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Effect of Soil-Applied Protoporphyrinogen Oxidase Inhibitor Herbicides on Soybean Seedling Disease

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EFFECT OF SOIL-APPLIED PROTOPORPHYRINOGEN OXIDASE INHIBITOR HERBICIDES ON SOYBEAN SEEDLING DISEASE

by

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Seedling disease is one the most economically important diseases of soybean in the United States. It is commonly caused by *Fusarium* spp., *Rhizoctonia solani*, *Pythium* spp., and *Phytophthora sojae*, alone, or together as a disease complex. Fungicide seed treatments continue to provide the most consistent management of seedling diseases. Soil-applied protoporphyrinogen oxidase (PPO) inhibitor herbicides are used preemergence in soybean production to manage several broadleaf weeds. Applications of PPO-inhibitors can result in phytotoxic injury to soybean when environmental conditions are not favorable for soybean growth. These environmental conditions can favor seedling disease development as well. In this thesis, two studies were conducted to determine the effect of soil-applied PPO-inhibitors on soybean seedling disease development in Nebraska under field and controlled conditions.

The first study assessed the effect of two PPO-inhibitors and a fungicide seed treatment on seedling disease and yield in 9 soybean fields in Nebraska. PPO-inhibitor injury occurred at 7 of 9 locations with inconsistent effects on seedling disease, where

increases in root rot severity of 6.6–28.1% were observed at 5 of 9 locations, decreases of 4.7–10.9% at two locations, and no effect at three locations. None of these effects impacted yield at any of the locations. Fungicide seed treatment did not reduce root rot severity at any location; however, it increased yield at two locations.

The second study investigated the effect of PPO-inhibitors on seedling disease caused by *Fusarium solani* under controlled conditions. Disease pressure was consistent throughout this study, with root rot severities ranging 32.1–38.9%. PPO-inhibitor injury occurred in all experiments with severities ranging 7.0–33.0%. Sulfentrazone alone increased root rot severity 9–12%.

There was an effect of PPO-inhibitors on seedling disease development, although results were inconsistent, indicating a need for further research. PPO-inhibitors should continue to be used in part of an integrated weed management program and fungicide seed treatments should be used in fields that have a history of seedling disease.

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DEDICATION

For Edward Anthony Hillman III

This morel is for you, Eddie

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Chapter One

Literature Review

1.1 Soybean

Soybean [*Glycine max* (L.) Merr.] is a leguminous species originating from East Asia that was first domesticated as a crop during the eleventh century B.C. (Hymowitz, 1990). Soybean was first introduced to the United States in 1765 as a forage crop and it was not until the 1920's that cultivation as a grain crop gained popularity (Hymowitz & Shurtleff, 2005). At the beginning of the 1940's, the total harvested soybean hectares for grain first exceeded the total hectares grown for forage (Hymowitz, 1990). Soybean is one of the most important crops worldwide, covering an estimated 6% of the world's arable land and is primarily processed into soybean meal and oil (Hartman et al., 2011). Nearly 98% of soybean meal produced is used in livestock feeds, while 95% of the oil is consumed as edible oil (Hartman et al., 2011). In 2017, the United States harvested a record 36.2 million hectares of soybean (USDA-National Agricultural Statistics Service, 2018). The United States is the second largest soybean producer in the world, encompassing nearly 30% of the total harvested soybean hectares, preceded only by Brazil and followed by Argentina, India, and China respectively (USDA-Foreign Agricultural Service, 2018). Soybean production is an important part of Nebraska's economy, as it is the fourth largest

soybean producing state in the United States, with over 2.2 million hectares were harvested in 2017, behind Illinois, Iowa, and Minnesota (USDA-National Agricultural Statistics Service, 2018)

1.2 Soybean Seedling Diseases

Soybean production is limited by many biotic stressors, including a number of diseases that impact overall plant health and subsequent yield. Seedling diseases have the second highest impact of all diseases affecting soybean, causing an average annual loss of over 1.3 million metric tons over a four-year time span (Koenning & Wrather, 2010). There are four common soilborne pathogens that can cause disease alone or as a complex that contribute to seedling disease in soybean; *Fusarium* spp., *Rhizoctonia solani*, *Pythium* spp., and *Phytophthora sojae* (Datnoff & Sinclair, 1988). Seedling diseases contribute to yield losses through reduced stand establishment, lower seedling vigor, and plant death.

1.2.1 *Fusarium* spp.

Fusarium spp. are true fungi in the phylum Ascomycota (Geiser et al., 2013). Several fungal species in this genus are important plant pathogens, which have large host ranges and are found throughout the world. There are multiple *Fusarium* species that are known to contribute to soybean seedling root rot, with *F. oxysporum* and *F. solani* being the most common (Killebrew et al., 1993; Diaz-Arias et al., 2013). *Fusarium* survives in soil as long term survival spores called chlamydospores (Nelson, 2015). Chlamydospores, along with macroconidia and microconidia, are capable of infecting plants and causing disease (Nelson, 2015). *Fusarium* infection in soybean is often favored by soil

temperatures below the optimum temperature for soybean germination (15 °C), water stress, and no-till cropping systems (Nelson, 2015). Symptoms of Fusarium root rot include seed decay and brown discoloration of the cortical and vascular portions of the root. When root rot is severe, the seedling may develop foliar symptoms such as wilting, stunting, yellowing, and defoliation (Nelson, 2015). There is currently no known resistance in soybean germplasm to the *Fusarium* spp. that contribute to seedling disease.

1.2.2 *Rhizoctonia solani*

Rhizoctonia solani is a true fungus in the phylum Basidiomycota (Sneh et al., 1996). *R. solani* is a common pathogen with a large host range and found throughout the world. *Rhizoctonia solani* isolates are grouped based on anastomosis reactions between hyphal cells and the number of nuclei per hyphal cell (Nelson et al., 1996; Arakawa & Inagaki, 2014). There are 14 anastomosis groups (AGs), which differ in both their ecology and host range (Arakawa & Inagaki, 2014). Several AGs have been noted to cause two different diseases in soybean, aerial web blight and seedling root rot (Rupe & Spurlock, 2015; Yang, 2015). Aerial web blight is caused by AG 1-IA and AG 1-IB while seedling root rot can be caused by AG-4, AG-5, AG-1-IB and AG-2-2-IIIB (Ploetz, et al., 1985; Liu & Sinclair, 1992; Nelson et al., 1996; Dorrance et al., 2003; Rupe & Spurlock, 2015). *R. solani* survives in the soil as long term survival structures called sclerotia (Yang, 2015). Hyphae emerging from sclerotia directly infect seedlings (Yang, 2015). Seedling disease is favored by sandy soils with damp but not oversaturated soil conditions, pH levels greater than 6.6, and warmer soil temperatures (>20 °C; Dorrance et al., 2003; Yang, 2015). Symptoms of postemergence damping-off are visible typically before the V1 growth stage and include reddish-brown lesions on the hypocotyl at the soil line

(Yang, 2015). When infected plants survive, lesions can grow and result in cortical rot, which can cause plants to yellow and be stunted (Yang, 2015). There is currently no known resistance to *Rhizoctonia solani* in soybean germplasm.

1.2.3 *Pythium* spp.

Pythium spp. are in the phylum Heterokonta and are water molds which are not true fungi (Dick et al., 1999). *Pythium* spp. are one of the most common and important causal agents of seed rot, root rot, and seedling damping off for many plant species around the world (Rothrock et al., 2015). *Pythium* is a diverse genus with nearly 100 species capable of causing plant disease, 13 of which are known to be associated with soybean disease (Dorrance et al., 2004; Broders et al., 2007). Many of the 13 species associated with soybean are capable of infecting corn (*Zea mays*) and cereal grain crops which are commonly used in rotation with soybeans in the Midwest (Broders et al., 2007). *Pythium* spp. primarily survive in the soil as long-term survival structures called oospores that act as infectious spores when soybean seedlings are present (Hendrix & Campbell, 1973; Rothrock et al., 2015). Structures called sporangia that are produced by mycelia of *Pythium* spp. contain infectious zoospores, which are capable of swimming through saturated soils to soybean roots (Rothrock, et al., 2015). In general, saturated soils and cool soil temperatures (10–20 ^{o}C) favor infection by *Pythium* spp., however, it has been documented that some species are favored by warmer soil temperatures (Bainbridge, 1970; Martin, 1996). In addition to seed rot, symptoms on affected seedlings include yellow to tannish-brown lesions on the roots and rotting of the hypocotyl, as well as reduced root development and stunted roots (Rothrock, et al., 2015). The hypocotyl may swell and the cortex of the roots can slough off when removing plants from the soil

(Rothrock, et al., 2015). There is currently no known resistance in soybean germplasm to the *Pythium* spp. that contribute to seedling disease.

1.2.4 *Phytophthora sojae*

Phytophthora sojae is also a water mold in the phylum Heterokonta (Dick et al., 1999). *Phytophthora sojae* consists of 55 described races that differ in phenotype based on their reactions with specific resistance (R) genes (Schmitthenner, 2015). Subspecies grouping is based on virulence which in turn is based on 15 Rps genes found in soybean differentials (Dorrance & McClure, 2001). *Phytophthora. sojae* primarily survives in the soil as long-term survival structures called oospores that act as infectious spores when soybean seedlings are present (Schmitthenner, 2015). Structures called sporangia that are produced by mycelia of *P. sojae* contain infectious zoospores which are capable of traveling through saturated soils to soybean roots (Schmitthenner, 2015). Saturated soils with warm soil temperatures (>20 ^{o}C) favor germination of spores and infection by *P. sojae* (Schmitthenner, 2015). *Phytophthora sojae* infection in the early soybean growth stages has similar root rotting symptoms as the other seedling disease causing pathogens (Schmitthenner, 2015). Unlike *Fusarium* spp., *Pythium* spp., and *Rhizoctonia solani*, infection by *Phytophthora sojae* can result in symptoms that include stunting, root rot, and stem rot at any stage of development (Dorrance et al., 2009). Symptoms of stem rot include browning of the exterior surface of the lower stem with brown discoloration of the vascular tissue and leaves wilting while still remaining attached (Schmitthenner, 2015). There is resistance to *P. sojae* currently available in commercial soybean cultivars with both resistance genes (Rps) identified as well as ratings on the relative level of partial resistance (tolerance).

1.3 Seedling Disease Management

1.3.1 Early History of Seed Treatments for Plant Protection

Seed treatment fungicide use can be traced back to 60 AD when cereal growers used wine as a protective seed coating against disease (Russel, 2005). Compounds and mixtures commonly used for seed treatments were first developed throughout the sixteenth to early nineteenth century and included brine, arsenic, copper sulphate, phenylmercury acetate, and copper oxide (Russel, 2005). It was not until the 1940's that chemical seed treatments gained widespread popularity as the chemical protectant industry grew, due to the rise of the petroleum industry and chemistries originally developed for World War II (Russel, 2005). This led to new chemistries, such as dithiocarbamates and aromatic hydrocarbons, being introduced as seed protectants (Russel, 2005). An increase in research and development of fungicide products in the 1960's and 1970's led to the introduction of several of the seed treatment fungicide chemistries currently used today, such as phenylamides and dicarboximides (Russel, 2005). Fungicides with systemic properties were also first developed during this period, allowing for management of post infection disease (Russel, 2005). Due to an increase in regulatory procedures in response to environmental concerns and public perception, the production and release of new chemistries slowed over the next few decades (Russel, 2005).

Consolidation of companies in the crop protection chemical industry in the 1990's resulted in only a few companies currently dominating the seed treatment market (Munkvold, 2009). Due to pressures to meet the growing population and food needs, seed

treatment use has rapidly increased. This resulted in the annual value of the global seed treatment market doubling from $1 to $2 billion from 2002 to 2008 (Munkvold, 2009).

1.3.2 Fungicide Seed Treatment in Soybean

Fungicide seed treatment use in soybean has increased rapidly in recent years, as only 8% of soybean seed in the United States was treated in 1996 compared to 30% in 2008 (Munkvold, 2009). By 2015, this amount had more than doubled, with 75% of soybean seed treated (Gaspar et al., 2016). There are several factors that contribute to the increased use of seed treatments. One factor is that producers are planting earlier into cool (<15 ^{o}C) and often wet soils in an attempt to increase yield potential (Conley & Santini, 2007). Planting into cool and wet soils slows soybean emergence and increases the potential for infection by seedling disease causing pathogens (Dorrance et al., 2009). Another factor is an increase in seed costs to close to $50 per unit (140,000 seeds) which represents nearly 36% of a producer's total annual variable operating expenses (USDA-Economic Research Service, 2016). A third factor is soybean commodity prices dramatically increasing between 2007 and 2013, which allowed for the economic justification of investing into additional inputs, such as seed treatments (USDA-National Agricultural Statistics Service, 2018).

1.3.3 Fungicide Seed Treatment Efficacy and Specificity to Pathogens

Fungicide seed treatments have been shown to have success in managing seedling diseases, although this success has not been consistent, due to variable disease pressure and environmental conditions not favorable for disease development across locations (Bradley et al., 2001; Dorrance & McClure, 2001; Dorrance et al., 2003). Active

ingredients of fungicide seed treatments differ in their relative efficacy on seedling disease causing pathogens (Munkvold, 2009). This has resulted in packaging multiple active ingredients with different modes of action together, which are sold as combination products (Gaspar et al., 2016).

Fungicides are grouped based on their mode of action which refers to the specific fungal cellular process affected by the active ingredients. The Fungicide Resistance Action Committee (FRAC) designates groups based on modes of action (MOA) and there are multiple MOAs commercially labeled for seed treatments of soybean. The most common MOAs include methyl benzimidazole carbamates (MBC, FRAC Group 1), demethylation inhibitors (DMI, Group 3), phenylamide acylanilides (Group 4), succinate dehydrogenase inhibitors (SDHI, Group 7), quinone outside inhibitors (QoI, Group 11), phynlpyrroles (Group 12), aromatic hydrocarbons (Group 14), thiazole carboxamides (Group 22), and Multi Site Action (Groups M3 and M4). For the purpose of this review, as it is the basis for the scope of the research to follow, only Groups 4, 7, and 12 will be discussed in detail.

Phenylamide acylanilides (Group 4) are divided into four subclasses including: acylalanines, butyrolactones, thiobutyrolactones, and oxazolidinones (Cohen & Coffey, 1986). Metalaxyl and mefenoxam (metalaxyl-m) are the most common fungicides in the acylalanine subclass and have been commonly used as soybean seed treatments. These fungicides have been rated ‘excellent’ for management of *Pythium* spp. and *Phytophthora sojae* and are ‘not recommended’ for management of *Fusarium* spp. and *Rhizoctonia* spp. in Nebraska (Jackson-Ziems et al., 2017). These fungicides were first introduced in 1977

for use as seed treatments, with the discovery of metalaxyl, and were used as an effective management tool of diseases caused by *Phytophthora* spp., *Pythium* spp., and *Pseudoperonospora* spp. (Gisi & Cohen, 1996). These fungicides disrupt nucleic acid synthesis through inhibition of the activity of the RNA polymerase I system (Yang et al., 2011). In particular, metalaxyl inhibits uridine incorporation into the RNA chain thus blocking rRNA synthesis at the level of uridine transcription (Yang et al., 2011).

SDHI (Group 7) fungicides were first introduced for use in agriculture in the 1960's with their main activity being against basidiomycete fungi such as *Rhizoctonia* spp. and rust pathogens (Avenot & Michailides, 2010). More recently developed SDHI fungicides are known to have a broader spectrum of control with activity against a diverse group of fungal pathogens (Stammler, et al., 2007). There are SDHI compounds labeled for use in soybean for both seed treatment of seedling diseases and foliar application for stem and foliar diseases. Some common SDHI active ingredients used as soybean seed treatments include carboxin, fluopyram, fluxapyroxad, penflufen, and sedaxane. These fungicides have been rated 'not specified on label' for management of *Pythium* spp. and *Phytophthora sojae,* 'not recommended' for management of *Fusarium* spp., and 'excellent' for management of *Rhizoctonia* spp. in Nebraska (Jackson-Ziems et al., 2017). SDHIs bind specifically to the ubiquinone-binding site (Q-site) of the mitochondrial complex II (succinate dehydrogenase complex) which inhibits fungal respiration (Avenot & Michailides, 2010).

Phenylpyrrole (Group 12) fungicides were first introduced for use in agriculture in the 1960s and have a broad spectrum of activity against fungal species among ascomycetes,

basidiomycetes, and deuteromycetes (Koch & Leadbeater, 1992). Fludioxonil is the only phenylpyrrole fungicide seed treatment used as a soybean seed treatment. These fungicides have been rated 'not recommended' for management of *Pythium* spp. and *Phytophthora sojae,* 'fair to very good' for management of *Fusarium* spp., and 'good' for management of *Rhizoctonia* spp. in Nebraska (Jackson-Ziems et al., 2017). Phenylpyrrole fungicides target the osmotic signal transduction pathway which controls the osmotic pressure of the mitochondrial membrane (Jespers et al., 1994).

1.3.4 Cultural Practices for Management of Seedling Diseases

There are cultural practices that can be used as tools in addition to fungicide seed treatments, to help manage seedling diseases. The adoption of no-till practices in soybean production has led to increased crop debris on the soil surface, which limits the drying and warming of the soil in the spring (Broders, et al., 2007). When soils remain cool and wet, germination and growth of soybean seedlings is prolonged (Broders, et al., 2007). Cool and wet soil conditions also favor infection by *Pythium* spp. and *Fusarium* spp. which increases pathogen inoculum in the soil (Pankhurst et al., 1995; Nelson, 2015; Rothrock et al., 2015). In fields with a history of seedling disease, tillage can be useful in producing an environment that favors soybean growth and is less conducive to infection and disease development (Thomson et al., 1971; Schlub & Lockwood, 1981). For diseases favored by saturated soils, such as *Pythium* spp. and *Phytophthora sojae*, increasing soil drainage is a helpful tool for disease management as it reduces the movement of zoospores (Rothrock et al., 2015; Schmitthenner, 2015). Finally, since infection by *Fusarium* spp. and *Pythium* spp. is favored by cool and wet soils, delaying

planting until when soils warm, can reduce infection and disease development (Rothrock et al., 2015; Nelson, 2015). None of these tools are entirely effective in disease management and an integrated approach on a field-by-field basis is required with targeted options related to site-specific disease issues.

