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Root Rot Pathogens of Wheat in South Dakota and Their Affect on Seed Germination and Seedling Blight in Spring Wheat Cultivars

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ROOT ROT PATHOGENS OF WHEAT IN SOUTH DAKOTA AND THEIR AFFECT ON SEED GERMINATION AND SEEDLING BLIGHT IN SPRING WHEAT CULTIVARS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the Master in Plant Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidates are necessarily the conclusions of the major department.

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ABBREVIATIONS

Bs	*Bipolaris sorokiniana*
CRR	common root rot
FCR	fusarium crown rot
Fg	*Fusarium graminearum*
Ggt	*Gaeumannomyces graminis tritici*
HRSW	hard red spring wheat
LSD	least significant difference
T	treated
UT	untreated

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ABSTRACT

ROOT ROT PATHOGENS OF WHEAT IN SOUTH DAKOTA AND THEIR AFFECT ON SEED GERMINATION AND SEEDLING BLIGHT IN SPRING WHEAT CULTIVARS

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Crown rot and common root rot are the important root diseases in wheat (*Triticum aestivum* L.) and other cereals causing significant germination and yield losses in the Northern Great Plains and other parts of the world. *Bipolaris sorokiniana* (Bs) and *Fusarium graminearum* (Fg) cause common root rot and crown rot respectively, are the important wheat root pathogens that can affect seed germination, seedling establishment and impact crop productivity. A survey was conducted in the year 2014 and 2015 to study the distribution and the prevalence of root rot pathogens in South Dakota. Out of 31 and eight roots samples collected in 2014 and 2015, respectively, *F. graminearum* was the major pathogen recovered in both years. All the collected samples harbored *F. graminearium*, and 50% of the samples produced *B. sorokiniana*. In 2014, 125 isolates of *F. graminearum* and 62 isolates of *B. sorokiniana* were recovered from 31 root samples and in 2015, 38 isolates of *F. graminearum* and eight isolates of *B. sorokiniana* were recovered from eight root samples. The fungus *Gaeumannomyces graminis tritici* associated with Take-all was not recovered from the collected samples in both years. Further, we studied the effect of *B. sorokiniana* and *F. graminearum* infested seed on germination and seedling establishment (blight) of 11 HRSW wheat cultivars under greenhouse and field conditions (Brookings and Volga). Seeds of 11 hard red spring

wheat cultivars HRSW cultivars, Advance, Brick, Briggs, Forefront, Oxen, Prevail, Russ, Select, SD4189, SD4215, and Traverse were infested individually with *B. sorokiniana* and *F. graminearum* by spraying with their respected spore suspension. Infested seed from all 11 cultivars were planted in paper cups (10 seeds/cup) filled with sterile vermiculite, using a complete randomized design. Seed germination and seedling blight data was recorded 10 and 20 days' post planting. The percent germination losses when the seed was infested with *F. graminearum* ranged from 4 to 33% while the seedling survival rate of the cultivars varied from 48 to 87% and the seedling blight ranged from 7-27% but when seed was infested with *B. sorokiniana*, percent germination varied from 2-17% with 58 to 96% seedling survival rate and 0-16% seedling blight. We further, planted 100 seeds of seven (2015) and 11 (2016) HRSW cultivars with six different treatments in a split plot design experiment in three replications at two field locations, Brookings and Volga. The treatments included were uninfested seed + untreated (T1), unifested + treated with fungicide (T2), infested (*B. sorokiniana*) + treated (T3), infested (*B. sorokiniana*) + untreated (T4), infested (*F. graminearum*) + treated (T5), infested (*F. graminearum*) + untreated (T6). Seed germination and seedling blight data were recorded after the germination for three consecutive weeks. Wheat cultivars varied in seed germination and seedling blight to both the pathogens; however, low seed germination was observed in *F. graminearum* infested seed as compared to *B. sorokiniana* infested seed at both locations in both years. Cultivars Russ (72%) and Oxen (80%) were highly affected for seed germination and seedling blight to both pathogens whereas Forefront (92%), Select (95%) and Briggs (88%) had the highest germination and the higher seedling survival rate as compared to the other cultivars both under greenhouse and field conditions. The

percent germination losses when the seed was infested with *F. graminearum* ranged from 17-35% while the seedling survival rate of the cultivars varied from 92-99%. In case of the seed infested with *B. sorokiniana*, germination losses ranged from 2-15% with the only highest germination loss observed in Russ cultivar (32%) with the survival rate of all the cultivars ranged from 91-97%. Fungicide treatment (T3 and T5) significantly increased the seed germination from 14-37% and the seedling blight was also reduced in almost all the cultivars. In another experiment, where oat kernels were used as a source of inoculum, reduction in percent seed germination was observed however, it was not significant.

Introduction

In the growing world economy, progress continues along with the fight against hunger. According to the recent reports from the FAO, 795 million people in the world are undernourished in the recent years of 2014-2016 (http://www.fao.org/3/a-i4646e.pdf). Feeding this undernourished population and an increasing world population, enough food should be produced every year so as to reduce the percentage of these figures. Among the different field crops, wheat is the principal cereal crop grown all over the world to feed the growing population. Wheat (*Triticum aestivum* L.) is one of the most important crops grown worldwide and considered as a staple food for 1/3rd of the world population. The United States of America stand fourth in wheat production after China, India and Russia in the year 2016 with the average production of about 2.3 billion bushels with an area planted of about 43.8 million acres. In South Dakota, area planted in 2016 was 2.27 million acres with the total production of 111.28 million bushels (USDA-NASS, 2016).

Disease in the field crops affects the crop production significantly. Losses due to plant diseases can range from 30 to 40% affecting the major crops worldwide. The major groups of pathogens include bacteria, fungi, oomycetes, viruses, nematodes and parasitic plants. Of these biotic pathogen groups, fungal pathogens are ranked first and cause the majority of plant diseases.

Wheat can succumb to many root, leaf, and head diseases and they significantly impact the crop productivity worldwide. Of these, root diseases can cause average yield losses ranging from 3-4% depending on the cultivar susceptibility level, pathogen virulence and suitable weather for disease development (Draper et al., 2000). The major root rot pathogens in wheat are *Fusarium graminearum* Schwabe (Crown rot)*, Bipolaris*

sorokiniana (Sacc.) Shoemaker (Common root rot)*, and Gaeumannomyces graminis* f. sp. *tritici* (Ito & Kuribayashi) Dreschsler ex Datur (Take-all) respectively. Of these three pathogens, *B. sorokiniana* and *F. graminearum* also affect seed germination and seedling blight. In general, root diseases can be managed through deployment of resistant cultivars, seed treatment and cultural practices. In South Dakota, very limited information on the prevalence of root diseases pathogens, their impact on seed germination and seedling blight, and reaction of spring wheat cultivars grown in the state is available. Information on root rot pathogens and status of wheat cultivars to root rot diseases in a particular region is essential in devising the disease management strategies.

The objectives of this study are 1) to survey wheat fields across the state for root rot pathogens in South Dakota and 2) effect of commonly prevalent root rot pathogens on spring wheat cultivars/lines seed germination and seedling blight.

CHAPTER-1

1 Literature Review:

1.1 The Host-Wheat

Wheat (*Triticum aestivum* L.) is the major cereal crop grown worldwide for food, feed and other products. In 2016, 546 million acres of wheat were planted with the production of 27.31 billion bushels worldwide. The United States alone accounts for 43.89 million acres with a production of 2.27 billion bushels thus ranked fourth in wheat production in the world in 2016 (USDA-FAS, 2016). In the US, six classes, hard red spring, hard red winter, soft red winter, soft white, hard white, and durum are produced and they are utilized for hard rolls, flat breads, tortillas, general purpose flour, cereal, pizza crust, cookies etc. (http://www.uswheat.org/wheatClasses).

Wheat ranks third in the United States after corn and soybean production. Nearly half of the wheat produced in the United States is exported outside as the wheat harvested has been dropped in the last decade because of the declining return on this crop. (http://www.ers.usda.gov/topics/crops/wheat.aspx). This declining trend in wheat production is because of many reasons that include changes in the food consumption, better market prices for other crops like corn and soybean, decrease in wheat market price due to over production and less demand overseas.

In South Dakota winter hard red and spring hard red wheat are primarily produced with the production of 111.28 million bushels of wheat which was produced on about 2.27 million acres in the year 2016. (http://usda.mannlib.cornell.edu/usda/current/SmalGraiSu/SmalGraiSu-09-30-2016.pdf).

In the recent years, an increase in temperatures and drought conditions in South Dakota led to the increased occurrence of the root diseases like common root rot, crown rot and take-all in the wheat crop. Because of the high temperature the crop started maturing early as a result of which many wheat fields of the South Dakota have been observed with white heads with no seed in them. In addition, there were fields planted with spring wheat last year with the susceptible cultivars to Fusarium head blight that can cause crop to be more prone to root rot diseases in winter wheat. In general, the extent of yield loss and distribution of pathogens in this crown and root rot complex in South Dakota are not well understood.

1.2 Worldwide distribution of root rot pathogens affecting the seed germination

In the world, several surveys have been conducted in various countries, including Australia, Argentina, Brazil, Canada, Iran, the Unites States, Turkey, to assess the severity of the root rot pathogens impacting cereal grain production (Hekimhan et al., 2004). Among the major root diseases Fusarium crown rot (FCR) and common root rot (CRR) diseases cause significant germination and yield losses worldwide (Burgess et al., 2001).

The survey for these fungi has been done in different parts of the world such as Pacific Northwest (Cook 1968; Smiley and Patterson, 1996; Smiley et al., 2005), Canadian Prairies (Bailey et al., 1995; Hall and Sutton, 1998; Fernandez and Jefferson, 2004; Fernandez et al., 2007; Fernandez et al., 2009), Texas Panhandle (Specht and Rush, 1988), Southeastern Idaho (Strausbaugh et al., 2004); upper coastal plain area of Mississippi (Gonzalez and Trevathan, 2000), eastern Australia (Backhouse et al., 2004), South Australia (Fedel-Moen and Harris, 1987), Queensland Australia (Wildermuth, et

al., 1997), United Kingdom (Pettitt et al., 2003), Turkey (Tunali et al., 2008), north west Iran (Saremi et al., 2007), Brazil (Diehl, 1979).

Among the root diseases, common root rot in wheat in the prairie provinces of Canada from the year 1969-1971, loss of 5.7% or 30 million bushels was reported reducing the significant overall wheat production in the country (Ledingham et al., 1973).

In Turkey, first study was reported on the yield trial for 3 years under the marginal conditions for the dryland root rot complex which comprises of common root rot (*B. sorokiniana*) and the *Fusarium* spp. (*F. graminearum* and *F. culmorum*). The yield losses due to root diseases caused by these pathogens were higher in the winter wheat followed by triticale and barley was observed. The yield loss varied from year to year and was 15%, 35% and 27% in consecutive years (Hekimhan et al., 2004).

A Survey conducted in the Southeastern Idaho documented the prevalence of *B. sorokiniana* and *F. culmorum* from the soil and root samples in 81 wheat and 52 barley fields in 13 counties. They also did the nematode soil assay which revealed that 96% of the fields had lesion nematodes (mostly *Pratylenchus neglectus*) and 78% had stunt nematodes (*Tylenchorhynchus* spp.). Both the results from greenhouse and soil assay indicated that with the disturbance in the soil to simulate disking reduced the severity of Fusarium root rot (Strausbaugh et al., 2004).

In Northwestern Minnesota, severity of the common root rot has been documented to be associated with the root rot pathogens, dominated with *B. sororkiniana* but they also found an association of *F. graminearum* and *F. culmorum* with root rot complex (Windels and Holen, 1989). In most cases *B. sorokiniana* was recovered from the lesioned sub crown internode.

Sultana and Rashid (2012) reported the effect of wheat seed infected with various levels of *B. sorokiniana* on the germination. The prevalence of the pathogen was highest in the shriveled seeds about 65%, 42% in black pointed seeds and 30% in healthy looking seeds. The percent germination failure was as high as 87% in the case of seeds infected with the fungus and 24% germination loss in the healthy looking seeds.

1.3 Pathogens associated with root diseases

1.3.1 *Fusarium graminearum*

Fusarium graminearum Schwabe (group II) (= *G. zeae* (Schwein.) Petch) is the most important root rot pathogen causing crown root rot or Fusarium foot rot of wheat in the United States. Additionally, this pathogen is responsible for the major disease of wheat called Fusarium Head Blight (Scab) which caused an epidemic in 1990's. The yield losses due to this epidemic were more than $3 billion dollars. In addition to this, there are other Fusarium spp., capable of causing root rot diseases of wheat such as *F. culmorum* (W.G. Smith) Sacc., *F. pseudograminearum* O'Donnell & T. Aoki, *F. avenaceum* (Fries) Sacc., *F. equiseti* (Corda) Sacc., *F. acuminatum* Ellis & Everh., *F. oxysporum* Schltdl. emend. Synder & Hansen which are widely prevalent in the semi-arid regions (Burgess et al., 2001; Cook 2010 and Paulitz et al., 2002).

The pathogen responsible for crown rot varies from region to region as it depends upon the weather conditions in which the pathogen can survive. For example, *F. culmorum* is the major species responsible for crown rot in the Northwest United States while *F. graminearum* was prevalent in the warmer portion of the regions of South Central Washington (Cook 1980). *F. avenaceum* and *F. acuminatum* were more prevalent

in the cooler regions of Canada and United States (Pettitt et al., 2003; Hall and Sutton, 1998).

1.3.1.1 Description and significance of *Fusarium graminearum*

1.3.1.1.1 Taxonomy and Classification

In the 18th century, a German scientist named J.H.F. link gave the name *"Fusarium*" based on the morphological characteristics of the fungal macroconidia, which were fusiform or spindle shaped. Later on, the *Fusarium* genus was divided into several species (Leslie and Summerell, 2006) and *F. graminearum* was distinguished from other species based on the morphological shape of macroconidia and production of sexual stage. The sexual stage of *F. graminearum* was described by Fries in 1821 while the asexual stage or the anamorph stage was described by Schwabe later in 1838.

1.3.1.1.2 Description of the pathogen

Fusarium graminearum Schwabe (group II) is an ascomycete fungus which belongs to the Kingdom- Fungi, Phylum- Ascomycota, Order- Hypocreales, Family- Nectriaceae, Genus- *Fusarium* and Species- *graminearum*. The anamorph stage produces macroconidia which are hyaline, long, slender, with 5 or more septa with a well-developed foot cell which is the typical feature of *F. graminearium*. It produces carmine red color on the half strength potato dextrose agar (PDA) medium (Nelson et al., 1983). The telomorph stage is named as *Gibberella zeae* that produces bright blue colored perithecia which bear 6-8 ascospores in a sac like structure called ascus of 4-10 µm wide x 50-80 µm long. Ascospores are multi-septate, hyaline, and ellipsoidal with 3.3-6.5 x 13-17 µm size (Cook 2010).

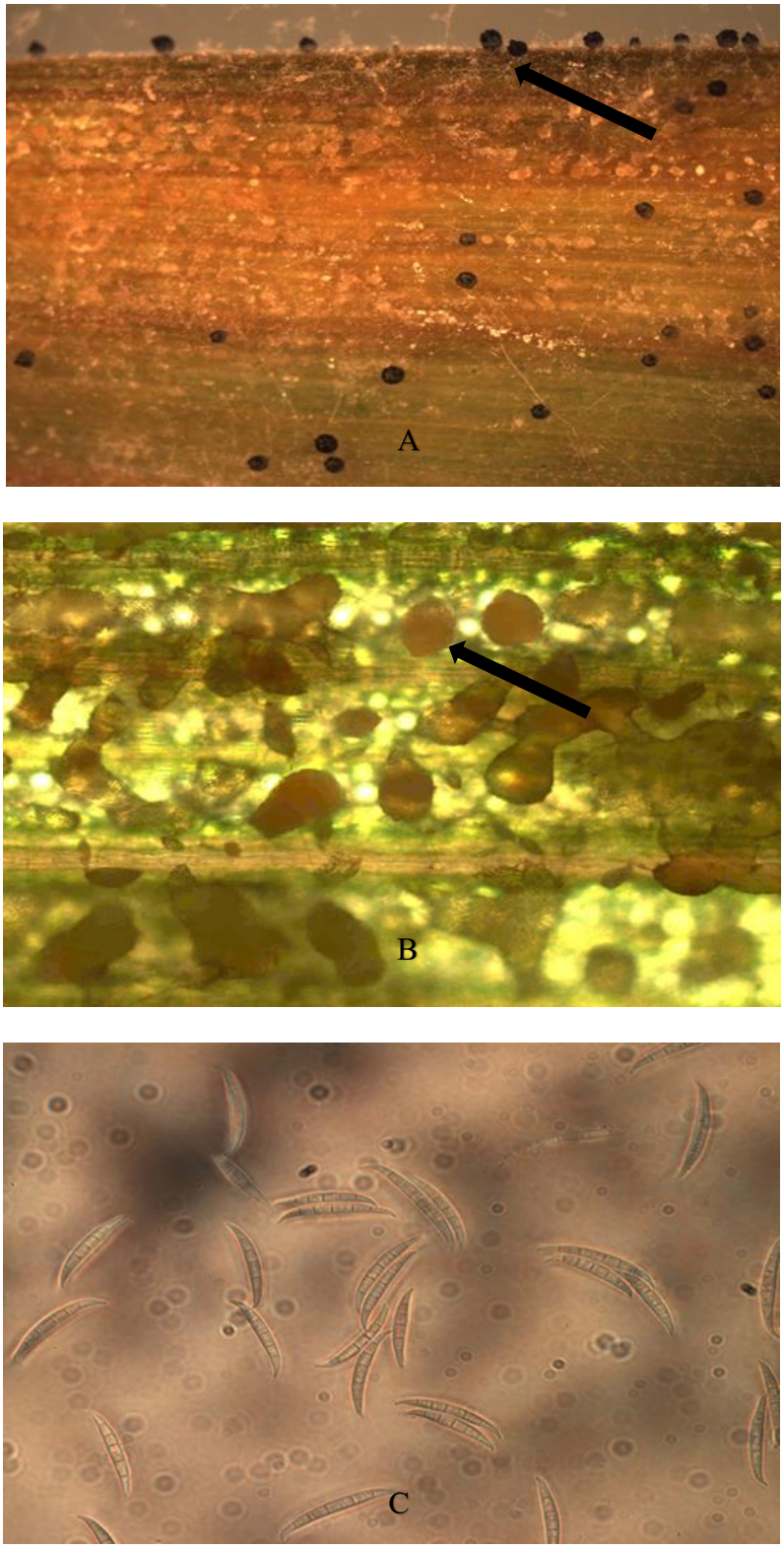

Figure 1.1 (Top to bottom) *F. graminearum,* perithecia (A), sporodochia on wheat leaf (B), and macroconidia (C)

1.3.1.2 Host Range

Fusarium graminearum has a very broad host range. Along with wheat (*Triticum aestivum* L.), it also infects rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), maize (*Zea mays* L.), dry beans (*Phaesolus vulgaris* L.), and soybean (*Glycine max* L.) (Goswami et al., 2004; Bilgi et al., 2011; Borders et al., 2007).

1.3.1.3 Symptoms of Crown rot

The fungus infects the crown root portion of the plant, thus continuously decaying the crown root portion. The infected area turns into dark brown to black and spread in the sub-crown root internode area. In mature plants, there is poor seed set in heads thus leading to production of white heads and hence reducing yield of the small grains worldwide. Sometimes the above ground symptoms are not visible if the weather conditions are not very conducive for disease development. It requires warm and moist weather conditions (20-25 °C) for the development of the typical symptoms.

1.3.1.4 Disease cycle of Fusarium crown rot

For the fungal survival and spore's dissemination, it survives on infected seed and infested crop residue. Thus for disease development, infected seed and infested crop residue are the important sources of inoculum (Cook 1981). In case of *F. graminearum* group II, the spore production is both sexually in the form of perithecia (ascospores) as well as asexually in the form of sporodochia (conidia). The fungus survives generally both in the mycelial, peritheicial form on crop residue and in the soil in the form of chlamydospores (Cook, 1981; Paulitz et al., 2002). The chlamydospores of *F. graminearum* group I are less hardy as compared to the chlamydospores of *F. culmorum*

because of the greater sensitivity to the heat stress as a result of which *graminearum* group I was rarely detected in the Northwest of the U.S.A. (Sitton and Cook, 1981).

1.3.2 *Bipolaris sorokiniana*

The fungus *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur (anamorph: *B. sorokiniana* (Sacc.) Shoemaker), is an ascomycete and an important pathogen causing common root rot in wheat and barley (Mathre et al., 2003). Additionally, the fungus is also involved in causing spot blotch, seedling blight and kernel blight in wheat. An estimated average loss of about 5.7% was reported in the prairie provinces of Canada during three years from 1969-1971 disease survey (Ledingham et al., 1973).

1.3.2.1 Description of the pathogen

Cochliobolus sativus (Ito et Kurib) Drechs. ex Dastur [anamorph: *B. sorokiniana* (Sacc.) Shoem. syn: *Helminthosporium sativum* P.K. & B.] belongs to Kingdom- Fungi, Phylum- Ascomycota, Order- Pleosporales, Family- Pleosporaceae, Genus- *Cochliobolus*, Species- *sativus*. The fungus is heterothallic and the sexual stage of this fungus is rarely observed under field conditions and has only been observed in Zambia (Raemaekers 1988). The conidia of *B. sorokiniana* are thick walled, elliptical shaped conidia (60-120 µm × 12-20 µm) with about 5 to 9 cells (Fig 1.2). The mycelium has different colors ranging from white, light grey to dark grey depending upon the isolate (Kumar et al., 2002).

Figure 1.2 Conidiospore of *B. sorokiniana*

1.3.2.2 Host range

The fungus *Bipolaris sorokiniana* has a wide host range which include cereals; barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.) etc. and many non-cereal grasses across the world (Jones 1983). Forty-five plant genera that include Asteraceae, Brassicaceae, Lineaceae and Solanaceae were reported to be susceptible to this pathogen (Harding 1979).

1.3.2.3 Symptoms of Common root rot (CRR)

Because of the difficulty to diagnose the disease due to absence of above ground symptoms common root rot can go unnoticed. The major distinctive symptom of the common root rot is the dark brown discoloration of the sub crown internode portion of the root. This necrosis in extreme cases extend upwards and can reach tillers bypassing the leaves and thus leading to plant death prior to maturity (Mathre 2003). Affected plants look stunted and, produce fewer tillers, shriveled grains resulting in premature death. Generally, the diseased plants are randomly distributed in the field.

