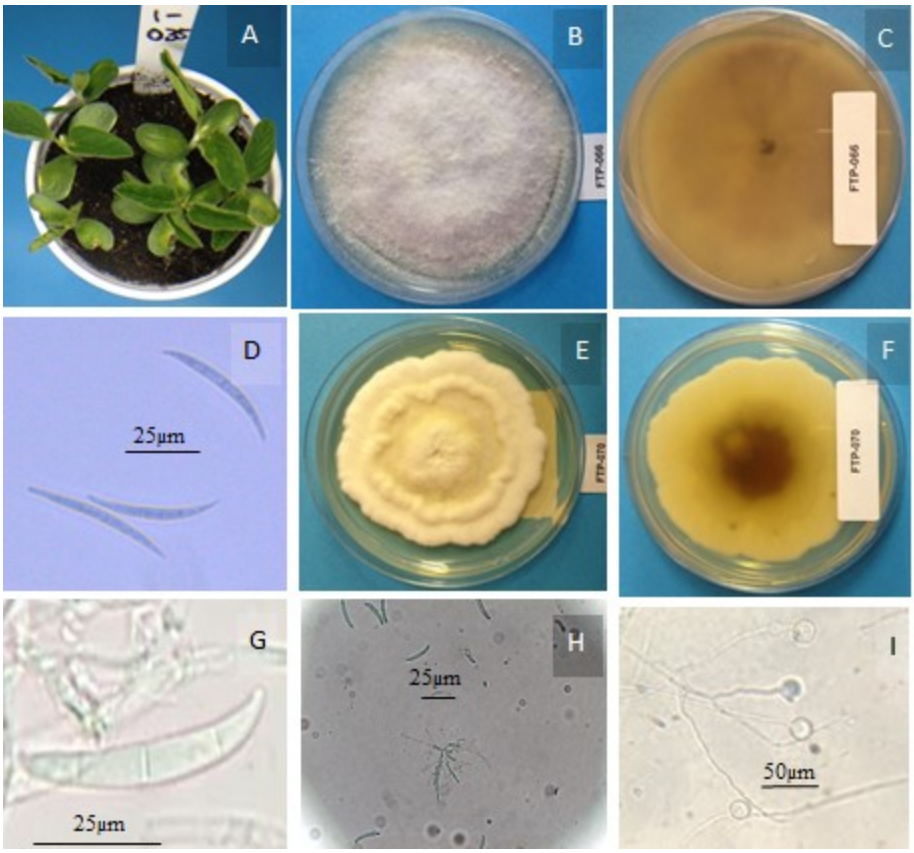

Figure 4.4 Aggressiveness and morphological characteristics of *Fusarium solani*.

Soybean plants (14 days after seeding) growing in soil inoculated with isolate 1-035 (A). Slightly curved to almost straight macroconidia (30µm) with 3-5 septa (D, G). White dense mycelium with a central spore mass and dark brown pigmentation (C) becoming lighter and creamy at the periphery and creamy slow growing mycelium with distinct growth rings and a light brown pigmentation that is darker at the center PDA (B, E). Microconidia formed abundantly on false heads and long monophialides (H). Smooth oval chlamydospores intercalary in the hyphae (I) on PDA.

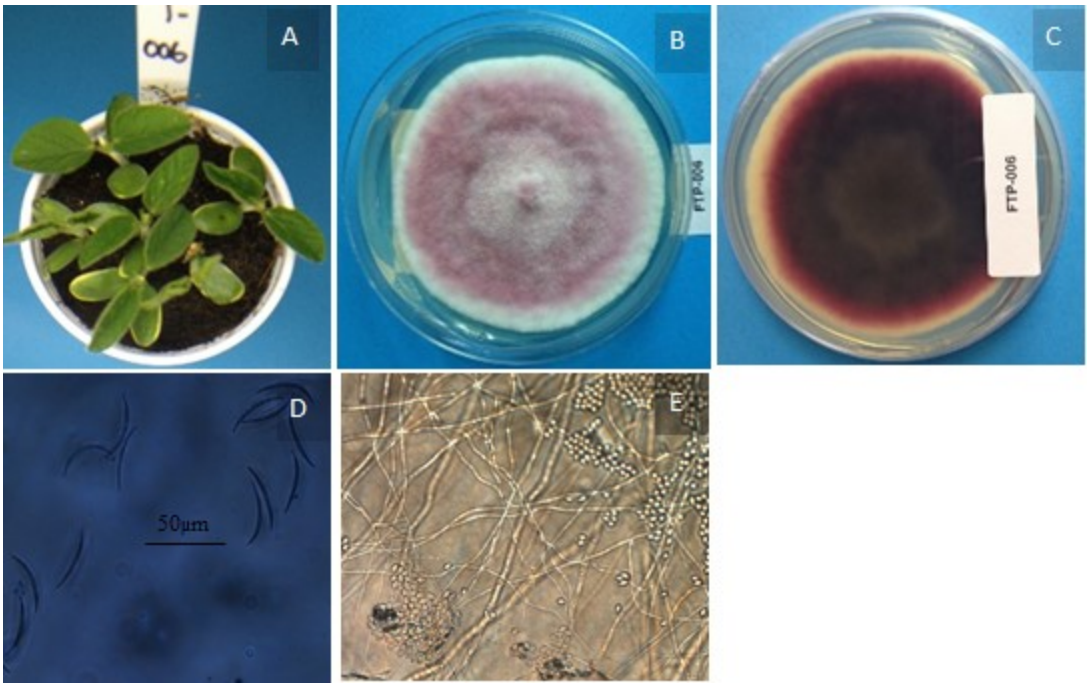

Figure 4.5 Aggressiveness and morphological characteristics of *Fusarium oxysporum*. Soybean plant (14 days after seeding) growing in soil inoculated with isolate 1-006 (A). Rose burgundy dense mycelium with a central spore mass (B) and a burgundy-greyish pigmentation that is creamy at the periphery on PDA (C). Numerous bright orange sporodochia on the PDA. Macroconidia on CLA slender with thin walls (D). Abundant oval microconidia (E).

