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Integrated management of Phytophthora stem and root rot of soybean and the effect of soil-applied herbicides on seedling disease incidence

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By

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University of Nebraska, 2019

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Soybean seedling diseases and Phytophthora stem and root rot (PSRR; caused by *Phytophthora sojae*) are two of the most economically important diseases in North Central U.S. Remarkable differences in disease incidence occur each year, which demonstrate that abiotic and biotic factors must interact for disease onset and development. During 2017 and 2018, field studies were conducted to (i) address the efficacy of seed treatment and genetic resistance for PSRR management on soybean population, canopy coverage (CC), and yield, and (ii) investigate potential interactions between pre-emergence (PRE) herbicides and the incidence of seedling diseases in alluvial soils in Nebraska.

Despite field history, PSRR developed in only four of six environments studied. Commercial seed treatment had a positive effect on plant population density, CC, and yield in at least three environments. Compared to non-treated control, seed treatment increased emergence between 11,600 to 53,700 plants ha^{-1} and early-season CC between 0.7 to 1.2%. Under high disease pressure, management programs using moderately resistant cultivars improved yields when compared to moderately susceptible cultivars. By contrast, minimum yield differences were detected between *Rps*1k and *Rps*1c genotypes, except in one environment. While a weak to moderate correlation was observed between CC and

incidence of *P. sojae* symptomatic plants, a moderate to strong association was found between CC and yield.

Across multiple environments, PRE herbicides chlorimuron-ethyl, metribuzin, saflufenacil, sulfentrazone, and flumioxazin had no impact on seedling root rot (disease severity index; DSI) when compared to the non-treated control. Similarly, no significant differences between PRE herbicides were detected on plant population, plant height, and yield. Community composition depicting primary pathogenic genera *Fusarium*, *Phytophthora*, *Pythium,* and *Rhizoctonia* did not occur at random but rather varied across environments and DSI classes. In two of the three environments, *Phytophthora* structured approximately 22% of primary pathogenic genera, whereas, *Rhizoctonia* recovery was low (<5.5%). These results suggest compatibility of PRE herbicides programs in late-planted soybeans with a history of seedling diseases.

Collectively, the research presented in this thesis furthers our knowledge on the management of soilborne pathogens in soybeans and offers insights into new avenues of research.

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DEDICATION

To my late friend Eddy…

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. Literature review

General introduction

This thesis is divided into four chapters. The first chapter contains the literature review that supports the research hypothesis. The second chapter reports the agronomic performance of seed treatment in combination with genetic resistance to *Phytophthora sojae* on soybean population, canopy coverage, and yield in Nebraska and Iowa. The third chapter describes a two-year field study conducted to address the effect of soil-applied pre-emergence herbicides on the incidence of seedling diseases caused by *Pythium*, *Phytophthora*, *Rhizoctonia*, and *Fusarium* species. The last chapter presents the general conclusions for this thesis, suggestions on management practices based on results gathered by the present investigation, and a discussion on possible future research topics.

1.1. Soybean: an overview

Soybean (*Glycine max* L. Merr.) (Fabaceae: Phaseoleae) is a leguminous plant cultivated as a major source of protein and oil. In 2016, production was estimated at 334 MMT worldwide (FAO 2016). About 50 countries grow soybeans in the world but production is geographically concentrated within the U.S., Brazil, Argentina, and China, which together account for almost 90% of the world's production (Wilcox 2004). The U.S. has been the world's leading producer with a cultivated area of 33.7 million hectares and production estimated at 117.2 MMT in 2016. Soybean production in the North Central region has increased due to changes in management systems, improved genetics, and

expanded soybean acreage. Annual yield increases in the order of 35.1 and 24.9 kg ha^{-1} for irrigated and rainfed agrosystems have positioned Nebraska among the top yielding states nationwide with estimated 4102.3 and 3866.9 kg ha^{-1} in 2016 and 2017, respectively (Specht et al. 1999; USDA-NASS 2018). Soybean exports are important for Nebraska's economic growth; roughly 50% of production is exported to other countries (http://nebraskasoybeans.org/topics/international-marketing).