1.3.2.4 Disease cycle of Common root rot (CRR)

The infection process starts from the conidia present in the leftover infested crop debris or the root infection can begin from the diseased seed (Valjavec-Gratian and Steffenson, 1997; Piening 1997). The fungus produces dark brown lesions on the outer coleoptile tissue and/or on the leaf base, which further can merge and give rise to larger areas of necrotic brown tissues. This causes poor seedling emergence or may even lead to seedling death. The conidia can be dispersed to long distances and they are able to germinate on the host tissue under moist conditions. Infection process begins from the formation of appressoria which colonize the seedling roots (Weste 1975). Infection tends to increase during the drought stress, high temperature (20-30 °C) or flooding (Stein 2010).

1.4 Interaction between the common root rot and crown rot

Many interaction studies involving the crown rot and common root rot have been done to see how the pathogens of these two diseases interact when both are present in the same crop causing root rot. The study was conducted late back in 1940's when Ledingham (1942) co-inoculated wheat roots with *Helmithosposrum sativus* and *F. culmorum*. Experiment was conducted both in greenhouse as well as in vitro and an antagonist response was observed in the seedling emergence in the inoculated seedlings. Also inhibition in the conidial germination in the film of clear agar was observed on a microscopic slide.

Tinline (1976) conducted a greenhouse study to test the multiple infections of the sub crown internode of wheat by common root rot fungi in Canada. The wheat plants were inoculated with three *Fusarium* spp. (*F. acuminatum*, *F. culmorum* and *F.*

sulphureum (Schltd.) Link) and *Cochliobolus sativus*. The results showed the interaction between *C. sativus* species and the *Fusarium* spp. was antagonistic despite repeated inoculation of *C. sativus.* Scardaci and Webster (1981) observed antagonistic response and reported 39% reduction in seedling blight when barley plants were inoculated with *F. graminearum* followed by inoculation with *B. sorokiniana* after 21 days. A significant lower level of disease was observed when plants were inoculated with *F. graminearum* alone at planting (Mean Disease rating (MDR) = 0.69) as compared to the plants inoculated 21 day's post planting (MDR= 3.06). Also the pathogen, which was inoculated, first was re-isolated more frequently as compared to the one that was inoculated after 21 days.

Not only the antagonistic response was observed with these pathogens but there was positive correlation also observed when wheat seedlings were inoculated with both *F. acuminatum and B. sorokiniana* simultaneously. A significant increase in the infection of the wheat seedlings was observed when they were inoculated simultaneously (Fernandez et al., 1985). Fernandez and Jefferson (2004) surveyed the fungal population in roots and crowns of common and durum wheat in Saskatchewan. They found a negative correlation between the isolation of the *B. sorokiniana* and *Fusarium spp*. (*F. culmorum, F. equiseti*) and *G. graminis*. The major pathogens recovered from the discolored sub-crown internodes and crowns or lower culms of common and durum wheat across that region were *B. sorokiniana*, followed by *Microdochium bolleyi* and the *Fusarium* spp.

Recently a study on the interaction between *F. pseudograminearum* and *B. sorokiniana* in wheat stems was conducted on the population dynamics using the real-

time qPCR technique (Moya-Elizondo et al., 2011). They observed that even in the presence of high and low rates of *F. pseudograminearum* inoculum, the populations of *B. sorokiniana* got reduced in field trials; however, *B. sorokiniana* inoculum did not affect *F. pseudograminearum* population. In these trials seedling counts were reduced significantly across the locations where fields were inoculated with *F. pseudograminearum*, *B. sorokiniana* alone and in combination of both at the rate of 19%, 12%, and 27% respectively.

1.5 Effect of environmental conditions on the pathogens

1.5.1 Soil Moisture

Disease severity and incidence depends upon many factors. Crown rot incidence is highly affected by soil moisture and nutrients. Higher soil moisture tends to have the greater crown root infection as compared to the drier soils. Also the high moisture conditions in the low lying areas help in the development of the fungus on the infected plants, therefore promoting higher risk of crown rot in wheat (Burgess et al., 1975; Klein et al., 1991).

1.5.2 Temperature

Temperature plays a crucial role in the survival of both *F. graminearum* and *B. sorokiniana* as well as in the spread of the disease. Poole et al. (2013) recently reported that there was a significant effect of temperature on the distribution and the prevalence of the *Fusarium* species. From the survey conducted in the Pacific Northwest of the United States, *F. pseudograminearum* was more prevalent in the regions of lower elevations with lower moisture and higher temperature while *F. culmorum* was more prevalent in the higher elevations and cooler temperatures.

1.6 Management of the Fusarium crown rot and Common root rot.

1.6.1 Tillage Operations

Tillage practices from conventional to zero also have a wide impact on the survival and increase in the inoculum of crown root rot and common root rot pathogens. Wildermuth et al. (1997) reported that the incidence of crown root rot and common root rot increased about 32.2% when crop stubble was retained as compared to 4.7% when it was removed. In contrast, the incidence of white heads was lower when stubble was retained as compared to the stubble removal treatment and thought to be due to availability of high moisture availability during planting and anthesis.

A study conducted in Texas showed that there was no significant difference in the distribution of the spores in the soil profile irrespective of the tillage operations. But the disease severity and the incidence were significantly higher in the conventional till plots as compared to no till plots possibly because of environmental conditions. It was observed that the samples collected during the dry weather period had more disease severity as compared to the samples collected during the high moisture conditions (Mathieson et al., 1990). There were other reports available on the effect of the tillage operations reducing the level of the *Fusarium* species in wheat roots (Bailey et al., 2001, Fernandez et al., 2007).

1.6.2 Seed Treatment

The fungal pathogens causing root diseases can be managed by using fungicide as a seed treatment to minimize root rot severity and thus increasing the yield. Several studies result indicated that the use of fungicide as a seed treatment could be one of the

effective ways to improve yield of crops especially in wheat and barley. Along with the yield advantage it also improves the test weight as well as 1000 kernel weight.

For example, seed treatment with Imazalil improved yield up to 6% and increased seed test weight in barley. In addition, Imazalil reduced the root rot severity index from 76 to 66 in barley (Hermann et al.,1990).

Stack and McMullen (1991) tested the effect of systemic fungicides as seed treatment against the common root rot pathogen *B. sorokiniana*. The sub crown root internode index (SCI) of common root rot significantly reduced with Triadimenol and difenoconazole in barley and wheat, increased 7-9 percent yield.

1.6.3 Planting date

There is no single control measure for the management of root rot pathogens. However, by following the recommended package of practices for the planting as well as cultivation of crop can reduce the losses to a greater extent. Both the early planting as well as late planting can increase the incidence of both pathogens (Cook 2001; Smiley et al., 2009).

1.6.4 Crop rotation

Crop rotation is one of the disease management strategy to reduce the incidence of crown rot and common root rot. Specifically, rotation with the non-cereals reduces the disease pressure and the inoculum density (Stein 2010). Rotation with the broadleaf crops limits the spread of the crown rot and common root rot in wheat. *F. graminearum* and *B. sorokiniana* have the ability to survive for at least two years. Thus to break this cycle of continuous infection a gap of non-cereal crop will reduce the inoculum of these

pathogens in the soil and therefore the disease incidence (Wiese 1991; Burgess et al., 2001; Cook 2010).

1.6.5 Biological control of root rot pathogens

Biological control agents play an important role in suppressing the root rot pathogens. Del Bello et al. (2003) reported that use of *Bacillus subtilis* and *Gliocladium roseum* as a biocontrol agent under greenhouse conditions can significantly reduce seedling blight caused by *B. sorokiniana*. However, similar results were not observed in their field study.

Bacon and Hinton (2007) evaluated the patented bacterial endophyte *Bacillus mojavensis* to control seedling blight caused by *F. graminearum* and related species. Germination of a highly scab (FHB) susceptible cultivar diseased seed increased from 77 to 97% and increase in seedling emergence from 20 to 82% in FHB susceptible wheat cultivar Norm when the seed was treated with the respective bacterium.

Use of the *Pseudomonas* sp. strain MKB 158 and chitosan also significantly reduced from 53-91% of seedling blight caused by *F. culmorum* (Khan et al., 2006).

Conjunctively screening test of biological control agents against Fusarium crown rot and Fusarium head blight in wheat was performed by Wang et al. (2015) and observed that *Pseudomonas fluorescens* LY1-8 performed well in both tests with 44.62% and 58.31% efficacy.

1.6.6 Host resistance

Use of resistant cultivars is the best option for management of crown rot and common root rot. However, there is no cultivar with complete resistance available to both the pathogens causing these diseases, but cultivars with partial resistance are available

that can be deployed as a management strategy (Cook 2010; Stein 2010). The reaction of hard red spring wheat to common root rot was determined under field conditions in the northern Great Plains. It was observed that none of the forty HRSW cultivars were immune to CRR. However, disease severity was lower in ND 722, AC Cadillac, HJ 98, Argent and Scholar throughout the study period which can be used as a source of resistance in breeding program (Tobias et al., 2009).

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Chapter 2

2 Distribution and prevalence of root diseases pathogens in South Dakota

Abstract

A survey was conducted in the years 2014 and 2015 to determine the distribution and the prevalence of root rot pathogens causing common root rot and crown rot, Take-all in wheat in South Dakota. Out of the 31 root samples collected in 2014, the major pathogens recovered was *Fusarium graminearum* and *B.* sorokiniana. In total, we recovered 125 isolates of *F. graminearum* and 62 isolates of *B. sorokiniana* were from 31 root samples. In addition, we also recovered other *Fusarium* species like *F. equiseti, Fusarium verticillioides, F. acuminatum, F. oxysporum, F. semitectum, F. dimerum* and *F. avenaceum* in very low frequency. In 2015, eight samples were collected from different fields in South Dakota showed similar trends. We recovered 38 isolates of *F. graminearum* and eight isolates of *B. sorokiniana* from the eight root samples. Our survey results suggest *F. graminearum* is the most prevalent crown rot pathogen of wheat in South Dakota. *G. graminis var. tritici* was not recovered in both the years suggesting that Take-all may not be a major problem causing root diseases in the region.

Key words: Common root rot, crown root rot, take-all, *Bipolaris sorokiniana, Fusarium graminearum, Gaeumannomyces graminis* var. *tritici.*

2.1 Introduction

Fusarium crown rot (FCR), common root rot (CRR) and Take-all are the important root diseases of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) that can reduce yield, germination, rotting of crown, sub crown and lower stems tissues (Cook 1981; Smiley et al., 1996; Paultitz, et al., 2002; Smiley et al., 2005). Fusarium crown rot is caused by *F. graminearum (*Mc Mullen et al., 1997)*, F. culmorum* (Cook 1968), *F. avenaceum* (Gorden 1933), *F. pseudograminearum* (Smiley and Patterson 1996) that are prevalent in the different parts of the world depending upon the climate they require for their growth. Among these, *F. graminearum* (= *G. zeae* (Schwein.) Petch) is the main pathogen associated with crown rot in North America (Mc Mullen et al., 1997). In contrast, common root rot is caused by *C. sativus* (Ito & Kuribayashi) Drechs. ex Dastur (anamorph: *Bipolaris sorokiniana* (Sacc.) Shoemaker) limiting the yield of wheat crop (Mathre et al., 2003) and the *G. graminis* (Sacc.) Oliver & Von Arx var. *tritici* causes the take-all (Weise 1987; Cook et al., 1995).

Root rot diseases can affect the yield of wheat crop depending upon the disease severity in that region. Tinline and Ledingham (1979) reported that the major yield losses due to these pathogens in wheat are in the range of 3.5% to 6.6%. Overall, the average losses due to root diseases range from 3-7% (Draper 2000). But significant higher yield losses have also been reported from different parts of the world. Fusarium crown rot decreased the yield as high as 35% in the commercial fields of the Pacific Northwest region of the United States (Smiley et al., 2005). The percent germination losses due to *F. graminearum* can be as high as 80% if the infested seed was planted (Wong et al., 2015).

An increasing trend in the incidence of root rot diseases has been observed in different parts of the United States. It is therefore important to know the prevalence of root rot pathogens in South Dakota. Thus, the objective of this study was to conduct the survey to see the prevalence and distribution of root rot pathogens of wheat across the state.

2.2 Materials and methods

2.2.1 Sampling

Wheat fields were sampled randomly at early milk stage in 2014 and 2015. Random wheat fields were chosen for sampling in different counties, 31 and eight roots samples were collected from different counties (Aurora, Beresford, Brule, Buffalo, Edmund, Groton, Hand, Hughes, Hyde, Ipswich, Jerald, Kingsburg, Miner, Potter, Sandborn, Selby Spink and Walworth) in 2014 and 2015 in South Dakota. In each field, 8-10 non-symptomatic root samples were obtained from random locations within the field and the date and location were recorded for each collected sample (Fig. 2.1).

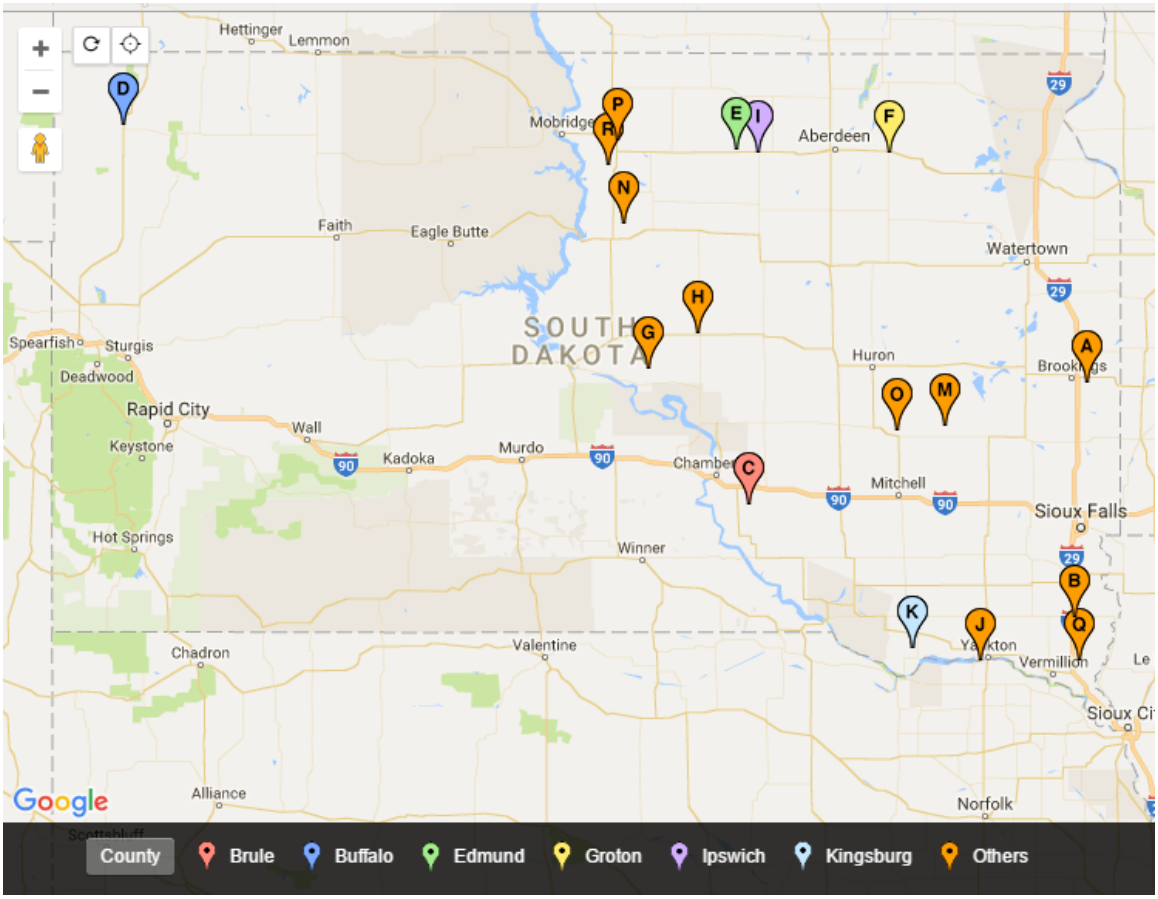

Figure 2.1 Map of the different counties of South Dakota from where root samples were collected randomly.

2.2.2 Preparation of the root samples

After the samples collection, roots were washed thoroughly under running tap water to remove the soil. The samples were dried overnight to remove the excess moisture from the roots. Once the roots were dried, the crown roots were excised from each root sample and were further cut into small segments for surface disinfection.

2.2.3 Surface disinfection of the root samples

The crown roots were surface disinfected with 5% bleach for 60 seconds and then washed with double distilled sterile water for 60 seconds. This disinfection process was done for isolating *Fusarium* species and *B. sorokiniana*. For Take-all, disinfection was done by washing the small root segments with 1% silver nitrate for 10 seconds and then washed with double distilled water for 60 seconds and excess moisture was removed prior to plating on the appropriate medium.

2.2.4 Plating

For the *Fusarium* species, about 40-50 root segments were plated on half strength Potato Dextrose Agar (PDA). The plates were placed under 12 hours' light and 12 hours' dark cycle at room temperature for the fungal recovery. The plates were examined after 7 days for *Fusarium* species identification. Further, cultures were transferred individually onto fresh PDA plates for pure cultures of the fungus. After obtaining the pure cultures, the plates were examined under the compound microscope for the identification of *Fusarium* species based on the fungal morphological characteristics (Leslie and Summerell 2006).

For *B. sorokiniana*, root segments were plated on water agar medium and the plates were kept under 12 hours' light and dark cycle at room temperature. They were

examined after 4 days under a stereoscope for the identification of the fungal pathogens growing on the roots segments. For take-all, root segments were plated on a specific medium modified SM-GGT7 under dark for 12 days. After 12 days, the dark black shining colonies if present were transferred to the wheat leaf agar medium for the fungal perithecial development.

2.2.5 Identification of the pathogens

After obtaining pure cultures of the pathogens, they were observed for growth and morphology, spore morphology, spore color, attachment of the spores with the mycelium under the compound microscope to identify the respective fungal species.

2.3 Results

In 2014, *F. graminearum* was isolated from all the 31 root samples (100%) collected from 17 counties. However, only 16 (51%) of the 31 root samples were infested with *B. sorokiniana.* We recovered 125 isolates of *F. graminearum* and 62 isolates of *B. sorokiniana* from 31 root samples (Table 2.1). In 2015, 8 samples collected from four counties in South Dakota showed the similar pattern of pathogens recovery as was in 2014. *F. graminearum* was recovered from all the eight samples while *B. sorokiniana* was recovered from three root samples. Thirty-eight isolates of *F. graminearum* and eight isolates of *B. sorokiniana* were recovered from the eight samples. *Gaeumannomyces graminis* var *tritici* causing Take-all in wheat was not recovered from any of the collected root sample in 2014 and 2015. Other *Fusarium* species recovered from the samples were *F. equiseti, F. verticillioides, F. acuminatum, F. oxysporum, F. semitectum, F. dimerum* and *F. avenaceum.*

Table 2.1 Recovery of root associated pathogens from root samples collected from South Dakota in 2014 and 2015

Year	Total Samples	Fungal Species	% Number of samples infected
2014	31	*Fusarium graminearum*	100
		Bipolaris sorokiniana	51
		Gaeumannomyces graminis var *tritici*	0
2015	8	*Fusarium graminearum*	100
		Bipolaris sorokiniana	50
		Gaeumannomyces graminis var *tritici*	0

Table 2.2 Other *Fusarium* species recovered from the root samples collected in 2014 and 2015

Year	Other ***Fusarium*** **Species recovered**
2014	*F. equiseti, F. verticillioides, F. acuminatum, F. oxysporum, F. semitectum, F. dimerum*
2015	*F. equiseti, F. dimerum, F. avenaceum, F. verticillioides*

2.4 Discussion

The results of our survey conducted in 2014 and 2015 in South Dakota showed that *F. graminearum* was the major pathogen responsible for crown rot in wheat followed by *B. sorokiniana* for common root rot. Along with *F. graminearum,* other *Fusarium* spp. were also recovered and that included *F. equiseti*, *F. verticillioides*, *F. acuminatum*, *F. oxysporum*, *F. semitectum*, *F. dimerum* and *F. avenaceum*. However, the frequency of these *Fusarium* species were minimal. These results indicate that the major pathogen responsible for the crown rot in wheat in South Dakota is *F. graminearum* and is distributed in all the 17 counties from where the root samples were collected.

Similar results were reported from surveys in New York (Kane et al., 1987) where winter wheat affected by foot and root rots showed presence of *F. graminearum* causing pre-emergence and post-emergence death of the seedlings and *B. sorokiniana* was also recovered. *F. graminearum* has been reported as major cause of crown rot in several regions in the US, California (Oswald 1950), and Minnesota (Warren et al., 1972) and countries Eastern Australia (Burgess et al., 1975; Burgess et al., 1981). Oswald (1950) reported that the major pathogens isolated from the root samples collected from 134 fields were *C. sativus*, *F. graminearum* and *G. graminis* var. *tritici* and *C. nivalis* attacked the sub crown internode, crown and basal culm tissue thus further developed brown discoloration and caused root damage. In another study from Minnesota, *F. graminearum* comprised of about 70% of the *Fusarium* species as compared to *F. culmorum* and *F. avenaceum* (Warren and Kommedahl, 1972) recovered from wheat root samples.

Our results suggest continuous monitoring of root rot pathogens is necessary for the diseases management in the region. The distribution and incidence of the root

pathogens information obtained in this study will help in developing diseases management strategies including identification of sources of resistance to these particular pathogens.