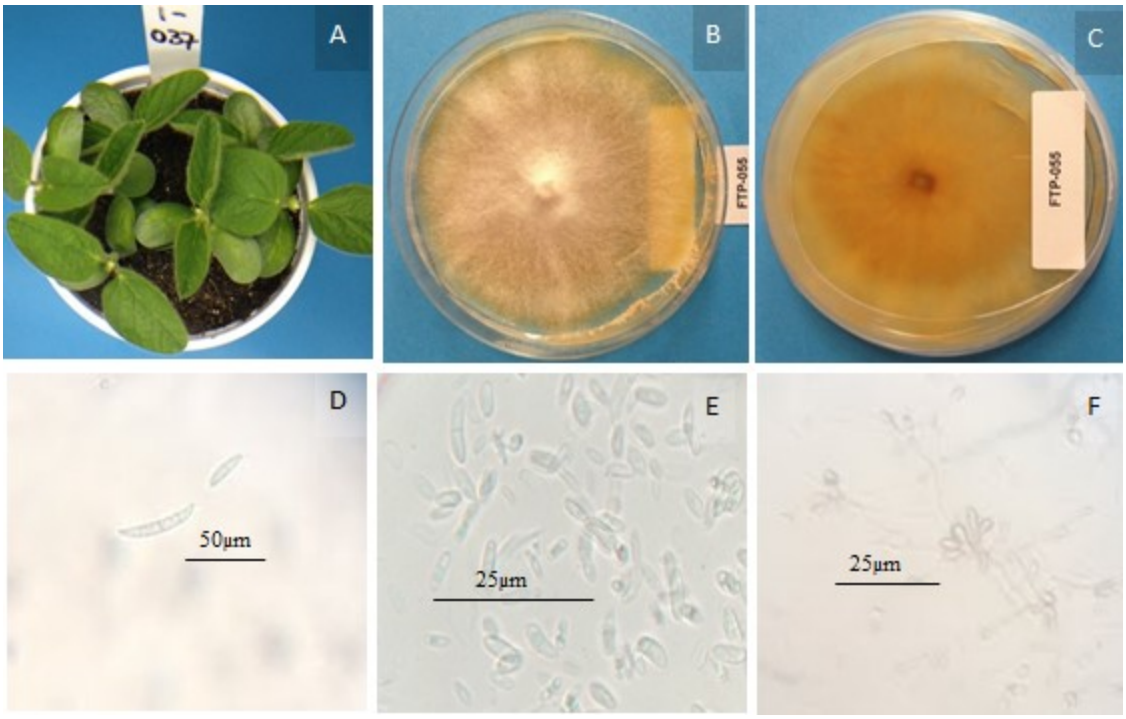

Figure 4.6 Aggressiveness and morphological characteristics of *Fusarium redolens.* Soybean plant (14 days after seeding) growing in soil inoculated with isolate 1-087(A). White mycelium, patchy dark brown rhizomatous pigmentation and abundant bright orange sporodochia on PDA (B, C). Macroconidia (50µm) long, slender and slightly curved with thin walls and distinct foot cells (D), borne on both mono- and polyphialides. Microconidia predominantly oval and single celled, but 2-celled oval microconidia also formed (E). Microconidia on false heads and abundant in the aerial mycelium (F).

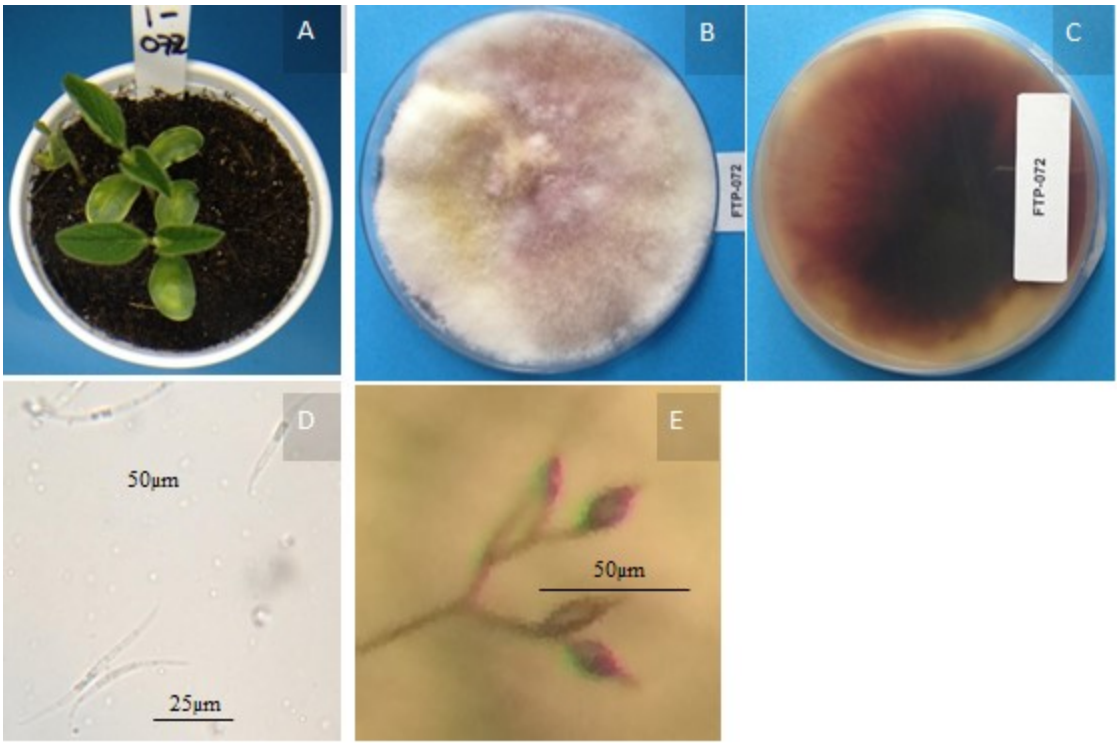

Figure 4.7 Aggressiveness and morphological characteristics of *Fusarium proliferatum.* Soybean plant (14 days after seeding) growing in soil inoculated with isolate 1-072 (A). White mycelium and light brown pigmentation on PDA (B, C). Macroconidia long, slender and slightly curved with thin walls (D). Microconidia borne on mono- and polyphialides (E).

Chapter 5: Management of Root Rot of Soybean in Alberta with Fungicide Seed Treatments and Genetic Resistance

5.1 Introduction

Soybean production is increasing in Alberta, Canada, with approximately 4,900 ha seeded to the crop in 2014 (Gabruch and Gietz 2014), and about 5,300 ha grown in 2015 (K.F. Chang, *personal communication*), mainly in the south of the province. While the production of soybean is still limited relative to other crops in Alberta, this crop could become an attractive choice for farmers given recent developments in breeding that have resulted in early maturing varieties, and also the ability of soybean to fix most of its nitrogen requirements while leaving significant amounts of the nutrient for subsequent crops. Unfortunately, the production of soybean faces a number of constraints including the occurrence of root rot disease, which has been found to be prevalent in most fields in Alberta (Chang et al. 2013., Nyandoro et al. 2014., Nyandoro et al. 2015). Fusaria were the most common fungi isolated from soybean roots collected in provincial surveys conducted in 2012 and 2013 (Chapter 4).