1.4 Weed Management in Soybean Cropping Systems

1.4.1 Integrated Weed Management

No tool is expected to provide total control thus, weed management requires an integrated approach (Swanton & Weise, 1991). Historically, tillage has been a helpful tool in weed management, as it disrupts weed seed beds and mechanically eliminates established weeds; however, the shift to no-till production systems has become increasingly common for potential benefits of reducing soil erosion and increasing moisture retention (Buhler & Oplinger, 1990; Johnson, 1994; Papendick et al., 1986). Crop rotation can be advantageous, as each crop has different emergence patterns, which allows them to compete with different weed species (Liebman & Dyck, 1993). Alternating crops consecutively, such as a corn-soybean rotation, allows for the use of diverse herbicide sites of action, different spray timings, and alleviates selection pressure of each of the management tools used (Liebman & Dyck, 1993). Recently use of cover crops, which are established in the fall and grown during the periods of the year that cash crops are absent, has increased in popularity in corn and soybean production systems throughout the United States (Sustainable Agriculture Research & Education/Conservation Technology Information Center, 2016; Werle, et al., 2017). In addition to several environmental benefits, such as prevention of erosion, increasing soil

carbon, and reducing nitrogen leaching, certain cover crops have shown potential in reducing winter annual weed densities (Werle et al., 2017). Utilizing a diverse herbicide program with multiple sites of action is necessary for effective weed management. Through the advent of herbicide resistant transgenic technologies, soybean producers have selective and non-selective herbicides to utilize (Young, 2006). Herbicides are the most commonly used tool for weed management in soybean production, with 95% of hectares in the United States treated with an herbicide in 2017 (USDA-National Agricultural Statistics Service, 2018). The overreliance on herbicides and specific sites of action has led to an increase in herbicide resistant weeds in soybean production systems (Norsworthy et al., 2012).

1.4.2 Herbicide-Resistant Soybean

The introduction of herbicide resistance traits in soybean began in 1996 with the commercialization of glyphosate-resistant (enzyme 5-enolypyruvyl-shikimate-3-phosphate synthase, EPSPS; Group 9) soybean, which dramatically changed weed management in soybean production systems worldwide (Young, 2006). Before glyphosate-resistant soybean, producers relied on scouting and identifying weeds and carrying out specific weed management strategies, comprised of cultural practices and selective herbicides (Green, 2014). After adopting glyphosate-resistant soybean, producers began to rely heavily on glyphosate for weed management, due to its performance and non-selective nature (Green, 2014). Reliance on glyphosate for weed management resulted in heavy selection pressure on weeds to evolve resistance (Dill et al., 2008). Due to a lack of new herbicide chemistries being developed, there is a need for

further development of tolerance traits to other herbicides. The development of soybean cultivars with resistance to several other herbicides, including glufosinate and dicamba, have been developed and introduced alongside glyphosate resistance in soybean in recent years (Green, 2014). These developments provide producers increased diversity in sites of action available for chemical weed management (Green, 2014).

1.4.3 Herbicide Resistant Weeds

The first documented incidence of herbicide resistance was reported in wild carrot (*Daucus carota* L.) in the 1950's, well before the introduction of herbicide resistant crops (Switzer, 1957). The first documented report of a herbicide resistant weed in Nebraska was in 1990 when a population of tall waterhemp (*Amaranthus rudis*) was determined to have resistance to photosystem II inhibitors (PSII; Group 5) (Anderson et al., 1996). Resistance to several other herbicide sites of action in multiple weed species have been documented in Nebraska, including acetolactate synthase inhibitors (ALS; Group 2), synthetic auxins (Group 4), EPSP synthase inhibitors (Group 9), protoporphyrinogen oxidase (PPO) inhibitors (Group 14), and 4-hydroxyphenylpyruvate dioxygenase inhibitors (HPPD; Group 27) (Heap, 2018). Eight weed species in Nebraska have been documented to have evolved herbicide resistance to at least one site of action (Heap, 2018). These include tall waterhemp (*Amaranthus rudis*; Groups 2, 4, 5, 9, 14, and 27*)*, palmer amaranth (*A. palmeri*; Groups 5, 9, and 27), giant ragweed (*Ambrosia trifida*; Group 9), common ragweed (*A. artemisiifolia*; Group 9), horseweed (*Conyza canadensis;* Group 9), kochia (*Kochia scoparia*; Groups 4, 5, and 9), shattercane (*Sorghum bicolor*; Group 2), and johnsongrass (*Sorghum halepense*; Group 2); (Heap, 2018).

With the increase of herbicide-resistant weeds, soybean producers are utilizing preemergent herbicides with residual activity, followed by postemergent herbicides (Sarangi et al., 2017). Relying on soil-applied preemergent herbicides with residual control is not a new concept as it was used commonly in weed management before the introduction of herbicide resistant soybean (Young, 2006). One particular group of herbicides, the PPO-inhibitors (Group 14) have been used effectively as a preemergent herbicide on several broadleaf weed species and is the focus of the herbicide component of the research in this thesis (Hager et al., 2002; Legleiter et al., 2009).

1.4.4 Protoporphyrinogen Oxidase Inhibitors

Protoporphyrinogen oxidase (PPO) inhibitors (Group 14) are cell membrane disrupting herbicides commonly used for broadleaf weed control in soybean production (Duke et al., 1991; Dayan et al., 1996). PPO-inhibitors were first commercialized in the 1960s for both preemergent and postemergent applications in soybean production (Matsunaka, 1976). These herbicides increased in popularity over time as they are less environmentally hazardous due to their rapid soil dissipation and low use rates (Taylor-Lovell et al., 2001). Preemergent PPO-inhibitors have been reported to be effective in control of tall waterhemp, with >85% control when used in a preemergent followed by postemergent herbicide program (Hager et al., 2002; Legleiter et al., 2009).

PPO-inhibitor herbicides consist of four chemical families: diphenylethers, N-phenylphthalimides, aryl triazinones, and trifluoromethyl uracils (Duke et al., 1991; Hao et al., 2011). The most common preemergent PPO-inhibitors used in soybean production include flumioxazin, sulfentrazone, and saflufenacil (Knezevic, et al., 2017). Flumoxazin is a member of the N-phenylphthalimide family and its commercial products include:

Valor SX®, Valor XLT®, Gangster®, Enlite®, Envive®, and Fierce® (Knezevic, et al., 2017). Sulfentrazone is a member of the triazinone family and commercial products include Spartan®, Authority First®, Authority Assist®, Authority MTZ®, Authority XL®, Authority Elite®, and Sonic® (Knezevic, et al., 2017). Saflufenacil is a member of the trifluoromethyl uracil family and commercial products include: Sharpen®, Optill®, Optill PRO®, and Verdict® (Knezevic, et al., 2017). Soil-applied PPO-inhibitors have residual properties in which the active ingredients are present and effective in the soil for several weeks after application (Taylor-Lovell et al., 2001).

PPO-inhibitors target the inhibition of protoporphyrinogen oxidase, an enzyme in the biosynthetic pathway leading to chlorophyll and heme production (Hao et al., 2011). Inhibition of the PPO enzyme leads to an accumulation of protoporphyrinogen-IX, which is oxidized to produce the photosensitive protoporphyrin IX (Jacobs & Jacobs, 1982). As protoporphyrin IX reacts with light, singlet oxygen molecules are released, which cause lipid peroxidation and cell death. (Jacobs & Jacobs, 1982).

PPO-inhibitors are selective herbicides which means that soybeans can metabolize the active ingredients and do not typically cause crop injury; however, when environmental conditions are unfavorable for soybean germination and emergence phytotoxicity and stand reduction can occur as a result of PPO-inhibitor injury (Taylor-Lovell et al., 2001). Soil temperature, organic matter composition, soil pH, and moisture events can have an affect on crop injury (Taylor-Lovell et al., 2001). Soils below the optimum temperature for soybean germination and emergence (15 °C) favor PPO-inhibitor injury, as it slows seedling growth, which results in extended exposure to the herbicides (Taylor-Lovell et

al., 2001). Adsorption of PPO-inhibitors to the soil is affected by soil organic matter and soil pH (Grey et al., 1997). Soils with higher organic matter have increased opportunities for adsorption of herbicides and often require higher application rates to deliver the intended dose necessary for adequate weed control (Grey et al., 1997). Alternatively, soils with low organic matter have increased herbicide concentration available (Grey et al., 1997). Additionally, adsorption of PPO-inhibitors to soil is greater when pH values are low (Grey et al., 1997). Injury can also occur when there is a precipitation or irrigation event at the time of emergence, which can result in splashing the herbicides from the soil onto the hypocotyl and cotyledons (Wise et al., 2015).

Common symptoms of PPO-inhibitor injury include callused tissue on the hypocotyl and the stem at the soil surface, shortened internodal length, phytotoxic chlorosis and necrosis of leaf and cotyledon tissues (Figure 1), and a slowed growth rate resulting in stunted plants (Li et al., 1999; Hulting et al., 2001). Several studies suggest differences in soybean varietal response to sulfentrazone (Swantek & Oliver 1996; Dayan et al., 1997; Li et al., 1999; Hulting et al., 2001; Reilling et al., 2006). Tolerance to sulfentrazone in soybean has been shown to be controlled by a single gene with tolerance dominant to sensitivity (Swantek, et al., 1998). There has been limited research on varietal sensitivity differences for flumioxazin (Taylor-Lovell et al., 2001; Mahoney et al., 2014). Currently, few seed companies rate their commercial varieties for sensitivity to PPO-inhibitors.

1.5 Interactions Between PPO-inhibitor Herbicides and Disease

Environmental conditions that can favor PPO-inhibitor injury, such as cool soil temperatures (<15 °C) and wet weather conditions through emergence can also favor

infection by *Fusarium* spp. and *Pythium* spp. as well as seedling disease development (Bainbridge, 1970; Taylor-Lovell et al., 2001; Nelson, 2015). Multiple studies have investigated interactions between PPO-inhibitor herbicides and *Fusarium* spp., *Rhizoctonia solani*, and *Pythium* spp.; however, only *Fusarium virguliforme* (causal pathogen of sudden death syndrome) and *R. solani* (causal pathogen of seedling root rot and damping off) have been studied in soybean. There are no known studies investigating PPO-inhibitor herbicide interactions with soybean seedling disease caused by *Phytophthora sojae*. Recently, studies have been conducted to investigate interactions of preemergent herbicides, including sulfentrazone and flumioxazin, with several fungicide seed treatments. This research resulted in no observed effects on yield, however, and no disease parameters were evaluated (Barlow et al., 2018).

There are no known studies on PPO-inhibitor herbicide effects on diseases caused by other *Fusarium* spp. associated with soybean seedling disease in any crop. Studies investigating the effects of several PPO-inhibitor herbicides on sudden death syndrome (SDS) and its causal pathogen, *F. virguliforme* have had variable results. Lactofen, a postemergent PPO-inhibitor, was observed to significantly reduce conidial germination, myecelial growth, and sporulation of *F. virguliforme in vitro* compared to the non-treated control (Sanogo et al., 2000). In additional experiments under controlled conditions in the greenhouse, lactofen was observed to significantly reduce SDS disease severity and isolation frequency of *F. virguliforme* isolation compared to the non-treated control (Sanogo et al., 2000). In similar field experiments, lactofen had no significant affect while acifluorfen, a postemergent PPO-inhibitor, significantly increased SDS severity

compared to the non-treated control (Sanogo et al., 2001). More recent research investigated two preemergent PPO-inhibitors, sulfentrazone and flumioxazin, and their effects on SDS severity and yield. There were no significant affects of either PPO-inhibitor herbicide on SDS severity or yield (Kandel et al., 2018).

Studies investigating the effects of several PPO-inhibitor herbicides on soybean seedling disease caused by *R. solani* have had variable results. Acifluorfen was observed to reduce the colony radius of AG-IA and AG-IB isolates *in vitro* compared to the non-treated control (Black et al., 1996). In studies conducted under controlled conditions in the greenhouse, acifluorfen (1X and 2X of its labeled rate) was observed to significantly increase disease severity compared to the non-treated control (Bradley et al., 2002). Acifluorfen was observed to significantly increase disease severity compared to the non-treated control at 2 of 6 non-inoculated field locations (Bradley et al., 2002). Lactofen was observed to have no significant affect on disease severity in *R. solani* inoculated field experiments with low levels of disease severity (Harikrishnan & Yang, 2002).

There has been no research investigating the effects of PPO-inhibitor herbicides on soybean seedling disease caused by *Pythium* spp.; however, limited research has investigated the effects of PPO-inhibitors on root rot caused by *Pythium* spp. in sugarcane. Three PPO-inhibitor herbicides (azafendin, flumioxazin, and sulfentrazone) were observed to significantly reduce mycelial growth of several *Pythium* spp. *in vitro* compared to the non-treated control (Daugrois et al., 2005). In studies conducted under controlled conditions in the greenhouse, full rates of soil-applied flumioxazin and sulfentrazone were observed to significantly decrease sugarcane root colonization by *P.*

arrhenomanes compared to the non-treated control (Daugrois et al., 2005). In the same greenhouse studies the 0.1X rate of flumioxazin applied to the foliage was observed to significantly decrease sugarcane root colonization by several *Pythium* spp. compared to the non-treated control (Daugrois et al., 2005). The 0.1x rate of sulfentrazone applied to the foliage was observed to significantly decrease sugarcane root colonization by *P. arrhenomanes* compared to the non-treated control (Daugrois et al., 2005). Alternatively, the 0.1X rate of soil-applied sulfentrazone significantly increased sugarcane root colonization by *Pythium* spp. compared to the non-treated control (Daugrois et al., 2005). Full rates of all three PPO-inhibitors had no affect on sugarcane root rot severity while the 0.1X rate of flumioxazin applied to the foliage significantly decreased root rot severity compared to the non-treated control (Daugrois et al., 2005).

From the literature available, PPO-inhibitor herbicides have been observed to have an interaction with root rot seedling diseases caused by *F. virguliforme*, *R. solani*, and *Pythium* spp. in either soybean or sugarcane. Across published studies, a total of five PPO-inhibitors have been evaluated (acifluorfen, azafendin, flumioxazin, lactofen, and sulfentrazone), four of which are labeled for use in soybean production. Further, there is a need to investigate preemergent PPO-inhibitors as 4 of 5 published studies on soybean have been on postemergent PPO-inhibitors (lactofen and acifluorfen). Only one of the studies investigated flumioxazin and sulfentrazone and their effects on soybean seedling diseases. These studies produced variable results, which presents a clear need for additional studies investigating PPO-inhibitor interactions with the predominant soybean seedling disease causing pathogens.

1.6. Research Objectives

The objectives of this research were to (1) determine the effects of two soil-applied PPO-inhibitor herbicides (flumioxazin and sulfentrazone) on soybean seedling root rot severity, plant population, and yield; and (2) determine the effect of fungicide seed treatment on soybean root rot severity, plant population, and yield in the presence of PPO-inhibitor induced injury.

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1.8 Figure 1

Figure 1. Symptoms of PPO-inhibitor injury include chlorosis and necrosis on soybean cotyledon and hypocotyl. Symptomatic seedlings indicated by arrows. Healthy seedling indicated by star.

Chapter Two

Effect of preemergent protoporphyrinogen oxidase inhibitor herbicides and fungicide seed treatment on soybean seedling disease and yield in Nebraska

2.1 Introduction

Seedling diseases of soybean [*Glycine max* (L.) Merr.] are caused by several pathogens including *Fusarium* spp., *Rhizoctonia solani*, *Pythium* spp. and *Phytophthora sojae* and can result in significant annual yield losses in the North Central Region of the United States. Disease can be caused by one pathogen or a disease complex (Datnoff & Sinclair, 1988). Seedling disease is considered the second most important soybean disease in the United States causing an average annual loss of over 1.3 million metric tons over a four year span (Koenning & Wrather, 2010). The end result can be reduced plant vigor and a decrease in stand establishment when infections are severe and environmental conditions are favorable for disease development.

Management of seedling disease is difficult, as these pathogens are capable of persisting for many years in soil absent of a host (Nelson, 2015; Rothrock, et al., 2015; Schmitthenner, 2015; Yang, 2015). Effective management requires knowledge of the disease history of the field to select management strategies. However, there are limited

cultural practices to manage seedling disease, depending on the causal pathogen. Fungicide seed treatment has become the primary tool used for seedling disease management and the use of fungicide seed treatments in soybean in the United States has increased from 8% of total seed treated in 1996 to 75% in 2015 (Munkvold, 2009; Gaspar et al., 2016).

Fungicide seed treatments can have broad spectrum fungicidal activity while others are specific for management of one pathogen. Often, several fungicides with specific fungicidal activity are packaged together as combination products targeting several pathogens (Munkvold, 2009; Gaspar et al., 2016). Previous research showed varying responses of fungicide seed treatments on stands and yield, depending on disease pressure, soil characteristics, and environmental conditions (Dorrance et al., 2003; Poag et al., 2005; Gaspar et al., 2015; Gaspar et al., 2016).

In addition to managing seedling diseases, another challenge for soybean producers is the effective management of weeds. The introduction and rapid adoption of glyphosate resistant crops, including corn (*Zea mays* L.) and soybean since 1996 has led to an increase in glyphosate resistant weeds in soybean production areas (Norsworthy et al., 2012). Currently, there are 16 weed species that have evolved resistance to glyphosate in the United States, six of which have been reported in Nebraska (Heap, 2018). With the increase of herbicide resistant weeds, soybean producers are relying heavily on preemergent herbicides that have residual properties, followed by application of postemergent herbicides (Sarangi et al., 2017). Protoporphyrinogen oxidase-inhibitors are commonly used in preemergent herbicide programs in soybean due to their ability to

effectively control several broadleaf weeds; however, these herbicides have the potential to injure soybean, especially in cool and wet conditions (Taylor-Lovell et al., 2001; Hager et al., 2002; Reiling et al., 2006; Legleiter et al., 2009; Belfry et al., 2016). Additionally, injury can occur when there is precipitation or irrigation at or near the time of emergence, which splashes droplets of the herbicide onto the hypocotyl and cotyledons (Wise et al., 2015; Figure 1). Examples of preemergent PPO-inhibitors used in soybean include flumioxazin, saflufenacil, and sulfentrazone.

Cool soil temperatures (<15 °C) and wet soil conditions that favor PPO-inhibitor injury are also conducive for development of seedling disease caused by *Fusarium* spp. and *Pythium* spp. (Bainbridge, 1970; Taylor-Lovell et al., 2001; Nelson, 2015). Multiple studies have investigated interactions between PPO-inhibitors and *Fusarium* spp., *R. solani*, and *Pythium* spp.; however, only *F. virguliforme* O'Donnell & T. Aoki [causal pathogen of sudden death syndrome (SDS)] and *R. solani* have been studied in soybean (Black et al., 1996; Sanogo et al., 2000; Sanogo et al., 2001; Bradley et al., 2002; Harikrishnan & Yang, 2002; Kandel et al., 2018). There is no known published research on PPO-inhibitor effects on soybean seedling disease caused by *Pythium* spp. and *Phytophthora sojae*; however, Daugrois et al. (2005) studied this effect of disease caused by *Pythium* spp. in sugarcane (*Saccharum officinarum*). These studies have produced inconsistent results, where several PPO-inhibitors were observed to both increase and decrease disease severity. Recently, studies have been conducted to investigate interactions of preemergent herbicides including sulfentrazone and flumioxazin, with several fungicide seed treatments in soybean. This research resulted in no observed

effects on yield, however, no disease parameters were evaluated (Barlow et al., 2018). There is a need for evaluation of the effects of preemergent PPO-inhibitors on seedling diseases of soybean.