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Chapter 3

1 Effect of *Fusarium graminearum* and *Bipolaris sorokiniana* on seed germination and seedling blight in spring wheat cultivars in South Dakota

Abstract

Bipolaris sorokiniana (Bs) and *Fusarium graminearum* (Fg) are important wheat root pathogens that can effect seed germination, seedling establishment and can impact crop productivity. In this study, we studied the effect of *B. sorokiniana* and *F. graminearum* infested seed on germination and seedling establishment (blight) in 11 wheat cultivars both under greenhouse conditions and field conditions for two years (2015-2016). The treatments included, uninfested seed + untreated (T1), uninfested + treated with fungicide (T2), infested (*B. sorokiniana*) + treated (T3), infested (*B. sorokiniana*) + untreated (T4), infested (*F. graminearum*) + treated (T5), infested (*F. graminearum*) + untreated (T6). In each treatment, 100 seeds were planted in a single row at SDSU experimental stations at Volga and Brookings. All the treatments and the cultivars were randomized prior to planting and were planted in split plot design. For greenhouse study, 100 seeds were planted in paper cups (10 seeds/cup) for each treatment. Seed germination and seedling blight data was recorded after the germination for 3 consecutive weeks. Cultivars Russ (72%) and Oxen (80%) were highly affected for seed germination and seedling blight to both pathogens whereas Forefront (92), Select (95) and Briggs (88) had the higher germination and the higher seedling survival rate as compared to the other cultivars both under greenhouse and field conditions. The percent germination losses when the seed was infested with *F. graminearum* ranged from 17-35% while the seedling survival rate of the cultivars varied from 92-99%. In case of the seed infested with *B. sorokiniana*,

germination losses ranged from 2-15% with the only highest germination loss observed in Russ cultivar (32%) with the survival rate of all the cultivars ranged from 91-97%. Fungicide treatment (T3 and T5) significantly increased the seed germination from 14-37% and the seedling blight was also reduced in almost all the cultivars. In case of second experiment where oat kernels were used as a source of inoculum, reduction in percent seed germination was observed however, it was not significant.

Key words: *Bipolaris sorokiniana, Fusarium graminearum,* seed germination, seedling blight.

3.1 Introduction

Fusarium crown rot (FCR) and common root rot (CRR) are the important root diseases of wheat and barley that cause poor seed germination, seedling emergence, rotting of the root, crown, sub-crown and lower stem tissues thus impacting the crop productivity in the US Northern Great Plains (Cook 1981; Stack 1992; Paulitz et al., 2002; Strausbaugh et al., 2004; Fernandez et al., 2009).

The Fusarium crown rot (FCR) complex involves different *Fusarium* species which includes *F. graminearum* Schwabe (group II) (= *Gibberella zeae* (Schwein) Petch), *F. pseudograminearum* (O'Donnell & Aoki) (=*F. graminearum* group I, =*Gibberella coronicola*), (Paulitz et al., 2006; Pettitt et al., 2003), *F. acuminatum* Ellis & Ever., *F. culmorum* (Wm. G. Sm.) Sacc., *F. avenaceum* (Fr.) Sacc. (= *Gibberella avenacea*) and *Microdochium nivale* (Fr.) Samuels & I. C. Hallett (= *Monographella nivalis*) (Paulitz et al., 2002; Backhouse et al., 2004; Fernandez et al., 2004; Gonzalez et al., 2004). The disease caused yield loss up to 35% in the Pacific Northwest parts of the United States (Smiley et al., 2005). Kane et al. (1987) reported yield reduction up to 24% in winter wheat plots planted with seed infested by *F. graminearum* in New York.

Common root rot (CRR) of wheat is caused solely by *B. sorokiniana* (Sacc.) Shoemaker (= *C. sativus* (S. Ito & Kuribayashi) Drechsler ex Dastur) in the Northern Great Plains and Canadian Prairies (Gordon and Sprauge 1941; Cook 1968; Stack 1992; Fernandez et al., 2004; Moya-Elizondo et al., 2010). The yield losses up to 35% have been reported due to common root rot in wheat (Machacek 1943). However, the losses can vary from region to region. For example, in Australia yield loss of 13.9 to 23.9% were observed in susceptible cultivars (Wildermuth et al., 1992).

Grey and Mathre (1988) reported that *F. culmorum* reduced the plant emergence in 12 spring barley cultivars. But they did not observe the effect of inoculation on the grain yield when compared with the hand thinned control. Wong et al. (1992) reported that *F. graminearum* reduced the seed germination, seedling emergence and yield in wheat in Manitoba. There was a significantly lower seedlings survival rate, seedling root infection when the plants were inoculated with *F. acuminatum* as compared to the non-inoculated wheat plants (Mergoum et al., 1997). The seed germination and the seedling blight losses can be reduced through using fungicides as seed treatment in wheat (Jones 1999).

Given the current scenario of the increasing yield losses, there is a need to study how root pathogens affects the seed germination and seedling emergence in wheat cultivars grown in South Dakota. The specific objective of this study was to study the effect of *F. graminearum* and *B. sorokiniana* on seed germination and seedling blight in hard red spring wheat cultivars grown in South Dakota.

3.2 Materials and Methods

3.2.1 Experiment in the greenhouse

Eleven hard red spring wheat varieties, Advance, Brick, Briggs, Forefront, Oxen, Prevail, Russ, Select, SD 4189, SD 4125 and Traverse were evaluated in the greenhouse. The germination of all the cultivars was tested using paper towel method prior to planting (Rao et al., 2006). These cultivars seed were infested individually with the spore suspension of *F. graminearum* (Fg) isolate SD Fg41 and the *B. sorokiniana* (Bs) isolate SD40. For infesting the seed, fresh cultures of *F. graminearum* and *B. sorokiniana* were prepared and spore concentration was adjusted to 100K/ml and 3K/ml respectively. The spore suspension was sprayed with a hand held sprayer onto the seed of all 11 cultivars and the seed was dried by placing it on paper towel overnight on a lab bench. After the seed infestation, confirmatory test was done to see if the seed was infested 100% with the respective fungus by plating 100 infested seed of each cultivar on half strength PDA (Fig. 3.1). The plates were kept under 12 hours light and 12 hours' dark cycle at room temperature for 7 days. After 7 days, carmine red color colonies for the *F. graminearum* and olive green colonies for *B. sorokiniana* were observed. The data was recorded on the number of seeds infested with the fungus. The slides of the both fungi were prepared from the infested seed and observed under the microscopes for the fungal identity confirmation. One hundred seed of each cultivar were planted in paper cups (10 seed/cup) in a completely randomized design along with the un-infested 100 seeds as a control. The experiment was conducted in the greenhouse in 2014 and 2015. There were three treatments, T1 (un-infested seed served as a check), T2 (infested with *F. graminearum*) and T3 (infested seed with *B. sorokiniana*). The plants were watered daily and fertilized

as needed. The germination and the seedling blight data was recorded after 10 and 20 days of planting, respectively.

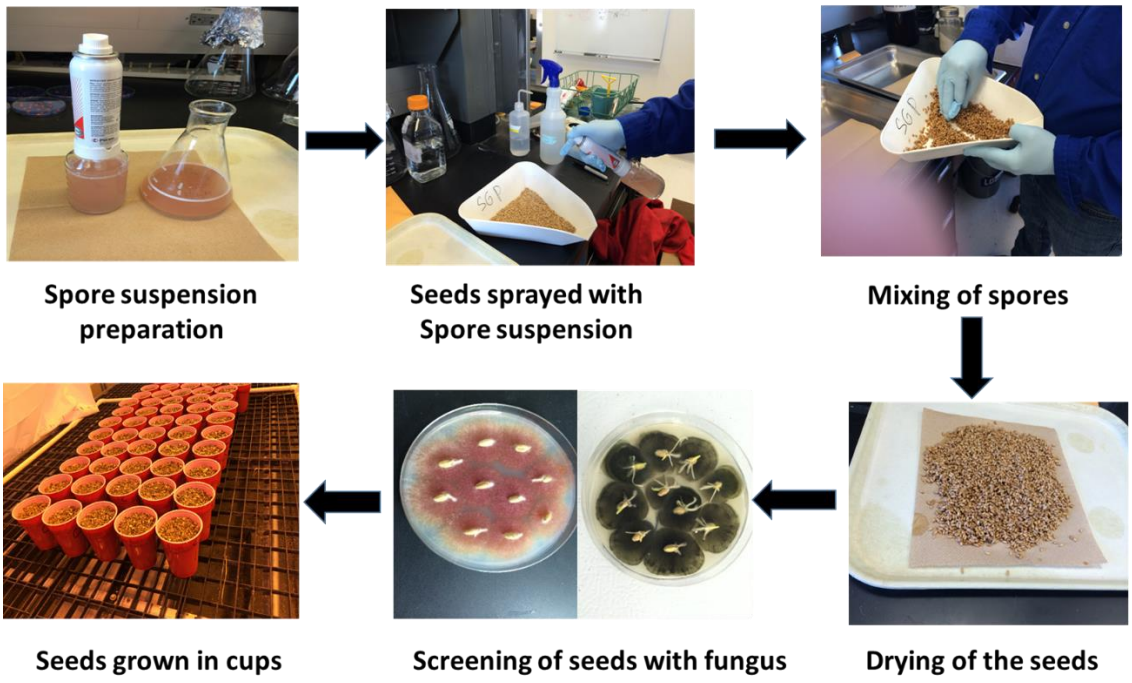

Figure 3.1 Schematic presentation of the experiment conducted in greenhouse

3.2.2 Experiment in the field

In 2015, seven cultivars Advance, Briggs, Forefront, Oxen, Russ, Prevail and one experimental line SD 4215 were evaluated under field conditions. In 2016, eleven cultivars, (Advance, Briggs, Forefront, Oxen, Russ, Prevail, Traverse, Select, Brick, SD4215 and SD4189) were evaluated. Before planting in the field, germination test of all the cultivars was done using the paper towel method (Rao et al., 2006). Two different experiments were conducted where in the first experiment, the main effect is considered as the infested seed as a source of inoculum as mentioned in the greenhouse experiment. In the second experiment, infested oat kernels with the two pathogens were used as a source of inoculum.

In experiment one (Experiment-I), six treatments were included: T1 (control); uninfested and untreated seed (UT+ Uinf); T2: uninfested seed and treated with the fungicide Raxil (T + Uinf); T3: infested with *B. sorokininana* and treated with fungicide [T + Inf (Bs)]; T4: infested seed with *B. sorokiniana* and untreated [UT + Inf (Bs)]; T5: infested seed with *F. graminearum* and treated with fungicide [T + Inf (Fg)] and T6: infested seed with *F. graminearum* and untreated with fungicide [UT + Inf (Fg)].

In second experiment (Experiment-II), the source of inoculum was oat kernels infested with *F. graminearum* or *B. sorokiniana*. The treatments used in this experiments were: Untreated and uninfested (T1), uninfested and fungicide treated seed (T2), fungicide treated uninfested seed + oat infested with *B. sorokiniana* (T3), untreated uninfested seed + oat kernels infested with *B. sorokiniana* (T4), fungicide treated uninfested seed + oat kernels infested with *F. graminearum* (T5) and untreated uninfested seed + oat kernels infested with *F. graminearum* (T6). Before infesting the oats kernels with the respective fungus, they were autoclaved under wet cycle to eliminate any fungal infection. The conidial suspension of *F. graminearum* and *B. sorokiniana* was prepared and adjusted to 100K/ml and 3K/ml respectively. Five grams of infested oat kernels were placed along with the seed of each treatment in a row.

Experiments were planted by hand in three replications in a split plot design at two locations, Brookings and Volga in 2015 and 2016. One hundred seeds were planted manually in a 5’ 3’ row. The treatments and cultivars were randomized and each plot consists of cultivars and each cultivar had six treatments. The germination data was recorded when the plants started to come out. The stand counts data were taken for three consecutive weeks post planting, respectively.

Figure 3.2 Taking germination notes after one week of planting

Figure 3.3 One field plot replication with six treatments in Volga, SD [T1 (UT+ Uinf), T2 (T +Uinf), T3 (T + Inf (Bs)), T4 (UT + Inf (Bs)), T5 (T+Inf (Fg)) and T6 (UT+Inf (Fg)]

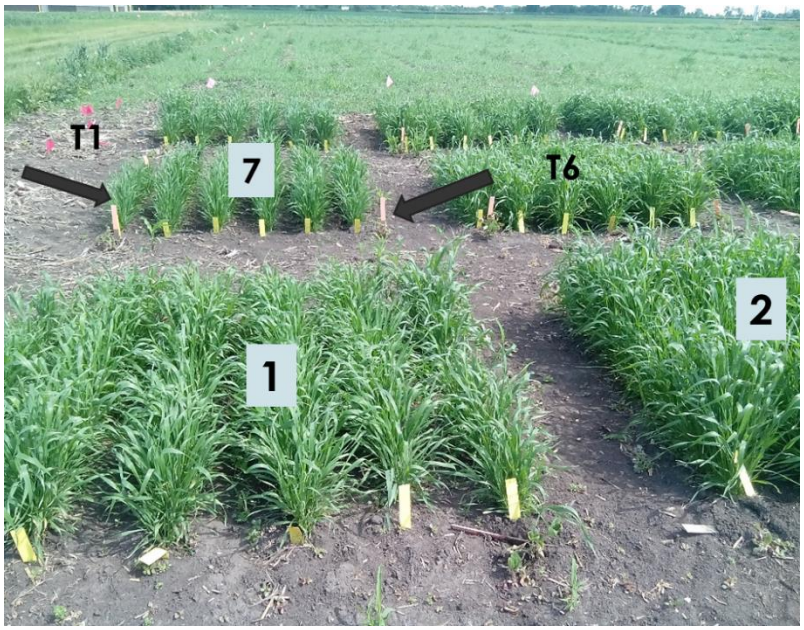

Figure 3.4 Experiment planted in Brookings and Volga with three replications and six treatments

Figure 3.5 Field experimental plot at maturity in Volga in the year 2015

3.3 Data analysis

Data was analyzed using SAS 9.4 (SAS Institute, Cary, NC, U.S.A) software. PROC GLM was used to find out the descriptive statistics on the percent seed germinated and percent seedling survival. As the data was calculated in percentage, so the data was transformed using the arcsine transformation. T-test was conducted to compare the cultivars if they were different for treatments.

3.4 Results

3.4.1 Effect of *Fusarium graminearum* and *Bipolaris sorokiniana* on seed germination and seedling blight in the greenhouse environment

3.4.1.1 Greenhouse season-I

The seed germination and seedling establishment was affected in seed infested with *F. graminearum* with loss in germination ranged from 6-27% while the seedling survival rate of the cultivars ranged from 45 to 87% and the seedling blight range from 7-28% (Fig. 3.6, Fig. 3.7). The seed germination and seedling survival in control was ~100 percent. The cultivars were significantly different in germination when the seed was infested with *F. graminearum*. Russ (73%) and Oxen (75%) were more susceptible showing more reduction in germination whereas Forefront (94%), Brick (92%) and Select (91%) had higher germination (Fig. 3.6). The percent survival of all the cultivars was significantly lower ($P < 0.05$) when the seed was infested with *F. graminearum* as compared to uninfested seed and seed infested with *B. sorokiniana* (Fig. 3.7).

In the case of seed infested with *B. sorokiniana*, the germination losses ranged from 2-10% with the highest germination loss in Russ (32%) (Fig. 3.8). The seedling survival rate of in trial ranged from 43% to 96% and seedling blight varied from 0-25%

(Fig. 3.9). Seed germination was significantly reduced in almost all the cultivars with the Russ (68%) being the most susceptible cultivar to seed germination in the trial. On the other hand, cultivars Forefront (98%), Select (91%), Brick (98%) and Prevail (98%) showed least impact of *B. sorokiniana* infestation and had the higher germination. Overall, there were higher germination losses and more seedling blight in case of *F. graminearum* infested seed as compared to *B. sorokiniana* infested seed treatments (Fig. 3.10, 3.11).

3.4.1.2 Greenhouse season-II

In the second run of greenhouse experiment similar results were obtained. The cultivars were more prone to seed germination and seedling blight when the seed was infested with *F. graminearum* as compared to *B. sorokiniana* (Fig. 3.16, 3.17). The cultivars Russ and Oxen were highly affected for seed germination and seedling blight with both pathogens. The cultivars Forefront, Select and Briggs had the highest germination and the higher survival rates as compared to the other cultivars. The percent germination losses when the seed was infested with *F. graminearum* ranged from 3-39% while the seedling survival rate of the cultivars ranged from 53- 86% and the seedling blight range from 8-26% (Fig. 3.12, Fig. 3.13). The lowest (61%) germination was recorded in Russ cultivar and the highest (97%) germination was observed in Forefront cultivar.

In case of seed infested with *B. sorokiniana*, the germination losses ranged from 2-24% with the highest germination loss was observed in Russ cultivar (24%) (Fig. 3.14). The survival rate in the trial ranged from 74 to 96% while the seedling blight varied from 0-7% (Fig. 3.15). Seed germination was significantly reduced in almost all the cultivars,

with the least germination observed in Russ cultivar. The cultivars Forefront, Select, Brick and Prevail had the highest germination as compared to other cultivars. Similar trend in treatment effect was observed in both greenhouse experiments, where higher germination losses and more seedling blight in case of *F. graminearum* infested seed as compared to *B. sorokiniana* infested seed (Fig 3.16, 3.17).

In both greenhouse experiments, out of the eleven cultivars evaluated for seed germination and seedling blight, a significant difference was observed among the cultivars. The cultivars differed significantly for the germination and the seedling survival when the seed was either infested with *F. graminearum* or *B. sorokiniana* ($P < 0.05$). However, germination was lower in case of *F. graminearum* infested seed as compared to the *B. sorokiniana* infested seed.

3.4.2 Field Study-2015

3.4.2.1 Effect of infested seed as a source of inoculum (Experiment-I)

The fungus *F. graminearum* significantly affect the germination of almost all the cultivars at both locations Brookings and Volga ($P < 0.05$) (Fig. 4.1, 4.3). Cultivars Russ and Oxen were susceptible to both pathogens while Advance, Forefront and Prevail were resistant and had the highest germination as compared to other cultivars (Fig. 4.2, 4.4). The percent germination loss due to *F. graminearum* ranged from 8-28% in Brookings and 26-42% in Volga, respectively. There was no significant difference observed for the percent seedling survival in all the cultivars. There was an increase in germination when the seed was treated with fungicide (4-13%) as compared to the untreated seed (control). Fungicide treatment improved the germination in case of infested seed ranging from 6-26% at Brookings and 23-47% at Volga. The germination losses in case of *B. sorokiniana*

infested seed were not much prominent as were observed with *F. graminearum* (ranged from 4-15%) at both locations (Fig. 4.6, 4.8). Use of fungicide as a seed treatment improved the seed germination from 2-13% whether the seed with or without the fungus (Fig 4.5, 4.7).

3.4.2.2 Effect of infested oats kernels as a source of inoculum (Experiment-II)

F. graminearum significantly affect the germination of almost all the cultivars in the field at both locations Brookings and Volga ($P < 0.05$) (Fig 4.9,4.11). The cultivars Russ and Oxen were susceptible to both pathogens while Advance, Forefront and Prevail had the highest germination as compared to other cultivars (Fig 4.10, 4.12). The percent germination losses due to *F. graminearum* ranged from 2-4% in Brookings and 1-15% in Volga respectively. There was no significant difference observed for the percent seedling survival in all the cultivars. There was an increase in germination when the treatment included the fungicide treatment (2-8%) as compared to the control. Fungicide treatment improved the germination in case of the infested seed ranging from 3-8% at Brookings and 1-22% at Volga. The germination losses in case of the *B. sorokiniana* infested seed were ranged from 7-24% at Brookings and 2-9% at Volga (Fig. 4.14, 4.16). Use of fungicide as a seed treatment improved the germination from 2-13% whether the seed was with or without the fungus (Fig. 4.13, 4.15).

3.4.3 Field study-2016

3.4.3.1 Effect of infested Seed as a source of inoculum (Experiment-I)

Similar trend among the treatments was observed in 2016 experiment as was seen in 2015. The fungus *F. graminearum* significantly affect the germination in almost all the cultivars in Brookings and Volga ($P < 0.05$) (Fig. 4.17, 4.19). The cultivars Russ and

Oxen were susceptible to both these pathogens while Advance, Forefront, Prevail, and SD4189 had the highest germination as compared to other cultivar (Fig. 4.18, 4.20). The percent germination losses due to *F. graminearum* ranged from 3-11% in Brookings and 6-22% in Volga respectively. But there was no significant difference observed for the percent seedling survival in all the cultivars. All the 11 cultivars had the higher survival rates thus no significant seedling blight. There was an increase in germination when the treatment included the fungicide treatment (1-13%) as compared to the control. Fungicide treatment improved the germination in case of the infested seed ranging from 1-12% at Brookings and 6-22% at Volga. The germination losses in case of *B. sorokiniana* infested seed (ranged from 3-9%) were not as high as with *F. graminearum* at both locations (Fig. 4.22, 4.24). Use of fungicide as a seed treatment improved the germination from 2-15% (Fig. 4.21, 4.23).

3.4.3.2 Effect of Infested oats kernels as a source of inoculum (Experiment-II)

A similar pattern of reduced germination was observed as was noted in experiment I (Fig. 4.25,4.26). However, treatments and cultivars did not differ significantly for seed germination and seedling survival in the year 2016 ($P>0.05$). The germination losses were recorded from 1-8 percent. And almost all 11 cultivars had more than 95% seedling survival rates.

3.4.4 Comparison of greenhouse and field study

Both the greenhouse and field experiments reflect a similar pattern of germination of the seven HRSW cultivars to both pathogens. Germination losses were significantly higher in the *F. graminearum* infested seed as compared to the *B. sorokiniana* infested seed; however, percent seed germination ranges were different between the greenhouse

and field experiments. There was a similar trend in seed germination of all the cultivars at both locations and years. Higher germination losses were observed in 2015 as compared to 2016. Also, higher germination losses were observed in Volga as compared to Brookings (Fig. 4.27).

3.5 Discussion

The results obtained from the greenhouse experiments indicated that there were significant differences in response to seed germination and seedling blight in the cultivars when the seed was infested with *F. graminearum* and *B. sorokiniana*. *F. graminearum* significantly reduced the germination and caused seedling blight in most of the cultivars as compared to the *B. sorokiniana* which had less severe impact on seed germination and seedling blight. Our results validate the variability for resistance to seed germination and seedling blight exist in cultivars as reported by several studies from different parts of the United States (Grey and Mathre, 1987; Wong et al., 1992; Hill and Blunt.,1994; Mergoum et al.,1997; Galli et al., 2005).

The experiment conducted in the greenhouse showed that the percent germination and seedling survival of all the cultivars was also significantly lower when the seed was infested with *F. graminearum* and *B. sorokiniana* as compared to uninfested seed (control). The percent seed germination losses were higher when the seed was infested with *F. graminearum* range from 6-27% and the seedling blight range from 7-28%, while the germination losses range from 2-10% and seedling blight vary from 0-25% in case of seed infested with *B. sorokiniana.* The seed germination was significantly reduced in most of the cultivars with the least germination was observed in Russ cultivar and the highest germination was recorded in Forefront, Select, Brick and Prevail.