1.1.1. Seedling diseases in the U.S. and Nebraska

The rapid expansion of cultivated area has also been accompanied by an increase in the incidence of soybean diseases which have a direct impact on grain production and quality (Hartman et al. 2015). From 1996 through 2007, yield losses to soybean diseases were estimated at 13.5% of attainable production in the U.S. (Koenning and Wrather 2010). Among the most yield-limiting maladies are seedling diseases that cause poor crop establishment and reduced plant stand. In 2009, seedling diseases exclusively were responsible for an estimated loss of 1.51 MMT in soybean production in the U.S. (Koenning and Wrather 2010). In Nebraska, the economic impact related to soybean diseases are estimated at $21.67 per-acre basis (Allen et al. 2017), which a significant portion is attributed to the occurrence of seedling diseases caused by a complex of pathogens, including *Pythium* spp., *Phytophthora sojae*, *Fusarium* spp., and *Rhizoctonia solani* (Giesler 2017; Parikh et al. 2018; Rojas-Flechas et al. 2017).

Symptoms of seedling diseases include seed decay, pre- and post-emergence damping-off, blight, and root rot. Water soaking and systemic vascular discoloration can also be observed in seedlings infected by oomycetes such as *Pythium* and *Phytophthora*. Emerged seedlings may also show soft brown to reddish-colored rot of lateral, taproot and

hypocotyl, which culminate in stunted plant development and stand failure (Schmitthenner and Dorrance 2015).

Risk factors for increased seedling disease include prolonged periods of saturated conditions associated with cooler soil temperatures (Han et al. 2017; Martin and Loper 1999), reduced tillage (Workneh et al. 1998), and compaction. Shifts on pathogen aggressiveness (Jackson et al. 2004; Stewart et al. 2014) and reduced crop genetic diversity upon the introduction of biotech traits available only in genetically interrelated parents (Mueller et al. 2018; Sneller 2003) can also magnify disease epidemics. In addition, abiotic stresses caused by pre-emergence herbicide (Bradley et al. 2002; Carson et al. 1991; Duncan and Paxton 1981) and physical injury can increase soybean susceptibility to pathogen infection.

1.1.1.1. *Fusarium* spp.

Fusarium belongs to the Eukarya domain, kingdom Fungi, phylum Ascomycota, class Sordariomycetes, order Hypocreales, family Nectriaceae, and genus *Fusarium*. *Fusarium* species incite several diseases of soybean including Fusarium wilt, sudden death syndrome caused by *F. virguliforme* O'Donnell & T. (Aoki et al. 2003), and seedling blight and root rot caused by a number of species, including *F. solani* (Mart.) Sacc., and *F. oxysporum* Scheldt. (Killebrew et al. 1993; Leslie et al. 1990; Rizvi and Yang 1996). Other species associated with soybeans in the U.S. include *Fusarium graminearum*, *F. acuminatum*, *F. commune*, *F. equiseti*, *F. armeniacum*, *F. proliferatum*, *F. redolens*, and *F. cerealis* (Abdelmagid et al. 2018; Bienapfl et al. 2010; Días Arias et al. 2013; Ellis et al. 2012). Infected tissue displays external lesions on taproot and adventitious roots, cortical decay, and vascular reddening (Hartman et al. 1999).

Besides being a cosmopolitan soil inhabitant, *Fusarium* species are also considered a common member of fungal communities associated with plant rhizosphere. *Fusarium* species produce three types of spores: chlamydospores, macroconidia, and microconidia (Nelson et al. 1994). Chlamydospores are ovoid, thick-walled overwintering structures filled with lipid-like material that allow for pathogen survival during starvation and adverse conditions. Plant infection occurs by direct penetration through mycelial contact or haustoria invagination from germinated spores, and indirectly through wounding resulted from secondary root development, injury from nematodes, insects, and farming equipment (Garret 1970; Summerell et al. 2003). Cool temperatures (10-15 °C) and saturated soils conditions are conducive to infection, although some species and strains are capable of causing disease under a range of soil temperature and moisture (Ellis et al. 2011).

Management of Fusarium root rot can be challenging but research suggests soybean cultivars differ in susceptibility to the disease (Zhang et al. 2010). Seed treatments reduce infection at the seedling stage (Broders et al. 2007b; Ellis et al. 2011) and cultivation practices that reduce soil compaction and promote drainage reduce disease severity, as can also the maintenance of optimum pH levels and macro and micronutrient levels.