Therefore, the objectives of this research were to: (1) determine the effects of soil-applied PPO-inhibitor herbicides on soybean seedling disease severity and yield; (2) determine the effect of fungicide seed treatment on soybean seedling disease severity and yield when PPO-inhibitor injury occurs.

2.2 Materials and Methods

2.2.1 Experimental locations

Field experiments were established at nine locations in Nebraska, including four in 2016 and five in 2017 (Table 1). The Lincoln and Mead experiments in 2017 were conducted on University of Nebraska research sites and all other experiments were conducted in producer fields. Location-specific information, including planting and harvest dates, tillage practices, irrigation information, and soil characteristics (soil type, organic matter content, and soil pH) are included in Table 1. Plots were planted with a 4-row cone planter at a depth of 3.8 cm with a seeding rate of 308,881 seeds/ha. Plots were four rows wide (76 cm row spacing) and 5.2–10.0 m long, depending on location.

2.2.2 Experimental design

Experiments were arranged as a randomized complete block design with four replications at each location. Treatments consisted of a factorial arrangement of preemergence herbicide program by seed treatment by cultivar. There were three herbicide programs, which consisted of sulfentrazone (Spartan® 4F, FMC Corporation,

Philadelphia, PA, 0.56 L/ha), flumioxazin (Valor® SX, Valent U.S.A LLC, Walnut Creek, CA, 175 g/ha), and a no herbicide treatment control. All locations, except Tekamah, received a glyphosate (Roundup® PowerMax, Monsanto Company, St. Louis, MO, 2.25 L/ha) with ammonium sulfate (NRich, American Plant Food Corporation, Galena Park, TX, 20.4 g/L) application at the time of planting for control of weeds. Herbicides were applied using a CO_2 pressurized back-pack sprayer with a 3 m wide hand-held boom with six XR8002-VS nozzles (TeeJet Technologies Illinois LLC, Urbandale, IL) on 50.8-cm spacing delivering 140 L/ha at 275 kPa. All applications were made 2–5 days after planting (DAP) (Table 2). Seeds received either no seed treatment or a fungicide seed treatment (ST) of mefenoxam (Apron® XL, Syngenta Crop Protection LLC, Basel, Switzerland, 0.64 mg a.i./seed) + fludioxonil (Maxim® 4FS, Syngenta Crop Protection LLC, 0.0076 mg a.i./seed) + sedaxane (Vibrance®, Syngenta Crop Protection LLC, 0.0076 mg a.i./seed). Seed treatments were applied as a slurry before planting, using an IMER Minuteman II portable cement mixer (IMER U.S.A. Inc., Hayward, CA) at a total volume of combined product rate of 3.26 mL/kg seed including water. Two cultivars, P22T41R2 and P28T08R (DuPont Pioneer®, Johnston, IA) were planted at all locations. Cultivars were selected based on ratings of sensitivity to saflufenacil and sulfentrazone from screenings performed by DuPont Pioneer® (DuPont Pioneer, 2017). P22T41R2 (sensitive) is listed as a cultivar that has a high potential for crop injury from sulfentrazone and saflufenacil, while P28T08R (tolerant) is listed as a cultivar with tolerance.

2.2.3 Data collection

Phytotoxicity (PPO-inhibitor Injury). Phytotoxicity was rated in all experimental units at one time between the cotyledon (VE) and first trifoliate (V1) growth stages (Fehr et al., 1971). Phytotoxicity was rated visually for total plot incidence on a continuous 0–100% scale.

Plant Population. Plant population was assessed at three times, the VE-V1, V2-V4, and at R8 (full maturity) growth stages. Plant population was calculated by counting the total number of live plants in 3.1 m of each of the two center rows of each plot. Counts from each row were combined to result in one value per plot. Data were converted to plants/ha for analysis.

Vigor. Plots were visually assessed for vigor at the V2-V4 growth stages. Vigor was assessed per plot on 0-100% continuous scale for relative greenness and plot uniformity.

Root Rot, Plant Biomass, and Plant Height Evaluation. At the V1-V4 growth stages, six plants were selected haphazardly from each plot and dug with a shovel from the outer two rows. Root systems from each plot were washed free of soil and evaluated on site for root rot severity. For this evaluation, root rot severity is described as the total percent area of the root system that is discolored with the typical browning symptoms of root rot. The six root systems were rated collectively on a 0-100% continuous scale for a single root rot severity value per plot. The roots from each plot were cut from the aboveground portion at the cotyledon scar and their collective fresh root biomass (g) was measured. The collective fresh aboveground biomass (g) of the six plants was also measured. In 2017, the height of six additional haphazardly chosen plants from the center two rows were measured. Heights were measured from soil surface to the node of the uppermost

developed trifoliate. An average height (cm) was calculated giving one height value per plot.

Pathogen Isolation. To determine the most common seedling disease causing pathogen for each field, roots were transported to the laboratory for pathogen isolation. For the isolation process, roots were selected only from the plots that did not receive a seed treatment. Two of the four replications at each site were then randomly selected and roots were pooled within each replication. Once pooled, symptomatic roots with lesions were cut into 2.5-cm segments and placed under running tap water for 20 minutes to remove debris. Five washed root segments were placed on six plates of water agar (Bacto™ Agar, Becton, Dickinson, and Company, Franklin Lakes, NJ) media as well as six plates each of two selective media: water agar + streptomycin sulfate (200 mg a.i./L) and corn meal agar (BBL™ Corn Meal Agar, Becton, Dickinson, and Company) + pentachloronitrobenzene (50 mg a.i./L) + benomyl (10 mg a.i./L) + pimaricin (5 mg a.i./L) + ampicillin (250 mg a.i./L) + rifampicin (10 mg a.i./L). Fungal growth was monitored and characterized into three categories: *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., using morphological identification characteristics outlined by Watanabe (1937).

Non-Seedling Disease Monitoring. Visual assessment of SDS was made at Chapman, a second was performed 7 days later (R6). Disease Incidence (DI), Disease Severity (DS) and a calculated Disease Index (DX) were recorded for each plot. Disease incidence was assessed as the % of plants with leaf symptoms, in increments of 5. Disease Severity was recorded on a 1-9 scale, in increments of 0.5 (Gibson et al., 1994). Disease Index (DX)

was calculated using the DI and DS scores with the following equation: *DX = DI * DS/9* (Gibson et al., 1994). Disease index has a range of 0 (no disease) to 100 (all plants prematurely dead at or before R6). Disease incidence of stem canker (*Diaporthe phaseolorum* var. *caulivora*) was rated at Auburn using a continuous percentage scale of 0-100% of total plants affected in the two center harvest rows at the R6 growth stage.

Grain Yield. All plots were trimmed to uniform length within each experiment for harvest. All locations were trimmed to 9.1 m plot lengths except for Lincoln and Mead which were trimmed to 4.6 m lengths due to smaller plot lengths planted at these locations. The center two rows of each plot were mechanically harvested at maturity using an ALMACO small-plot combine (Almaco, Nevada, IA) equipped with a HarvestMaster (Juniper Systems Inc, Logan, UT) grain gauge. Yield data were calculated and adjusted to 13% moisture for comparison.

Environmental Data. Soil temperature (°C) was collected at 10-cm depth using a bimetal thermometer (Taylor Precision Products, Oak Brook, IL) from the experimental area at planting at each location (Table 2). Rainfall amounts were collected from Stratus RG202 rain gauges (Stratus, Seattle, WA) at each location established at planting (Table 2).

2.2.4 Data analysis

Analysis of variance was performed using PROC GLIMMIX in SAS version 9.4 (SAS Institute Inc., Cary, NC) to determine the effect of herbicide program (Herbicide), seed treatment (ST), cultivar (Cultivar), and their interactions on response variables. Cultivar, seed treatment, herbicide program, and location were treated as fixed effects

and replication was treated as a random effect. Mean separation was performed using Fisher's protected LSD at $\alpha = 0.05$. Whenever necessary, data for the response variable was log transformed prior to analyses in order to satisfy Gaussian assumptions of normality and homogeneity of variance. Contingency table analyses were conducted on fungal characterization data from each experiment location using the GGPLOT2 package (Wickham, 2016) in R version 3.5.1 (2018-07-02).

2.3 Results

There were significant Location X Herbicide, Location X ST, and Location X Cultivar interactions for the response variables ($\alpha = 0.05$) and therefore data is presented by location.

Phytotoxicity (PPO-inhibitor Injury). Applications of sulfentrazone and flumioxazin resulted in symptoms of phytotoxic injury at all locations except Cordova where no phytotoxicity was observed (Figure 1). Sulfentrazone and flumioxazin applications resulted in phytotoxicity 3.6–28.1% higher than the no herbicide control ($P < 0.05$) at 5 of 9 locations (Auburn, Chapman, Clearwater, Lincoln, and Tekamah; Tables 3 and 4). At Schuyler, sulfentrazone and flumioxazin applications resulted in phytotoxicity incidences 13.1–20.4% higher than the no herbicide control across non-seed treated plots while sulfentrazone resulted in 11.9% more phytotoxicity across fungicide seed treated plots ($P = 0.05$; Figure 2A). The tolerant cultivar resulted in 4.8–11.5% higher phytotoxicity than the sensitive cultivar ($P < 0.05$) at 3 of 9 locations (Auburn, Chapman, and Tekamah; Tables 3 and 4). At Ord, sulfentrazone and flumioxazin applications resulted in 5.7–16.1% more phytotoxicity than the no herbicide control within both

cultivars ($P < 0.01$; Table 3, Figure 2B). Fungicide seed treatment resulted in 4.6% higher phytotoxicity than no seed treatment within the tolerant cultivar ($P = 0.03$) at Ord as well (Table 3, *data not shown*). There were no significant effects on phytotoxicity at Mead (Table 3).

Plant Populations at VE - V1 Growth Stages. Sulfentrazone and flumioxazin applications resulted in 17,700–19,100 plants/ha less than the no herbicide control ($P = 0.04$) at Clearwater (Tables 5 and 6). The fungicide seed treatment resulted in 19,100–43,100 plants/ha more than no seed treatment ($P < 0.05$) at only two locations, Auburn and Ord (Tables 5 and 6). The sensitive cultivar resulted in 20,900–30,200 plants/ha more than the tolerant cultivar ($P < 0.01$) at 4 of 9 locations (Chapman, Clearwater, Cordova, and Schuyler; Tables 5 and 6). When sulfentrazone was applied at Tekamah, the tolerant cultivar resulted in 41,400 plants/ha more than the sensitive cultivar ($P = 0.02$; Table 5, data not shown). Across fungicide seed treated plots at Mead, the sensitive cultivar resulted in 47,500 plants/ha more than the tolerant cultivar ($P = 0.03$; Table 5, data not shown). There were no significant effects at Lincoln for plant population at VE - V1 growth stages (Table 5).

Plant Populations at V2 - V4 Growth Stages. At Chapman, sulfentrazone and flumioxazin applications resulted in 18,000–20,600 plants/ha less than the no herbicide control at V2 - V4 growth stages ($P < 0.05$; Tables 7 and 8). At Schuyler, sulfentrazone applications resulted in 15,300 plants/ha more than flumioxazin ($P < 0.05$; Tables 7 and 8). The fungicide seed treatment again resulted in 20,500–35,300 plants/ha more than no seed treatment ($P < 0.01$) at Auburn and Ord (Tables 7 and 8). At Chapman, the sensitive

cultivar resulted in 21,300 plants/ha more than the tolerant cultivar ($P < 0.01$; Tables 7 and 8). There was a significant Cultivar X ST interaction ($P < 0.05$) at 4 of 9 locations (Clearwater, Cordova, Lincoln, and Mead) where the sensitive cultivar resulted in 23,300 plants/ha more than the tolerant cultivar across non-seed treated plots at Cordova and 27,800–46,600 plants/ha more across fungicide seed treated plots at Lincoln and Mead (Table 7, *data not shown*). At Clearwater, no seed treatment resulted in 17,600 plants/ha more than the fungicide seed treatment within the sensitive cultivar (*data not shown*).

Plant Populations at R8 Growth Stage. Only at Ord did the fungicide seed treatment significantly increase populations at the R8 growth stage where it resulted in 55,200 plants/ha more than no seed treatment ($P < 0.001$; Tables 9 and 10). The sensitive cultivar resulted in 12,700–40,900 plants/ha more than the tolerant cultivar ($P < 0.01$) at 5 of 9 locations (Chapman, Cordova, Lincoln, Schuyler, and Tekamah; Tables 9 and 10). Across fungicide seed treated plots at Mead, the sensitive cultivar resulted in 40,700 plants/ha more than the tolerant cultivar ($P = 0.03$; Table 9, *data not shown*). At Auburn within the sensitive cultivar, the fungicide seed treatment resulted in 60,900 plants/ha more than the no seed treatment when flumioxazin was applied ($P < 0.01$; Table 9, *data not shown*).

Plant Vigor. Sulfentrazone and flumioxazin applications resulted in significantly lower vigor than the no herbicide control at Schuyler and Tekamah with reductions of 8.9–27% ($P < 0.01$; Tables 11 and 12). Additionally, at Schuyler sulfentrazone applications resulted in 18.1% higher vigor than flumioxazin (Table 12). The fungicide seed treatment resulted in 7.1 - 17% higher vigor than no seed treatment ($P \leq 0.05$) at Ord

and Tekamah (Tables 11 and 12). Across non seed treated plots at Chapman, sulfentrazone and flumioxazin applications resulted in 23–24% lower vigor than the no herbicide control (P = 0.05; Table 11, *data not shown*). There was a significant Herbicide X Cultivar interaction (P < 0.01) at Auburn and Clearwater where the tolerant cultivar resulted in 9% higher vigor than the sensitive cultivar when no herbicide was applied and 22% higher vigor when sulfentrazone was applied at Auburn (Table 11, *data not shown*). At Clearwater the no herbicide control resulted in 20% higher vigor than when sulfentrazone was applied within the sensitive cultivar and 17% higher vigor than when both sulfentrazone and flumioxazin were applied within the tolerant cultivar (P < 0.01; Table 11, *data not shown*). Additionally, at Clearwater the tolerant cultivar resulted in 7% higher vigor than the sensitive cultivar when no herbicide was applied (P < 0.01; Table 11, *data not shown*). At Auburn, the tolerant cultivar resulted in 7–16% higher vigor than the sensitive cultivar across both non seed treated plots and fungicide seed treated plots (P = 0.03; Table 11, *data not shown*). Additionally at Auburn, the fungicide seed treatment resulted in 12% higher vigor than no seed treatment within the sensitive cultivar (P = 0.03; Table 11, *data not shown*).

Root Rot, Plant Biomass, and Plant Height Evaluation. Sulfentrazone and flumioxazin applications resulted in 6.6%–10% higher root rot severity than the no herbicide control (P < 0.01) at Chapman (Tables 13 and 14). Across non-seed treated plots, sulfentrazone and flumioxazin applications resulted in 21.2–28.1% higher root rot severity than the no herbicide control at Cordova (Figure 3A) while sulfentrazone application resulted in 10.9% lower root rot severity at Mead (P < 0.05; Figure 3B).

Additionally, across fungicide seed treated plots, flumioxazin application resulted in 12.9% higher root rot severity than the no herbicide control ($P < 0.05$) at Cordova (Figure 3a). The sensitive cultivar resulted in 5.9%–7.9% higher root rot severity than the tolerant cultivar ($P < 0.05$) at Auburn and Tekamah (Tables 13 and 14). Within the sensitive cultivar, flumioxazin application resulted in 25.4% higher root rot than the no herbicide control at Cordova (Figure 4A) while sulfentrazone application resulted in 4.7% lower root rot severity than the no herbicide control at Lincoln ($P < 0.05$; Figure 4B). Alternatively, within the tolerant cultivar, sulfentrazone application resulted in 15.2% higher root rot severity than the no herbicide control at Cordova (Figure 4A) while flumioxazin application resulted in 6.4% higher root rot severity than the no herbicide control at Lincoln ($P < 0.05$; Figure 4B). There was a significant Herbicide X ST X Cultivar interaction ($P = 0.03$) at Schuyler where within the sensitive cultivar, flumioxazin applications resulted in 13% higher root rot than the no herbicide control across fungicide seed treated plots (Figure 5). There were no significant effects at Clearwater and Ord for root rot severity (Table 13).

At Lincoln, sulfentrazone applications resulted in 0.8 g more root biomass than when flumioxazin was applied ($P = 0.04$; Tables 15 and 16). The fungicide seed treatment resulted in 0.5–0.8 g less root biomass than no seed treatment ($P \leq 0.01$) at Cordova and Lincoln (Tables 15 and 16). Within the sensitive cultivar, the no herbicide control resulted in 1.2 g more root biomass than flumioxazin at Chapman and 2.8 g more root biomass than sulfentrazone at Clearwater ($P \leq 0.05$; Table 15, *data not shown*). At Chapman, when no herbicide was used, the sensitive cultivar resulted in 1.0 g more root

biomass than the tolerant ($P \leq 0.05$; *data not shown*). Within the tolerant cultivar, the fungicide seed treatment resulted in 2.3–2.4 g more root biomass than no seed treatment when flumioxazin was used at Schuyler and when no herbicide was used at Tekamah ($P = 0.05$; Table 15, *data not shown*). Additionally, within the tolerant cultivar the no herbicide control resulted in 2.1–2.3 g more root biomass than when flumioxazin was applied across non-seed treated plots at Schuyler and fungicide seed treated plots at Tekamah ($P = 0.05$; Table 15, *data not shown*).

Sulfentrazone application resulted in 1.1–1.3 g less aboveground biomass than the no herbicide control at Auburn and Tekamah and 1.3–2.9 g less aboveground biomass than flumioxazin application ($P < 0.05$) at 3 of 9 locations (Auburn, Chapman, and Tekamah) (Tables 17 and 18). The fungicide seed treatment resulted in 1.5 g more aboveground biomass at Auburn and 2.8 g less biomass at Cordova compared to no seed treatment ($P \leq 0.01$; Tables 17 and 18). The fungicide seed treatment resulted in 2.3–8.3 g more aboveground biomass than no seed treatment when sulfentrazone applications were made at Clearwater and when no herbicide was used at Mead ($P = 0.05$; Table 17, *data not shown*). Additionally across non-seed treated plots, sulfentrazone application resulted in 8.4–9.1 g less aboveground biomass than flumioxazin and the no herbicide control at Clearwater while resulting in 2.3 g more aboveground biomass than the no herbicide control ($P = 0.05$) at Mead (Table 17, *data not shown*).