In the field study, similar treatments trend was observed as was in the greenhouse on seed germination but the seedling survival rate was not significantly different in the cultivars showing that there was minimal seedling blight in the field. The trend of the reduced seed germination observed in the field was similar to the greenhouse experiment and validated our greenhouse results, however, there were no significant differences observed for the seedling survival in all the cultivars under field conditions. The treatment that included fungicide as seed treatment improved the seed germination in the range of l4-37% and suggests a promising method to improve seed germination and seedling survival caused by these two pathogens. Similar results were obtained in Minnesota (Jones l999), who reported that the use of the surface sterilized seed increased the seed germination up to 32% and also the seed treatment with maneb- or thiabendazole-containing fungicide combinations significantly reduced seedling blight and improved crop stands derived from the *Fusarium*-damaged seed lot. The results of other studies conducted in different parts of the Unites States supported our results (Jones l999; Galli et al., 2005).

In the second experiment where infested oat kernels were used as a source of inoculum showed the similar treatments trend but they were not significantly different. The percent germination losses were lower as compared to the experiment I, where seed was infested directly. Further, there were no significant differences observed for the percent seedling survival indicating that there was no significant seedling blight observed under the field conditions. Experiments conducted at two different locations over the two years resulted the similar trend of reduction in seed germination but there were more germination loss observed in Brookings as compared to in Volga in 2016. The soil

moisture was lower in Brookings as compared to Volga and that may have triggered more germination losses in Brookings as compared to in Volga.

Our study in greenhouse and field experiments results shows a significant reduction in seed germination of the hard red spring wheat cultivars with the seed infested with *F. graminearum* and *B. sorokiniana*. Use of fungicide as a seed treatment can reduce the germination losses caused by these two pathogens. Also weather conditions may play an important role in the disease incidence so the management practices should be adopted accordingly.

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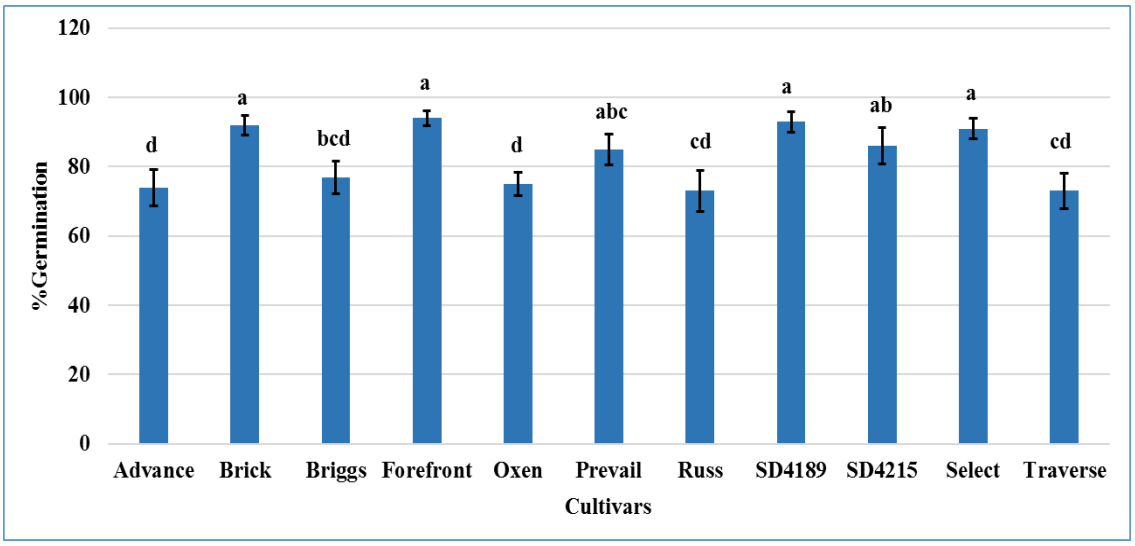

Figure 3.6 Effect of *Fusarium graminearum* on seed germination of 11 HRSW cultivars

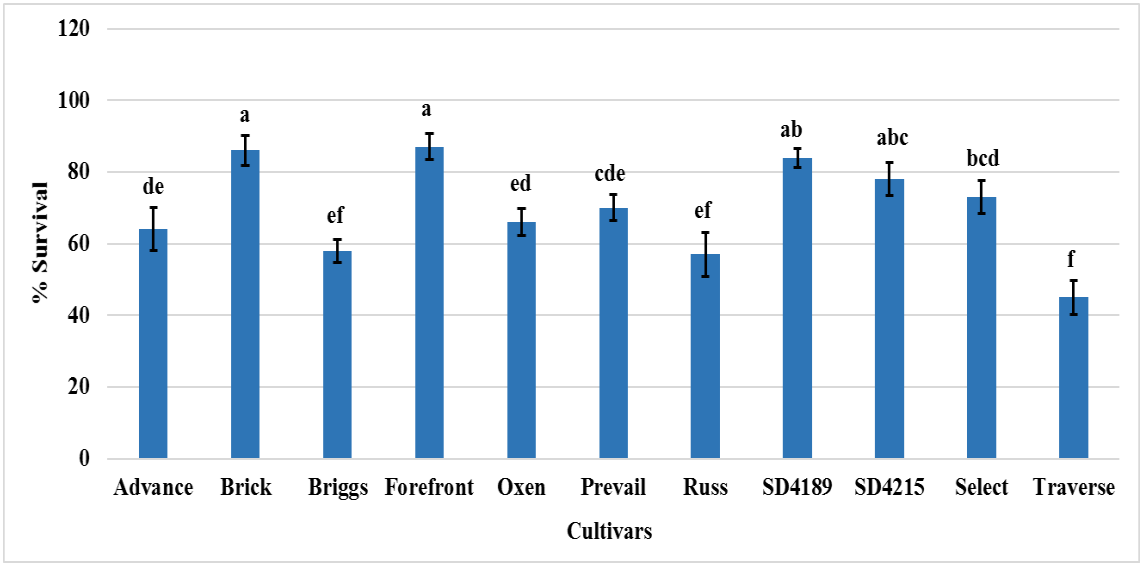

Figure 3.7 Effect of *Fusarium graminearum* on seedlings survival of 11 HRSW cultivars

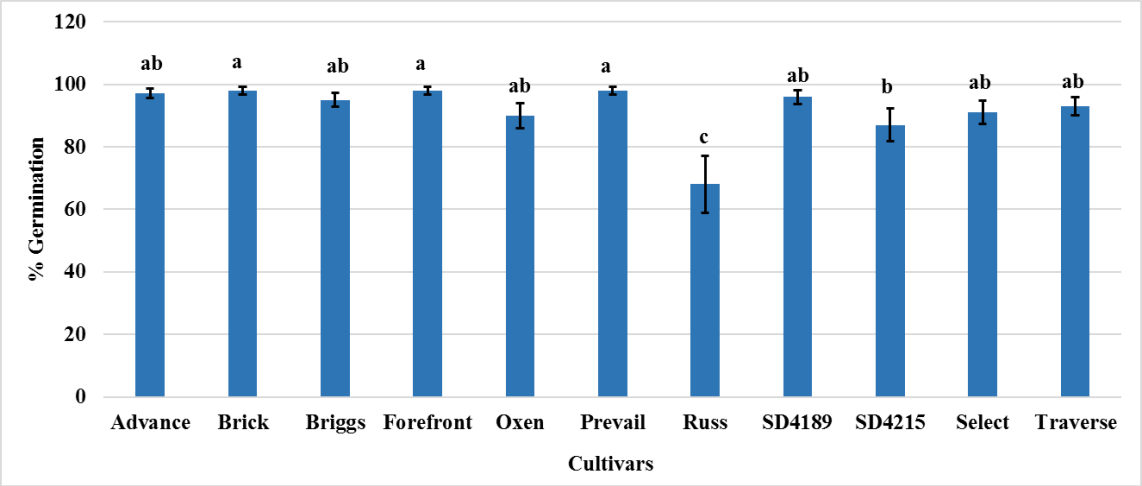

Figure 3.8 Effect of *Bipolaris sorokiniana* on seed germination of 11 HRSW cultivars

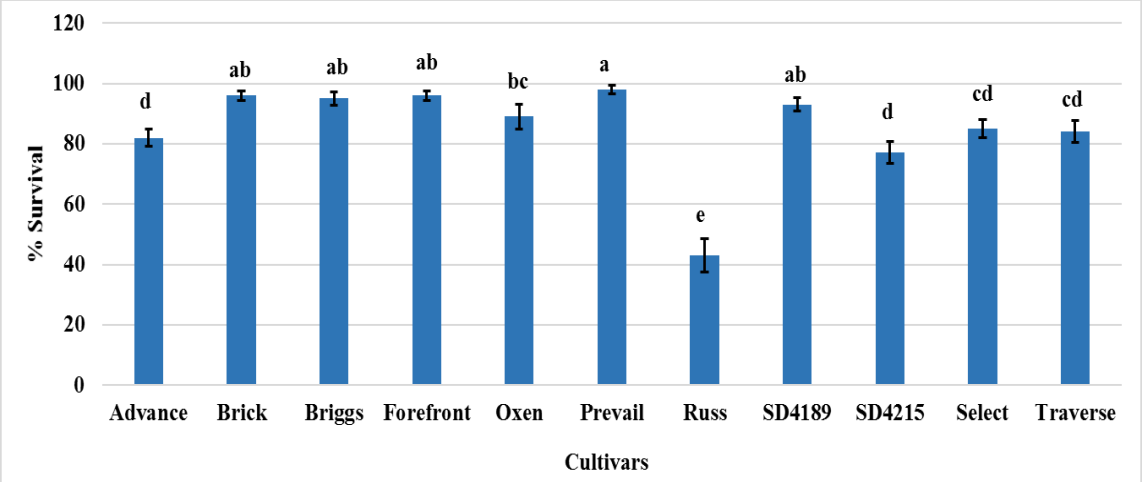

Figure 3.9 Effect of *Bipolaris sorokiniana* on seedlings survival of 11 HRSW cultivars

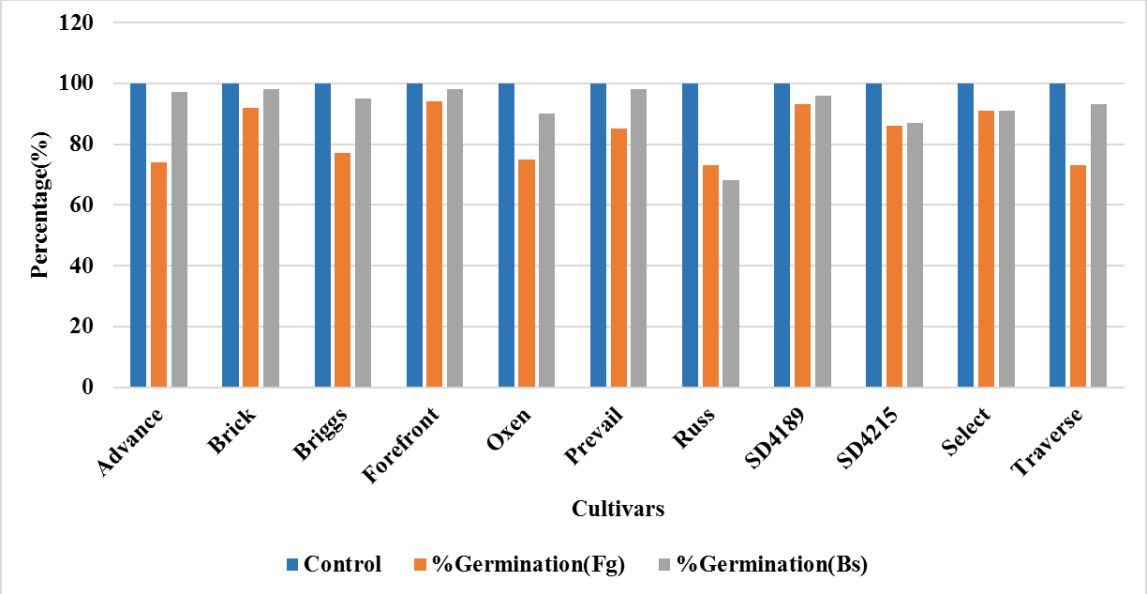

Figure 3.10 Comparison of percent seed germination affected by *Fusarium graminearum* and *Bipolaris sorokiniana* in 11 HRSW cultivars

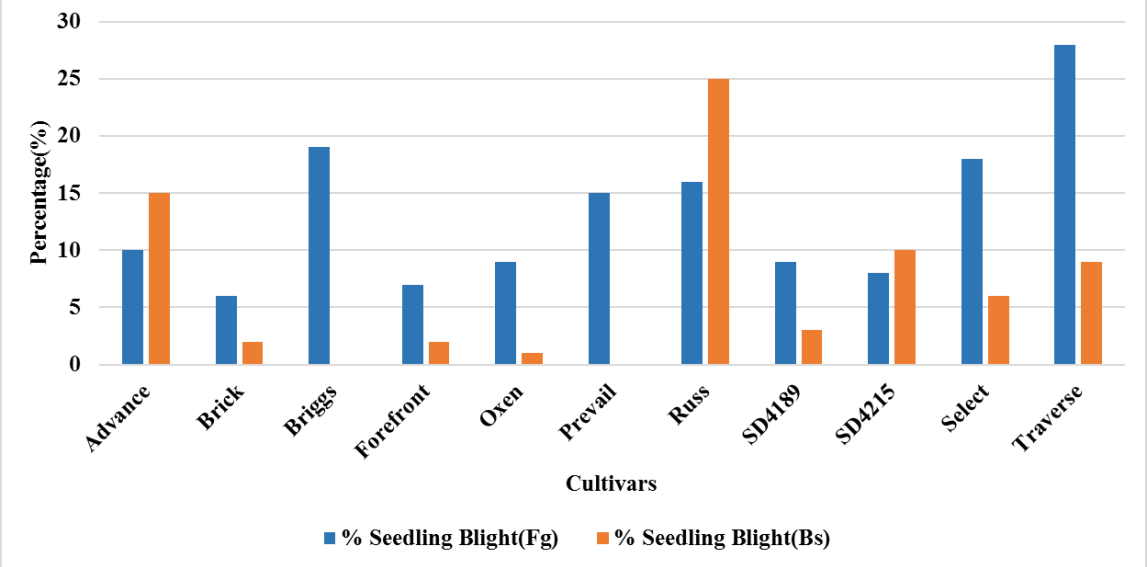

Figure 3.11 Comparison of percent seedling blight affected by *Fusarium graminearum* and *Bipolaris sorokiniana* in 11 HRSW cultivars

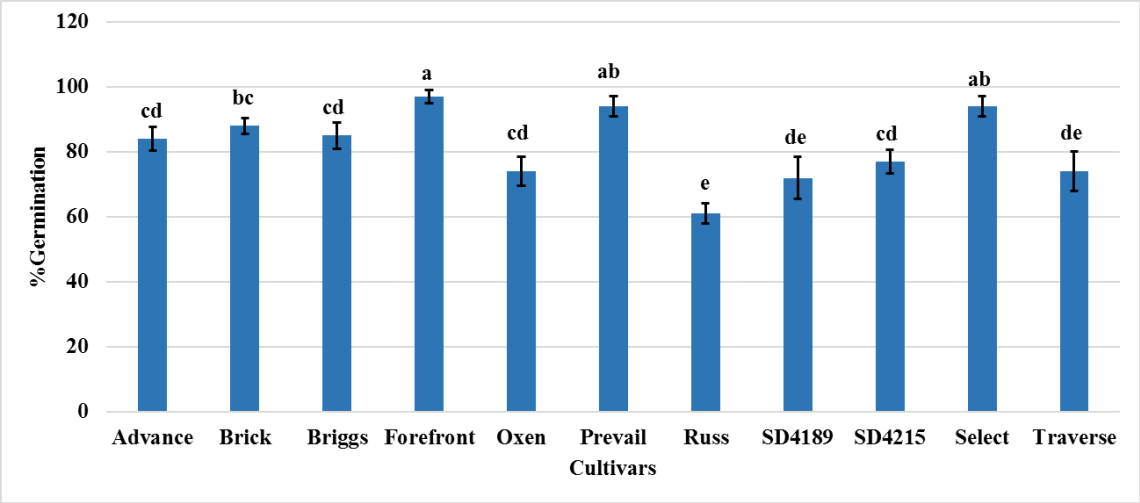

Figure 3.12 Effect of *Fusarium graminearum* on seed germination of 11 HRSW cultivars (Greenhouse Experiment-II)

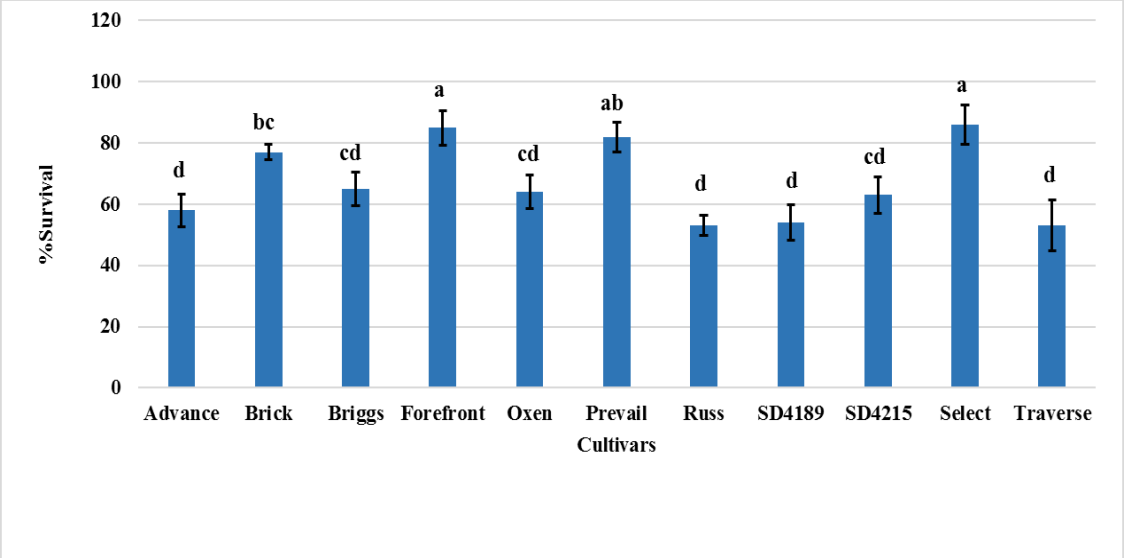

Figure 3.13 Effect of *Fusarium graminearum* on seedling survival of 11 HRSW cultivars (Greenhouse Experiment-II)

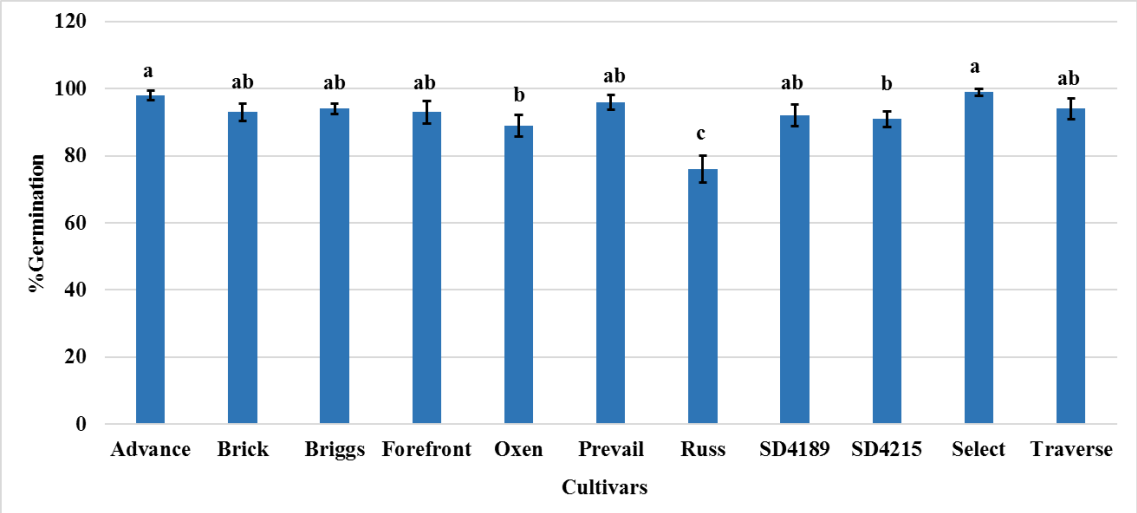

Figure 3.14 Effect of *Bipolaris sorokiniana* on seed germination of 11 HRSW cultivars (Greenhouse Experiment-II)

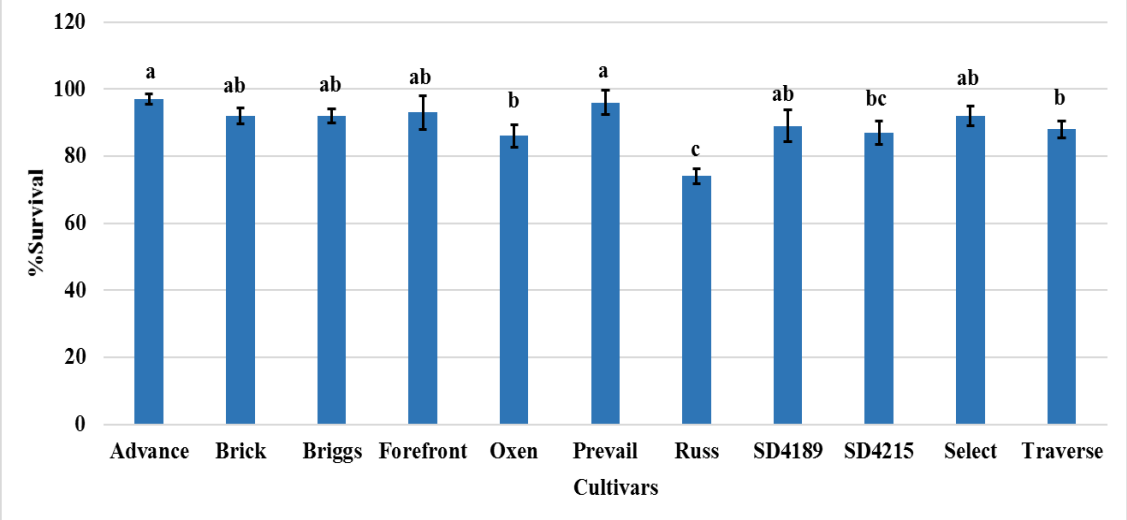

Figure 3.15 Effect of *Bipolaris sorokiniana* on seedling survival of 11 HRSW cultivars (Greenhouse Experiment-II)