Root rot in pulse crops causes crop stand thinning, which in turn may lead to a reduction in yields. The disease is favored by environments characterized by elevated soil moisture and depressed soil temperatures during the crop establishment phase (Roy et al. 1989., Scherm and Yang 1996). Surveys focusing specifically on root rot in soybean crops have identified a high incidence and severity of the disease in southern Alberta (Chang et al. 2013., Nyandoro et al. 2014., Nyandoro et al, 2015). The main causal agents of root rot of soybean in Alberta appear to be species of *Fusarium* (Chapter 4), including *F. proliferatum, F. avenaceum* and *F. acuminatum*. Infection by *Fusarium* species reduces root mass in infected soybean plants at all

stages of development (Rupe et al. 1993., Roy et al. 1997., Navi and Yang 2008). A reduction in root mass results in reduced water and nutrient uptake by the plant, and affected crops have reduced yields. It is critical, therefore, to find ways to mitigate the impact of root rot of soybean to ensure the viability of the crop in Alberta.

Earlier experiments have shown that infection of the plant can take place within 2 weeks of planting (Gao et al. 2006). This early infection favors rapid colonization of the xylem tissue, which is a necessity for successful disease development (Huang and Hartman. 1998). As such, it is critical to protect the seed and seedling from infection during crop establishment, during which plants are most prone to infections that will have a negative impact on later plant growth and development. While no single strategy has been shown to control root rot completely, moderate soil moisture, planting into warm soils, tillage management and delaying the seeding date (Von Qualen et al. 1989., Wrather et al. 1995., Vick et al. 2003), along with good general crop management practices, can be effective in significantly reducing root infection. In addition, the disease also may be managed by planting soybean cultivars that are partially resistant to root rot, since partial genetic resistance has been reported in some soybean genotypes (Hnetkovsky et al. 1996., Hartman et al. 1997., Njiti et al. 1997., Iqbal et al. 2002., Mueller et al. 2002). Azoxystrobin and fludioxonil have been reported to have significant activity against *Fusarium* species (Bradley 2008).

The objectives of this study were to: (1) evaluate a suite of 12 soybean genotypes for resistance or tolerance to root rot, and (2) compare the efficacy of fungicide seed treatments for control of this disease. The ultimate goal was to identify effective strategies for the management of soybean root rot in Alberta.

5.2 Materials and methods

5.2.1 Plant materials

Twelve soybean genotypes were compared for resistance or tolerance to root rot in greenhouse and field experiments. Glyphosate-tolerant genotypes were selected to reduce the amount of labor required for weed control in the field experiments. These included the genotypes 90M01, 900Y61, 900Y71 and 900Y81(Pioneer Hi-Bred Ltd, Chatham, Ontario, Canada) as well as TH27005RR, TH29002RR, TH32004R2Y, LS003R, LS005RR, NSC Portage, OAC Prudence and Tundra (Fabian Seed Farms Inc., Tilley, Alberta, Canada). The genotype TH29002RR was used in the fungicide seed treatment experiments.

5.2.2 Fungicide seed treatments

Various fungicides were used alone or as mixtures to treat soybean seeds before planting in both field and greenhouse conditions. The fungicides assessed included: Vibrance (sedaxane, 500 g/L, Syngenta, Calgary, AB) applied at 50 mL/100 kg seed + Apron XL (metalaxyl, 33.3%, Syngenta) applied at 100 mL/100 kg seed; Apron Maxx RTA (0.73% fludioxonil + 1.10% metalaxyl-M and S-isomers, Syngenta) applied at 325 mL/100 kg seed; Vitaflo 280 (15.59% carbathiin + 13.25% thiram, Chemtura, Elmira ON) applied at 260 mL/100 kg seed; Rancona Summit (0.902% ipconazole + 1.443% metalaxyl, Arysta Life Scinces, Cary NC) applied at 260 mL/100 kg seed + Apron XL (100 mL/100 kg seed); Trilex EverGol (13.3% protioconazole + 13.3% penflufen + 28.35% metalaxyl, Bayer, Mississauga, ON) applied at 300 mL/100 kg seed; EverGol Energy (7.18 % protioconazole + 3.59 % penflufen + 5.74 % metalaxyl, Bayer) applied at 300 mL/100 kg seed; Rancona Summit + Maxim 480 (40.3% fudioxonil, Syngenta) applied at 260 mL/100 kg seed; Rancona Summit + Maxim at 480 mL/100kg seed + Vibrance applied at

260 mL/100 kg seed; and Vitaflo 280 + Apron XL applied at 260mL/100kg seed. The fungicides were mixed with water to the consistency of slurry prior to use. The soybean seeds (2 kg for each fungicide preparation) were placed in a plastic bottle, and the fungicide slurry was distributed evenly inside the bottle, above the seeds. The plastic bottle was then gently rotated to achieve an even fungicide coating on the seeds.

5.2.3 Inoculum preparation

Fusarium avenaceum was selected as a representative pathogen for all experiments, since it is among the fungi most commonly associated with root rot of soybean in Alberta (Chapter 4). A virulent isolate of *F. avenaceum* grown on wheat grain was used as the inoculum. This isolate had been originally recovered from soybean roots displaying severe symptoms of root rot and was highly virulent on soybean (K.F. Chang, Alberta Agriculture and Forestry, *unpublished data*). The fungus was cultured at room temperature (RT) on potato dextrose agar (PDA) medium in 100 mm-diameter Petri dishes. The wheat grain was soaked in water overnight, drained, placed in 1 L bags and autoclaved at 120° C for 90 min. The *F. avenaceum* cultures produced on the Petri dishes were cut into ~2-mm^2 pieces with a sterilized scalpel and the contents of each dish were mixed with 1 kg of the wheat grain. The inoculated grain was incubated for 21 days at RT to allow colonization by the pathogen. The grain was then dried at 25°C for 4 days, milled through a 2-mm gauge sieve and stored at 5° C.

5.2.4 Evaluation of fungicide treatments: field experiments

The efficacy of the various fungicide treatments was evaluated in field trials located at the Crop Diversification Centre South (CDCS), Alberta Agriculture and Forestry, Brooks, AB whose soils are generally brown chermozomic clay-loams. The experiments were replicated at two sites (Lendrum and McLeod with no documented history of root rot) in 2012 and 2013. The