The tolerant cultivar resulted in 1.6–3.6 g more aboveground biomass than the sensitive cultivar ($P < 0.05$) at 6 of 9 locations (Auburn, Chapman, Cordova, Mead, Ord, and Tekamah; Tables 17 and 18). At Clearwater, the tolerant cultivar resulted in 22 g

more aboveground biomass than the sensitive cultivar when sulfentrazone was applied ($P < 0.01$; Table 17, data not shown). Within the sensitive cultivar at Clearwater, sulfentrazone application resulted in 10.6–11.2 g less aboveground biomass than flumioxazin and the no herbicide control ($P < 0.01$; Table 17, *data not shown*). At Lincoln, the tolerant cultivar resulted in 1.8–4.8 g more aboveground biomass than the sensitive cultivar across both non seed treated and fungicide seed treated plots ($P = 0.02$; Table 17, *data not shown*). At Schuyler, the tolerant cultivar resulted in 17.4–22.9 g more aboveground biomass than the sensitive cultivar across fungicide seed treated plots when either sulfentrazone or flumioxazin applications were made ($P = 0.02$; Table 17, *data not shown*). Additionally, at Schuyler, within the tolerant cultivar the no herbicide control resulted in 22 g more aboveground biomass than when flumioxazin applications were made across non-seed treated plots ($P = 0.02$; *data not shown*). At all 5 locations in 2017, the tolerant cultivar resulted in 1.0–16.0 cm more than the sensitive cultivar ($P < 0.05$; Tables 19 and 20).

Pathogen Isolation. A total of 309 fungal isolates with morphological characteristics of *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. were isolated from symptomatic roots from all experiment locations (Table 21). *Fusarium* spp. were the most commonly isolated organism (n = 186), *Pythium* spp. were the second most commonly isolated organism (n = 111), and very few *Rhizoctonia* spp. isolates were recovered from the experiment locations (n = 12; Table 21). Of the three pathogen groups, *Fusarium* spp. were the most commonly isolated at 6 of the 9 locations (Auburn, Chapman, Clearwater, Lincoln, Mead, and Ord), *Pythium* spp. were the most common at 2 locations (Cordova

and Tekamah) while *Rhizoctonia* spp. were most common at Schuyler (Table 21). Mead had the highest number of isolates recovered (n = 70) while Schuyler had the least number of isolates recovered (n = 18; Table 21). Figure 6 displays the relative fungal isolate characterization of each of the 9 locations. A Pearson chi-square value of 136.82 was calculated from the contingency table analysis and the relationship between location and isolation frequency was determined to be significant ($P < 0.0001$). Figure 7 displays the relative fungal isolate characterization of each root rot severity class. Classes include "High" (Cordova), "Moderate" (Auburn, Chapman, Mead, Ord, and Tekamah), and "Low" (Clearwater, Lincoln, and Schuyler). Root rot severity classes were established by separating locations that were significantly different ($P < 0.001$) from each other in root rot severity means of the non-seed treated plots at each location (data not shown). A Pearson chi-square value of 47.35 was calculated from the contingency table analysis and the relationship between root rot severity classes and isolation frequency was determined to be significant ($P < 0.0001$). The locations in the "Low" class had the least amount of isolation recovery (16–23 isolates) and had no one pathogen isolated far more than the others (Figure 7). The locations in the "Moderate" class had intermediate to high amount of isolation recovery (30–70 isolates) and all locations had *Fusarium* spp. as the dominant pathogen isolated except for Tekamah which had *Pythium* spp. as the dominant pathogen (Figure 7). The "High" class consisted of only the Cordova location and had an intermediate amount of isolation recovery (45 isolates) with *Pythium* spp. being the dominant pathogen isolated (Figure 7).

Non-Seedling Disease Monitoring. The sensitive cultivar resulted in significantly higher SDS scores than the tolerant cultivar at both SDS evaluation timings. Within the sensitive cultivar, mean incidence (DI) was 7.0% with mean severity (DS) of 44.0 resulting in a mean index (DX) of 34.9 compared to within the tolerant cultivar where DI was 5.5%, DS was 7.5, which resulted in a DX of 4.6 ($P < 0.05$). At Auburn, the sensitive cultivar resulted in significantly higher stem canker incidence than the tolerant cultivar with 5.6% compared to 2.8% ($P < 0.01$).

Grain Yield. Sulfentrazone and flumioxazin applications resulted in 455 kg/ha and 406 kg/ha less than the no herbicide control ($P < 0.01$) at Tekamah (Tables 22 and 23). The fungicide seed treatment resulted in 167 kg/ha and 223 kg/ha more than the no seed treatment control ($P < 0.05$) at Auburn and Ord (Tables 22 and 23). The tolerant cultivar yielded 384–463 kg/ha more than the sensitive cultivar at Auburn and Chapman yet yielded 148–368 kg/ha less than the sensitive cultivar at Ord and Schuyler ($P \leq 0.01$; Tables 22 and 23). Within the sensitive cultivar at Mead, the fungicide seed treatment resulted in 163 kg/ha more than no seed treatment ($P = 0.03$; Table 22, *data not shown*). There were no significant effects at Clearwater, Cordova, and Lincoln for yield (Table 22).

2.4 Discussion

In this study, preemergence PPO-inhibitor herbicides resulted in greater phytotoxicity at the VE-V1 growth stages compared to the non-treated control at 7 of 9 locations. As was observed by the significant location effect ($P < 0.0001$), environment of each location was one factor determining the level of phytotoxicity resulting from PPO-

inhibitor applications. Another factor that influenced the level of phytotoxicity observed at locations was herbicide rate. Specific rates for sulfentrazone and flumioxazin used in this study were selected based on soil characteristics of the 2016 locations and were chosen for consistency throughout experiments. Location specific rates were later determined using soil characteristic information such as soil type, organic matter, and pH. Based on this information, 7 of 9 locations received lower than the recommended label rate of sulfentrazone which resulted in less herbicide concentration available in the soil solution. Clearwater was the only location that received the recommended rate of sulfentrazone, while Chapman received a higher rate than recommended. The rate of flumioxazin used throughout the study was appropriate for all locations based on the label recommendations. The elevated rate of sulfentrazone delivered, in combination with precipitation events up to 7 days post-emergence, could help explain why phytotoxicity was observed at higher levels at Chapman than at any other location (Tables 2 and 4). Although precipitation events occurred after emergence at 4 of 9 locations, three of these locations (Auburn, Clearwater, and Cordova) received either the recommended rate or lower of sulfentrazone which reduced the risk of crop injury.

PPO-inhibitor phytotoxicity was observed at 8 of 9 locations, yet sulfentrazone and flumioxazin applications had significant effects on plant population at only three locations (Table 5). At Chapman, where phytotoxicity was highest, PPO-inhibitors resulted in lower early season plant populations; however, there was no corresponding effect on yield. At Clearwater and Schuyler, where phytotoxicity was generally lower, PPO-inhibitor effects on plant populations were inconsistent and also had no effect on

yield. In contrast, sulfentrazone and flumioxazin applications resulted in phytotoxicity and lower yields at Tekamah without any significant effect on plant populations. This lack of effect on yield from changes in planting populations may be due to soybean plants being able to compensate for reduced populations and adjust to the growing space available (Carpenter & Board, 1997). In general, applications of PPO-inhibitors impacted plant growth through lower vigor, root biomass, and aboveground biomass at several of the locations that phytotoxicity was observed.

PPO-inhibitors had either a significant effect or interactions with seed treatments and cultivars on root rot severity at 5 of 9 locations. Phytotoxicity due to PPO-inhibitors was not consistently associated with significant PPO-inhibitor effects on root rot severity. Root rot severity was greater with herbicide applications at Cordova and Chapman, yet these locations had either no observed phytotoxicity (Cordova) or highest phytotoxicity (25% ave. at Chapman). In general, phytotoxicity was observed at lower incidences at the three other locations where applications of either sulfentrazone or flumioxazin resulted in a significant effect on root rot severity.

Rainfall events close to emergence was a useful factor in determining the effect of sulfentrazone and flumioxazin applications on root rot severity. Only at Lincoln and Mead, locations that had no rainfall through emergence, did sulfentrazone result in lower root rot severity. Conversely at Chapman and Cordova, locations with high amounts of rainfall through emergence, sulfentrazone and flumioxazin resulted in higher root rot severity with either sulfentrazone or flumioxazin applications (Tables 2 and 13). Higher root rot severities with herbicide applications were observed at locations that had

moderate to high levels of root rot (Chapman, Cordova, and Mead) as well as low levels (Lincoln and Schuyler). At Schuyler, isolation recovery was also low (16 isolates) and half of these were *Rhizoctonia* spp. This is similar to results observed in previous field studies performed by Bradley et al. (2002), which reported significant increases in Rhizoctonia root rot at low frequency across several locations when applications of non-PPO-inhibitor preemergent herbicides were made. None of the locations that had significant herbicide effects on root rot severity resulted in corresponding effects on yield. Even at Chapman, where the PPO-inhibitor applications resulted in higher root rot severity and lower plant populations than the no herbicide control, there was no impact on yield. It is possible that the differences in root rot observed at Chapman were reflective of early season symptoms of SDS infection. This supports findings recently reported by Kandel et al. (2018), which did not result in any effect of preemergent PPO-inhibitor herbicides on SDS severity or yield; and this research did not evaluate root rot levels in the experiments.

Pathogen isolation in isolates from nearly 40% of the total plated root segments. *Fusarium* spp. were the most abundant isolated organisms throughout the entire experiment and were the most abundant at 6 of 9 locations. The large percentage of *Fusarium* spp isolates at locations with lower disease severity could mean that some of the *Fusarium* spp. present in the roots were non-pathogenic saprophytes. There did not appear to be any relationship between frequency of isolation and herbicide affect on root rot severity. In the “Low” root rot class (Clearwater, Lincoln, and Schuyler), where no one dominant pathogen was isolated consistently, both higher and lower root rot

severities were observed when herbicide applications were made, yet there were no affects on yield. In the "Moderate" root rot class (Auburn, Chapman, Mead, Ord, and Tekamah) where *Fusarium* spp. were the dominant pathogen isolated at 4 of the 5 locations, sulfentrazone applications resulted in lower root rot severity at Mead while both PPO-inhibitors resulted in higher root rot at Chapman. Neither effects were reflected on yield. At 3 of 5 locations (Auburn, Mead, and Ord) where root rot levels were moderately high, the fungicide seed treatment did result in higher yield. At Cordova, the only location within the "High" root rot class, where *Pythium* spp. were the dominant pathogen isolated, PPO-inhibitors resulted in higher root rot severity yet showed no impact on yield.

Fungicide seed treatment did not have a consistent effect on phytotoxicity as there were significant seed treatment interactions at only two locations. Results from these two locations differed, however, as fungicide seed treatment showed higher phytotoxicity at Ord yet lower at Schuyler, compared to no seed treatment. In general, the inconsistency and overall lack of fungicide seed treatment effects on phytotoxicity were expected as the fungicide active ingredients used in this study do not typically result in any phytotoxicity in soybean. Fungicide seed treatment showed increased plant populations and yield at 2 of 9 locations (Auburn and Ord) (Figure 8) and differences in root rot severity due to fungicide were nearly significant ($P = 0.06$) at Auburn yet not significant at Ord ($P = 0.29$; Table 14). Pathogenic inoculum can vary spatially within a plot and the selection of six plants from the outer two rows may not have been able to fully represent the disease levels throughout the entire plot at these locations. SDS was present at Chapman in 2016

and the fungicide seed treatment did not show a significant effect on plant population, root rot severity, or yield. The fungicides used as the seed treatment in this study are not recommended for control of *F. virguliforme* and would not be expected to affect infection and disease development (Jackson-Ziems et al., 2017).

Cultivars showed differences in phytotoxicity at 4 of 9 locations and unexpectedly, the tolerant cultivar had higher levels of phytotoxicity than the sensitive at these locations. The tolerant cultivar had a higher field emergence score than the sensitive, which would have resulted in earlier emergence and increased risk of injury at locations with precipitation events near emergence (Dupont-Pioneer, 2018). Previous research has shown that when environmental conditions are adverse to crop growth, it is possible to observe phytotoxicity in cultivars with tolerance to PPO-inhibitors (Taylor-Lovell et al., 2001; Li et al., 1999; Hulting et al., 2001). The observations of PPO-inhibitor injury occurring in both the sensitive and tolerant cultivars indicate the importance of screening cultivars for sensitivity to PPO-inhibitors. Cultivars resulted in inconsistent differences in plant populations as the sensitive cultivar was observed to have higher populations than the tolerant at 5 of 9 locations and only resulted in increased yield at Schuyler. The sensitive cultivar yielded more than the tolerant at Ord and there was a trend of higher populations for the sensitive cultivar at R8 growth stage, though non-significant ($P = 0.10$). The tolerant cultivar yielded more than the sensitive at 2 of 9 locations (Auburn and Chapman) and were likely due to genetic differences between cultivars to late season non-seedling disease development. Stem canker was observed at low incidences at Auburn and the sensitive cultivar had significantly higher incidences. As was noted

earlier, SDS was observed throughout plots at Chapman and the sensitive cultivar had significantly higher incidence and severity of SDS which appeared to impact yield.

Some of the results observed in this study suggest the potential for PPO-inhibitor applications to increase root rot severity. There is a need for additional research on the interactions of PPO-inhibitors, fungicide seed treatments, and cultivar sensitivity and their effects on soybean seedling diseases and yield. Further research should be located in fields with history of high levels of disease pressure, as we observed a response in root rot severity from PPO-inhibitor injury more often at locations with moderate to high amounts of root rot. Research should be conducted with high disease pressure under controlled conditions as well to produce varying levels of PPO-inhibitor injury, which will serve to better understand the effect of these herbicides in absence of injury. For future studies aiming to determine the effect of cultivar sensitivity to PPO-inhibitors on seedling disease, it would be beneficial to compare many sensitive and tolerant cultivars as a comparison of just two cultivars is inadequate. It is also critical for studies to isolate and identify the species and groups of pathogens present, as pathogen populations may vary significantly by location, which was demonstrated by this study.

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2.6 Figures

Figure 1. Symptoms of PPO-inhibitor injury include chlorosis and necrosis on soybean cotyledon and hypocotyl. Symptomatic seedlings indicated by arrows. Healthy seedling indicated by star.

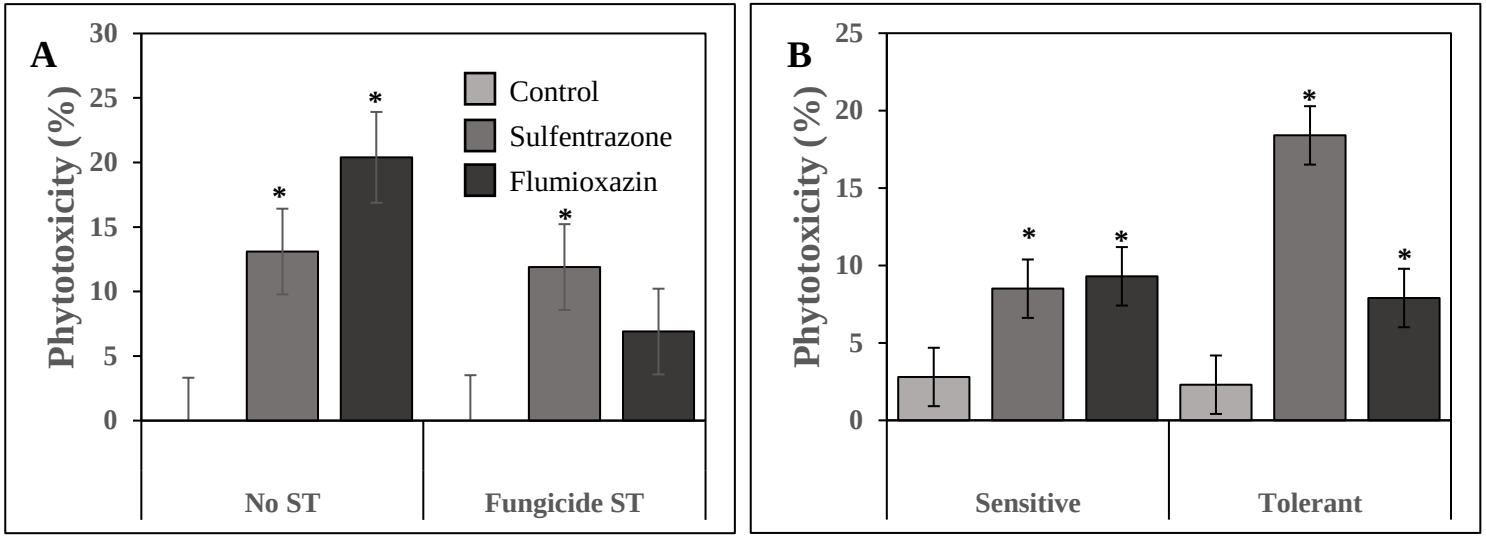

Figure 2. (A) Phytotoxicity for herbicide treatments within seed treatments (P = 0.05) at Schuyler in 2016. (B) Phytotoxicity for herbicide treatments within cultivars (P < 0.01) at Ord in 2017. Vertical bars represent the mean for each parameter and lines extending from each bar represent the standard error of mean. Some chlorosis was observed in few control plots resulting in a non-zero standard error. Fungicide seed treatment (ST) consisted of mefenoxam (Apron XL®, Syngenta Crop Protection LLC, 0.64 mg a.i./seed) + fludioxonil (Maxim 4FS®, Syngenta Crop Protection LLC, 0.0076 mg a.i./seed) + sedaxane (Vibrance®, Syngenta Crop Protection LLC, 0.0076 mg a.i./seed). Phytotoxicity was rated visually for total plot incidence on a linear 0-100% scale from VE-V1 growth stages. *indicates significant difference at $\alpha = 0.05$ level.