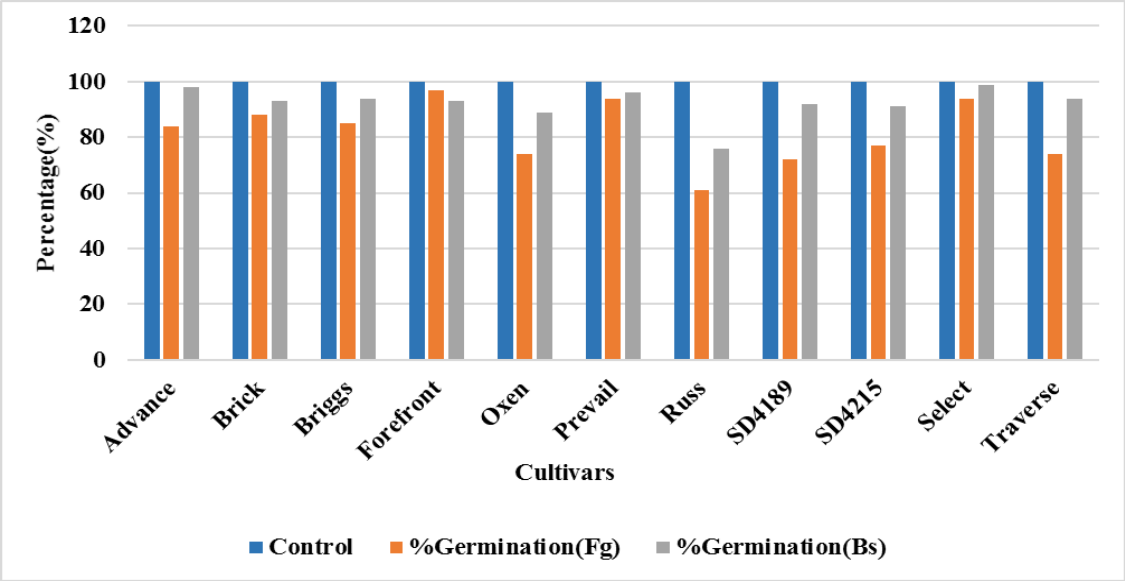

Figure 3.16 Comparison of percent seed germination affected by *Fusarium graminearum* and *Bipolaris sorokiniana* in 11 HRSW cultivars (Greenhouse Experiment-II)

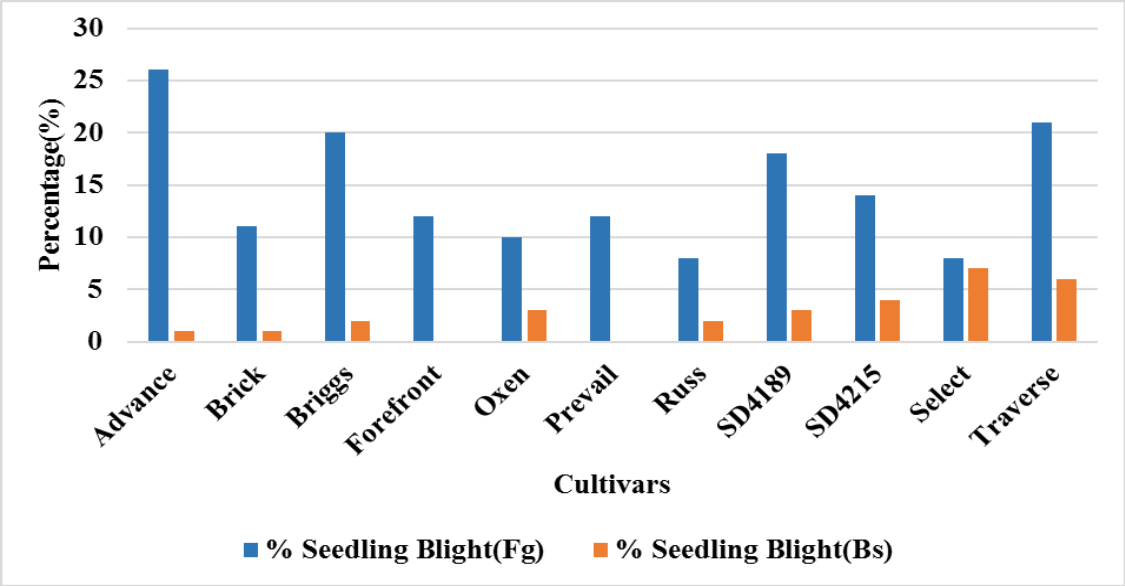

Figure 3.17 Comparison of percent seedling blight affected by *Fusarium graminearum* and *Bipolaris sorokiniana* in 11 HRSW cultivars (Greenhouse experiment- II)

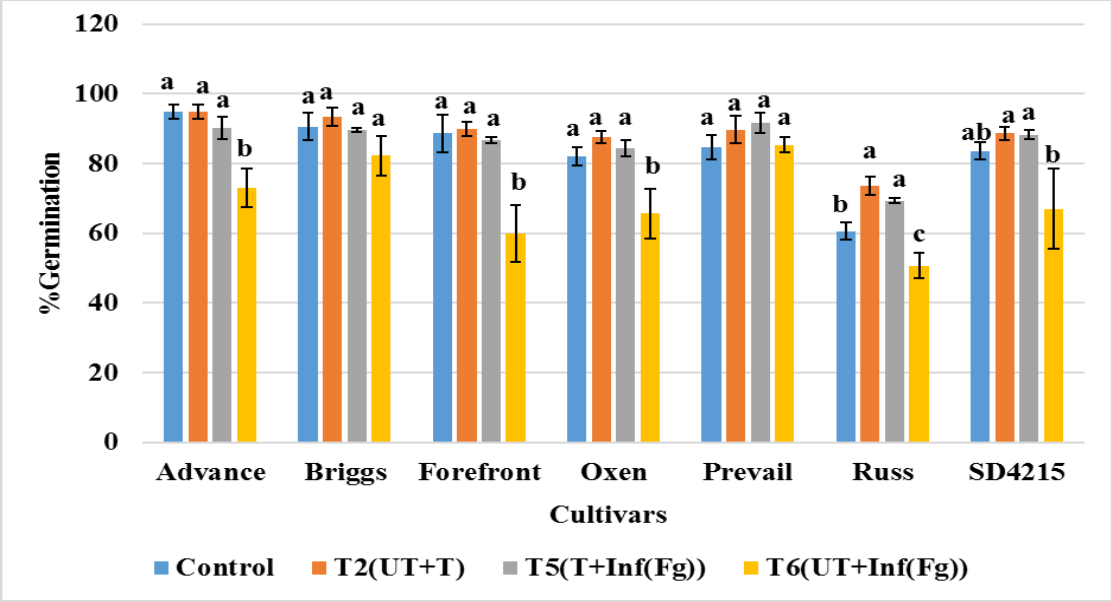

Figure 4.1 Effect of *Fusarium graminearum* on seed germination of seven HRSW cultivars planted in Brookings in 2015 (Experiment-I)

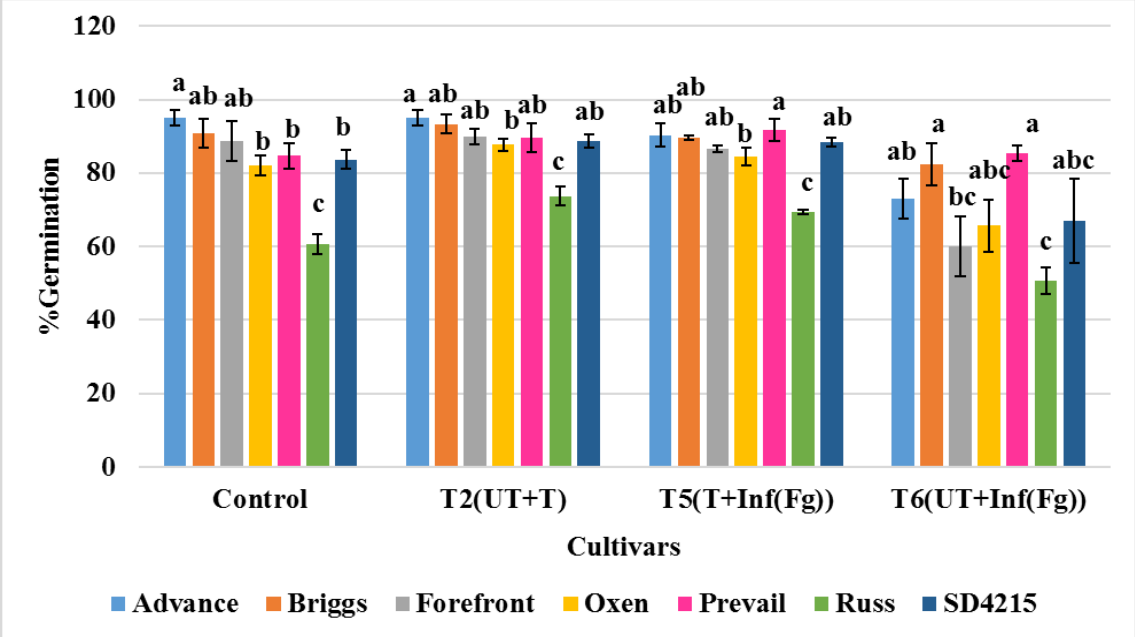

Figure 4.2 Differences in seven HRSW cultivars seed germination to different treatments planted in Brookings in 2015 (Experiment-I)

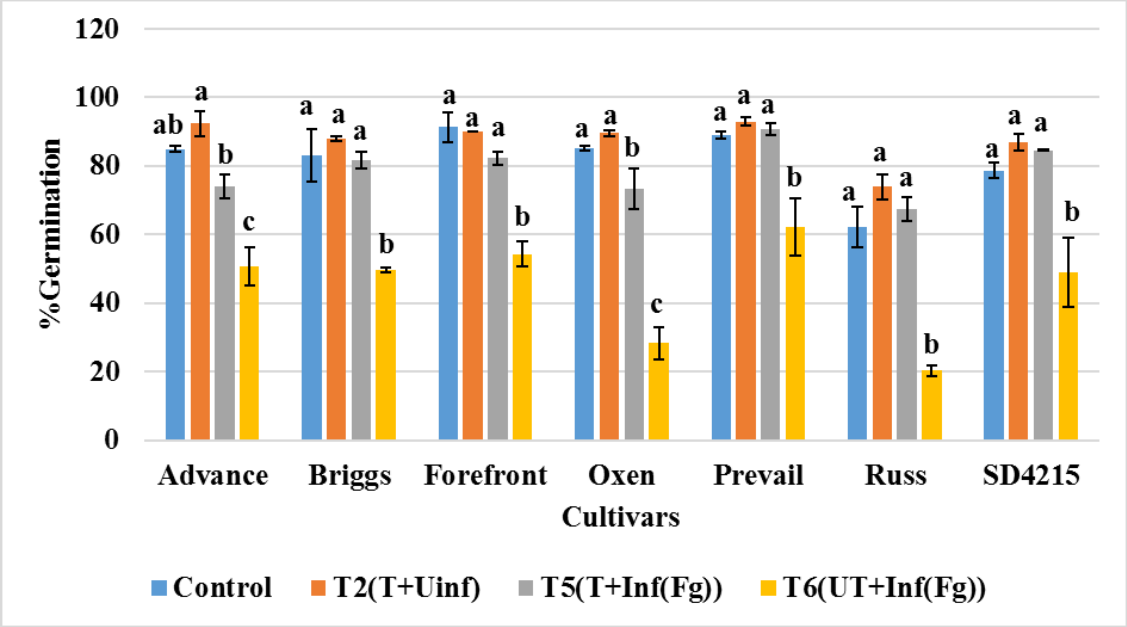

Figure 4.3 Effect of *Fusarium graminearum* on seed germination of HRSW cultivars planted in Volga in 2015 (Experiment-I)

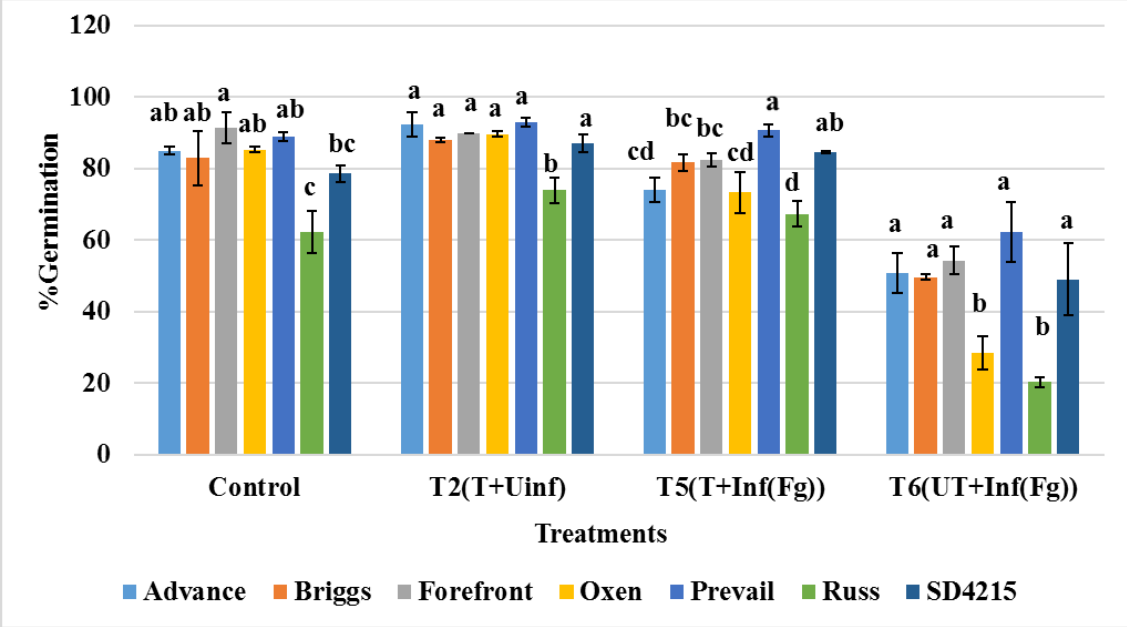

Figure 4.4 Differences in the cultivars seed germination to different treatments planted in Volga in 2015 (Experiment-I)

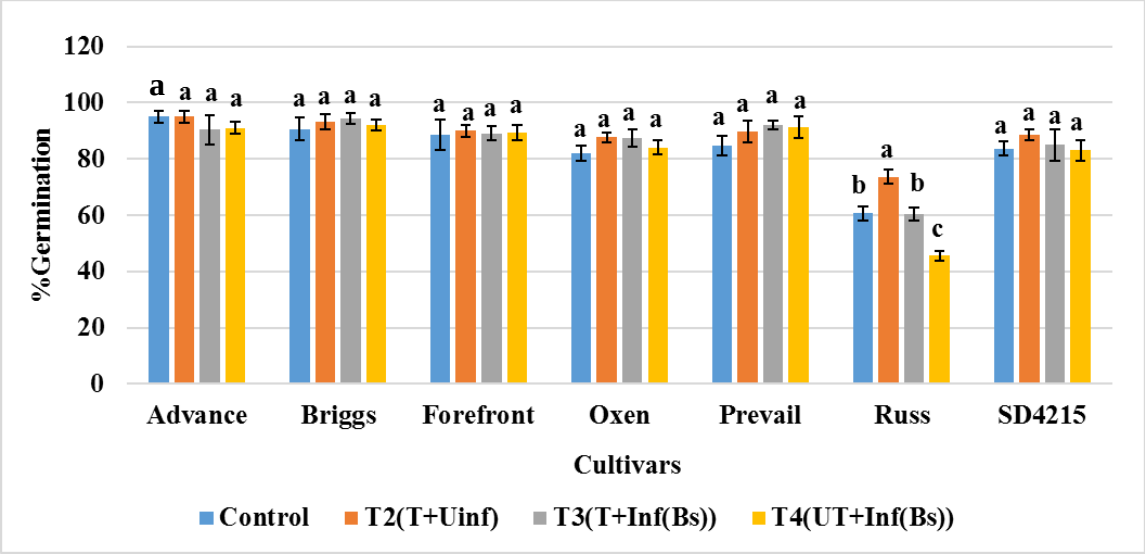

Figure 4.5 Effect of *Bipolaris sorokiniana* on seed germination of HRSW cultivars planted in Brookings in 2015 (Experiment-I)

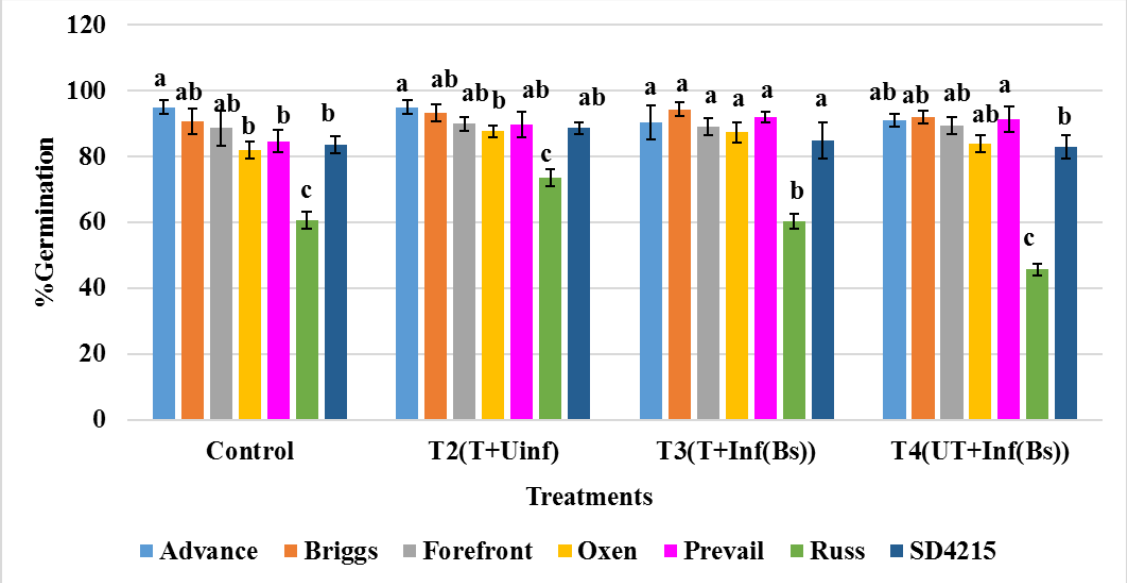

Figure 4.6 Differences in the cultivars seed germination to different treatments planted in Brookings in 2015 (Experiment-I)

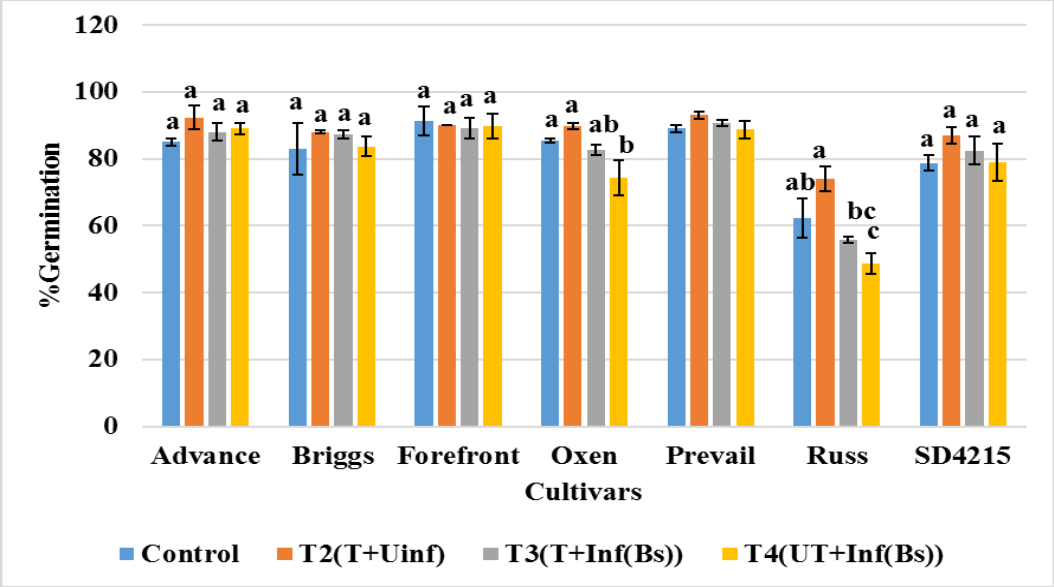

Figure 4.7 Effect of *Bipolaris sorokiniana* on seed germination of HRSW cultivars planted in Volga in 2015 (Experiment-I)

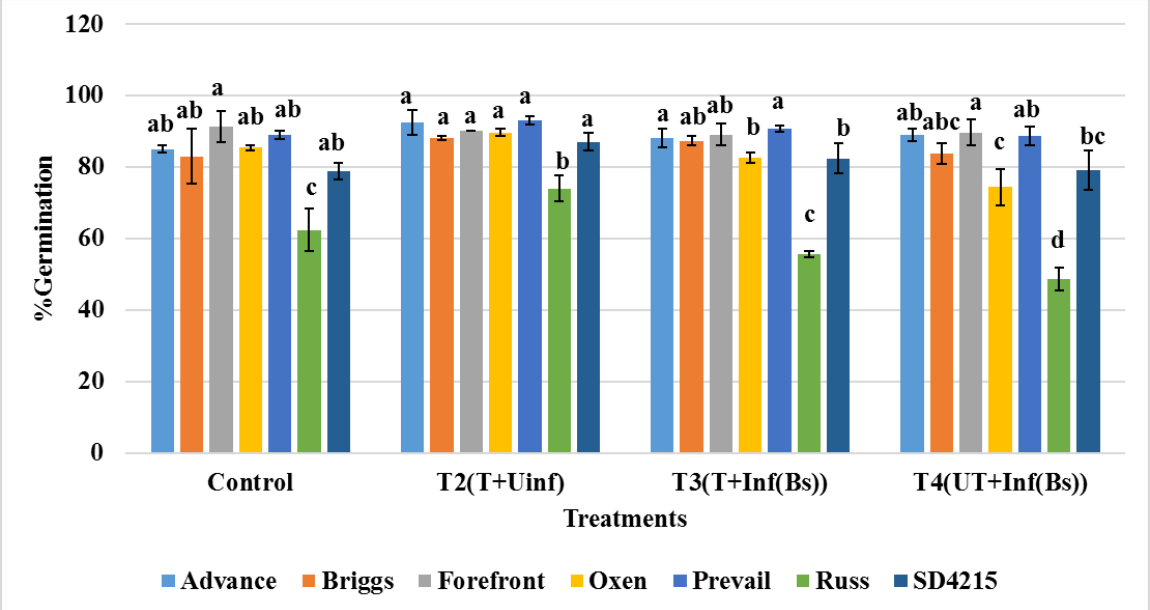

Figure 4.8 Differences in the cultivars seed germination for the different treatments planted in Volga in 2015 (Experiment-I)