1.1.1.2. *Rhizoctonia solani*

Rhizoctonia species belong to Eukarya domain, kingdom Fungi, phylum Basidiomycota, class Agaricomycetes, order Ceratobasidiaceae, family Cantharellales, and genus *Rhizoctonia*. The pathogen *Rhizoctonia solani* Kühn (syn. *Thanatephorus cucumeris* (A. B. Frank) Donk) is recognizably a major causal agent of damping-off in soybeans in the U.S. (Doupnik 1993). Symptoms include seed decay, root rot, hypocotyl rot, crown rot,

stem canker, post-emergence damping-off, and foliar web blight (Yang and Hartman 2015).

Isolates of *R. solani* are classified based on culture hyphae compatibility reaction into 14 different anastomosis groups (AGs) (AG 1 to 13 and AG-BI) (Carling et al. 1999; Ogoshi 1987). Individual AGs have different host preferences and geographic distributions but multiple AGs have been reported to infect soybeans. Ajayi-Oyetunde and Bradley (2017) reported AG-2-2IIIB was the most aggressive on soybean roots, whereas, AG-4 displayed greater aggressiveness on the hypocotyl. Other AGs including AG-2-1, AG-2-2, AG-7, and AG-11 have been isolated from soybean seedlings in Iowa and Arkansas (Rizvi and Yang 1996; Rothrock et al. 1993). Morphologically, young vegetative hyphae are multinucleate and hyaline but turn brown with age. Septate hyphae branch at right angles with constricted insertion points. Isolates produce thick-walled, brown sclerotia that accumulate dark pigments during incubation (Yang and Hartman 2015). In nature, *R. solani* reproduces asexually and exists primarily as vegetative mycelium colonizing debris or as sclerotia that function as hardened long-term surviving structure. Unlike other pathogens, *R. solani* can infect soybeans in a wide temperature range (20-32℃) (Boosalis 1950; Dorrance et al. 2003b; Lewis and Papavizas 1977).

Management of Rhizoctonia seedling diseases relies on an integrated approach that combines fungicide seed treatments (Dorrance et al. 2003b; Xue et al. 2007) and agronomic practices that encourage seedling development. Minimizing soil compaction can reduce disease incidence in dry beans (Harveson et al. 2005). Despite of the benefits on soil health and activity of antagonistic organisms, crop rotation may have minimal effect for management of Rhizoctonia diseases because of the wide range in susceptible

hosts, including corn (*Zea mays* L.), crucifers, sugar beet (*Beta vulgaris* L.), potato (*Solanum tuberosum* L.), cotton (*Gossypium* spp.), cucumber (*Cucumis sativus* L.), chickpeas (*Cicer arietinum* L.), canola (*Brassica napus* L.), rice (*Oryza sativa* L.), and weeds (Anderson 1982; Harveson 2011). In terms of genetic resistance, no complete resistance has been found in the soybean germplasm, but cultivars exhibit different tolerance levels to the disease (Bradley et al. 2001; Zhao et al. 2005).

1.1.1.3. *Pythium* spp.

Pythium species belong to the Eukarya domain, kingdom Straminipila, phylum Oomycota, class Peronosporomycetes, order Peronosporales, family Pythiaceae, and genus *Pythium* (Beakes et al. 2014). The genus *Pythium* consists of many important plant pathogens. Several species have been isolated from disease soybean seedlings around the world, but common species found in the North Central U.S. include *Pythium irregulare*, *P. torulosum*, *P. sylvaticum, P. oopapillum, P. heterothallicum, P. ultimum* var. *ultimum*, and *P. aphanidermatum* (Broders et al. 2009; Radmer et al. 2017; Zitnick-Anderson and Nelson Jr. 2015). Infected seedlings display a soft mushy rooting tissue on the cotyledon, radicle, and hypocotyl, as well as root rot and early-season post-emergence damping-off. Lesions may vary from yellow to tan to brown in color and infected tissue are usually water soaked (Rothrock et al. 2015).