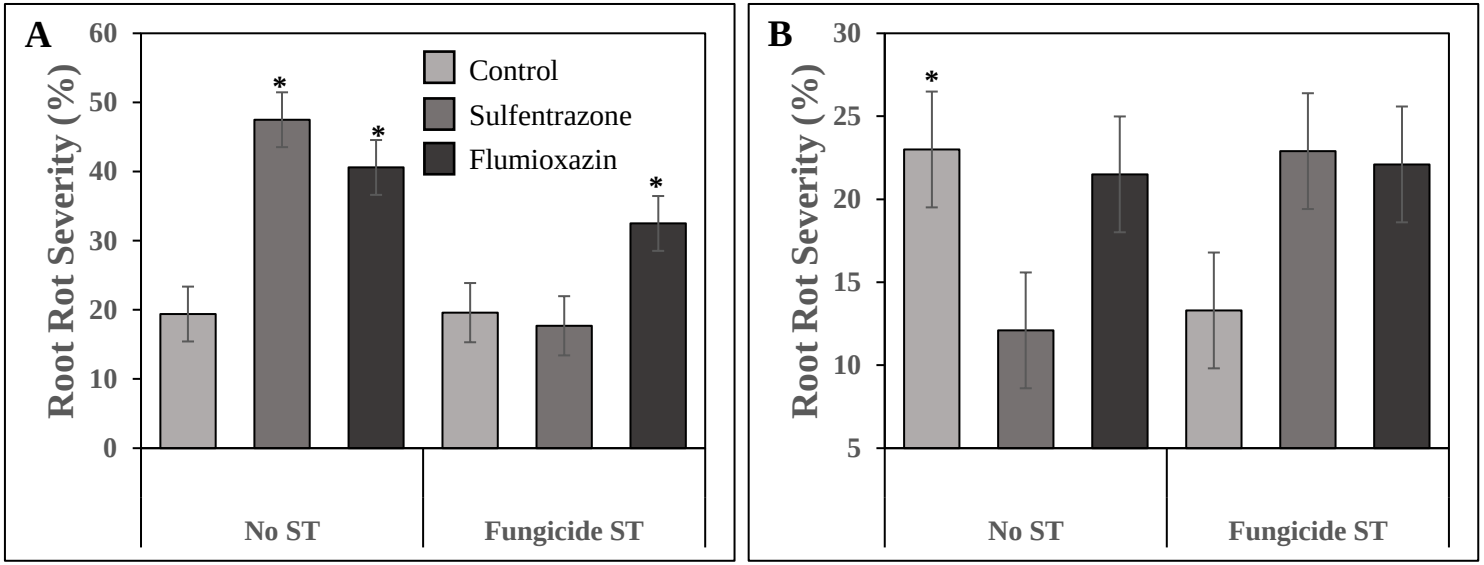

Figure 3. (A) Root rot severity for herbicide treatments within seed treatments ($P < 0.01$) at Cordova in 2016. (B) Root rot severity for herbicide treatments within seed treatments ($P < 0.05$) at Mead in 2017. Vertical bars represent the mean for each parameter and lines extending from each bar represent the standard error of mean. Fungicide seed treatment (ST) consisted of mefenoxam (Apron XL®, Syngenta Crop Protection LLC, 0.64 mg a.i./seed) + fludioxonil (Maxim 4FS®, Syngenta Crop Protection LLC, 0.0076 mg a.i./seed) + sedaxane (Vibrance®, Syngenta Crop Protection LLC, 0.0076 mg a.i./seed). Root Rot Severity was rated visually for total percent discolored area of six root systems of plants dug from each plot from V1-V4 growth stages. *indicates significant difference at $\alpha = 0.05$ level.

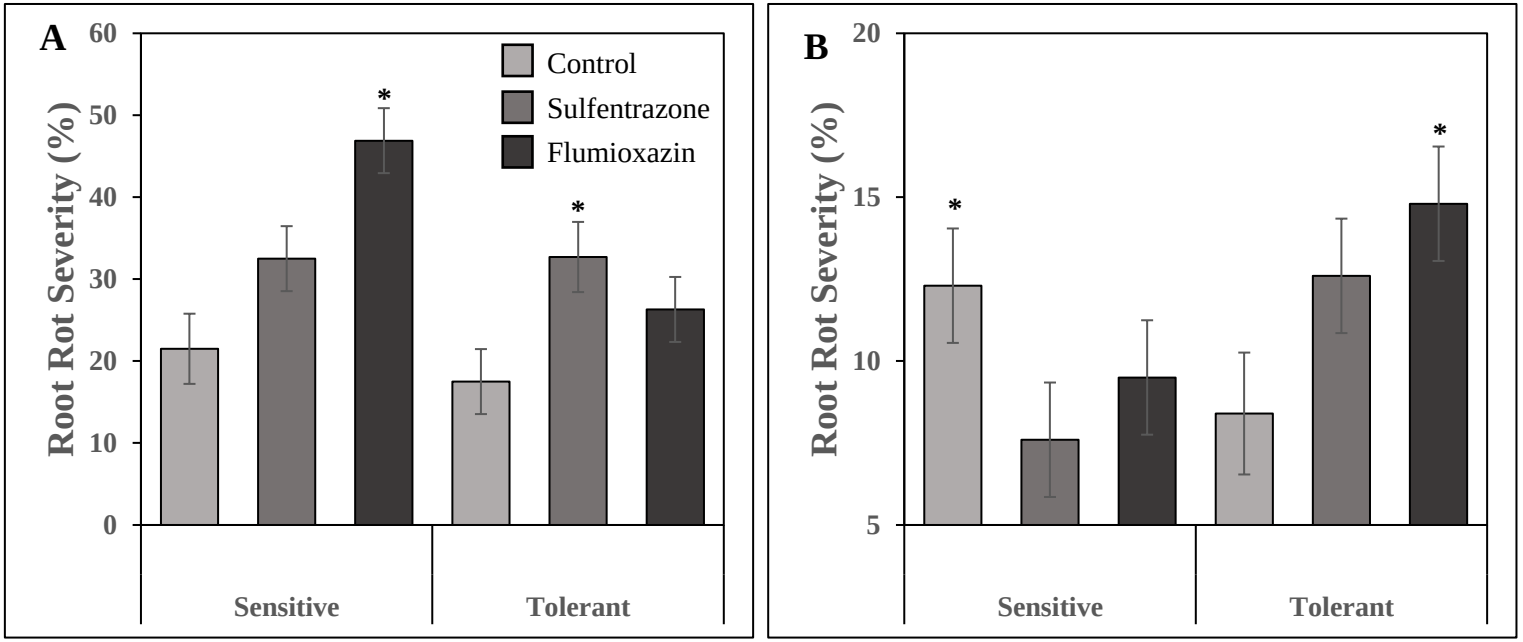

Figure 4. (A) Root rot severity for herbicide treatments within cultivars ($P < 0.05$) at Cordova in 2016. (B) Root rot severity for herbicide treatments within cultivars ($P < 0.01$) at Lincoln in 2017. Vertical bars represent the mean for each parameter and lines extending from each bar represent the standard error of mean. Root Rot Severity was rated visually for total percent discolored area of six root systems of plants dug from each plot from V1-V4 growth stages. *indicates significant difference at $\alpha = 0.05$ level.

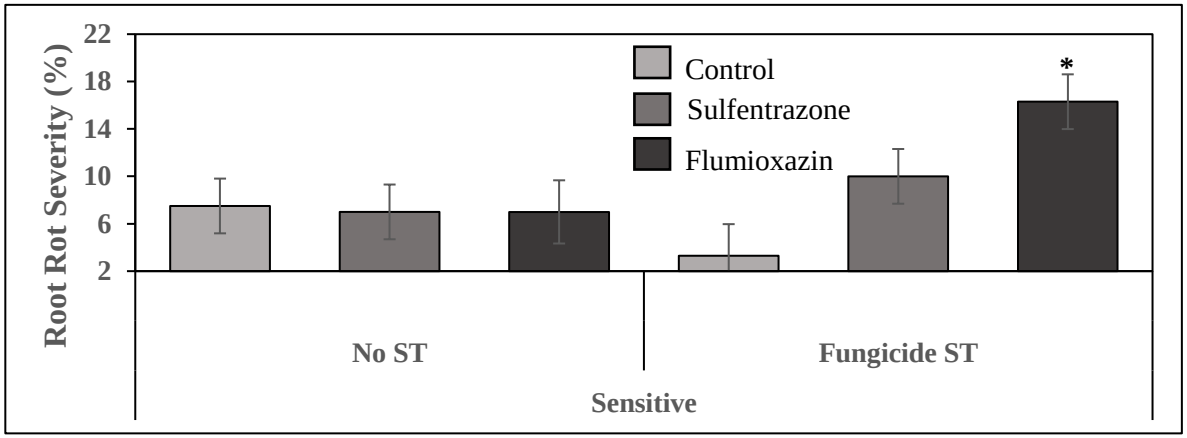

Figure 5. Root rot severity for herbicide treatments within seed treatments within the sensitive cultivar ($P < 0.05$) at Schuyler in 2016. Vertical bars represent the mean for each parameter and lines extending from each bar represent the standard error of mean. Root Rot Severity was rated visually for total percent discolored area of six root systems of plants dug from each plot from V1-V4 growth stages. *indicates significant difference at $\alpha = 0.05$ level.

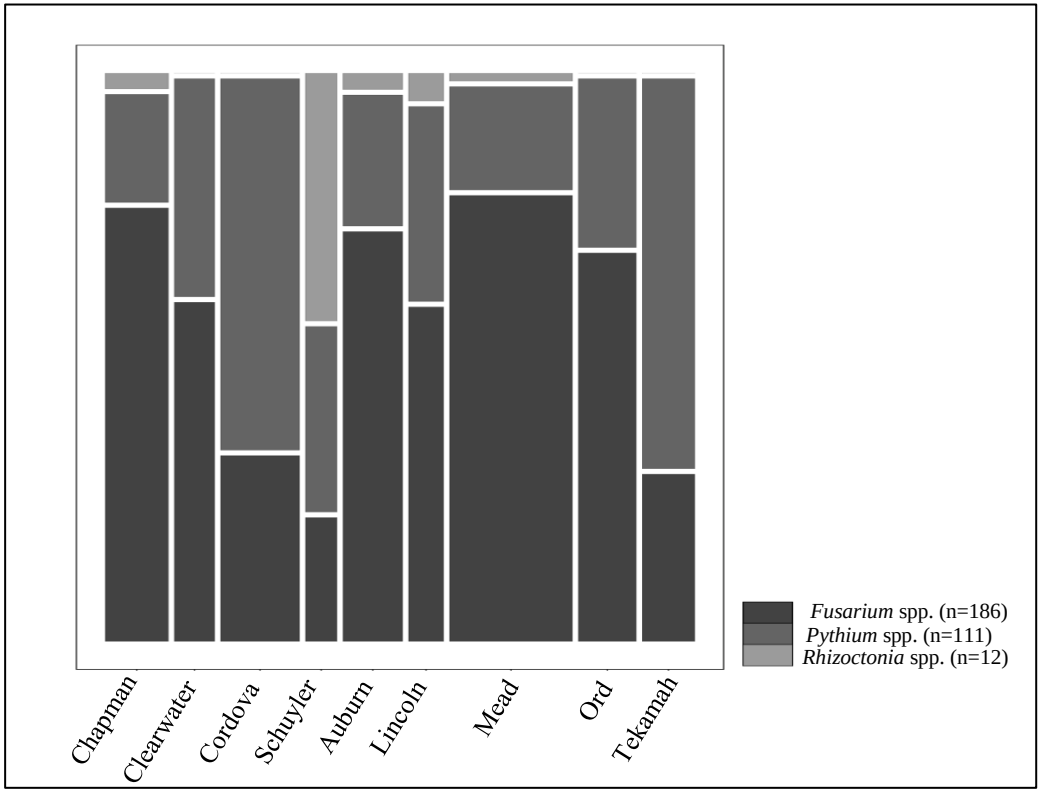

Figure 6. Proportions of fungal isolates at each Nebraska location in 2016 and 2017. The width of each bar corresponds to the relative number of isolates recovered at each location. The wider the bar, the more isolates recovered. A Pearson chi-square value of 136.82 was calculated from the contingency table analysis and the relationship between location and isolation frequency was determined to be significant ($P < 0.0001$).

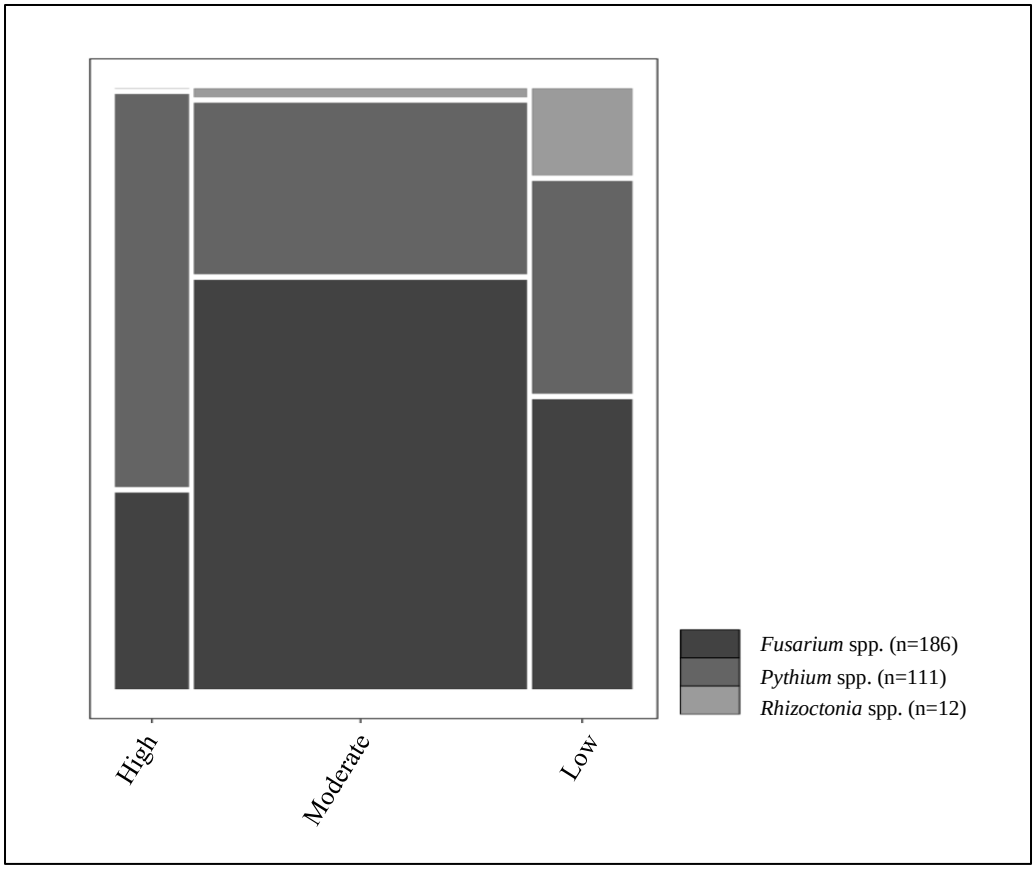

Figure 7. Proportions of fungal isolates for the root rot severity classes: High (Cordova), Moderate (Auburn, Chapman, Mead, Ord, and Tekamah), and Low (Clearwater, Lincoln, and Schuyler). The width of each bar corresponds to the relative number of isolates recovered at each location. The wider the bar, the more isolates recovered. A Pearson chi-square value of 47.35 was calculated from the contingency table analysis and the relationship between root rot severity classes and isolation frequency was determined to be significant ($P < 0.0001$).

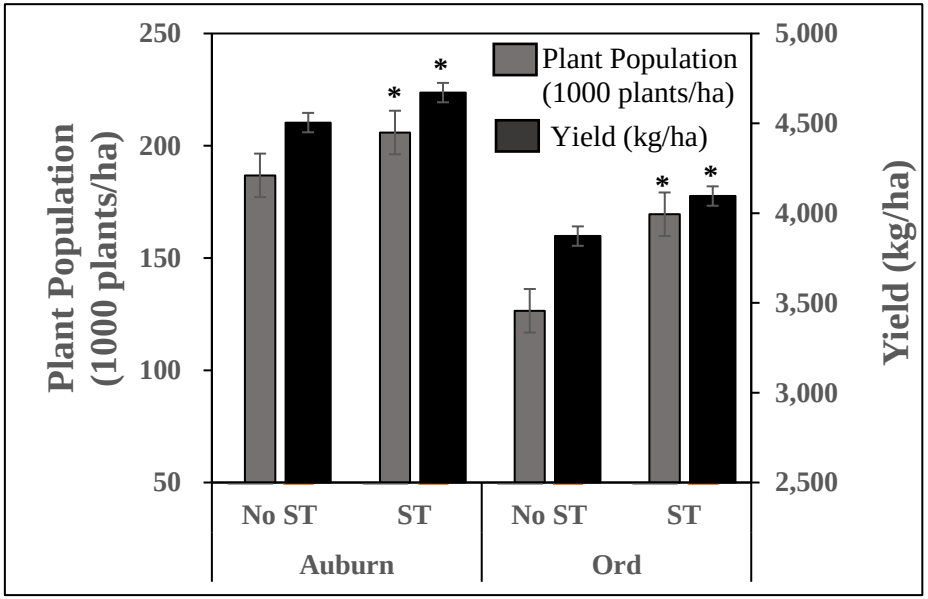

Figure 8. ST effect for plant population (1000 plants/ha) at VC-V2 growth stage and yield for Auburn ($P = 0.04$) and Ord ($P < 0.0001$ and $P < 0.01$, respectively) in 2017. Vertical bars represent the mean for each parameter and lines extending from each bar represent the standard error of mean. Populations were determined by counting the total live plants in 3.1 m of each of the two center rows of each plot. Counts from each row were combined to result in one value per plot. The center two rows of each plot were mechanically harvested at maturity. Yield data were calculated and adjusted for 13% moisture for comparison. Data*indicates significant difference at $\alpha = 0.05$ level.

2.7 Tables

Table 1. Descriptive information regarding soil type, organic matter, pH, planting date, tillage, irrigation, and harvest date for Nebraska research locations in 2016 and 2017.

Year	Location[a]	Soil Type	Organic Matter (%)	pH[c]	Planting Date	Tillage[d]	Irrigation[e]	Harvest Date
2016	Chapman	Loamy sand	1.2	7.1	5-May	Yes	Yes	5-Oct
	Clearwater	Silt loam	1.3	6.9	13-May	Yes	Yes	18-Oct
	Cordova	Silt loam	3.2	6.2	6-May	No	Yes	10-Oct
	Schuyler	Loam	2.6	6.7	19-May	No	Yes	21-Oct
2017	Auburn[b]	Silty clay loam	3.9	6.8	9-May	No	Yes	18-Oct
	Lincoln	Silty clay loam	3.0	6.9	31-May	No	No	1-Nov
	Mead	Silty clay loam	2.4	5.9	1-Jun	No	Yes	6-Nov
	Ord	Clay loam	1.5	5.8	15-May	Yes	Yes	20-Oct
	Tekamah	Silty clay loam	3.7	6.7	8-May	No	Yes	19-Oct

[a] Previous crop was corn for all locations.
[b] Auburn location had a cereal rye cover crop over winter that was terminated on 21-Apr.
[c] pH was determined by adding 10 g of soil to 10 mL water.
[d] Locations that received tillage were disked in the spring before planting.
[e] All Locations with irrigation were center pivot systems other than Mead which was furrow flood irrigation. Irrigation was not applied until after flowering at all locations except Ord which received 1.25 cm on 30-May.