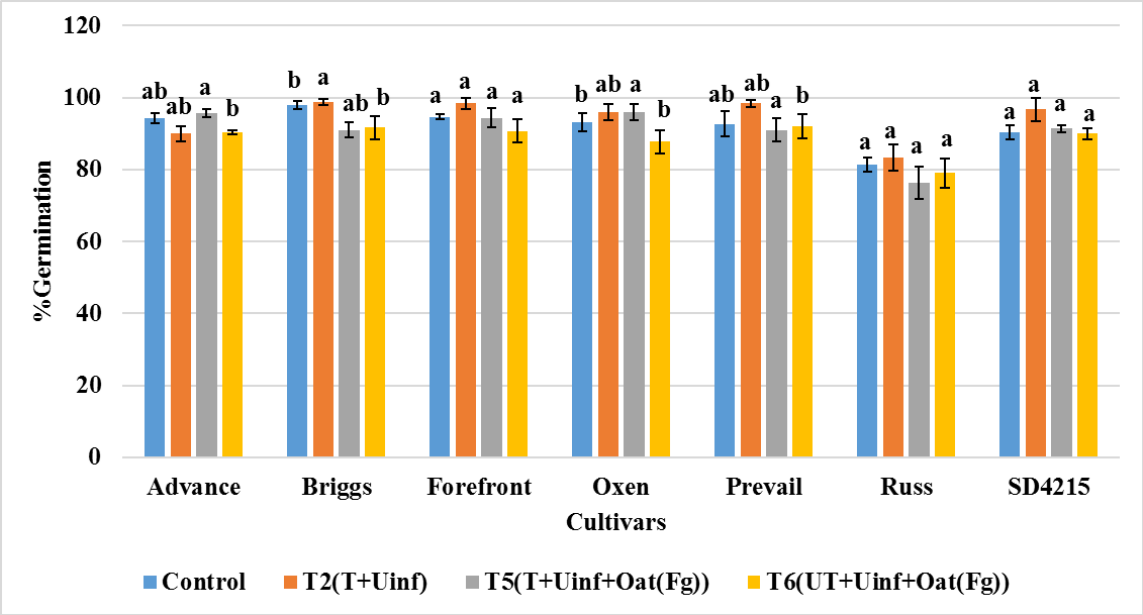

Figure 4.9 Effect of *Fusarium graminearum* on the seed germination of HRSW cultivars planted in Brookings in 2015 (Experiment-II)

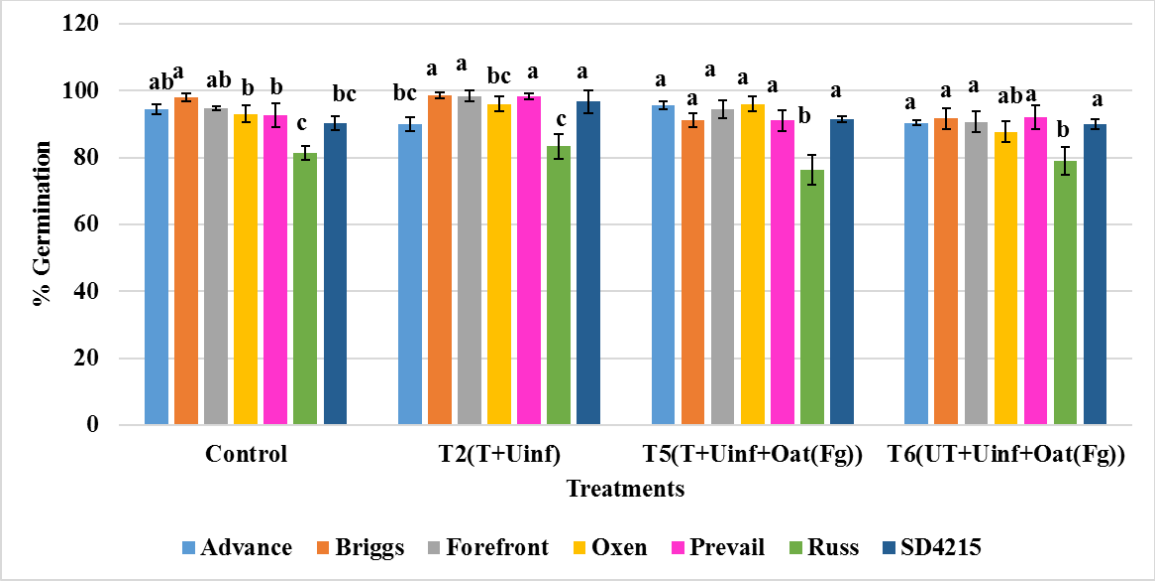

Figure 4.10 Differences in the cultivars for seed germination to different treatments planted in Brookings in 2015 (Experiment-II)

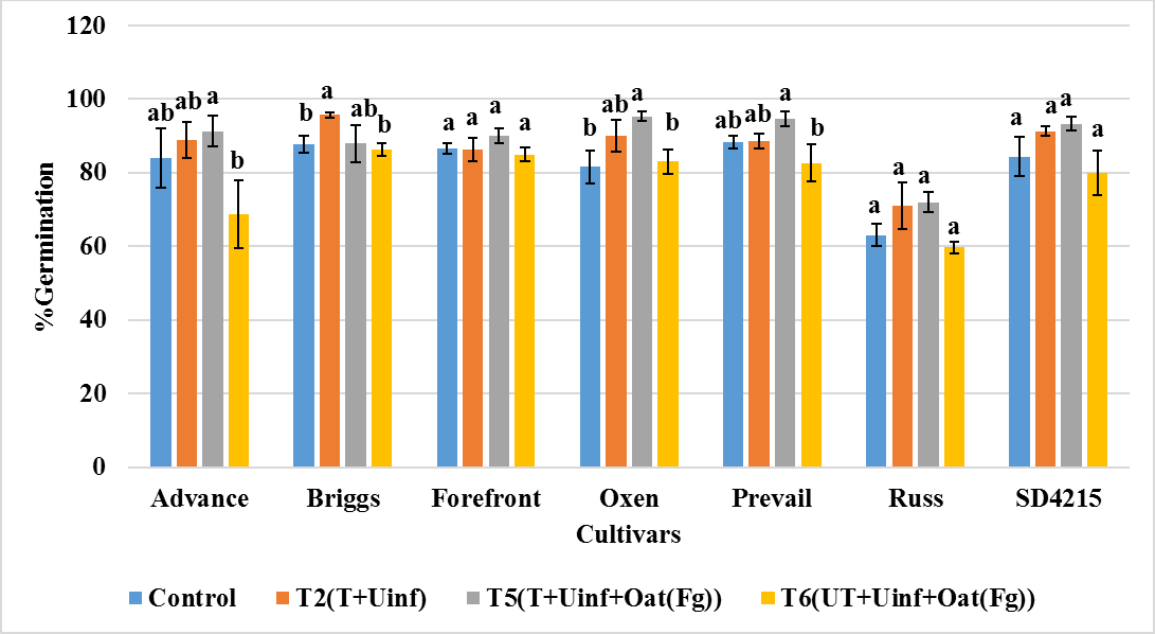

Figure 4.11 Effect of *Fusarium graminearum* on seed germination of HRSW cultivars planted in Volga in 2015 (Experiment-II)

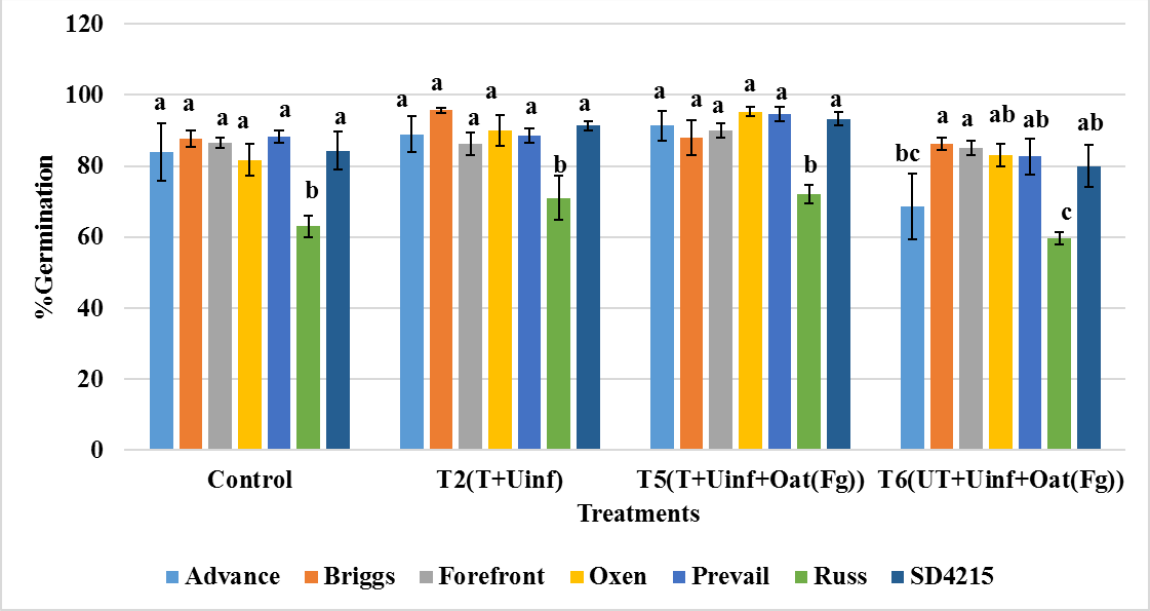

Figure 4.12 Differences in the cultivars seed germination seed to different treatments planted in Volga in 2015 (Experiment-II)

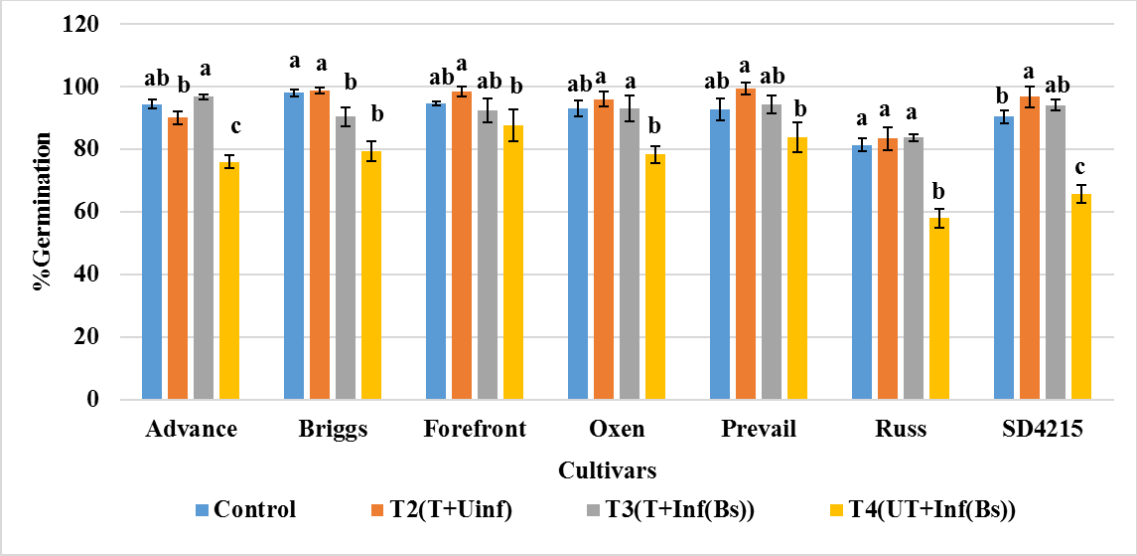

Figure 4.13 Effect of *Bipolaris sorokiniana* on seed germination of HRSW cultivars planted in Brookings in 2015 (Experiment-II)

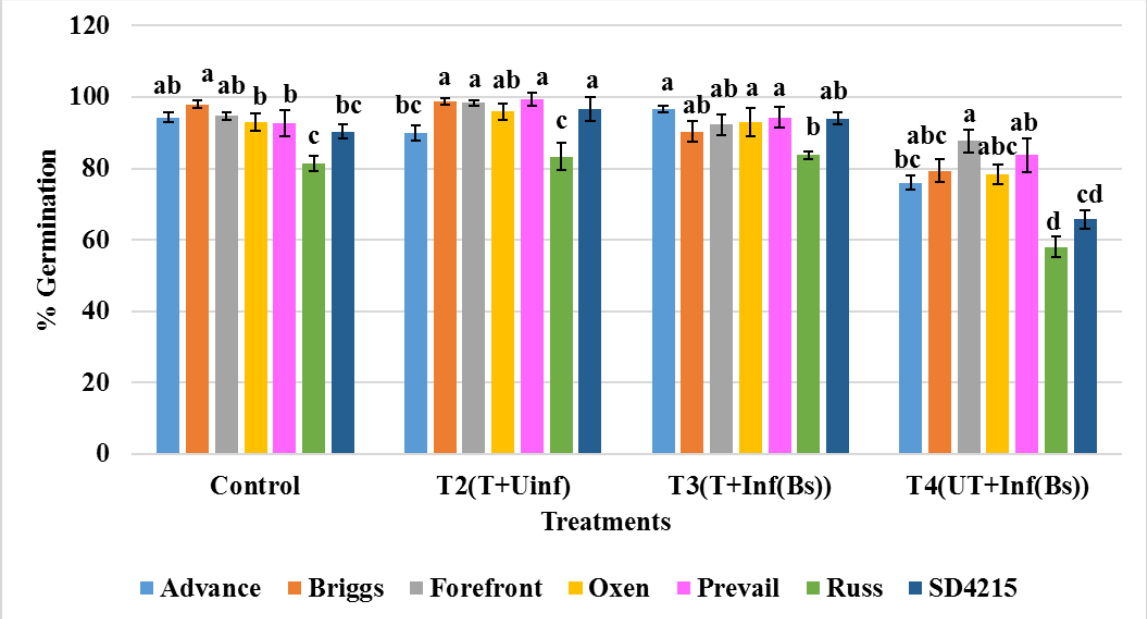

Figure 4.14 Differences in the cultivars seed germination to different treatments planted in Brookings in 2015 (Experiment-II)

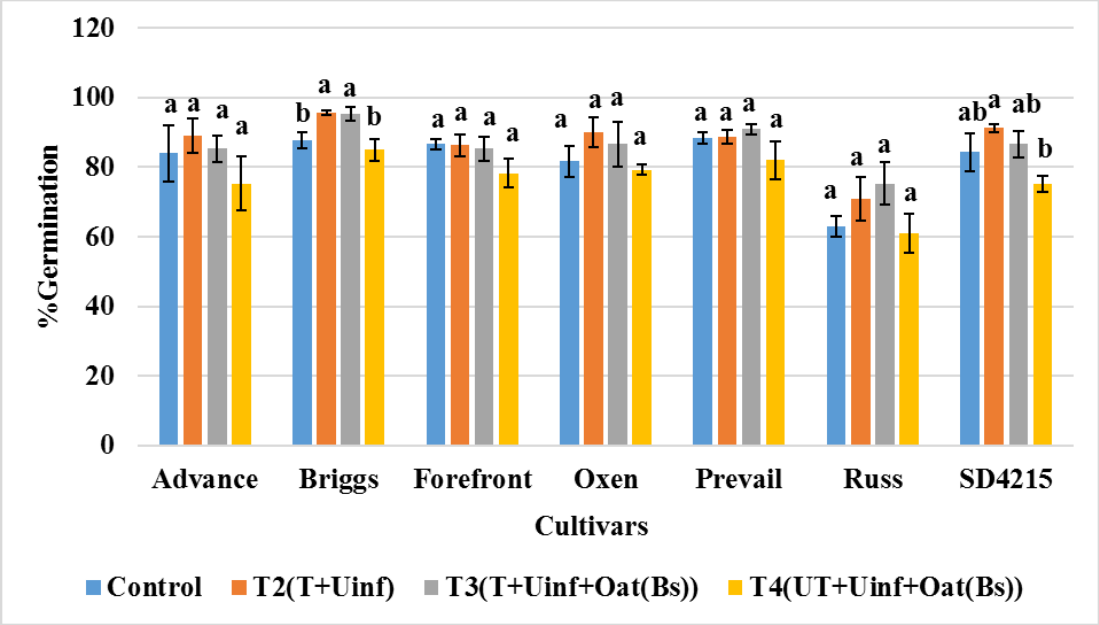

Figure 4.15 Effect of *Bipolaris sorokiniana* on seed germination of HRSW cultivars planted in Volga in 2015 (Experiment-II)

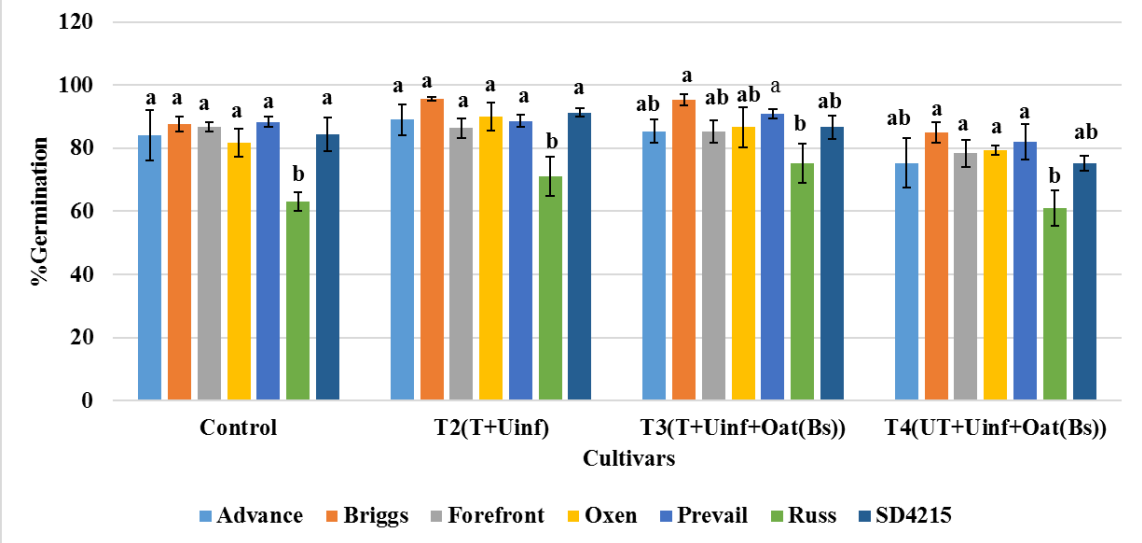

Figure 4.16 Differences in the cultivars seed germination to different treatments planted in Volga in 2015 (Experiment-II)

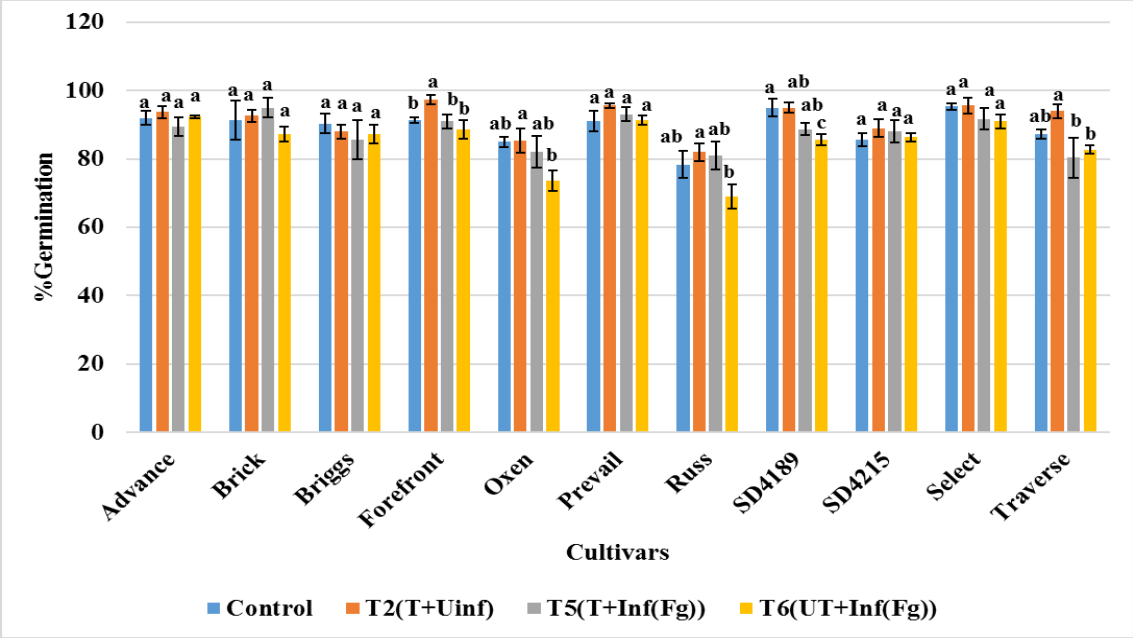

Figure 4.17 Effect of *Fusarium graminearum* on seed germination of HRSW cultivars planted in Brookings in 2016 (Experiment-I)