The cell wall of many oomycetes is composed of cellulose and β-1, 3 glucan with minimal amounts of chitin, which is a distinctive characteristic that separate Oomycota (ex. *Pythium*, *Phytophthora*, *Aphanomyces*, *Bremia, Peronospora, Plasmopara*, etc.) from Fungi (ex. *Fusarium*, *Rhizoctonia*, *Paecimolyces*, *Sclerotinia*, etc.) (Fry and Grünwald 2010). *Pythium* species reproduce asexually by means of either hyphae or hyphal swellings

and sexually via antheridia and oogonia. Sporangia can be globular to ovoid, with or without internal proliferation; some may have apical papilla but lack the apical thickening characteristic of those species of *Phytophthora* (Ho 2018). Plant infection may occur shortly after planting when dormant, overwintering pathogen propagules germinate in response to chemical signaling exudates released by roots (Donaldson and Deacon 1993). Differences in temperature determine the aggressiveness of *Pythium* species in soybeans. For example, some species are favored by cool soil temperatures (5 to 10°), such as *Pythium ultimum* var. *ultimum* and *P. irregulare*, *P. macrosporum*, while others thrive in warmer conditions (25 to 30°), such as *P. aphanidermatum* (Thomson et al. 1971; Wei et al. 2010).

Management of Pythium seed and seedling rot and damping-off can be difficult depending on environmental conditions prevailing at emergence. No definite genetic resistance is commercially available for disease management, however, differences in disease tolerance have been reported in the soybean germplasm (Bates et al. 2008; Ellis et al. 2013; Rod et al. 2018). Seed treatments provide protection during initial developmental stages, but certain species and isolates exhibit different sensitivity to fungicides (Radmer et al. 2017). In high-risk areas, increasing oomycide (metalaxyl, mefenoxam, and ethaboxam) rates during seed treatment may be necessary to prolong seedling protection (Jackson-Ziems et al. 2017). Choice of proper planting time can be utilized for the management of Pythium root rot and damping-off in soybeans. Chilling or freezing temperatures during imbibition and emergence predispose seedlings to *Pythium sylvaticum* infection (Serrano and Robertson 2018), but a tradeoff on plant productive components (e.g. number of productive nodes and pods) and yield exists as planting is delayed.

1.1.1.4. *Phytophthora* spp.

Phytophthora species belong to the Eukarya domain, kingdom Straminipila, phylum Oomycota, class Peronosporomycetes, order Peronosporales, family Peronosporaceae, and genus *Phytophthora* (Beakes et al. 2014). *Phytophthora sojae* (syn. *P. megasperma* var. *sojae* A. A. Hildebr., *P. megasperma* f. sp. *glycinea* Kuan & Erwin, *P. sojae* f. sp. *glycines* Faris et al.) is a soilborne pathogen and principal causal agent of Phytophthora stem and root rot (PSRR) in soybeans. The disease was first observed affecting soybeans in Indiana in 1948 and Ohio in 1951 (Bernard et al. 1957) and was initially thought be to caused by *Phytophthora cactorum* (Skotland 1955), but later renamed to *P. sojae* in a comprehensive report by Kaufmann and Gerdemann (1958). Since its emergence, substantial economic losses due to PSRR have been reported in North America. In 1994, yield losses to PSRR were estimated at 5.7 MMT and increased to 9.4 MMT in 2014 (Allen et al. 2017; Wrather et al. 1997). Worldwide, *P. sojae* has been detected in Canada (Hildebrand 1959), Japan (Tsuchiya et al. 1978), Australia (Pegg et al. 1980), Hungary (Kövics 1981), Argentina (Barreto et al. 1991), China (Shen and Su 1991), and Brazil (Costamilan et al. 1996), Korea Republic (HyeongJin et al. 1998), Italy, and other countries (Schmitthenner 1999).

Recently, a second *Phytophthora* species, *P. sansomeana* E.M. Hansen & Reeser, has also been described as a seedling pathogen in soybeans (Hansen and Hamm 1983; Hansen et al. 2009). *Phytophthora sansomeana* has been recovered from symptomatic soybean seedlings in Arkansas, Illinois, Indiana, Iowa, Kansas, Michigan, Nebraska, Ohio, and Wisconsin and abroad in Canada and China (Phibbs et al. 2014; Rojas-Flechas et al. 2017; Tang et al. 2010; Zelaya-Molina et al. 2010). The pathogen has also been detected in

soil or isolated from hosts other than soybeans in New York, North Carolina, Pennsylvania, Oregon, and abroad in Japan (Coffua et al. 2016; Pettersson et al. 2017; Rahman et al. 2014; Rojas-Flechas et al. 2017).