Table 2. Soil temperature at planting, rainfall from planting to emergence, growing season rainfall, date of herbicide application, date of emergence, and record of precipitation events at Nebraska research locations in 2016 and 2017.

Location (Year)	Soil Temp.[a] (°C)	Rainfall to Emergence (cm)	Total Rainfall (cm)	Herbicide Application Date	DAP[b]	Emergence	Precipitation Events (DAE)[c]						
Chapman (2016)	17.8	18.7	42.9	7-May	2	20-May	-12	**-10**	**-9**	**-4**	**+3**	**+5**	**+7**
Clearwater (2016)	12.9	8.7	35.0	17-May	5	27-May	**-4**	-2	**+1**	+3	.	.	.
Cordova (2016)	18.6	12.6	46.2	10-May	4	20-May	**-10**	**-9**	-4	**+3**	+5	**+6**	**+7**
Schuyler (2016)	16.2	4.1	51.9	24-May	5	3-Jun	-9	-8	**-7**	-6	-3		
Auburn (2017)	21.9	8.3	51.8	11-May	2	22-May	-6	-5	**-4**	**-3**	-2	0	**+5**
Lincoln (2017)	22.4	0.0	73.2	3-Jun	3	8-Jun	.	.	.	.	.	.	.
Mead (2017)	21.6	0.0	69.2	3-Jun	2	8-Jun	.	.	.	.	.	.	.
Ord (2017)	18.6	8.9	53.2	18-May	3	7-Jun	-20	**-19**	**-18**	**-9**[d]	-1	.	.
Tekamah (2017)	19.9	11.7	56.6	12-May	4	22-May	**-6**	**-5**	-4	**-3**	**-2**	.	.

[a]Soil Temp. = soil temperature recorded at 10 cm depth at planting.
[b]Number of days after planting (DAP) herbicide application was made at each location
[c]Rainfall events from planting to emergence at each location. Reported as days after emergence (DAE). Negative values indicate days before emergence after planting, positive values indicate days after emergence. Precipitation events greater than 1 cm are in bold.
[d]Ord received 1.25 cm irrigation on 30-May.

Table 3. Phytotoxicity at VE-V1 growth stages recorded in Nebraska field experiments performed in 2016 and 2017 and the probability of rejecting null hypothesis (P > F) observed based on analysis of variance (ANOVA) tests for the effects of preemergence herbicides (Herbicide), seed treatment (ST), cultivar (Cultivar) and their interaction.

		2016				2017				
Factor	df[a]	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	2	**< 0.0001**[b]	**< 0.0001**	.[c]	**< 0.0001**	**< 0.0001**	**0.03**	0.73	**< 0.0001**	**< 0.01**
ST	1	0.95	0.10	.	**0.03**	0.31	0.22	0.53	0.37	0.21
Herbicide X ST	2	0.98	0.40	.	**0.05**	0.45	0.49	0.19	0.18	0.64
Cultivar	1	**< 0.01**	0.07	.	0.56	**< 0.001**	0.37	0.10	0.08	**0.03**
Herbicide X Cultivar	2	0.09	0.33	.	0.32	0.12	0.67	0.72	**< 0.01**	0.32
Cultivar X ST	1	0.23	0.83	.	0.73	0.61	0.97	0.45	**0.03**	0.91
Herbicide X ST X Cultivar	2	0.40	0.98	.	0.08	0.14	0.08	0.89	0.08	0.85

[a]Degrees of freedom (df) for each of the main effects and interactions.
[b]significant effects and interactions in **bold** ($\alpha = 0.05$).
[c] "." denotes data were not recorded. No Phytotoxicity was observed at Cordova.

Table 4. Phytotoxicity (%) at VE-V1 growth stages in Nebraska field experiments in 2016 and 2017 and the effect of preemergence herbicide treatments (Herbicide), seed treatment (ST) and cultivar (Cultivar).

		2016				2017				
Factor	Treatment	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	Control	0.0 b[a]	0.1 c	.[b]	0.0	1.7 c	3.1 b	5.2	2.5	3.9 b
	Sulfentrazone	28.1 a	8.9 a	.	12.5	15.3 a	6.7 a	6.3	13.4	15.2 a
	Flumioxazin	24.7 a	4.8 b	.	13.7	10.8 b	7.0 a	6.0	8.6	16.2 a
ST	Control	17.7	5.8	.	11.2	9.9	6.4	6.2	7.5	13.5
	Fungicide	17.5	3.5	.	6.1	8.6	4.8	5.5	8.8	10.0
Cultivar	Sensitive	11.9 b	3.3	.	9.3	6.8 b	5.0	4.8	6.8	8.6 b
	Tolerant	23.3 a	5.9	.	8.0	11.7 a	6.2	6.8	9.5	14.9 a

[a]Values followed by the same letter indicate no significant difference ($P < 0.05$) determined using Fisher's protected LSD. When interactions are significant ($P < 0.05$), significant main effects are not displayed (Table 3).
[b] "." denotes data were not recorded. No phytotoxicity observed at Cordova.

Table 5. Plant populations recorded at VE-V1 growth stages in Nebraska field experiments performed in 2016 and 2017 and the probability of rejecting null hypothesis (P > F) observed based on analysis of variance (ANOVA) tests for the effects of preemergence herbicides (Herbicide), seed treatment (ST), cultivar (Cultivar) and their interaction.

		2016				2017				
Factor	df[a]	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	2	0.07	**0.04**	0.23	0.50	0.17	0.88	0.74	0.14	0.54
ST	1	0.77	0.70	0.63	0.92	**0.04**	0.33	0.87	**< 0.0001**	0.09
Herbicide X ST	2	0.29	0.91	0.66	0.50	0.57	0.55	0.32	0.45	0.76
Cultivar	1	**< 0.001**[b]	**< 0.0001**	**< 0.01**	**< 0.001**	0.34	0.38	**< 0.001**	0.84	0.45
Herbicide X Cultivar	2	0.25	0.67	0.64	0.72	0.61	0.24	0.86	0.61	**0.02**
Cultivar X ST	1	0.10	0.45	0.35	0.92	0.45	0.06	**0.03**	0.81	0.47
Herbicide X ST X Cultivar	2	0.67	0.27	0.61	0.58	0.78	0.58	0.52	0.88	0.47

[a]Degrees of freedom (df) for each of the main effects and interactions.
[b]significant effects and interactions in **bold** ($\alpha = 0.05$).

Table 6. Plant populations (1000 plants/ha) at VE-V1 growth stages recorded in Nebraska field experiments performed in 2016 and 2017 and the effect of preemergence herbicide treatments (Herbicide), seed treatment (ST) and cultivar (Cultivar).

		2016				2017				
Factor	Treatment	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	Control	160.0	229.2 a	220.1	179.8	205.1	278.4	290.9	156.7	226.2
	Sulfentrazone	143.7	210.0 b	220.2	177.1	184.3	278.0	285.1	151.8	225.8
	Flumioxazin	143.0	211.5 b	232.6	171.8	199.5	273.7	291.4	135.5	216.1
ST	Control	149.9	218.2	222.6	176.5	186.8 b	272.6	289.7	126.5 b	215.4
	Fungicide	148.0	215.6	226.0	175.9	205.7 a	280.8	288.6	169.5 a	230.0
Cultivar	Sensitive	162.3 a[a]	231.9 a	236.7 a	186.7 a	200.6	273.0	304.8	148.9	219.5
	Tolerant	135.5 b	201.7 b	211.9 b	165.8 b	192.0	280.3	273.5	147.1	225.9

[e]Values followed by the same letter indicate no significant difference ($P < 0.05$). When interactions are significant ($P < 0.05$), significant main effects are not displayed (Table 5).

Table 7. Plant populations recorded at V2-V4 growth stages in Nebraska field experiments performed in 2016 and 2017 and the probability of rejecting null hypothesis (P > F) observed based on analysis of variance (ANOVA) tests for the effects of preemergence herbicides (Herbicide), seed treatment (ST), cultivar (Cultivar) and their interaction.

		2016				2017				
Factor	df[a]	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	2	**0.02**[b]	0.50	0.91	**0.03**	0.18	0.25	0.14	0.66	0.90
ST	1	0.74	0.66	0.61	0.28	< **0.01**	0.50	0.31	< **0.001**	0.91
Herbicide X ST	2	0.38	0.91	0.95	0.47	0.67	0.22	0.42	0.64	0.27
Cultivar	1	< **0.01**	< **0.001**	0.11	0.44	0.19	0.08	< **0.0001**	0.80	0.73
Herbicide X Cultivar	2	0.06	0.92	0.92	0.10	0.08	0.44	0.31	0.90	0.12
Cultivar X ST	1	0.30	**0.01**	**0.04**	0.96	0.48	**0.04**	< **0.01**	0.71	0.75
Herbicide X ST X Cultivar	2	0.47	0.32	0.77	0.34	0.76	0.84	0.62	0.49	0.71

[a]Degrees of freedom (df) for each of the main effects and interactions.
[b]significant effects and interactions in **bold** ($\alpha = 0.05$).

Table 8. Plant populations (1000 plants/ha) at V2-V4 growth stages recorded in Nebraska field experiments performed in 2016 and 2017 and the effect of preemergence herbicide treatments (Herbicide), seed treatment (ST) and cultivar (Cultivar).

		2016				2017				
Factor	Treatment	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	Control	177.6 a[a]	195.4	205.3	188.3 ab	204.0	262.6	287.5	137.8	210.7
	Sulfentrazone	159.6 b	192.1	206.4	196.2 a	190.7	275.8	280.5	145.2	209.4
	Flumioxazin	157.0 b	187.3	203.3	180.8 b	205.7	263.4	293.6	135.6	205.7
ST	Control	165.8	192.9	203.4	186.0	189.9 b	264.9	284.5	121.9 b	208.1
	Fungicide	163.7	190.3	206.6	190.8	210.3 a	269.7	289.9	157.2 a	209.1
Cultivar	Sensitive	175.4	204.0	200.0	186.7	204.8	273.8	301.7	140.6	210.2
	Tolerant	154.1	179.2	210.0	190.2	195.5	260.8	272.7	138.4	207.0

[a]Values followed by the same letter indicate no significant difference ($P < 0.05$). When interactions are significant ($P < 0.05$), significant main effects are not displayed (Table 7).

Table 9. Plant populations recorded at R8 growth stage in Nebraska field experiments performed in 2016 and 2017 and the probability of rejecting null hypothesis (P > F) observed based on analysis of variance (ANOVA) tests for the effects of preemergence herbicides (Herbicide), seed treatment (ST), cultivar (Cultivar) and their interaction.

		2016				2017				
Factor	df[a]	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	2	0.39	0.91	0.38	0.37	0.87	0.61	0.31	0.56	0.23
ST	1	0.82	0.62	0.70	0.48	**0.02**	0.06	0.28	< **0.001**	0.42
Herbicide X ST	2	0.78	0.88	0.81	0.60	0.26	0.30	0.46	0.34	0.25
Cultivar	1	< **0.001**[b]	0.13	< **0.01**	< **0.0001**	0.06	< **0.01**	< **0.01**	0.10	< **0.01**
Herbicide X Cultivar	2	0.06	0.13	0.95	0.41	0.49	0.12	0.76	0.89	0.08
Cultivar X ST	1	0.35	0.35	0.29	0.14	0.56	0.21	**0.03**	0.87	0.09
Herbicide X ST X Cultivar	2	0.98	0.12	0.79	0.99	< **0.01**	0.95	0.74	0.44	0.74

[a]Degrees of freedom (df) for each of the main effects and interactions.
[b]significant effects and interactions in **bold** ($\alpha = 0.05$).

Table 10. Plant populations (1000 plants/ha) at R8 growth stage recorded in Nebraska field experiments performed in 2016 and 2017 and the effect of preemergence herbicide treatments (Herbicide), seed treatment (ST) and cultivar (Cultivar).

		2016				2017				
Factor	Treatment	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	Control	160.8	205.3	194.0	170.7	185.3	226.0	240.9	132.1	204.6
	Sulfentrazone	151.1	205.1	194.6	169.7	181.4	231.3	236.4	125.8	191.2
	Flumioxazin	153.3	202.6	204.1	162.9	184.9	228.9	249.5	124.9	198.7
ST	Control	154.4	205.8	198.9	169.5	176.1	224.6	246.1	116.4 b	195.6
	Fungicide	155.7	202.9	196.3	166.0	191.6	232.9	238.4	138.8 a	200.7
Cultivar	Sensitive	166.1 a[a]	208.8	209.2 a	188.2 a	190.2	235.1 a	254.6	132.7	208.2 a
	Tolerant	144.0 b	199.9	186.0 b	147.3 b	177.5	222.4 b	229.9	122.5	188.2 b

[a]Values followed by the same letter indicate no significant difference ($P < 0.05$). When interactions are significant ($P < 0.05$), significant main effects are not displayed (Table 9).

Table 11. Vigor at V2 - V4 growth stages recorded in Nebraska field experiments performed in 2016 and 2017 and the probability of rejecting null hypothesis (P > F) observed based on analysis of variance (ANOVA) tests for the effects of preemergence herbicides (Herbicide), seed treatment (ST), cultivar (Cultivar) and their interaction.

		2016				2017				
Factor	df[a]	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	2	< **0.001**[b]	< **0.0001**	0.46	< **0.0001**	< **0.001**	0.89	0.86	0.23	< **0.01**
ST	1	0.58	0.35	0.57	0.76	< **0.001**	0.49	0.48	< **0.0001**	**0.05**
Herbicide X ST	2	**0.05**	0.35	0.30	0.70	0.12	0.31	0.43	0.98	0.34
Cultivar	1	0.78	**0.05**	0.21	0.44	< **0.0001**	0.42	0.08	0.06	0.34
Herbicide X Cultivar	2	0.10	< **0.01**	0.06	0.59	< **0.01**	0.21	0.19	0.35	0.62
Cultivar X ST	1	0.27	0.61	0.18	0.44	**0.03**	0.91	0.90	0.80	0.34
Herbicide X ST X Cultivar	2	0.87	0.10	0.30	0.70	0.95	0.89	0.50	0.42	0.09

[a]Degrees of freedom (df) for each of the main effects and interactions.
[b]significant effects and interactions in **bold** ($\alpha = 0.05$).

Table 12. Vigor (%) at V2-V4 growth stages recorded in Nebraska field experiments performed in 2016 and 2017 and the effect of preemergence herbicide treatments (Herbicide), seed treatment (ST) and cultivar (Cultivar).

		2016				2017				
Factor	Treatment	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	Control	78.1	84.7	75.4	85.8 a[a]	68.8	68.2	68.8	53.8	67.5 a
	Sulfentrazone	61.3	66.3	70.6	76.9 b	58.6	69.1	66.9	53.1	54.1 b
	Flumioxazin	64.4	73.8	73.8	58.8 c	60.6	67.4	66.8	47.5	56.6 b
ST	Control	67.1	74.0	74.2	74.2	59.0	67.3	68.7	43.1 b	55.8 b
	Fungicide	68.8	75.8	72.4	73.5	66.4	69.3	66.3	59.8 a	62.9 a
Cultivar	Sensitive	68.3	72.9	75.3	74.7	57.0	67.1	64.4	48.3	57.7
	Tolerant	67.5	76.9	71.3	72.9	68.4	69.4	70.5	54.6	61.0

[a]Values followed by the same letter indicate no significant difference ($P < 0.05$). When interactions are significant ($P < 0.05$), significant main effects are not displayed (Table 11).

Table 13. Root rot severity recorded in Nebraska field experiments performed in 2016 and 2017 and the probability of rejecting null hypothesis (P > F) observed based on analysis of variance (ANOVA) tests for the effects of preemergence herbicides (Herbicide), seed treatment (ST), cultivar (Cultivar) and their interaction.

		2016				2017				
Factor	df[a]	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	2	**< 0.01**[b]	0.18	**< 0.001**	**0.03**	0.68	0.37	0.41	0.69	0.17
ST	1	0.74	0.17	**< 0.001**	0.16	0.06	0.82	0.85	0.29	0.50
Herbicide X ST	2	0.24	0.69	**< 0.01**	0.25	0.67	0.73	**0.02**	0.50	0.74
Cultivar	1	0.25	0.17	**0.02**	0.07	**0.03**	0.10	0.39	0.26	**< 0.01**
Herbicide X Cultivar	2	0.36	0.65	**0.03**	0.67	0.60	**< 0.01**	0.77	0.22	0.35
Cultivar X ST	1	0.74	0.82	0.85	0.60	0.51	0.45	0.40	0.23	0.87
Herbicide X ST X Cultivar	2	0.32	0.08	0.93	**0.03**	0.15	0.75	0.33	0.77	0.53

[a]Degrees of freedom (df) for each of the main effects and interactions.
[b]significant effects and interactions in **bold** ($\alpha = 0.05$).

Table 14. Root rot severity (%) at V1-V4 growth stages recorded in Nebraska field experiments performed in 2016 and 2017 and the effect of preemergence herbicide treatments (Herbicide), seed treatment (ST) and cultivar (Cultivar).

		2016				2017				
Factor	Treatment	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	Control	14.7 b[a]	7.8	19.5	7.6	16.9	10.3	18.1	14.3	14.1
	Sulfentrazone	24.7 a	10.6	32.6	9.5	19.7	10.1	17.5	12.5	13.4
	Flumioxazin	21.3 a	9.8	36.6	12.4	18.4	12.1	21.8	14.4	18.8
ST	Control	19.8	10.4	35.8	8.8	20.8	11.0	18.9	14.8	16.3
	Fungicide	20.6	8.4	23.3	10.8	15.8	10.7	19.4	12.7	14.6
Cultivar	Sensitive	18.8	8.5	33.6	8.5	21.3 a	9.8	17.9	12.6	19.4 a
	Tolerant	21.7	10.3	25.5	11.1	15.4 b	11.9	20.4	14.9	11.5 b

[a]Values followed by the same letter indicate no significant difference ($P < 0.05$). When interactions are significant ($P < 0.05$), significant main effects are not displayed (Table 13).