Lendrum site (SE 27-18-14 W4 land location) has loam soil (33% sand-25% clay-42% silt), while McLeod site (SW 23-18-14-W4 land location) has clay loam soil (35% sand-28% clay-37% silt). The soil was prepared by disc harrowing; with the granular herbicide Edge (ethalfluralin) incorporated at 17 kg/ha for pre-planting weed control. In crop weed control was achieved by spraying 2.6 L/ha of Round Up (glyphosate) HC at the 4th trifoliate leaf stage. Treatments were arranged in a randomized complete block design with four replications. Each plot consisted of four 6 m long rows spaced 25 cm apart with 60 cm bare space between plots. A total of 75 soybean seeds were planted per row with a one-row V-belt push planter (AMALCO, Allan Machine Company, Iowa, U.S.A.). The seeds were mixed with 30 mL of *F. avenaceum* inoculum per 75 seeds (prepared as described above) prior to sowing. The seeds were inoculated with granular *Bradyrhizobium japonicum* (Novozymes BioAg, Saskatoon, Canada) inoculum at 5g/row and pathogen inoculum (on wheat grain) at 30 mL/row at planting by adding them along on the planter's V- belt at planting. Fungicide-untreated uninoculated seed and fungicide-free *F. avenaceum*-inoculated seeds were planted as the untreated and treated controls, respectively. The plots were seeded on May 21, 2012, and on May 31, 2013, and harvested October 05, 2012, and October 10, 2013. Seedling emergence was assessed by counting the seedlings in each plot 4 weeks after planting. The development of root rot was evaluated at the early pod development R3-stage (Purcell et al. 2013) of plant growth. Briefly, the roots of 12 randomly selected plants were dug out from the two middle rows of each plot. The roots were taken back to the laboratory, where they were washed under running water and rated visually for root rot severity on a 0-4 scale (Chang et al. 2007), where: 0 = normal root color, 1 = 1-25% root discoloration, 2 = 26-50% root discoloration, 3 = 51-75% root discoloration, and 4 = 76-100% root discoloration. Nodulation rates were also scored on a scale of 0-4, where: 0 = no nodules, 1 =1-5 nodules, 2 =

6-10 nodules, 3 = 11-15 nodules, and 4 = >15 nodules on each individual root system. The plots were harvested at maturity with a small plot combine and the grain was dried at 30°C for 48 h and then weighed.

5.2.5 Evaluation of fungicide treatments: greenhouse experiments

The efficacy of the seed treatments also was assessed under greenhouse conditions. Soybean seeds were planted in 500 mL cups filled with potting mix (Sun Gro Horticulture Canada Ltd., Seba Beach, AB), with the potting mix compacted with the flat base of a glass jar to create a smooth surface. Each cup was inoculated by evenly spreading a 5 mL aliquot of *F. avenaceum* inoculum on the surface of the potting mix. The seeds were then evenly placed directly on the inoculum at a density of 7 seeds per cup and covered with 100 mL of potting mix. Treatments were replicated 5 times, with each cup serving as a replicate. The treatments were arranged in a completely randomized design and placed in a greenhouse that was maintained at approximately 25°C with a 12-h photoperiod (natural light supplemented with artificial lighting). No fertilizer was added. The cups were watered and maintained at field capacity for 14 days. On day 14, seedling emergence was assessed by counting the number of seedlings in each cup (experimental unit) and seedling shoot length measured. After 14 days from the date of planting, the seedlings were gently dug out from the potting mix, and the roots were washed under running water and visually rated for root rot as described above. After the disease assessment, the seedlings were cut to separate the roots from the shoots and dried at 35°C for 36 h. Dry root and shoot mass were weighed separately for each plant.

5.2.6 Soybean root rot resistance study

Experiments were conducted under both field and greenhouse conditions to assess the impact of *F. avenaceum* on the soybean genotypes listed in Section 5.2.1. The experiments were

arranged in a split-plot design replicated 4 times with inoculation as the main plots and soybean genotypes as the fixed effects. The field experiments were established at the Lendrum and McLeod field sites in 2012 and 2013. The seeding and harvest dates in each year were as given above for the fungicide field experiment; the size of the individual plots and planting procedures also were as above, except that the number of seeds per row varied for individual genotypes based on the results of a preliminary germination test. The target was 75 plants per row. Data (crop emergence, root rot severity, nodulation and grain yield) were collected in the same manner as in the fungicide field experiment. In addition to the field evaluation, the reaction of the soybean genotypes to *F. avenaceum* also was examined under greenhouse conditions. Inoculation of the potting mix, planting and greenhouse conditions were as described above for the fungicide greenhouse experiment, except that a split-plot design was used with inoculum level as the main plots and genotypes as the fixed variables. In the greenhouse experiments, data on seedling emergence and height were collected while the plants were still growing in the potting mix, while the severity of seedling root rot, root dry mass and shoot dry mass were evaluated after the plants had been dug out from the potting mix.

5.2.7 Data analysis

Data were analyzed by year and site using the Proc Mixed in SAS v. 9.3 (SAS Institute, Inc. 2015). The percent yield loss was assessed for each treatment by comparison of the mean yield of the non-inoculated plots in the treatment with the mean yield of the inoculated plots. Analyses of variance were performed using the General Linear Model Procedure of SAS, with Duncan's New Multiple Range Test used for all means comparisons. Differences among means were considered significant at $P \leq 0.05$ unless specified otherwise.

5.3 Results

5.3.1 Seedling emergence

There was significant variation in soybean emergence in response to fungicide seed treatment ($P \leq 0.05$) relative to the uninoculated and inoculated controls under both field and greenhouse conditions. All fungicides had significantly lower seedling emergence compared with untreated, uninoculated soybeans under greenhouse conditions (Table 5.1). The Rancona Summit + Apron Maxx RTA, Rancona Summit, Apron Maxx RTA, Rancona Summit + Maxim + Vibrance, Vitaflo 280 and EverGol Energy treatments resulted in significantly greater seedling emergence than the Vibrance + Apron XL treatment or the inoculated control. Under field conditions, all treatments with the exception of Vibrance + Apron XL resulted in greater emergence compared with the inoculated control.

In the root rot resistance study, the emergence of all genotypes under field conditions was lower in the inoculated treatments vs. the uninoculated control (Table 5.2). The soybean genotype 90M01 had significantly greater emergence than all other genotypes except 900Y61 and 900Y81. The genotype 900Y71 had significantly lower emergence compared with all other genotypes, and LS003RR had lower emergence compared with 90M01, 900Y61, 900Y81 and TH27005RR. Under greenhouse conditions, the genotype 900Y81 had greater emergence compared with all other genotypes except Tundra and OAC Prudence. The genotype 900Y71 had lower emergence than all other genotypes except NSC Portland and LS005RR.

5.3.2 Root rot severity

All of the fungicide treatments tested in the field experiment significantly ($P \leq 0.001$) reduced soybean root rot severity in the *F. avenaceum*-inoculated plots, relative to the inoculated control (Table 5.1). The uninoculated control developed mild root rot while the inoculated control developed severe root rot. Root rot severity was lower when seed was treated with Rancona Summit + Maxim + Vibrance (0.7 disease severity) compared with Vitaflo 280 or with Vibrance + Apron XL. Under greenhouse conditions, all treatments except Vibrance + Apron XL reduced root rot severity compared with the inoculated control. Root rot severity was lowest on soybeans grown from uninoculated seeds, seeds treated with Rancona Summit + Maxim + Vibrance, Rancona Summit + Maxim or EverGol Energy. Plants grown from Vibrance + Apron XL treated seeds developed a greater disease severity than those treated with all of the other fungicides, but slightly less disease than the inoculated control.