At the seedling stage, both *Phytophthora* species cause root rot and damping-off but after the development of trifoliate leaves, PSRR occurs primarily as a root and stem rot associated with *P. sojae* (Schmitthenner and Dorrance 2015). Infected plants display light-chocolate to brown lesion that progresses upwards within the cortex and vascular tissue, followed by wilting and plant collapse (Figure 1.1). PSRR develops rapidly in fine-textured soils when warmer temperatures (25-30°C) and saturated soil conditions prevail.

The disease cycle starts when growing mycelium develops sporangia, after repeated soil saturation. *In vitro*, light, culture age, temperature, quality and quantity of washing

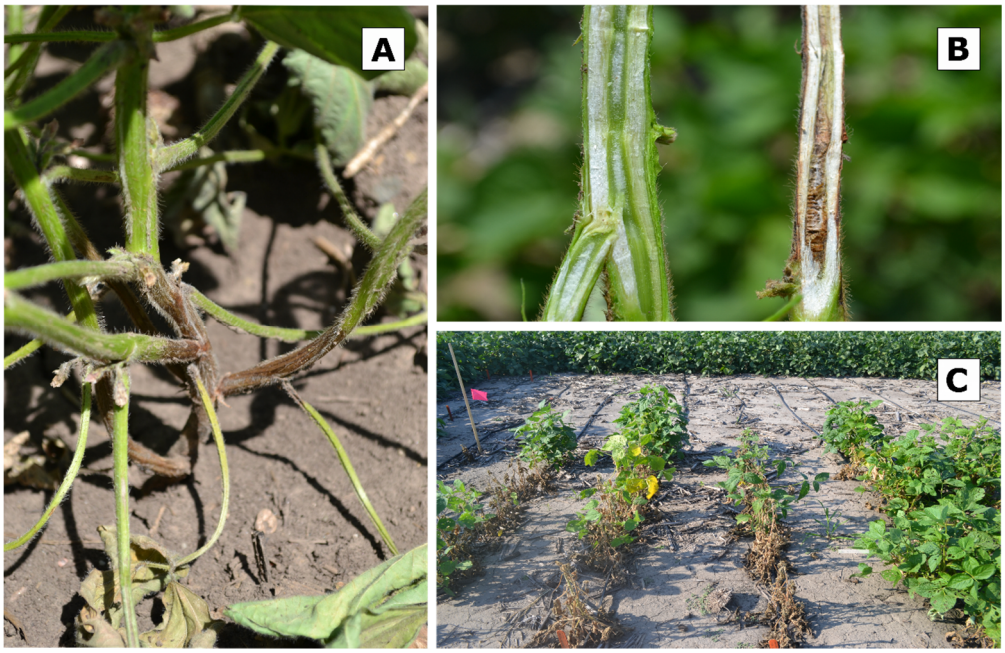


Figure 1. 1. Symptoms of Phytophthora stem and root rot (PSRR) of soybeans. **A,** Chocolate-colored lesion progressing upwards in the stem; **B,** Vascular discoloration in infected plant; **C,** Severe PSRR outbreak in breeding nursery in Lincoln-NE, August 2017. Detail on drip-irrigation providing continuous moisture for propagule development and dispersal.

solutions have been shown to interact with sporangial development and zoospore production (Eye et al. 1978; Schmitthenner and Bhat 1994). Single-celled, motile, biflagellate zoospores are chemotactically attracted to soybean roots by root exudates (genistein, daidzein, and other isoflavones). Upon zoospore encystment and germination, appressoria are formed at the point of penetration into host tissue, initiating infection process that will result in production in oospores in root tissue (Schmitthenner 1999; Tyler et al. 1996). *Phytophthora* species persist as well-adapted resting-structures called oospores in either crop residue or freely in the soil (Figure 1.2). The cycle is completed