Table 15. Root biomass at V1-V4 growth stages recorded in Nebraska field experiments performed in 2016 and 2017 and the probability of rejecting null hypothesis (P > F) observed based on analysis of variance (ANOVA) tests for the effects of preemergence herbicides (Herbicide), seed treatment (ST), cultivar (Cultivar) and their interaction.

		2016				2017				
Factor	df[a]	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	2	0.27	0.12	0.10	0.43	0.28	**0.04**	0.68	0.87	0.40
ST	1	0.60	0.26	**0.01**	0.53	0.07	< **0.01**	0.16	0.37	0.43
Herbicide X ST	2	0.58	0.06	0.87	0.47	0.29	0.81	0.17	0.97	0.10
Cultivar	1	0.32	0.06	0.75	0.29	0.66	0.23	0.78	0.28	0.27
Herbicide X Cultivar	2	**0.05**[b]	**0.03**	0.77	0.57	0.45	0.09	0.87	0.50	0.54
Cultivar X ST	1	0.49	0.70	0.65	0.12	0.31	0.76	1.0	0.37	0.64
Herbicide X ST X Cultivar	2	0.86	0.80	0.55	**0.05**	0.46	0.64	0.94	0.79	**0.05**

[a]Degrees of freedom (df) for each of the main effects and interactions.
[b]significant effects and interactions in **bold** ($\alpha = 0.05$).

Table 16. Root biomass (g) at V1-V4 growth stages recorded in Nebraska field experiments performed in 2016 and 2017 and the effect of preemergence herbicide treatments (Herbicide), seed treatment (ST) and cultivar (Cultivar).

		2016				2017				
Factor	Treatment	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	Control	7.8	11.9	5.9	12.2	4.0	3.0 ab	4.2	5.8	3.6
	Sulfentrazone	7.3	10.7	5.6	12.1	3.4	3.4 a	4.0	5.5	3.4
	Flumioxazin	7.4	11.2	5.4	11.5	3.6	2.6 b	4.3	5.5	3.2
ST	Control	7.6	11.0	5.9 a[a]	11.8	3.4	3.4 a	4.0	5.8	3.3
	Fungicide	7.4	11.5	5.4 b	12.1	4.0	2.6 b	4.4	5.4	3.5
Cultivar	Sensitive	7.6	10.8	5.6	11.7	3.8	2.8	4.2	5.3	3.3
	Tolerant	7.4	11.7	5.7	12.2	3.6	3.2	4.1	5.8	3.5

[a]Values followed by the same letter indicate no significant difference ($P < 0.05$). When interactions are significant ($P < 0.05$), significant main effects are not displayed (Table 15).

Table 17. Aboveground biomass recorded at V1-V4 growth stages in Nebraska field experiments performed in 2016 and 2017 and the probability of rejecting null hypothesis (P > F) observed based on analysis of variance (ANOVA) tests for the effects of preemergence herbicides (Herbicide), seed treatment (ST), cultivar (Cultivar) and their interaction.

		2016				2017				
Factor	df[a]	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	2	**0.04**[b]	0.72	0.11	0.46	**0.04**	0.60	0.60	0.36	**0.02**
ST	1	0.76	0.75	**0.01**	0.96	**< 0.001**	0.19	0.33	1.0	0.43
Herbicide X ST	2	0.12	**0.04**	0.41	0.49	0.53	0.64	**0.05**	0.22	0.07
Cultivar	1	**< 0.01**	**< 0.001**	**0.04**	**0.02**	**< 0.001**	**< 0.0001**	**< 0.0001**	**< 0.001**	**< 0.0001**
Herbicide X Cultivar	2	0.12	**< 0.01**	0.80	0.95	0.69	0.58	0.60	0.37	0.99
Cultivar X ST	1	0.20	0.60	0.27	0.20	0.16	**0.02**	0.87	0.81	0.27
Herbicide X ST X Cultivar	2	0.78	0.19	0.09	**0.02**	0.45	0.22	0.27	0.77	0.48

[a]Degrees of freedom (df) for each of the main effects and interactions.
[b]significant effects and interactions in **bold** ($\alpha = 0.05$).

Table 18. Aboveground biomass (g) at V1-V4 growth stages recorded in Nebraska field experiments performed in 2016 and 2017 and the effect of preemergence herbicide treatments (Herbicide), seed treatment (ST) and cultivar (Cultivar).

		2016				2017				
Factor	Treatment	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	Control	18.0 ab[a]	43.5	24.2	66.1	6.4 a	12.2	14.6	11.0	8.7
	Sulfentrazone	15.8 b	42.1	23.1	62.8	5.3 b	11.6	15.3	12.3	7.4
	Flumioxazin	18.7 a	44.4	21.5	60.9	6.6 a	11.5	14.9	11.6	8.9
ST	Control	17.4	43.7	24.4 a	63.2	5.3 b	12.2	14.7	11.6	8.2
	Fungicide	17.6	43.0	21.6 b	63.3	6.8 a	11.4	15.2	11.6	8.5
Cultivar	Sensitive	15.9 b	38.3	21.8 b	58.9	5.3 b	10.1	13.1 b	10.1 b	7.2 b
	Tolerant	19.1 a	48.3	24.2 a	67.6	6.9 a	13.4	16.7 a	13.2 a	9.5 a

[a]Values followed by the same letter indicate no significant difference ($P < 0.05$). When interactions are significant ($P < 0.05$), significant main effects are not displayed (Table 17).

Table 19. Plant height at V1-V4 growth stages recorded in Nebraska field experiments performed in 2017 and the probability of rejecting null hypothesis (P > F) observed based on analysis of variance (ANOVA) tests for the effects of preemergence herbicides (Herbicide), seed treatment (ST), cultivar (Cultivar) and their interaction.

Factor	df[a]	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	2	0.71	0.75	0.67	0.06	0.55
ST	1	0.65	0.38	0.34	0.18	0.46
Herbicide X ST	2	0.43	0.85	0.13	0.62	0.29
Cultivar	1	**< 0.0001**[b]	**< 0.0001**	**< 0.0001**	**0.03**	**< 0.0001**
Herbicide X Cultivar	2	0.27	0.72	0.62	0.27	0.89
Cultivar X ST	1	0.64	0.40	0.29	0.12	0.58
Herbicide X ST X Cultivar	2	0.31	0.63	0.18	0.27	1.0

[a]Degrees of freedom (df) for each of the main effects and interactions.
[b]significant effects and interactions in **bold** ($\alpha = 0.05$).

Table 20. Plant height (cm) at V1-V4 growth stages recorded in Nebraska field experiments performed in 2017 and the effect of preemergence herbicide treatments (Herbicide) seed treatment (ST) and cultivar (Cultivar).

Factor	Treatment	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	Control	35.4	53.1	58.5	15.1	48.4
	Sulfentrazone	34.8	51.7	58.7	14.2	46.8
	Flumioxazin	34.7	52.6	59.6	13.7	48.3
ST	Control	34.8	53.2	58.4	14.0	48.3
	Fungicide	35.2	51.8	59.4	14.7	47.3
Cultivar	Sensitive	31.4 b[a]	45.4 b	50.9 b	13.8 b	41.6 b
	Tolerant	38.6 a	59.6 a	67.0 a	14.9 a	54.0 a

[a]Values followed by the same letter indicate no significant difference ($P < 0.05$). When interactions are significant ($P < 0.05$), significant main effects are not displayed (Table 19).

Table 21. Total number of isolates recovered from symptomatic roots from V1-V4 growth stages at each Nebraska filed experiment location in 2016 and 2017.

	2016				2017					
	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah	All
Fusarium spp.	28	14	15	4	25	12	56	23	9	186
Pythium spp.	7	9	30	6	8	7	13	10	21	111
Rhizoctonia spp.	1	0	0	8	1	1	1	0	0	12
Total (n):	36	23	45	16	34	20	70	33	30	309

[a]Fungal growth characterized into three categories: *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., using morphological identification characteristics outlined by Watanabe (1937).

Table 22. Yield recorded in Nebraska field experiments performed in 2016 and 2017 and the probability of rejecting null hypothesis (P > F) observed based on analysis of variance (ANOVA) tests for the effects of preemergence herbicides (Herbicide), seed treatment (ST), cultivar (Cultivar) and their interaction.

		2016				2017				
Factor	df[a]	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	2	0.54	0.06	0.07	0.94	0.22	0.11	0.78	0.81	**< 0.01**
ST	1	0.68	0.63	0.99	0.54	**0.04**	0.67	0.49	**< 0.01**	0.35
Herbicide X ST	2	0.88	0.77	0.79	0.11	0.20	0.49	0.60	0.93	0.68
Cultivar	1	**< 0.01[b]**	0.83	0.25	**0.01**	**< 0.0001**	0.38	**< 0.01**	**< 0.0001**	0.47
Herbicide X Cultivar	2	0.51	0.77	0.57	0.45	0.18	0.49	0.32	0.61	0.51
Cultivar X ST	1	0.55	0.52	0.83	0.32	0.52	0.31	**0.03**	0.71	0.37
Herbicide X ST X Cultivar	2	0.98	0.17	0.85	0.64	0.49	0.69	0.31	0.65	0.23

[a]Degrees of freedom (df) for each of the main effects and interactions.
[b]significant effects and interactions in **bold** ($\alpha = 0.05$).

Table 23. Yield (kg/ha) recorded in Nebraska field experiments performed in 2016 and 2017 and the effect of preemergence herbicide treatments (Herbicide), seed treatment (ST) and cultivar (Cultivar).

		2016				2017				
Factor	Treatment	Chapman	Clearwater	Cordova	Schuyler	Auburn	Lincoln	Mead	Ord	Tekamah
Herbicide	Control	3,739	4,670	4,007	4,850	4,678	3,617	4,061	4,014	5,039 a
	Sulfentrazone	3,876	4,540	4,285	4,826	4,570	3,522	4,024	3,985	4,584 b
	Flumioxazin	3,747	4,666	4,208	4,838	4,513	3,476	4,070	3,951	4,633 b
ST	Control	3,764	4,637	4,167	4,821	4,504 b	3,526	4,032	3,872 b	4,703
	Fungicide	3,810	4,614	4,166	4,856	4,671 a	3,550	4,071	4,095 a	4,801
Cultivar	Sensitive	3,595 b[a]	4,620	4,109	4,912 a	4,356 b	3,562	3,968	4,167 a	4,790
	Tolerant	3,979	4,631	4,224	4,764 b	4,819 a	3,514	4,136	3,799 b	4,714

[a]Values followed by the same letter indicate no significant difference ($P < 0.05$). When interactions are significant ($P < 0.05$), significant main effects are not displayed (Table 22).

Chapter Three

Effect of preemergent protoporphyrinogen oxidase inhibitor herbicides on seedling disease caused by *Fusarium solani* under controlled conditions

3.1 Introduction

Seedling diseases of soybean [*Glycine max* (L.) Merr.] are caused by several pathogens including *Fusarium* spp., *Rhizoctonia solani*, *Pythium* spp. and *Phytophthora sojae* and can result in significant annual yield losses in the North Central Region of the United States. Seedling diseases are considered the second most important soybean disease in the United States, causing over a four year span an average annual loss of over 1.3 million metric tons (Koenning & Wrather, 2010). Disease can be caused by one pathogen alone or in combination as a multiple pathogen disease complex (Datnoff & Sinclair, 1988). Of the 22 *Fusarium* spp. that have been isolated from soybean plants, *F. acuminatum*, *F. graminearum*, *F. oxysporum*, and *F. solani* are the most common isolated species from soybean roots in North America (Nelson, 2015). Symptoms of seedling disease caused by *Fusarium* spp. include seed decay, brown discoloration of cortical and vascular root tissues, stunting of plants, wilting of foliage, and plant death.

Protoporphyrinogen oxidase-inhibitors (PPO-inhibitors) are commonly used preemergent herbicides in soybeans due to their effective control of several broadleaf weeds. These herbicides have the potential to injure soybean, especially in cool and wet conditions (Taylor-Lovell et al., 2001; Hager et al., 2002; Reiling et al., 2006; Legleiter et al., 2009). Symptoms of PPO-inhibitor injury include callused tissue on the hypocotyl and the stem near the soil surface, phytotoxic chlorosis and necrosis of cotyledon and leaf tissues (Figure 1), and shortened internodal length resulting in stunted plants (Li et al., 1999; Hulting et al., 2001). Examples of common preemergent PPO-inhibitors used in soybean include flumioxazin, saflufenacil, and sulfentrazone.

Cool soil temperatures (< 15 °C) and wet soil conditions that favor PPO-inhibitor injury are also conducive for development of seedling disease caused by *Fusarium* spp. (Bainbridge, 1970; Taylor-Lovell et al., 2001; Nelson, 2015). Multiple studies have investigated interactions between PPO-inhibitors and *Fusarium* spp., *R. solani*, and *Pythium* spp.; however, only *Fusarium virguliforme* O'Donnell & T. Aoki [causal pathogen of sudden death syndrome (SDS)] and *R. solani* have been studied in soybean under controlled conditions. Sonogo et al. (2000) observed applications of lactofen, a postemergent PPO-inhibitor, decrease SDS severity compared to the no herbicide control in greenhouse experiments. Alternatively, Bradley et al. (2002) observed that multiple rates of acifluorfen, a postemergent PPO-inhibitor, increased seedling disease severity of *R. solani* on soybean plants in greenhouse experiments. Due to the limited research investigating disease responses to PPO-inhibitors, there is a gap in understanding the effect of these herbicides on soybean seedling disease incidence and severity. Thus, there is a need for critical evaluation of the effects of preemergent PPO-inhibitors on seedling

disease caused by *Fusarium* spp. other than *F. virguliforme* under controlled conditions. *F. solani* was selected as the pathogen for this experiment due to the relatively large representation of *Fusarium* spp. among the isolates recovered from the field study portion of this research (Chapter Two).

Therefore, the objective of this research was to determine the effects of soil-applied PPO-inhibitor herbicides on soybean seedling disease caused by *Fusarium solani* under controlled conditions.

3.2 Materials and Methods

3.2.1 Experiment design and background

Three experimental runs were conducted consecutively under controlled conditions in a University of Nebraska greenhouse in Lincoln, Nebraska from December 10, 2018 - January 25, 2019.

Experiments were arranged as a randomized complete block design with six replications of each treatment. Treatments consisted of a 2-way factorial arrangement of preemergence herbicide program by fungal inoculation. The experiment was replicated three times with Experiment 1 planted on 12-10-18, Experiment 2 planted on 1-4-19, and Experiment 3 planted on 1-8-19. There were three herbicide programs which consisted of 6X the labeled rate of sulfentrazone (Spartan® 4F, FMC Corporation, Philadelphia, PA, 3.36 L/ha; 0.016 mg a.i.), 6X the labeled rate of flumioxazin (Valor® SX, Valent U.S.A LLC, Walnut Creek, CA, 1050 g/ha; 0.54 mg a.i.), and a no herbicide treatment control. The elevated herbicide rates were selected in order to achieve plant injury symptoms typical of those observed in field experiments conducted in Nebraska in previous years

(Chapter Two). Herbicide treatments were applied 3 days after planting (DAP) to the soil surface of each experimental unit within a spray chamber (Research Track Sprayer; DeVries, Hollandale, MN) in 140 L/ha carrier volume using a TP8001E flat-fan nozzle tip (TeeJet Technologies, Spraying Systems Co., Wheaton, IL) at a pressure of 241 kPa. There were two inoculum treatments including a no inoculum control (None) and inoculum of *F. solani*.

Non-seed treated seeds of a PPO-inhibitor sensitive soybean cultivar ('Pioneer P22T41R2'; Dupont-Pioneer, 2018) were planted into square plastic pots (10-cm width and 9-cm height). Pots were first filled with 500 mL of coarse vermiculite to 4-cm height (PALMETTO VERMICULITE Co. Inc., Woodruff, SC) and then if inoculated, 5.5 g/pot of inoculum was spread as an even layer on top of the vermiculite. Inoculum consisted of sterilized sorghum seed colonized by a Nebraska isolate of *F. solani* and was prepared using previously established methods (de Farias Neto, et al., 2006). Then, 100 mL (4-cm) of sterilized silty clay (16% sand, 43% silt, and 41% clay) soil with 2.7% organic matter and pH of 7.0 was added. Four seeds were sown into the soil at a depth of 3-cm resulting in the inoculum layer 1-cm below the seeds. The greenhouse was maintained at 22° C and daily temperatures were ± 2° C throughout the experiments based on air temperature recording at the top of pot level using a WatchDog data logger Model 450 (Spectrum Technologies, Inc., Plainfield, IL). Natural day length was approximately 9-10-h, and supplemental lighting was applied to establish 12-h daylength using 650 watt Lumigrow LED light fixtures (Lumigrow, Inc. Emeryville, CA). 25 mL of non-fertilized tap water was applied to the soil surface of each pot daily using a squeeze bottle.

3.2.2 Data collection

At 10 days after planting (DAP) two plants were selected randomly from each experimental unit for data collection (phytotoxicity severity, plant height, root rot severity, and root biomass), as not every experimental unit had successful germination of all four seeds. At the time of root evaluations all plants were gently removed from the vermiculite-soil mix and washed free of debris. The two randomly selected plants were retained and means of each response variable were averaged for evaluations while additional plants from each pot were discarded.

Phytotoxicity (PPO-inhibitor Injury). Shoot phytotoxicity severity was rated on a continuous 0-100% scale in all experimental units at 10 DAP when plants were between the cotyledon (VE) and unifoliate (VC) growth stages (Fehr et al., 1971). Typical phytotoxicity symptoms associated with PPO-inhibitor injury include chlorosis and necrosis on the hypocotyl and cotyledons (Figure 1).

Plant Height. Plant height (cm) was measured in all experimental units at 10 DAP between the VE - VC growth stages. Heights were measured from the soil surface to the uppermost node (cotyledon or unifoliate).

Root Rot Severity. Root rot severity was rated in all experimental units at the termination of each experiment, 14 DAP, between the VE - V1 growth stages. The root systems were gently removed from the vermiculite-soil mix and washed free of debris. Each root system was visually evaluated for total root rot severity which is the total percent area (0-100% continuous scale) of the root system that is discolored with the typical browning symptoms of root rot.

Root Biomass. The roots of two representative plants from each experimental unit were cut from the aboveground portion at the cotyledon scar at 14 DAP and a collective fresh root biomass (g) was recorded for each experimental unit.

3.2.3 Data analysis

Analysis of variance was performed using PROC GLIMMIX in SAS version 9.4 (SAS Institute Inc., Cary, NC) to determine the effect of herbicide program (Herbicide), Inoculation, and their interactions on response variables. Herbicide program and Inoculation were treated as fixed effects while replication was nested within experiment and treated as a random factor. Mean separation was performed using Fisher's protected LSD at $\alpha = 0.05$.