The various soybean genotypes exhibited varying levels of resistance to Fusarium root rot under both field and greenhouse conditions (Table 5.2). Severe root rot resulted in lower nodulation on inoculated soybeans (Figure 5.1). The soybean genotype LS003RR developed the lowest disease severity under field conditions, with an average disease severity rating of 1.2. Disease severity in the genotypes LS003RR, 900Y61, NSC Portland, TH29002RR and 90M01 was significantly lower than on TH32004R2Y. The genotype LS003RR also had significantly lower disease severity relative to TH27005RR and 900Y71. In the greenhouse, the genotypes 900Y81, OAC Prudence and Tundra developed the lowest disease severity relative to all other genotypes except 900Y61 and TH2500RR. Disease severity was higher in TH29002RR than on 900Y61, 900Y81, OAC Prudence and Tundra.

5.3.3 Root nodulation

The degree of root nodulation on fungicide-treated soybeans differed significantly from root nodulation on uninoculated soybeans (Table 5.1), as well as between genotypes (Table 5.2) in the field experiments. Root nodulation was only assessed in the field experiments since greenhouse experiments were maintained for only 14 days after planting which is not long enough for nodule development. Seeds that were not treated with fungicide but inoculated with the pathogen had the lowest nodule development in the field experiments. Treatment of seeds with Rancona Summit + Maxim + Vibrance, Rancona Summit + Maxim and EverGol Energy resulted in higher nodule development compared with the inoculated control (Table 5.1).

The various soybean genotypes grown in the *F. avenaeceum*-inoculated field plots showed significant differences in nodule development. The soybean genotype NSC Portland had the highest nodulation rating (1.35) among all genotypes except 90M01 (Table 5.2). TH32004R2Y had the lowest nodulation rating among all of the genotypes tested except TH27005RR, 900Y71 and LS005RR.

5.3.4 Root and shoot dry mass

In the greenhouse, root mass was greater in most of the fungicide treatments relative to the untreated control (this parameter was not measured in the field experiments). The exceptions were the Vibrance + Apron XL and Trilex EverGol treatments (Table 5.1). Soybeans grown from seeds treated with Rancona Summit + Apron Maxx RTA + Vibrance had greater root mass than plants grown from seeds treated with Vibrance + Apron XL, Trilex EverGol or Apron Maxx. All treatments except Vibrance + Apron XL produced a greater seedling shoot dry mass than the untreated control. The Rancona Summit + Maxim + Vibrance and Vitaflo 280 treatments

resulted in a greater shoot dry mass than the Trilex EverGol and Vibrance + Apron XL treatments.

In the comparison of soybean genotypes in the greenhouse, LS003RR had a greater root dry mass (0.19g/plant) than all other genotypes except 90M01, LS005RR and TH29002RR (Table 5.2). Root dry mass was lower in OAC Prudence and Tundra than in LS003RR, 90M01, LS005RR and TH29002RR. Shoot dry mass was greater in TH29002RR and 900Y61 than in 900Y81 or LS005RR.

5.3.5 Yield

All of the fungicide seed treatments in the field experiments had significantly lower seed yield gains than the uninoculated control, but had significantly greater yields than the inoculated control (Table 5.1). Seed treatment with Rancona Summit and Apron Maxx resulted in the highest yield gains among the fungicide seed treatments.

The yield losses varied significantly among the various soybean genotypes inoculated with *F. avenaceum* under field conditions (Table 5.2). At the Lendrum site, yield losses on 900Y81 were lower compared with losses on 90M01 and LS003RR. At the McLeod site, yield losses were greater on 900Y81 relative to all other genotypes except TH27005RR, TH29002RR and 900Y61.

5.4 Discussion

Fungicide seed treatments and the deployment of partially resistant soybean cultivars were evaluated as Fusarium root rot management strategies under greenhouse and field conditions. Treatment with Rancona Summit + Maxim, Rancona Summit, Apron Maxx RTA and EverGol provided the best protection against *F. avenaceum* under field conditions, while

Rancona Summit and Vitaflo 208 offered the best protection under greenhouse conditions. All of these products except Vitaflo 280 have metalaxyl as one of the active ingredients. Metalaxyl is a systemic fungicide with specific activity against Oomycetes (Hewitt 1998., Uesugi 1998), more so under conservation rather than conventional tillage (Guy and Oplinger 1989). Metalaxyl protects the seedling during the early stages of the infection process, including spore germination, tissue penetration and tissue colonization. Although formulated for the control of Oomycetes, metalaxyl has shown great control of Fusarium when used as a protectant against tuber rots in potatoes, probably through the stimulation of host defense mechanisms (Barak et al. 1984). Treatment with metalaxyl also has been shown to increase production of phytoalexins by the soybean tissue, with these plant defense compounds helping to boost disease resistance (Lazarovits and Ward 1982., Ward et al. 1980). Early infection is necessary for severe root rot development, since the young seedlings are most prone to effective colonization by the pathogen(s), which is necessary for successful disease development (Huang and Hartman 1998).

Plants that grew from seeds treated with Apron Maxx RTA produced high grain yields relative to the inoculated control treatment, despite moderate crop emergence and nodulation (Table 5.1). Apron Maxx RTA contains both fludioxonil and metalaxyl as active ingredients. Fludioxonil is a phenylpyrrole contact fungicide that has been reported to be effective against *Fusarium* spp. and *Rhizoctonia solani* in other experiments (Broders et al. 2007, Hewitt 1998, Kiewnick et al. 2001, Meyer et al. 2006, Uesugi 1998, Ernst et al. 2003). Seed treatment with fludioxonil resulted in reduced root lesion development compared with other seed treatments and untreated seeds, while those treated with azoxystrobin had higher disease severity (Ellis et al. 2011). Fludioxonil is an effective fungicide that slows down mycelial growth (Broders et al. 2007). However, *F. graminearum* mutants insensitive to fludioxonil readily developed in a

laboratory assay (Broders et al. 2007), which indicates a potential for future loss of the effectiveness of this fungicide in the absence of proper fungicide stewardship.

EverGol Energy and Trilex EverGol provided moderate levels of root rot control under both field and greenhouse conditions, with EverGol Energy seed treatment resulting in slightly higher seedling emergence. Both products combine three active ingredients: penflufen and metalaxyl plus prothioconazole (in EverGol Energy) or trifloxystrobin (in Trilex EverGol). Prothioconazole has been previously reported to have some activity against *Fusarium* spp. (Paul et al. 2008), and perhaps its inclusion in EverGol Energy resulted in the higher seedling emergence obtained with this treatment. Seed treatment with Rancona Summit + Maxim + Vibrance resulted in the lowest levels of root rot (Table 5.1) under both field and greenhouse conditions. This product combines four active ingredients: ipconazole, metalaxyl, fludioxonil, and sedaxane. Fludioxonil has been shown to be effective against important seedling pathogens such as *F. graminearum* on maize (Munkvold and O'Mara 2002). Vibrance (sedaxane) has a broad spectrum activity against soil borne fungi, including *R. solani*, *R. cerealis* and *Typhula incarnate*, and seed borne fungi, including *Ustilago nuda*, *Tilletia caries*, *Monographella nivalis* and *Pyrenophora graminea* (Zeun et al. 2013). However, it is not registered for the control of *Fusarium*, and Vibrance did not significantly control root rot and seedling emergence under either open field or greenhouse conditions. Nonetheless, the inclusion of Vibrance in a soybean seed fungicide treatment with Rancona Summit + Maxim did enhance root rot control. This suggests that the use of a broad spectrum of fungicides could reduce the contribution of other soilborne fungi to root rot development, which often results from infection by a complex of pathogens (Zeun et al. 2013).