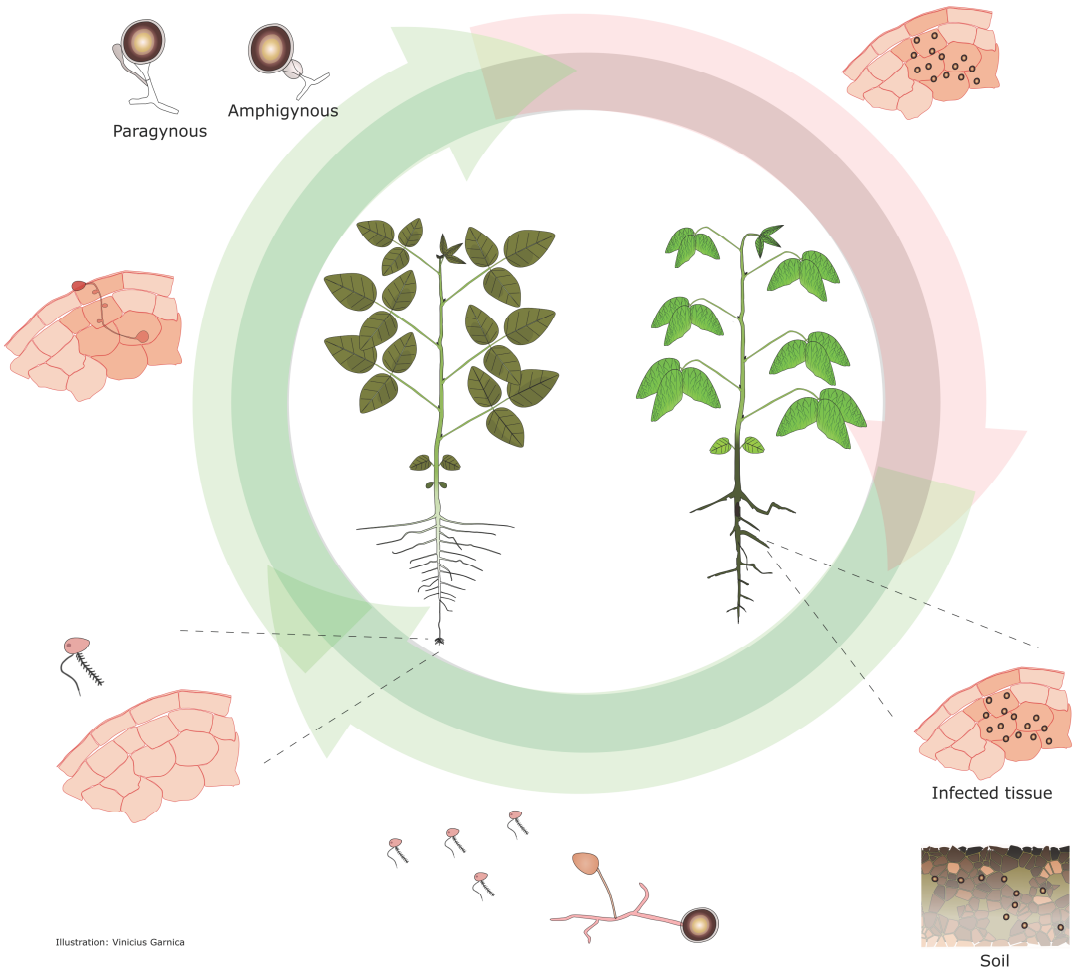


Figure 1. 2. Disease cycle of Phytophthora stem and root rot (PSRR) in soybean.

when oospores germinate to form mycelia that produce sporangia (Dorrance et al. 2007). In media, *P. sojae* is homothallic (self-fertile) with globose oogonium varying from 29.4 to 45.7, averaging 36.6 µm. Antheridia can be both paragynous and amphigynous (Figure 1.2). Sporangia are non-papillate and ovoid, ellipsoid, and sometimes obpyriform and vary from 23 to 88 µm long and 16 to 51 µm wide (Hildebrand 1959; Kaufmann and Gerdemann 1958). Both *Phytophthora* spp. mycelia are coenocytic, branching mostly at right angles and with a slight constriction at the base of each branch. Hyphae range from 3 to 9 µm wide and are slightly curled. As opposite to *P. sansomeana*, mycelial development of *P. sojae* is limited in full-strength potato dextrose agar (Schmitthenner and Dorrance 2015).