3.3 Results

Phytotoxicity (PPO-inhibitor Injury). Phytotoxic symptoms occurred at variable levels in all three experiments with the highest levels observed in Experiment 1. Although seldom observed, mild chlorosis not associated with herbicide injury was observed in some of the no herbicide control pots. Sulfentrazone and flumioxazin applications resulted in phytotoxicity 15.8–17.6% higher than the no herbicide control ($P < 0.0001$) (Tables 1 and 2).

Plant Height. Plant heights were not recorded for Experiment 1. Applications of sulfentrazone and flumioxazin slowed the growth of the soybeans and significantly affected plant height in Experiments 2 and 3. Applications of sulfentrazone resulted in 64–66% reduced plant heights compared to the no herbicide control within both inoculation treatments ($P < 0.02$; Figure 2). Applications of flumioxazin resulted in 23–

37% reduced plant heights compared to the no herbicide control within both inoculation treatments ($P < 0.02$; Figure 2).

Root Rot Severity. Root rot severity was fairly uniform across the three experiments with mean root rot severity ratings ranging from 32.1–38.9% in the *F. solani* inoculated pots (*data not shown*). Necrosis appearing similar to root rot symptoms, were observed at low levels (2.4–3.3%) on roots in the non-inoculated pots in all experiments (*data not shown*). Applications of sulfentrazone resulted in 9.2 and 12.0% more root rot severity compared the no herbicide control and flumioxazin respectively, across *F. solani* inoculated pots ($P < 0.05$) while there were no differences across non-inoculated pots (Figure 3). The *F. solani* inoculated pots resulted in 32.6% more root rot severity compared to the non-inoculated pots ($P < 0.0001$) (Tables 1 and 2).

Root Biomass. Applications of flumioxazin and sulfentrazone resulted in 29 and 41% less root biomass respectively, compared to the no-herbicide control across non-inoculated pots ($P < 0.01$; Figure 4). Across inoculated pots, applications of sulfentrazone resulted in 25% less root biomass compared to the no herbicide control ($P < 0.01$; Figure 4).

3.4 Discussion

Overall, sulfentrazone and flumioxazin resulted in significant plant injury on hypocotyls and cotyledons as well as significant reductions in plant heights throughout these experiments. This was expected as 6X the labeled rates of both sulfentrazone and flumioxazin were used to ensure consistent herbicide injury. Although soybeans under typical production practices would not receive such a high dose of these herbicides, the

symptoms observed throughout the experiments were similar to levels of PPO-inhibitor injury witnessed in soybean under adverse environmental conditions in Nebraska.

In general, higher amounts of injury and larger reductions in plant heights were observed when sulfentrazone was applied compared to flumioxazin applications. This could be related to the measure of sensitivity of the cultivar used in this study, which was rated to sulfentrazone specifically. Although flumioxazin is also a preemergent PPO-inhibitor used in soybean, these ratings have no indication of the relative sensitivity to this herbicide. Reductions in plant heights appeared to be related to slower plant growth. At the time of plant height evaluations, flumioxazin treatments were beginning to unfurl the unifoliate leaves (VC), sulfentrazone treatments were only at the cotyledon stage (VE), and the no herbicide control were at full VC. Sulfentrazone and flumioxazin applications reduced root biomass in all three experiments demonstrating that these herbicides at elevated rates can negatively impact root growth.

Although flumioxazin and sulfentrazone applications both resulted in injury and plant height reductions, only sulfentrazone resulted in higher root rot severity compared to the no herbicide control. This indicates a negative effect on seedling disease development caused by this particular *F. solani* isolate, when PPO-inhibitor injury occurs under controlled conditions.

The method for inoculation used in this study proved to be effective at producing consistent levels of disease throughout the three experiments and could be adopted for future greenhouse research. Infection by *F. solani* can result in reduced plant heights due to slowing growth and emergence. Root biomasses varied by experiment yet there was a

general trend of reduced root biomass in the inoculated pots compared to the non-inoculated pots.

Overall, PPO-inhibitors and seedling disease caused by *F. solani* had negative impacts on soybean seedling health and vigor under controlled conditions. There is some indication that injury induced by sulfentrazone can increase root rot in soybean; however, the evidence presented in this research is not conclusive that field applied rates of sulfentrazone and flumioxazin will influence seedling disease development.

Additional research on PPO-inhibitor herbicide effects on soybean seedling diseases would benefit soybean producers as they continue to face challenges associated with managing weeds and seedling diseases. Further research should utilize variable rates of herbicides to obtain different levels of injury under controlled conditions. This would help researchers understand if there is a threshold level of injury necessary for an increase in root rot. There is also a need to determine if PPO-inhibitors can increase root rot without visible injury occurring. In the field component of this research (Chapter Two), increases to root rot severity were observed at various incidences of PPO-inhibitor injury even when no injury occurred.

Seedling disease tends to be variable throughout soybean fields due to disease pressure not being uniformly distributed. Further research on the effects of high amounts of PPO-inhibitor injury on several levels of disease pressure under controlled conditions should be conducted to better understand this interaction.

Additional research conducted in high disease pressure environments at the field level would benefit the understanding of how the observed interactions can impact yield. Although this research investigated impacts on seedling disease caused by *F. solani*

alone, it is often the case that producers have several seedling disease causing pathogens in their fields. Therefore, it would be of benefit to conduct this type of research both under controlled conditions and in naturally infested fields with more than one of the seedling disease causing pathogens to mimic a scenario representative of what producers' face in Nebraska.

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3.6 Figures

Figure 1. Symptoms of PPO-inhibitor injury include chlorosis and necrosis on soybean cotyledon and hypocotyl. Symptomatic seedlings indicated by arrows. Healthy seedling indicated by star.

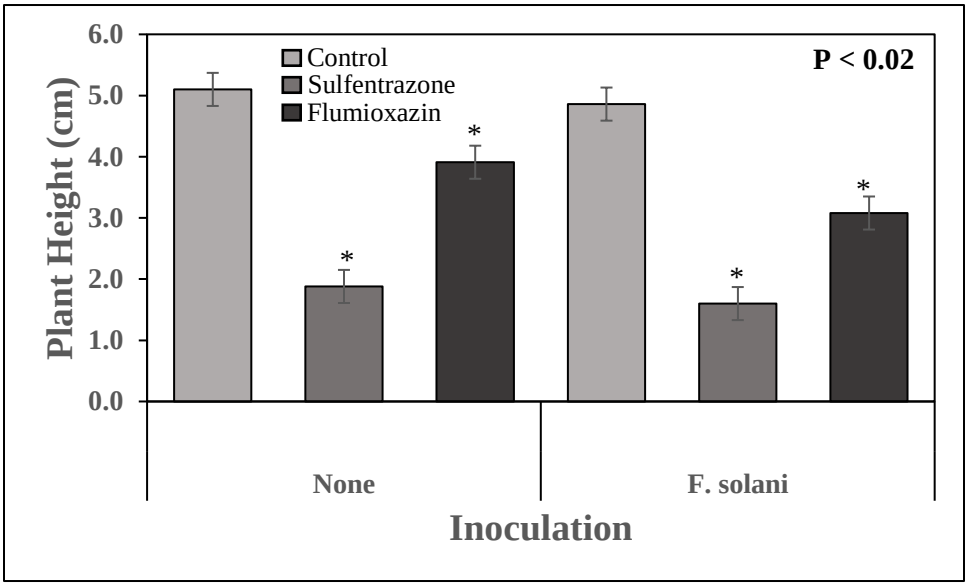

Figure 2. Herbicide X Inoculation interaction ($P < 0.02$) for plant height for Experiments 2 and 3. Vertical bars represent the mean for each parameter and lines extending from each bar represent the standard error of mean. Plant height (cm) was rated in all experimental units at one timing (10 DAP) between the VC - V1 growth stages. Heights were measured for two plants in each experimental unit from the soil surface to the uppermost node (cotyledon or unifoliate). *indicates significant difference from the no herbicide control within each inoculation type at $\alpha = 0.05$ level determined using Fisher's protected LSD.

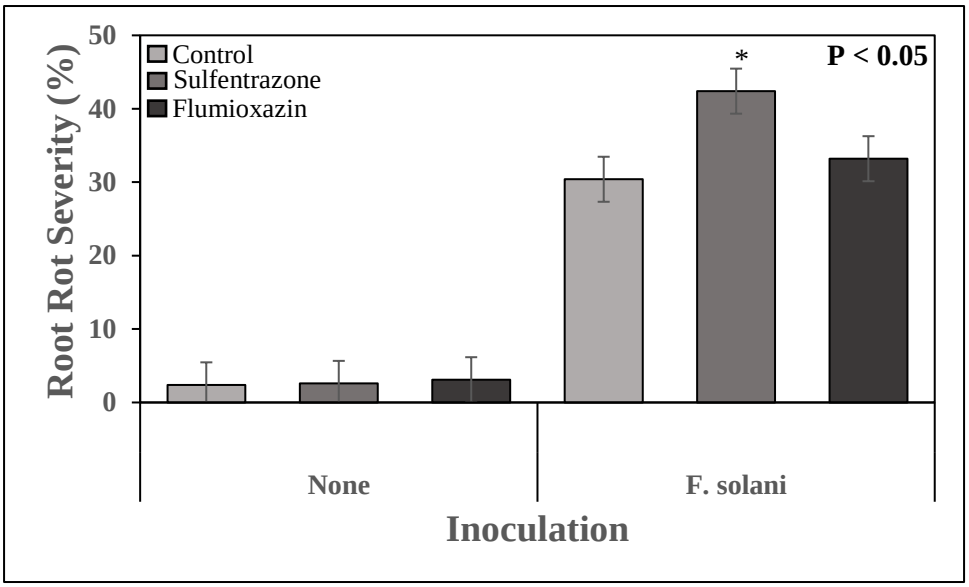

Figure 3. Herbicide X Inoculation interaction ($P < 0.05$) for root rot severity for all experiments. Vertical bars represent the mean for each parameter and lines extending from each bar represent the standard error of mean. Each root system was evaluated for total root rot severity which is the total percent area (0-100% linear scale) of the root system that is discolored with the typical browning symptoms of root rot between the VC-V2 growth stages. *indicates significant difference from the no herbicide control within each inoculation type at $\alpha = 0.05$ level determined using Fisher's protected LSD.

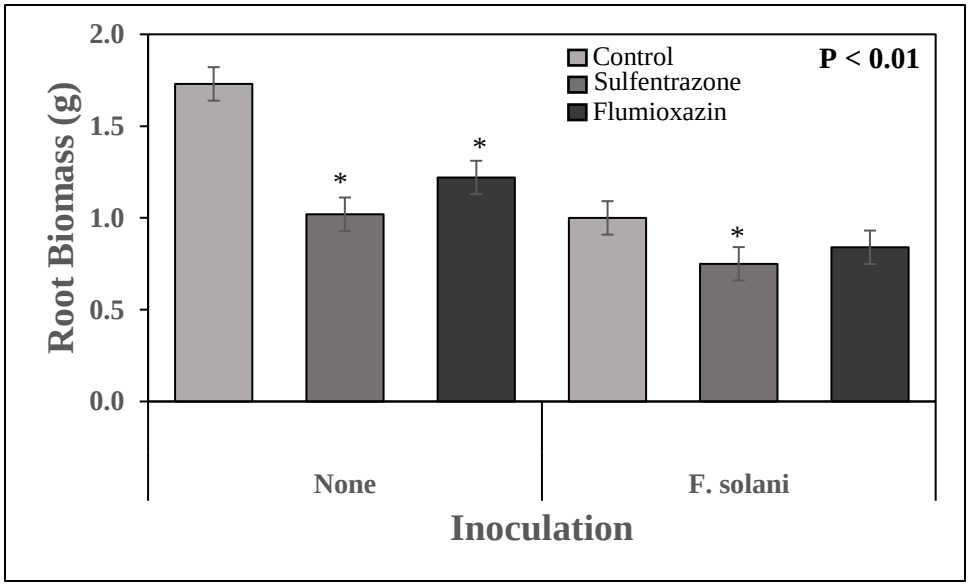

Figure 4. Herbicide X Inoculation interaction for root biomass ($P < 0.01$) for all experiments. Vertical bars represent the mean for each parameter and lines extending from each bar represent the standard error of mean. The roots of two plants from each experimental unit were cut from the aboveground portion at the cotyledon scar and a collective fresh root biomass (g) was measured between the VC-V2 growth stages. *indicates significant difference from the no herbicide control within each inoculation type at $\alpha = 0.05$ level determined using Fisher's protected LSD.

3.7 Tables

Table 1. Phytotoxicity, plant height, root rot severity, and root biomass under controlled conditions and the probability of rejecting null hypothesis (P > F) observed based on analysis of variance (ANOVA) tests for the effects of preemergence herbicides (Herbicide), Inoculation, Experiment, and their interactions.

Factor	df[a]	Phytotoxicity	Plant Height	Root Rot Severity	Root Biomass
Herbicide	2	**< 0.0001**[b]	**< 0.0001**	**0.02**	**< 0.0001**
Inoculation	1	0.50	0.10	**< 0.0001**	**< 0.0001**
Herbicide X Inoculation	2	0.74	**0.02**	**0.02**	**< 0.01**

[a]Degrees of freedom (df) for each of the main effects and interactions.
[b]Significant effects and interactions in **bold** ($\alpha = 0.05$)

Table 2. Phytotoxicity (%), plant height (cm), root rot severity (%), and root biomass (g) under controlled conditions and the effect of preemergence herbicides (Herbicide) and Inoculation.

Factor	Treatment	Phytotoxicity	Plant Height	Root Rot Severity	Root Biomass
Herbicide	Control	0.21 b	4.98	16.4	1.37
	Sulfentrazone	17.8 a	1.74	22.5	0.89
	Flumioxazin	16.0 a	3.50	18.2	1.03
Inoculation	None	10.8	3.54	2.7	1.32
	F. solani	11.8	3.28	35.3	0.87

[a]Values followed by the same letter indicate no significant difference ($P < 0.05$) determined using Fisher's protected LSD. When interactions are significant ($P < 0.05$), significant main effects are not displayed (Table 1).

Chapter Four

Thesis Conclusions

Seedling disease is one of the most economically important diseases affecting soybean in the United States and can be caused by several pathogens alone or in combination as a disease complex. Fungicide seed treatments continue to provide the most consistent management of soybean seedling diseases; however, effects on yield are variable depending on disease pressure and environmental conditions. With the prevalence of resistant weeds to glyphosate and other herbicide sites of action, soybean producers are utilizing soil-applied preemergent herbicides for effective management of several broadleaf weeds. Some preemergent herbicides, such as PPO-inhibitors, can result in injury to soybean when conditions are unfavorable for crop growth. These conditions also favor infection by some of the seedling disease causing pathogens and contribute to further disease development.

Since both seedling disease and the use of these herbicides are common in soybean production, there has been some research on the interactions between PPO-inhibitor injury and fungicide seed treatments and their effects on soybean seedling disease and yield. These experiments have been limited to seedling diseases caused by *Fusarium virguliforme* and *Rhizoctonia solani* and inconsistent results have provided a

need for further research to understand this relationship. As PPO-inhibitor injury and seedling disease often occur under similar environmental conditions, it is important to understand their relationship so that soybean producers can make sound management decisions as needed.

The greenhouse component of this thesis (Chapter Three) serves as the first known study on soil-applied PPO-inhibitor herbicides' effect on soybean seedling disease caused by *Fusarium solani*. Additionally, the large representation of *Fusarium* and *Pythium* spp. among the isolates recovered in the field component of this thesis (Chapter Two) suggests the seedling disease present at many of the locations were caused by these pathogens. It is also unique in that much of the research on herbicide and soybean seedling disease interactions has neglected to characterize the fungal and oomycete composition associated with infected roots from each field. With this descriptive information, researchers would be able to group locations consisting of similar seedling disease causing pathogen complexes and potentially identify which pathogens are more commonly associated with responses in disease development related to herbicide injury interactions.

Although not entirely comprehensive, experiments conducted for this research were located within fields consisting of soils representative of soybean fields in Nebraska. This is particularly helpful to Nebraska soybean producers as they now have a resource providing some understanding of the potential impact of this herbicide-disease relationship in fields similar to their own. The observed inconsistency in the herbicides' effects on root rot severity was likely related to the relative pathogen load of each field as

well as the diversity in location specific environmental conditions during critical periods of early season soybean emergence and growth and late season grain filling stages.

Sulfentrazone and flumioxazin applications resulted in soybean injury at 8 of 9 locations in this study, yet they reduced yield at only one location. This suggests producers should not be overly concerned of the impact of PPO-inhibitor injury on yield. To minimize stand loss producers should attempt to lessen the risk of PPO-inhibitor injury through ensuring uniform planting depth, adequate seeding row closure with good seed to soil contact, and as needed deploying tolerant cultivars in coarse textured fields with low organic matter. Fungicide seed treatment did not impact root rot severity at any location and increased yield at only 3 of 9 locations. The effect of fungicide seed treatments on yield remains heavily dependent on early season disease development and late season environmental stress.

There is still a need for further research to fully understand the impacts of PPO-inhibitor injury on seedling disease in soybean. Replication of this research throughout soybean growing regions of the United States would produce a robust dataset with varying soil characteristics and climatic factors which in turn could help identify situations where these herbicide effects could impact yield. There may also be value in conducting experiments similar to this with additional herbicide sites of action commonly used in preemergent programs in soybean as several are capable of causing injury when environmental conditions are unfavorable for soybean growth.

The goal of this research was to attempt to understand the relationship between PPO-inhibitor injury and seedling disease severity under both field and controlled conditions in Nebraska through three objectives: (1) determine the effects of soil-applied

PPO-inhibitor herbicides on soybean seedling disease and yield in the field; (2) determine the effects of soil-applied PPO-inhibitor herbicides on seedling disease caused by *Fusarium solani* under controlled conditions; and (3) determine the effect of fungicide seed treatment on soybean seedling disease and yield when PPO-inhibitor injury occurs.

In conclusion, though soil-applied PPO-inhibitors increased root rot severity in several fields and under controlled conditions, producers should continue to utilize these herbicides as one of several tools in an effective integrated weed management program. Furthermore, fungicide seed treatments should continue to be used in fields with history of seedling disease as environmental conditions play an important role in disease development and vary from year to year. As additional research provides further insight on the relationship between PPO-inhibitor injury and soybean seedling disease, producers in Nebraska and the United States will have a better understanding of when to use a fungicide seed treatment and in which fields they should take extra care in ensuring practices that will minimize PPO-inhibitor injury.