The effectiveness of seed treatments on yields is influenced by environmental conditions, with significant benefits observed in crops planted in soil that was below 15°C and which received less than 111 mm rain in the three-week period from one week pre-planting to two weeks after planting (Bradley 2008). Optimum conditions for the germination of *F. graminearum* macroconidia consist of >80% relative humidity at a temperature of about 20°C under darkness, based on *in vitro* studies (Beyer et al. 2004). Similarly, optimal conditions for vegetative growth of *F. graminearum* were determined to be 12 h of light alternating with 12 h of darkness at 25°C and 20°C, respectively (Leslie and Summerell 2006). The lower disease severity observed under greenhouse versus field conditions in this study therefore likely resulted from warmer greenhouse temperatures, which did not favor rapid seed infection prior to germination and seedling establishment.

Comparison of the impact of root rot on 12 soybean genotypes revealed varying levels of resistance to this disease, under both field and greenhouse conditions. The emergence of some cultivars, for example 900Y81, was low in the presence of *F. avenaceum*, and yet these cultivars did not suffer as severe yield losses as others (Table 5.2). In contrast, other cultivars had higher initial emergence rates, yet developed more severe symptoms of root rot and experienced higher yield losses. Superior emergence at the initial stages of crop development does not appear to guarantee higher yields at the end of the season. Some studies comparing the impact of *F. solani* on different soybean cultivars also revealed that while the roots of some genotypes may become infected to similar degrees, they may differ in the level of foliar disease severity later in crop development (Gray and Achenbach 1996). The results of the current study suggest that the cultivars evaluated may possess different levels of resistance/tolerance to Fusarium root rot, or at least to *F. avenaceum*. However, only one isolate was used in the screening, and while *F.*

avenaceum has been shown to be one of the most aggressive soybean root rot fungi in Canada, the results should be interpreted with caution (Zhang et al. 2010). Other studies have found variation in the severity of disease development on the same soybean cultivar following inoculation with different pathogenic *Fusarium* spp. (Zhang et al. 2010).

The results of this study show some benefits of the treatment of soybean seeds with fungicides before planting, and the planting of cultivars that exhibit at least some resistance to root rot. There is potential for the use of both fungicide seed treatments and resistant host genotypes in the management of root rot of soybeans. This observation is consistent with previous reports indicating that there is no single, foolproof control strategy for root rot (Weems et al. 2015). As such, an integrated approach that includes multiple strategies is needed to manage this disease.

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Table 5.1 Effect of fungicide seed treatments on root rot severity, seedling emergence, and plant growth parameters of the soybean genotype TH29002RR planted in soil or potting mix inoculated with *Fusarium avenaceum* under field ('Field') and greenhouse ('GH') conditions.

	Root rot (0-4)		Root nodulation	Seedling emergence (%)		Yield gain (t/ha)	Shoot length (mm) GH	Dry mass (g)	
Fungicide	Field 2013	GH (2012/13)	Field -2013	Field (2012/13)	GH (2012/13)	Field (2012/13)	(2012/13)	root GH (2012/13)	shoot GH (2012/13)
Uninoculated Control	0.63^{d}	0.11^{d}	2.63^{ab}	85.54^{a}	98.57^{a}	0.501^{a}	92^{a}	0.38^{a}	1.38^{a}
RanMaxm	0.81^{cd}	2.39^{c}	2.65^{ab}	81.58^{ab}	63.57^{bc}	0.217^{bc}	26^{b}	0.13^{bc}	0.39^{bc}
Rancona	1.00^{cd}	2.64^{bc}	2.02^{bc}	80.96^{ab}	68.57^{b}	0.232^{b}	23^{bc}	0.13^{bc}	0.39^{bc}
EvEnergy	0.88^{cd}	2.38^{c}	2.47^{ab}	80.46^{ab}	56.43^{bcd}	0.192^{bc}	24^{b}	0.11^{bcd}	0.33^{bc}
RanMaxmVib	0.63^{d}	2.36^{c}	3.39^{a}	79.79^{ab}	64.29^{bc}	0.099^{bc}	24^{b}	0.14^{b}	0.43^{b}
TriEverGol	0.75^{cd}	2.92^{b}	1.69^{bc}	79.21^{b}	44.29^{de}	0.184^{bc}	17^{cd}	0.08^{de}	0.29^{cd}
Vitaf280	1.01^{c}	2.79^{b}	1.90^{bc}	78.38^{b}	67.86^{b}	0.134^{bc}	25^{b}	0.12^{bc}	0.42^{b}
AprMax	0.99^{cd}	2.6b^{c}	1.92^{bc}	77.88^{b}	55.00^{cd}	0.2318^{b}	20^{c}	0.10^{cd}	0.32^{bc}
VitafApr	0.83^{cd}	2.81^{b}	1.70^{bc}	76.71^{b}	65.71^{bc}	0.195^{bc}	22^{bc}	0.12^{bc}	0.39^{bc}
VibraApr	1.59^{b}	3.56^{a}	1.70^{bc}	71.29^{c}	32.86^{ef}	0.143^{bc}	11^{e}	0.06^{e}	0.20^{de}
Inoculated	2.48^{a}	3.60^{a}	1.03^{c}	66.25^{c}	26.43^{f}	0.000^{c}	8^{e}	0.04^{e}	0.13^{e}

Data are the means of four replicates, pooled over two experiments. Values within a column followed by the same letter are not significantly different according to Duncan's New Multiple Range Test ($P \leq 0.05$). Seedling emergence was calculated as the percentage of seedlings emerged relative to the number of seeds planted. Root rot was evaluated on a scale of 0-4, where: 0 = normal root color, 1 = 1-25% root discoloration, 2 = 26-50% root discoloration, 3 = 51-75% root discoloration, and 4 = 76-100% root discoloration. Nodulation was evaluated in field experiments on a scale of 0-4, where: 0 = no nodules on the entire root system, 1 =1-5 nodules, 2 = 6-10 nodules, 3 = 11-15 nodules, and 4 = >15 nodules on each individual root system.

Table 5.2 Comparison of root rot severity, seedling emergence, and plant growth parameters among soybean genotypes planted in soil or potting mix inoculated with *Fusarium avenaceum* under open field ('Field') and greenhouse ('GH') conditions.