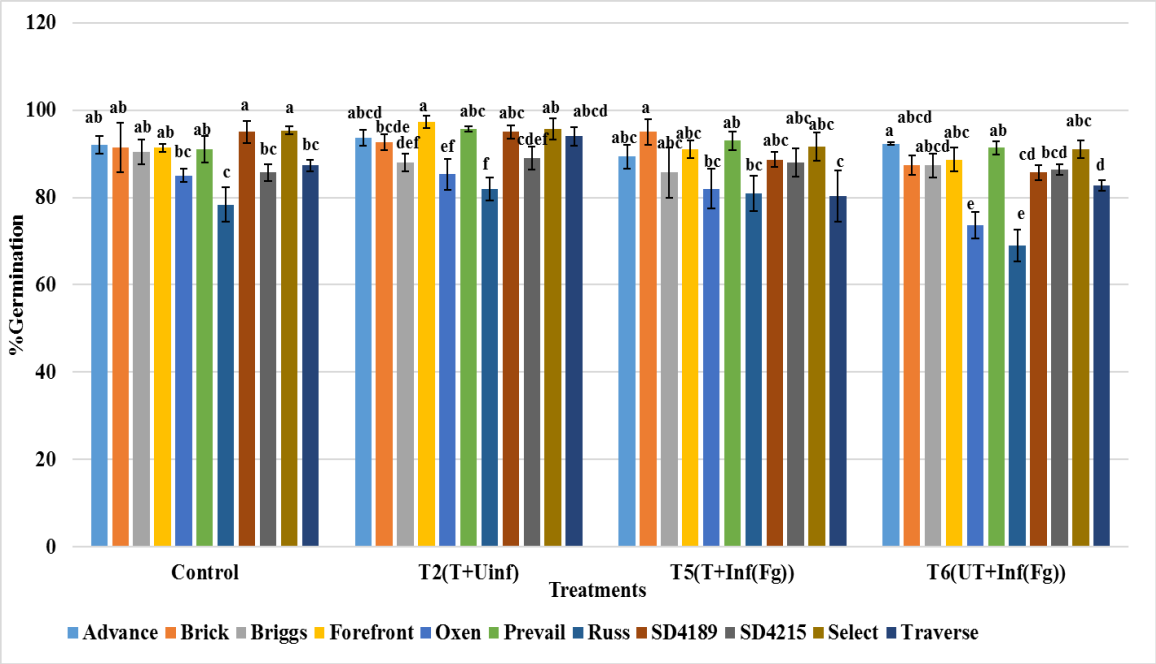

Figure 4.18 Differences in the cultivars seed germination to different treatments planted in Brookings in 2016 (Experiment-I)

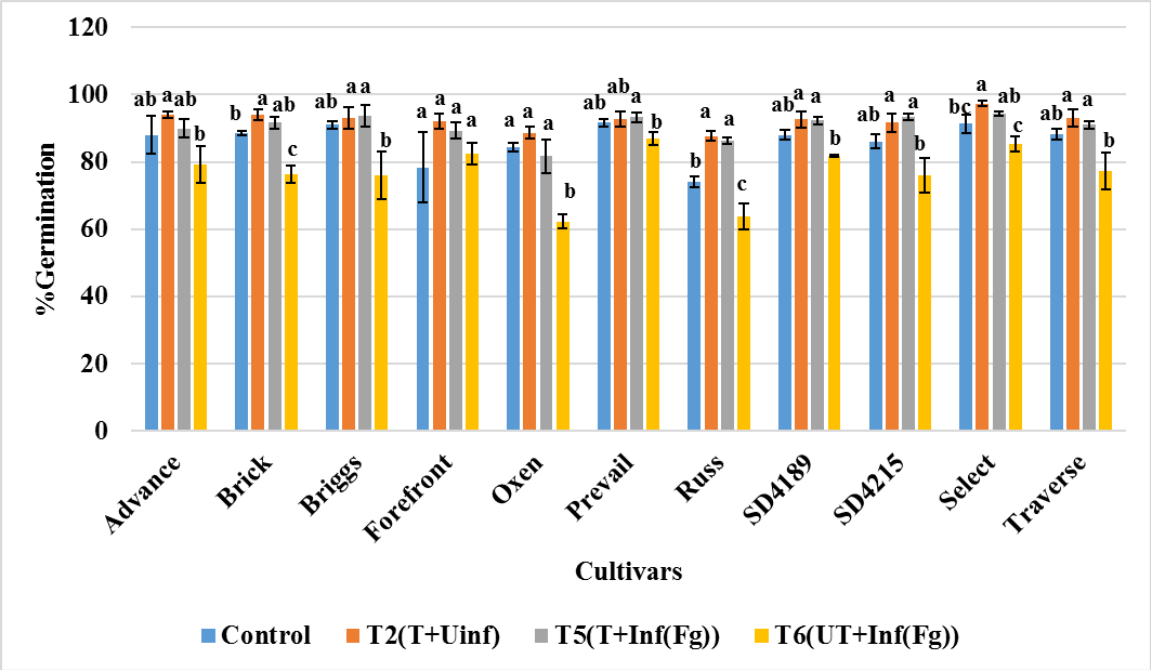

Figure 4.19 Effect of *Fusarium graminearum* on seed germination of 11 HRSW cultivars planted in Volga in 2016 (Experiment-I)

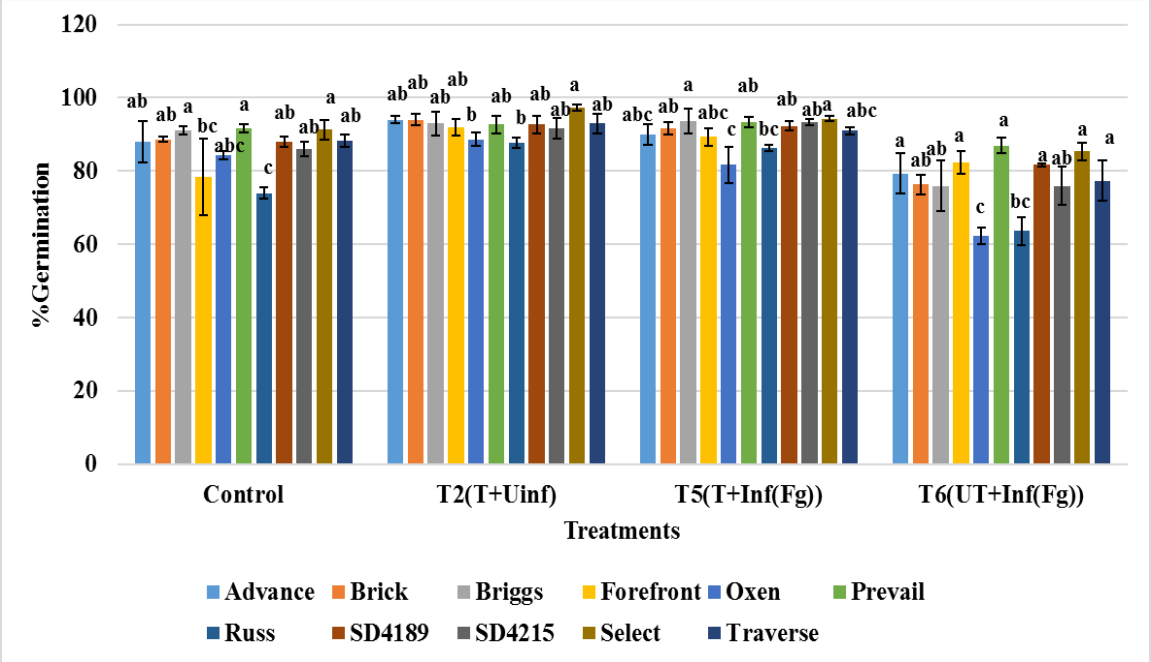

Figure 4.20 Differences in the 11 HRSW cultivars seed germination to different treatments planted in Volga in 2016 (Experiment-I)

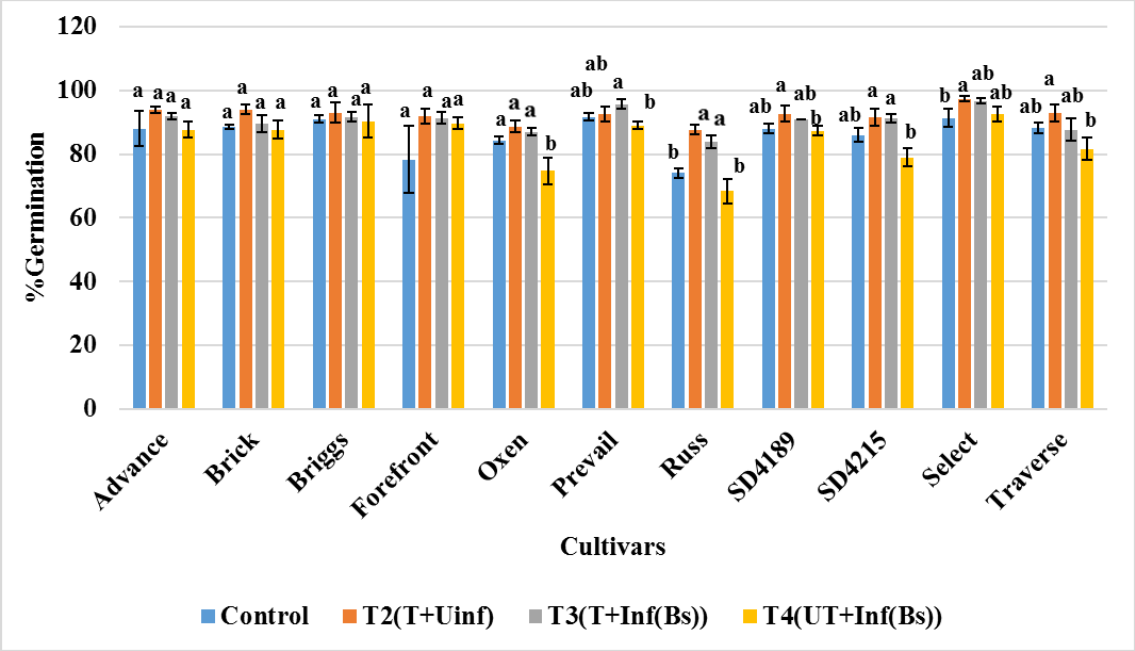

Figure 4.21 Effect of *Bipolaris sorokiniana* on seed germination of 11 HRSW cultivars planted in Brookings in 2016 (Experiment-I)

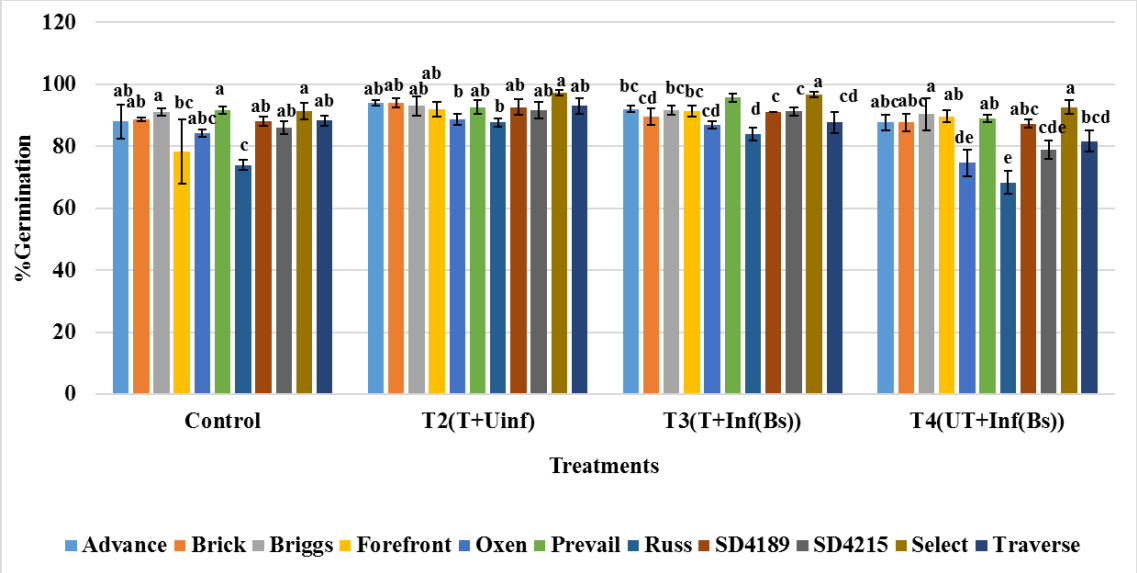

Figure 4.22 Differences in 11 HRSW cultivars seed germination to different treatments planted in Brookings in 2016 (Experiment-I)

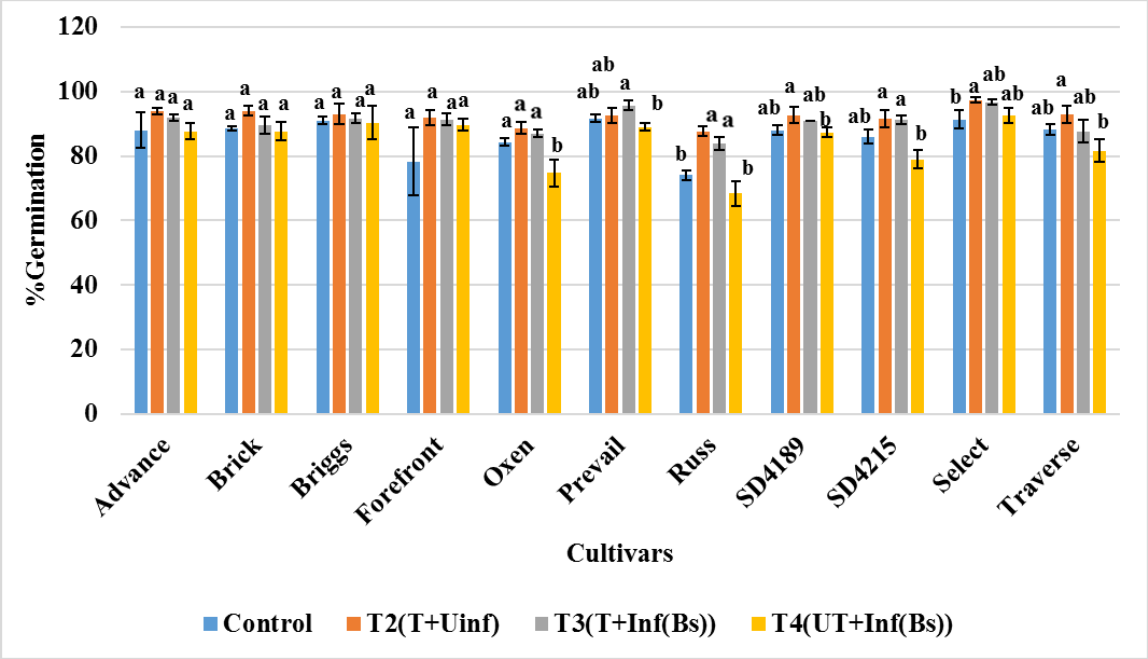

Figure 4.23 Effect of *Bipolaris sorokiniana* on seed germination of 11 HRSW cultivars planted in Volga in 2016 (Experiment-I)

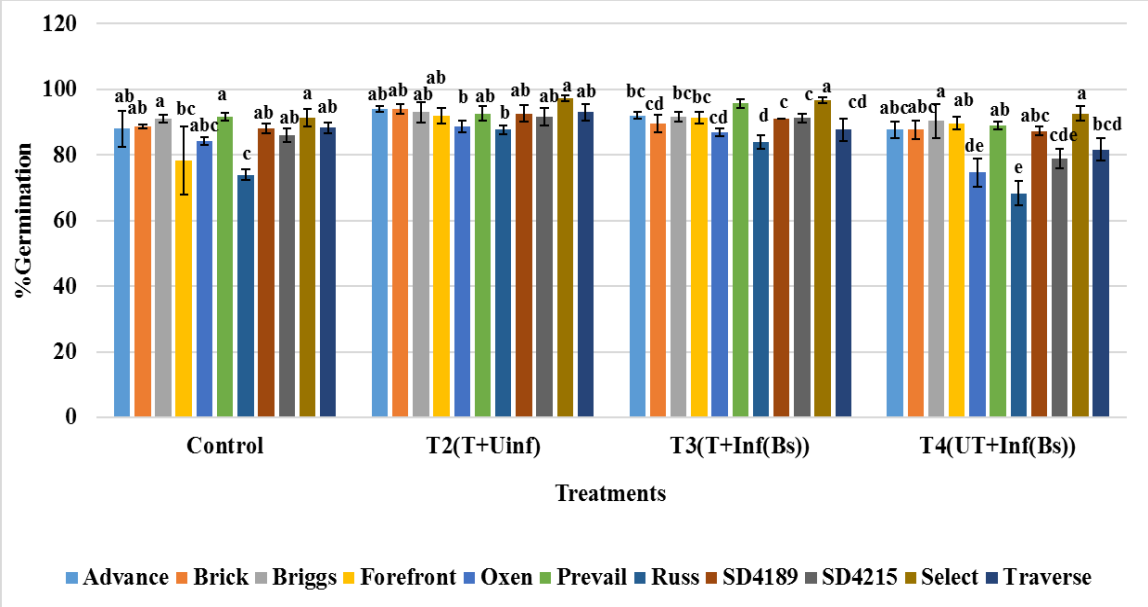

Figure 4.24 Differences in eleven HRSW cultivars seed germination to different treatments planted in Volga in 2016 (Experiment-I)

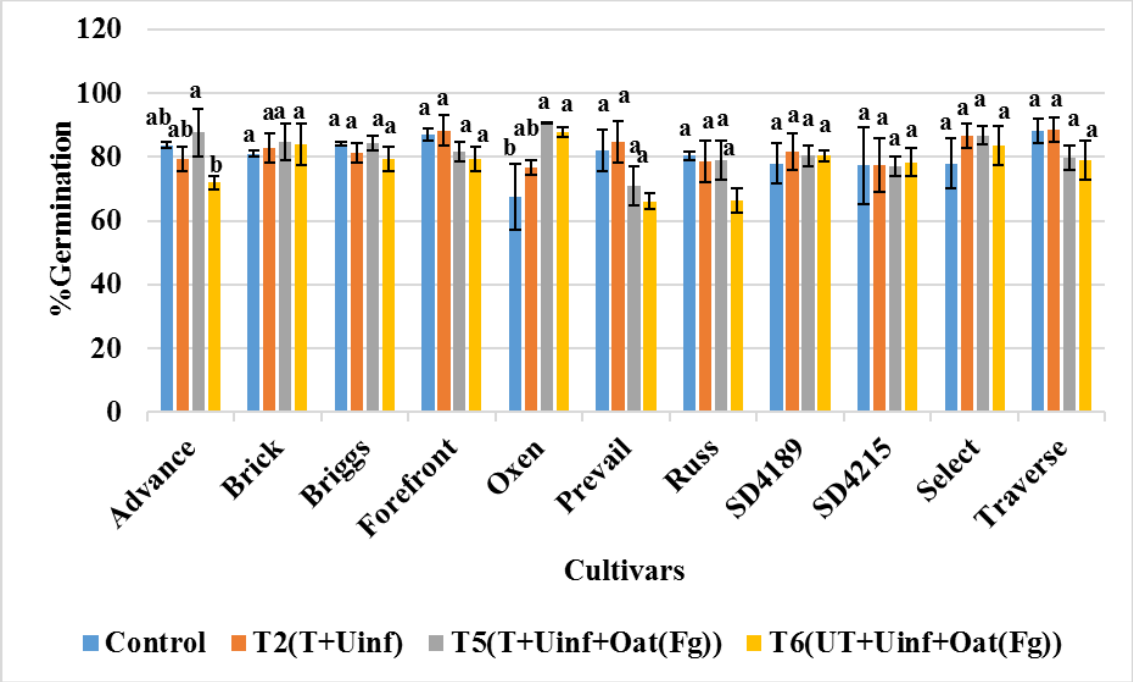

Figure 4.25 Effect of *Fusarium graminearum* on seed germination of 11 HRSW cultivars planted in Brookings in 2016 (Experiment-II)

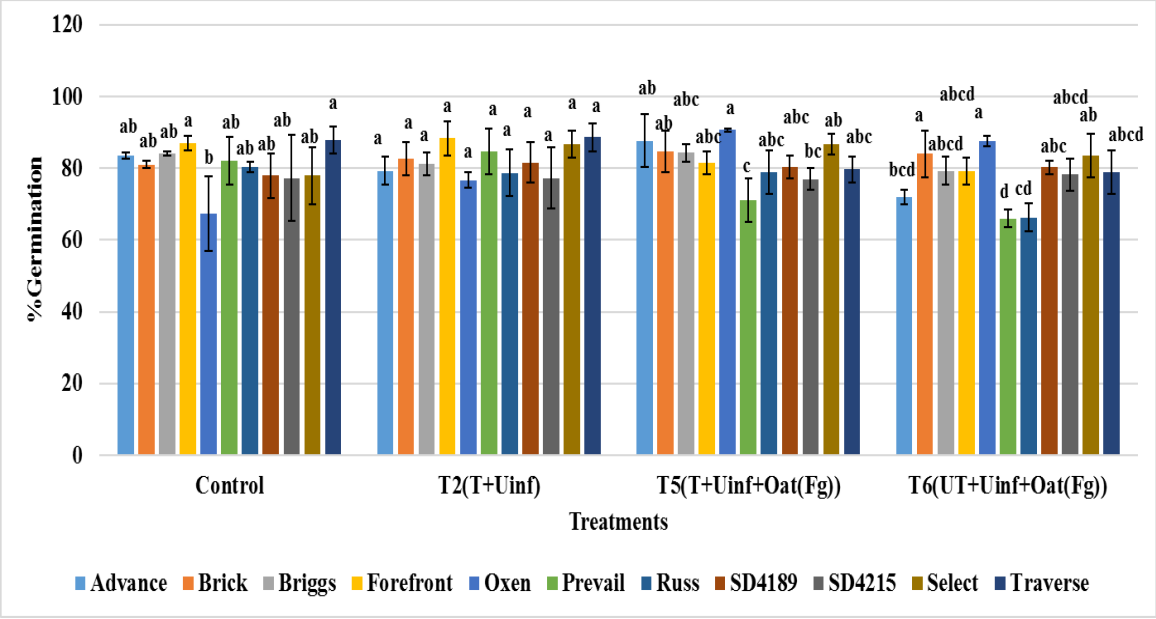

Figure 4.26 Differences in 11 HRSW cultivars seed germination to different treatments planted in Brookings in 2016 (Experiment-II)