In relation to host specificity, *P. sansomeana* infects Douglas-fir, alfalfa (*Medicago sativa* L.), corn (*Zea mays* L.), soybeans and weed species (Hansen et al. 2009; Zelaya-Molina et al. 2010). More recently, *P. sansomeana* was also reported causing root rot in field pea (*Pisum sativum* L.) in Canada (Chang et al. 2017), which potentially could discourage adoption of this crop in rotational programs for soybean producers in Nebraska. By contrast, *P. sojae* has a more limited host range which includes soybeans, Lima bean (*Phaseolus lunatus* L.), string bean (*Phaseolus vulgaris* L.), Cranesbill (*Geranium carolinianum* L.), and *Lupinus* spp. (Erwin and Ribeiro 1996).

PSRR is best managed by planting resistant cultivars, promoting soil drainage, and applying effective oomycides as seed treatment or in-furrow. Most seed companies operating in the North Central U.S. provide information about resistance genes (*Rps*) and levels of disease susceptibility of soybean cultivars (Giesler and Broderick 2016). Knowledge of predominant pathotypes within a field may help on the selection of which

resistance genes (*Rps*) to use but the high within-field pathotype diversity makes disease management based entirely on *Rps* resistance difficult. Selecting highly tolerant cultivars with stacked *Rps* genes has also been suggested as a control strategy in areas PSRR is endemic. In addition to genetic resistance, early-season seedling protection can be achieved with seed treatments that contain oomycides (e.g. metalaxyl, mefenoxam, ethaboxam, and oxathiapiprolin) in their formulation. Practices that promote soil drainage and minimize inoculum accumulation on soil surface (Anderson and Buzzell 1982; Workneh et al. 1998) should be integrated with genetic resistance and seed treatment for durable PSRR management.

1.1.2. Inherited host resistance and pathotype diversity of *Phytophthora sojae* in Nebraska

The use of resistant cultivars is the most effective tool for PSRR management (Schmitthenner 1999). Host resistance is expressed in two major ways: race-specific through *Rps* mediated resistance and non-race specific through quantitative trait loci (QTLs) (a.k.a. tolerance, field tolerance, partial resistance). Both are screened separately but occur simultaneously at varying levels of tolerance in combination to the presence/absence of *Rps* gene(s) for a given soybean line.

Since its discovery, numerous resistance genes have been identified in the soybean germplasm: *Rps*1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8, 9, 10, 11, 12, UN1, UN2, Yu25, YD25, YD29, YB30, ZS18, SN10, HN, HC18, JS, Q, and an unnamed *Rps* gene (Waseshiroge) (Bernard et al. 1957; Dorrance 2018; Li et al. 2017; Niu et al. 2017; Sahoo et al. 2017; Zhong et al. 2018). Among these, *Rps*1a, 1b, 1c, 1k and to some extent *Rps*3a and *Rps*6 are the primary resistance genes incorporated into commercial lines in the U.S.

(Robertson et al. 2009; Slaminko et al. 2010). Slaminko et al. (2010) reported that the most common resistance in the lines evaluated by the Variety Testing Program in Illinois between 2004 and 2008 were *Rps*1c, 1k, and *Rps*1a and to a much smaller degree *Rps*3a,1b and *Rps*7.

In Nebraska, soybean cultivars belonging to maturity groups II and III are recommended for cultivation. Among these maturity groups, the resistance genes *Rps*1k and *Rps*1c are the most widely available resistance available in commercial germplasm (Table 1.1). Currently, 8 to 39% of cultivars contain *Rps*1k resistance whereas that 41 to 53% carry the *Rps*1c resistance gene. A smaller percentage of *Rps*3a and *Rps*3a/1k cultivars is also available, particularly for maturity groups 2-2.9. However, the continuous deployment of the same *Rps* genes in elite, commercial soybean cultivars have increased the selection pressure and led to a shift on virulence of *P. sojae* populations across some producing regions. To date, more than 200 unique virulence pathotypes have been identified across soybean regions of the U.S. (Dorrance et al. 2016).

In Nebraska, the earliest comprehensive study documenting the virulence diversity of *P. sojae* was conducted in the early 1980s (White et al. 1983). At the time, *P. sojae* was

Table 1. 1. Genetic composition of soybean cultivars with *Phytophthora sojae* resistance genes (*Rps*) across maturity groups recommended for production in Nebraska.