Soybean genotype	Root rot (0-4)		Root nodulation	Seedling emergence (% of control)		Grain yield loss (%)		Seedling shoot length (mm)	Seedling dry mass (g) in GH	
	Field (2013)	GH (2012/13)	Field (2013)	Field (2013)	GH (2012/13)	Field Lendrum (2013)	Field McLeod (2013)	GH (2012/13)	Root (2012/13)	Shoot (2012/13)
TH32004R2Y	1.58^{a}	3.20^{ab}	0.87^{d}	75.08^{bcd}	50.48^{cde}	15.2^{bc}	14.6^{b}	46.3^{abc}	0.10^{b-e}	0.43^{ab}
TH27005RR	1.44^{ab}	3.02^{abc}	1.05^{bcd}	75.56^{bc}	45.57^{cde}	11.4^{bc}	17.8^{ab}	47.7^{abc}	0.09^{b-e}	0.59^{ab}
900Y71	1.44^{ab}	3.23^{ab}	1.02^{bcd}	54.85^{e}	26.67^{f}	13.1^{bc}	16.7^{b}	48.1^{abc}	0.08^{cde}	0.46^{ab}
900Y81	1.39^{abc}	2.59^{c}	1.14^{bc}	78.42^{ab}	75.48^{a}	5.5^{c}	30.2^{a}	24.5^{d}	0.03^{de}	0.17^{c}
LS005RR	1.38^{abc}	3.33^{ab}	0.96^{cd}	70.92^{cd}	42.86^{def}	18.7^{abc}	10.9^{b}	59.5^{a}	0.12^{abc}	0.38^{bc}
90M01	1.32^{bc}	3.39^{ab}	1.23^{ab}	82.542^{a}	47.86^{cde}	31.0^{a}	13.5^{b}	54.5^{ab}	0.17^{ab}	0.53^{ab}
TH29002RR	1.32^{bc}	3.39^{a}	1.15^{bc}	71.479^{cd}	45.48^{cde}	9.0^{bc}	22.4^{ab}	53.5^{ab}	0.11^{abcd}	0.62^{a}
NSC Portland	1.31^{bc}	3.36^{ab}	1.36^{a}	74.60^{bcd}	34.29^{ef}	18.6^{abc}	12.8^{b}	50.0^{abc}	0.05^{cde}	0.47^{ab}
900Y61	1.26^{bc}	2.91^{bc}	1.14^{bc}	77.33^{ab}	54.52^{bcd}	12.6^{bc}	22.6^{ab}	37.7^{c}	0.09^{b-e}	0.63^{a}
LS003RR	1.23^{c}	3.33^{ab}	1.12^{bc}	69.90^{d}	48.33^{cde}	23.2^{ab}	11.8^{b}	51.5^{ab}	0.19^{a}	0.43^{ab}
OAC Prud		2.67^{c}			60.48^{abc}			36.7^{c}	0.01^{e}	0.46^{ab}
Tundra		2.64^{c}			71.19^{ab}			41.7^{bc}	0.04cd^{e}	0.48^{ab}

Data for root rot severity, root nodulation and crop emergence under field conditions are the means of four replicates, pooled over two experimental sites in 2013. Seed yield data were presented separately for the Lendrum and McLeod, Alberta, sites because there was a strong environmental interaction. The experiments in 2012 were damaged by flooding. Data within a column followed by the same

letter are not significantly different according to Duncan's New Multiple Range Test ($P \leq 0.05$). Data for the greenhouse experiments was pooled together for 2012 and 2013. Seedling emergence was calculated as the percentage of seedlings emerged relative to the number of seeds planted. Root rot was evaluated on a scale of 0-4, where: 0 = normal root color, 1 = 1-25% root discoloration, 2 = 26-50% root discoloration, 3 = 51-75% root discoloration, and 4 = 76-100% root discoloration. Nodulation was scored on a scale of 0-4, where: 0 = no nodules on the entire root system, 1 =1-5 nodules, 2 = 6-10 nodules, 3 = 11-15 nodules, and 4 = >15 nodules on each root.