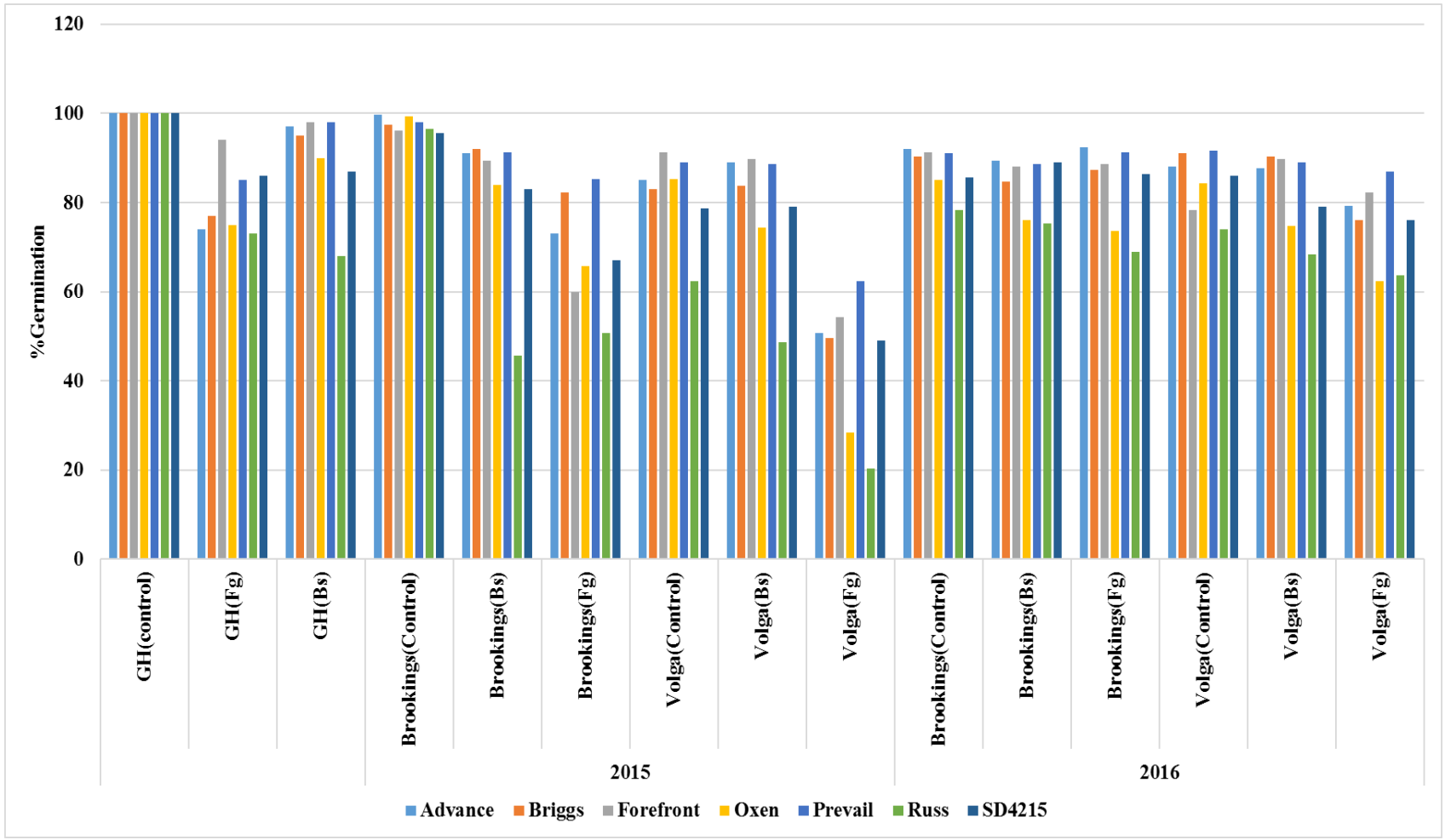

Figure 4.27 Comparison of greenhouse and field experiments results

Chapter 4

Conclusions

In this thesis research we have conducted an extensive survey in wheat growing region of South Dakota to monitor the prevalence of pathogens responsible for causing root rot diseases in wheat. Further, we studied the effect of *F. graminearum* and *B. sorokiniana* on seed germination and seedling blight in hard red spring wheat cultivars grown on considerable acreage in South Dakota. This valuable information would be useful for suggesting disease management strategies to the producers and reducing cost of production. Our study showed root rot pathogens like *F. graminearum* and *B. sorokiniana* were most common in the South Dakota and they effect the seed germination and seedling survival under greenhouse and field conditions.

In total 31 root samples were collected from 17 counties in 2014 and eight samples were collected from 4 counties in 2015. *F. graminearum* was recovered from all the counties (100%) in two years surveyed in this study; however, *B. sorokiniana* was less common in South Dakota and was recovered from 50% (n = 9) in 2014 and 50% (n = 4) in 2015.

Further we studied the impact of *F. graminearum* and *B. sorokiniana* infestation on seed germination and seedling survival. Our greenhouse and field studies results show that although *F. graminearum* caused some seedling blight but it had severe effect on seed germination. *B. sorokiniana* also affected seed germination however, the extent reduction in germination was lower than that by *F. graminearum.*

Variability in resistance to both *F. graminearum* and *B. sorokiniana* was observed in cultivars screened, where Russ and Oxen were highly affected for seed germination

and seedling blight to both pathogens; whereas, Forefront, Select and Briggs had the highest germination and the higher survival rates as compared to the other cultivars. Our results provide information to wheat growers that can help in selection of cultivars and minimize the chances of planting any susceptible cultivar.

Further our study shows that though fungicides are effective (14-37% increase in germination) in reducing damage caused by *F. graminearum* and *B. sorokiniana,* however, there effectiveness is realized if the seed is infected or inoculum is available in the field under suitable environmental conditions for disease development. Our study suggests disease management strategy against root rot pathogens should include disease resistant varieties and seed treatment depending on the seed quality and environmental conditions in the region. This approach will reduce farm loss, cost of production and increase farm sustainability.

Appendices

Appendix 1. Analysis of variance of the effect of *Fusarium graminearum* on the germination of the HRSW cultivars under greenhouse conditions during experiment I.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	6.75386773	6.75386773	253.86	<0.0001
Cultivar	10	1.17591970	0.11759197	4.42	<0.0001
Treatment*cultivar	10	1.00812619	0.10081262	3.79	0.0001

*Coefficient of variance (CV) = 18.65373, Mean = 83

Appendix 2. Analysis of variance of the effect of *Fusarium graminearum* on the seedling survival of HRSW cultivars under greenhouse conditions during experiment I.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	15.64567107	15.64567107	783.83	<0.0001
Cultivar	10	1.84579822	0.18457982	9.25	<0.0001
Treatment*cultivar	10	1.21470081	0.12147008	6.09	<0.0001

*Coefficient of variance (CV) = 18.09729, Mean = 69.81

Appendix 3. Analysis of variance of the effect of *Bipolaris sorokiniana* on the germination of the HRSW cultivars under greenhouse conditions during experiment I.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	1.40675165	1.40675165	50.50	<0.0001
Cultivar	10	1.52708652	0.15270865	5.48	<0.0001
Treatment*cultivar	10	0.50779993	0.05077999	1.82	0.0587

*Coefficient of variance (CV) = 16.06071, Mean = 91.90

Appendix 4. Analysis of variance of the effect of *Bipolaris sorokiniana* on the seedling survival of the HRSW cultivars under greenhouse conditions during experiment I.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	4.53203333	4.53203333	234.59	<0.0001
Cultivar	10	3.40674308	0.34067431	17.63	<0.0001
Treatment*cultivar	10	2.09038161	0.20903816	10.82	<0.0001

*Coefficient of variance (CV) = 13.83561, Mean = 85.27

Appendix 5. Analysis of variance of the effect of *Fusarium graminearum* on the germination of the HRSW cultivars under greenhouse conditions during experiment II.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	4.38565111	4.38565111	140.73	<0.0001
Cultivar	10	2.77864184	0.27786418	8.92	<0.0001
Treatment*Cultivar	10	1.59236021	0.15923602	5.11	<0.0001

*Coefficient of variance (CV) = 16.88779, Mean = 81.81

Appendix 6. Analysis of variance of the effect of *Fusarium graminearum* on the seedling survival of HRSW cultivars under greenhouse conditions during experiment II.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	11.00321848	11.00321848	278.49	<0.0001
Cultivar	10	2.97165084	0.29716508	7.52	<0.0001
Treatment*Cultivar	10	1.99458628	0.19945863	5.05	<0.0001

*Coefficient of variance (CV) = 22.39831, Mean = 67.27

Appendix 7. Analysis of variance of the effect of *Bipolaris sorokiniana* on the germination of the HRSW cultivars under greenhouse conditions during experiment II.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	0.89894975	0.89894975	35.36	<0.0001
Cultivar	10	2.10816924	0.21081692	8.29	<0.0001
Treatment*Cultivar	10	0.21611129	0.02161113	0.85	0.5811

*Coefficient of variance (CV) =13.84963, Mean = 92.27

Appendix 8. Analysis of variance of the effect of *Bipolaris sorokiniana* on the seedling survival of the HRSW cultivars under greenhouse conditions during experiment II.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	1.60271024	1.60271024	54.48	<0.0001
Cultivar	10	2.04858654	0.20485865	6.96	<0.0001
Treatment*Cultivar	10	0.16312552	0.01631255	0.55	0.8493

*Coefficient of variance (CV) = 15.52177, Mean = 89.63

Appendix 9. Analysis of variance of the effect of *Fusarium graminearum* on the germination of the HRSW cultivars at Brookings in 2015 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.72416441	0.24138814	27.55	<0.0001
Cultivar	6	0.91462367	0.15243728	17.40	<0.0001
Treatment*cultivar	18	0.18611962	0.01033998	1.18	0.3084

*Coefficient of variance (CV) = 8.137090, Mean = 81.70

Appendix 10. Analysis of variance of the effect of *Fusarium graminearum* on the seedling survival of the HRSW cultivars at Brookings in 2015 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.03710070	0.01236690	0.92	0.4382
Cultivar	6	0.19275271	0.03212545	2.38	0.0402
Treatment*cultivar	18	0.39115970	0.02173109	1.61	0.0882

*Coefficient of variance (CV) = 8.184080, Mean = 96.14

Appendix 11. Analysis of variance of the effect of *Bipolaris sorokiniana* on the germination of the HRSW cultivars at Brookings in 2015 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.06469360	0.02156453	2.91	0.0425
Cultivar	6	1.51059028	0.25176505	33.94	<.0001
Treatment*cultivar	18	0.14603822	0.00811323	1.09	0.3822

*Coefficient of variance (CV) = 7.206087, Mean = 84.92

Appendix 12. Analysis of variance of the effect of *Bipolaris sorokiniana* on the seedling survival of the HRSW cultivars at Brookings in 2015 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.02917185	0.00972395	0.60	0.6202
Cultivar	6	0.14473454	0.02412242	1.48	0.2022
Treatment*cultivar	18	0.34540900	0.01918939	1.18	0.3113

*Coefficient of variance(CV) = 8.831583, Mean = 96.83

Appendix 13. Analysis of variance of the effect of *Fusarium graminearum* on the germination of the HRSW cultivars at Brookings in 2015 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.24199259	0.08066420	8.86	<.0001
Cultivar	6	0.57217359	0.09536226	10.48	<.0001
Treatment*cultivar	18	0.22407984	0.01244888	1.37	0.1845

*Coefficient of variance(CV) = 7.309498, Mean = 91.52

Appendix 14. Analysis of variance of the effect of *Fusarium graminearum* on the seedling survival of the HRSW cultivars at Brookings in 2015 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.01304225	0.00434742	0.31	0.8200
Cultivar	6	0.13187296	0.02197883	1.55	0.1779
Treatment*cultivar	18	0.27641014	0.01535612	1.09	0.3898

*Coefficient of variance(CV) = 8.557089, Mean = 95.41

Appendix 15. Analysis of variance of the effect of *Bipolaris sorokiniana* on the germination of the HRSW cultivars at Brookings in 2015 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	1.16473654	0.38824551	39.09	<.0001
Cultivar	6	0.58287122	0.09714520	9.78	<.0001
Treatment*cultivar	18	0.28564580	0.01586921	1.60	0.0933

*Coefficient of variance(CV) = 7.891986, Mean = 88.559

Appendix 16. Analysis of variance of the effect of *Bipolaris sorokiniana* on the seedling survival of the HRSW cultivars at Brookings in 2015 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.00500171	0.00166724	0.10	0.9577
Cultivar	6	0.07380862	0.01230144	0.76	0.6018
Treatment*cultivar	18	0.24511550	0.01361753	0.85	0.6418

*Coefficient of variance(CV) = 9.123105, Mean = 95.54

Appendix 17. Analysis of variance of the effect of *Fusarium graminearum* on the germination of the HRSW cultivars at Volga in 2015 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	3.07581862	1.02527287	115.97	<.0001
Cultivar	6	0.83423391	0.13903898	15.73	<.0001
Treatment*cultivar	18	0.24833179	0.01379621	1.56	0.1039

*Coefficient of variance(CV) = 8.920928, Mean = 73.47

Appendix 18. Analysis of variance of the effect of *Fusarium graminearum* on the seedling survival of the HRSW cultivars at Volga in 2015 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.00463697	0.00154566	0.06	0.9796
Cultivar	6	0.10804159	0.01800693	0.72	0.6334
Treatment*cultivar	18	0.30031895	0.01668439	0.67	0.8258

*Coefficient of variance(CV) = 11.83174, Mean = 92.59

Appendix 19. Analysis of variance of the effect of *Bipolaris sorokiniana* on the germination of the HRSW cultivars at Volga in 2015 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.13378563	0.04459521	5.76	0.0017
Cultivar	6	1.20936992	0.20156165	26.04	<.0001
Treatment*cultivar	18	0.12994321	0.00721907	0.93	0.5451

*Coefficient of variance(CV) = 7.575953, Mean = 82.76

Appendix 20. Analysis of variance of the effect of *Bipolaris sorokiniana* on the seedling survival of the HRSW cultivars at Volga in 2015 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.05478112	0.01826037	1.03	0.3846
Cultivar	6	0.12748613	0.02124769	1.20	0.3183
Treatment*cultivar	18	0.16598908	0.00922162	0.52	0.9357

*Coefficient of variance(CV) = 9.816319, Mean = 93.91

Appendix 21. Analysis of variance of the effect of *Fusarium graminearum* on the germination of the HRSW cultivars at Volga in 2015 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.35033848	0.11677949	12.22	<.0001
Cultivar	6	0.75905480	0.12650913	13.24	<.0001
Treatment*cultivar	18	0.14736389	0.00818688	0.86	0.6291

*Coefficient of variance(CV) = 8.272665, Mean = 84.20

Appendix 22. Analysis of variance of the effect of *Fusarium graminearum* on the seedling survival of the HRSW cultivars at Volga in 2015 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.20553705	0.06851235	5.17	0.0032
Cultivar	6	0.04726176	0.00787696	0.59	0.7331
Treatment*cultivar	18	0.17921259	0.00995626	0.75	0.7435

*Coefficient of variance (CV) = 8.494477, Mean = 94.12

Appendix 23. Analysis of variance of the effect of *Bipolaris sorokiniana* on the germination of the HRSW cultivars at Volga in 2015 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.30317297	0.10105766	10.07	<.0001
Cultivar	6	0.65204792	0.10867465	10.83	<.0001
Treatment*cultivar	18	0.06941890	0.00385661	0.38	0.9861

*Coefficient of variance(CV) = 8.598603, Mean = 83.20

Appendix 24. Analysis of variance of the effect of *Bipolaris sorokiniana* on the seedling survival of the HRSW cultivars at Volga in 2015 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr
Treatment	3	0.11725246	0.03908415	2.45	0.0728
Cultivar	6	0.07123916	0.01187319	0.74	0.6161
Treatment*cultivar	18	0.20015767	0.01111987	0.70	0.7989

*Coefficient of variance(CV) = 9.193298, Mean = 94.75

Appendix 25. Analysis of variance of the effect of *Fusarium graminearum* on the germination of the HRSW cultivars at Brookings in 2016 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.21463171	0.07154390	10.62	<.0001
Cultivar	10	0.73874396	0.07387440	10.97	<.0001
Treatment*cultivar	30	0.20315234	0.00677174	1.01	0.4735

*Coefficient of variance(CV) = 6.616394, Mean = 88.45

Appendix 26. Analysis of variance of the effect of *Fusarium graminearum* on the seedling survival of the HRSW cultivars at Brookings in 2016 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.00630685	0.00210228	0.17	0.9191
Cultivar	10	0.20189592	0.02018959	1.59	0.1219
Treatment*cultivar	30	0.28823804	0.00960793	0.76	0.8028

*Coefficient of variance(CV) = 7.614764, Mean = 97.96

Appendix 27. Analysis of variance of the effect of *Bipolaris sorokiniana* on the germination of the HRSW cultivars at Brookings in 2016 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.19971105	0.06657035	8.62	<.0001
Cultivar	10	0.67874167	0.06787417	8.79	<.0001
Treatment*cultivar	30	0.20441663	0.00681389	0.88	0.6421

*Coefficient of variance(CV) = 7.009706, Mean = 89.22

Appendix 28. Analysis of variance of the effect of *Bipolaris sorokiniana* on the seedling survival of the HRSW cultivars at Brookings in 2016 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.00348400	0.00116133	0.08	0.9708
Cultivar	10	0.18826676	0.01882668	1.29	0.2462
Treatment*cultivar	30	0.29354612	0.00978487	0.67	0.8899

*Coefficient of variance(CV) = 8.168484, Mean = 97.82

Appendix 29. Analysis of variance of the effect of *Fusarium graminearum* on the germination of the HRSW cultivars at Brookings in 2016 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.07189118	0.02396373	1.80	0.1525
Cultivar	10	0.17322203	0.01732220	1.30	0.2413
Treatment*cultivar	30	0.45845744	0.01528191	1.15	0.3019

*Coefficient of variance(CV) = 10.22674, Mean = 80.71

Appendix 30. Analysis of variance of the effect of *Fusarium graminearum* on the seedling survival of the HRSW cultivars at Brookings in 2016 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.10753411	0.03584470	2.53	0.0627
Cultivar	10	0.13521453	0.01352145	0.95	0.4902
Treatment*cultivar	30	0.45033614	0.01501120	1.06	0.4067

*Coefficient of variance(CV) = 8.075647, Mean = 97.67

Appendix 31. Analysis of variance of the effect of *Bipolaris sorokiniana* on the germination of the HRSW cultivars at Brookings in 2016 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.02035967	0.00678656	0.46	0.7127
Cultivar	10	0.23734484	0.02373448	1.60	0.1197
Treatment*cultivar	30	0.17682326	0.00589411	0.40	0.9973

*Coefficient of variance(CV) = 10.79436, Mean = 80.84

Appendix 32. Analysis of variance of the effect of *Bipolaris sorokiniana* on the seedling survival of the HRSW cultivars at Brookings in 2016 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.03344677	0.01114892	0.74	0.5306
Cultivar	10	0.10524699	0.01052470	0.70	0.7228
Treatment*cultivar	30	0.48454888	0.01615163	1.07	0.3879

*Coefficient of variance(CV) = 8.394976, Mean = 97.39

Appendix 33. Analysis of variance of the effect of *Fusarium graminearum* on the germination of the HRSW cultivars at Volga in 2015 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.97056398	0.32352133	47.39	<.0001
Cultivar	10	0.47795476	0.04779548	7.00	<.0001
Treatment*cultivar	30	0.15317530	0.00510584	0.75	0.8144

*Coefficient of variance(CV) = 6.807581, Mean = 86.60

Appendix 34. Analysis of variance of the effect of *Fusarium graminearum* on the seedling survival of the HRSW cultivars at Volga in 2015 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.11783723	0.03927908	3.10	0.0307
Cultivar	10	0.14739857	0.01473986	1.16	0.3261
Treatment*cultivar	30	0.23900936	0.00796698	0.63	0.9241

*Coefficient of variance(CV) = 7.860818, Mean = 96.90

Appendix 35. Analysis of variance of the effect of *Bipolaris sorokiniana* on the germination of the HRSW cultivars at Volga in 2015 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.33420104	0.11140035	19.72	<.0001
Cultivar	10	0.54628708	0.05462871	9.67	<.0001
Treatment*cultivar	30	0.14804824	0.00493494	0.87	0.6538

*Coefficient of variance(CV) = 6.071507, Mean = 88.46

Appendix 36. Analysis of variance of the effect of *Bipolaris sorokiniana* on the seedling survival of the HRSW cultivars at Volga in 2015 (Experiment-I).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.07578390	0.02526130	1.95	0.1276
Cultivar	10	0.07460825	0.00746083	0.58	0.8298
Treatment*cultivar	30	0.27804077	0.00926803	0.71	0.8500

*Coefficient of variance(CV) = 7.980805, Mean = 96.80

Appendix 37. Analysis of variance of the effect of *Fusarium graminearum* on the germination of the HRSW cultivars at Volga in 2015 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.12282585	0.04094195	2.31	0.0819
Cultivar	10	0.15310430	0.01531043	0.86	0.5698
Treatment*cultivar	30	0.11518997	0.00383967	0.22	1.0000

*Coefficient of variance(CV) = 10.91286, Mean = 87.15

Appendix 38. Analysis of variance of the effect of *Fusarium graminearum* on the seedling survival of the HRSW cultivars at Volga in 2015 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.02082026	0.00694009	0.47	0.7014
Cultivar	10	0.17493942	0.01749394	1.19	0.3061
Treatment*cultivar	30	0.15330186	0.00511006	0.35	0.9991

*Coefficient of variance(CV) = 8.476281, Mean = 96.79

Appendix 39. Analysis of variance of the effect of *Bipolaris sorokiniana* on the germination of the HRSW cultivars at Volga in 2015 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.08641237	0.02880412	1.45	0.2351
Cultivar	10	0.21283102	0.02128310	1.07	0.3956
Treatment*cultivar	30	0.17655314	0.00588510	0.30	0.9998

*Coefficient of variance(CV) = 11.78686, Mean = 85.55

Appendix 40. Analysis of variance of the effect of *Bipolaris sorokiniana* on the seedling survival of the HRSW cultivars at Volga in 2015 (Experiment-II).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	0.01776877	0.00592292	0.48	0.6937
Cultivar	10	0.15934717	0.01593472	1.30	0.2408
Treatment*cultivar	30	0.34104993	0.01136833	0.93	0.5751

*Coefficient of variance(CV) = 7.768219, Mean = 96.67