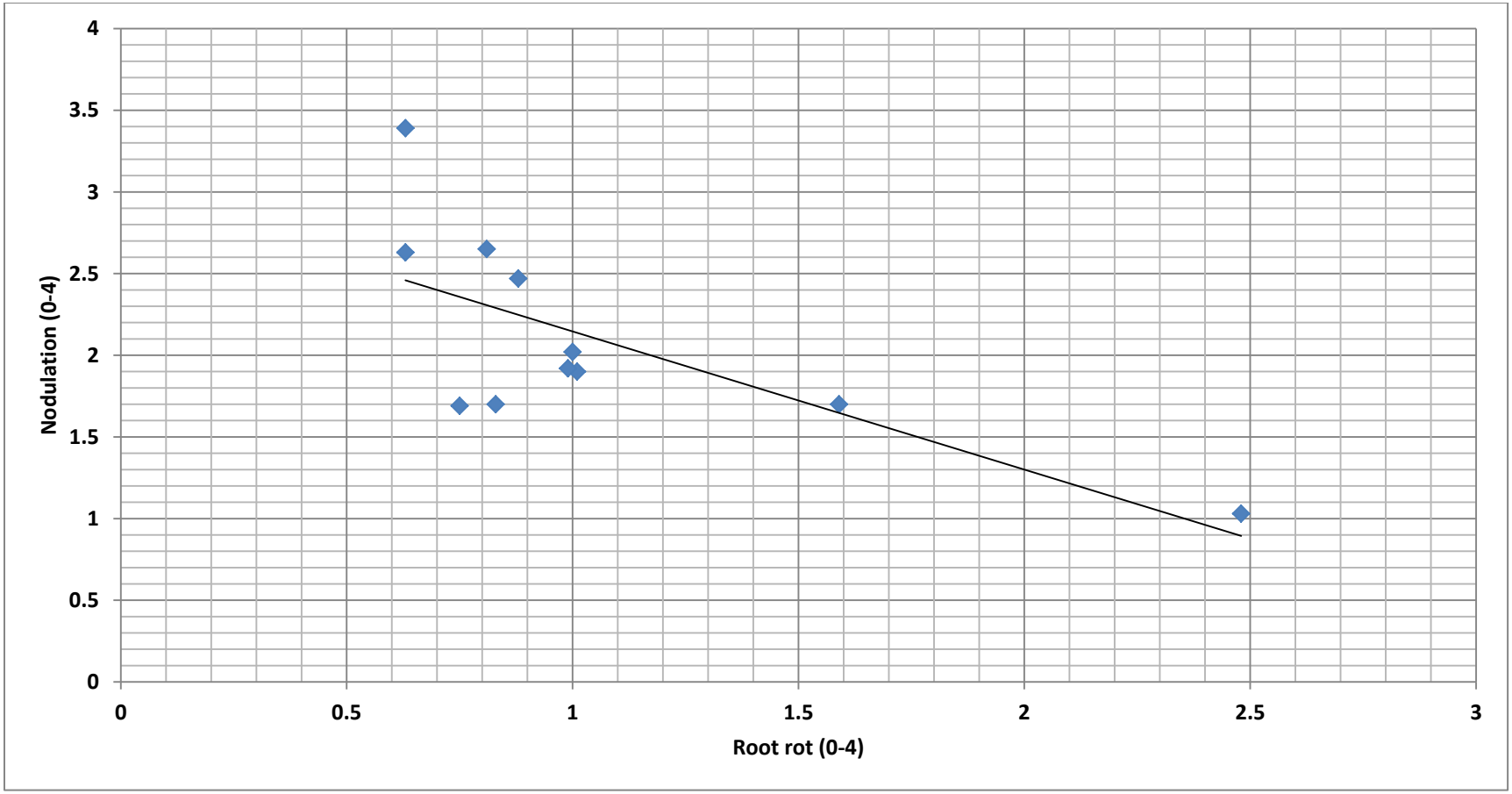

Figure 5.1 Visual representation of the relationship between root rot severity and nodulation on soybeans inoculated with *Fusarium avenaceum*. Average root rot severity is shown on a scale of 0-4, where: 0 = normal root color, 1 = 1-25% root discoloration, 2 = 26-50% root discoloration, 3 = 51-75% root discoloration, and 4 = 76-100% root discoloration. Average root nodulation is shown on a scale of 0-4, where: 0 = no nodules, 1 =1-5 nodules, 2 = 6-10 nodules, 3 = 11-15 nodules, and 4 = >15 nodules per root system.

Chapter 6: Overview and Future Directions

The objectives of this study were to evaluate the occurrence of root rot of soybean in Alberta, characterize the causative *Fusarium* species isolated from infected roots, and investigate the possibility of controlling the disease in an integrated manner with fungicidal seed treatments and resistant host genotypes. These objectives were addressed through disease surveys, recovery of Fusarium isolates from field-collected root samples, evaluation of the pathogenicity of selected isolates in bioassays, an assessment of fungicide efficacy, and resistance screening of a suite of soybean genotypes.

In the disease surveys carried out in 2012-2014, root rot was found in all soybean crops visited, with the highest prevalence in low lying areas of the fields where water accumulated during rainstorms. Root rot incidence levels of up to 100% were observed at some survey sites. Root nodulation by *Bradyrhizobium japonicum* tended to decline with increasing disease severity, indicating that a healthy root system is required for effective nodulation. There were, however, no data collected on the fungicidal seed treatments used by the farmers in the surveyed crops, nor on yields obtained from those same crops. Future research could examine the impact of root rot severity on yields in commercial cropping systems, as well as the influence of tillage practices on pathogen inoculum levels and disease development.

Fusarium species were common on soybean roots exhibiting symptoms of root rot. A total of seven species were recovered, with *F. avenaceum*, *F. solani*, *F. oxysporum*, and *F. acuminatum* found to be predominant. The wide spectrum of *Fusarium* species confirmed that soybean root rot is caused by a complex of fungi rather than a single species. It may be informative to examine the occurrence of *Fusarium* species and other fungi and fungal-like

microorganisms from soil as well as root samples, to obtain a more complete picture of the composition of the microflora in fields planted to soybean. This may provide an indication of overall soil health, and help identify possible interactions that might be exploited for better root rot control. The use of molecular methods, in addition to classical mycological approaches, to identify soybean-associated fungi should also be considered as a focus of future studies.

The aggressiveness of *Fusarium* spp. recovered from infected soybean roots varied significantly, with isolates causing mild to severe symptoms of root rot under controlled environmental conditions. Among the species identified, isolates of *F. acuminatum*, *F. proliferatum* and *F. avenaceum* were the most aggressive. In contrast, *F. solani*, a commonly isolated pathogen from soybean roots that is traditionally associated with root rot, caused variable levels of the disease on inoculated soybeans. In the current research, each *Fusarium* species was inoculated individually, in order to obtain clear information on the relative aggressiveness of the different species. Under natural field conditions, however, pathogen inoculum usually consists of a complex of species, including other fungal genera, bacteria, and even arthropods. Further research is needed to evaluate the interaction of Fusarium with other soil-borne microorganisms present in soybean fields. Such studies may help to identify synergistic or antagonistic effects, which could increase or decrease the effectiveness of pathogen inoculum. The isolates characterized in this study may represent a valuable resource for such future work, given that they have been identified to species and in some cases evaluated for aggressiveness characteristics.

The treatment of soybean seeds with fungicides significantly reduced pre-emergence damping-off, improving seedling emergence, nodule development, root and shoot dry mass accumulation. While root rot still developed, symptom severity was reduced. In the field

experiments, treatments consisting of Rancona Summit + Apron Maxx RTA, Rancona Summit, Apron Maxx RTA, and EverGol increased emergence rates. In the greenhouse experiments, the Rancona Summit and Vitaflo 280 treatments resulted in significantly higher seedling emergence. A mix consisting of Rancona Summit + Maxim + Vibrance was the most effective seed treatment, however, since it resulted in the least severe root rot under both field and greenhouse conditions. Soybeans treated with this fungicide mixture had the highest intensity of nodulation by *B. japonicum* in the field study, and the highest root and shoot mass under greenhouse conditions.

In the resistance screening studies, the best emergence rates in the presence of *F. avenaceum* were obtained for the soybean cultivar 90Y81 under both field and greenhouse conditions. This suggests that this cultivar may carry some resistance to *F. avenaceum*, and could prove to be a valuable resource in resistance breeding efforts. There was considerable variation in seedling emergence and disease development among the other genotypes evaluated, suggesting some diversity in the resistance of these genotypes. Nonetheless, additional resistance screening with a wider range of *Fusarium* species and isolates will be necessary to fully evaluate the potential for genetic resistance as a soybean root rot management tool. The research presented in this thesis has shown that root rot of soybean caused by *Fusarium* species represents a significant challenge to the production of this crop in Alberta. At the same time, the work has identified some fungicide and varietal management options to mitigate the impact of this disease. It may be worthwhile to conduct additional experiments in which soybean genotypes showing some resistance to *Fusarium* species are evaluated for root rot reaction in combination with various fungicidal treatments, in order to identify effective ways in which disease management strategies could be integrated.

Furthermore, additional research could include studies to better understand how abiotic stress factors can interact with fungal pathogens in the development of root rot. An investigation of the aggressiveness of soybean root-causing *Fusarium* species at varying soil temperature and moisture regimes, as well as at varying levels of salinity, may improve knowledge of the epidemiology of this pathosystem. Such knowledge may allow for the development of effective root rot forecasting models for soybean growers, and aid in the design of appropriate disease management strategies. An integrated approach will be needed to successfully manage soybean root rot in Alberta.

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