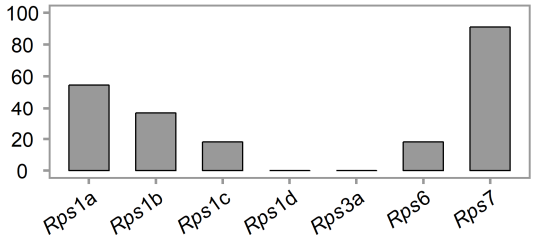
Maturity Group	Proportion (%) of *Rps* genes[z]									Total number
	1a	1c	1k	3a	3a/1c	3a/1k	6	7	None	
2-2.4	2	53	25	2	1	5	0	0	14	110
2.5-2.9	1	41	39	2	1	1	0	0	13	135
3-3.4	0	63	17	1	2	1	0	0	17	129
3.5-3.9	2	59	8	1	1	1	0	0	28	116

[z] Based on a survey conducted in July 2018 of AgriGold®, Asgrow®, Channel®, Credenz®, Golden Harvest®, Hefty®, Hoegemeyer®, Latham®, LG Seeds®, Mycogen®, NK®, Phillips®, Pioneer®, Seitec®, and Stine® seed catalogs.

geographically confined; only 12 isolates were baited across 468 fields sampled from 39 eastern counties of the state. Despite the limited number of isolates collected in that survey, pathotype 1 was the predominant virulence form followed by 3, 9, 4, 18 and 23 (White et al. 1983). Together, these isolates were able to defeat *Rps*1a, 1b, 1c, 6, and 7, but none of them were virulent on differentials 'PI 171.442', 'PI 103.091', indicating superior efficacy of *Rps*3a and *Rps*1d against *P. sojae* in Nebraska at the time (Table 1.2).

The pathotype diversity and geographical range of the pathogen increased rapidly as soybean acreage expanded in Nebraska. Between 2000 and 2004, a second state-wide was conducted to determine the virulence profile in Nebraska (Schimelfenig et al. 2005). Across 181 fields sampled, 52 located in north-central, western, south-central, south-eastern were positive for the pathogen, including in areas where the first survey was performed. In this second survey, pathotypes 3, 25, 28 and 33 were the dominant physiological races found across soybean regions in the state followed by 1, 4, 5, 8, 13, 43, and 44 (Schimelfenig et al. 2005). The same work indicated that *Rps*1a, 1b, 1c, 1k, and 3a

Table 1. 2. Proportion (%) of *Phytophthora sojae* isolates collected in a survey[x] between 1980 and 1981 in Nebraska by White et al. (1983) with virulence towards a particular *Rps* gene.



Pathotype[y]	Race[z]	Number of isolates	Isolate (%)
7	1	3	27.2
1a, 7	3	1	9.1
1a, 6, 7	9	1	9.1
1c, 7	14	1	9.1
1c	18	1	9.1
1a, 1b, 6, 7	23	4	36.3

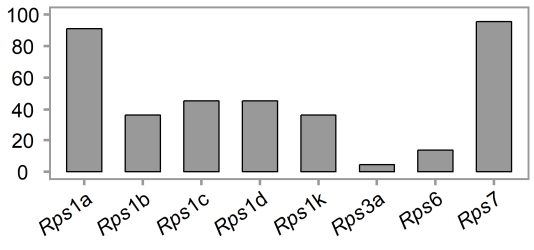
[x] Isolates were obtained from diseased seedlings in a soil bioassay using Harosoy (*Rps*7) as baiting cultivar.

[y] Pathotype identified by susceptible reaction using hypocotyl technique and *P. sojae* differentials: 'Harosoy' (*Rps*7), 'Harosoy63' (*Rps*1a+7), 'Sanga' (*Rps*1b), 'Mack' (*Rps*1c), 'Altona' (*Rps*6), 'PI171.442' (*Rps*3a), and 'PI103.091' (*Rps*1d), according to Laviolette and Athow (1983). Genetic information about soybean differentials was compiled from Dorrance et al. (2009) and Anderson and Buzzell (1992).

[z] According to Sugimoto et al. (2012).

provided disease immunity to approximately 73, 36, 33, 29 and 9%, respectively, of the pathogen population collected in the state at the time (Schimelfenig et al. 2005). Compared to the first survey conducted in the early 1980s, no major virulence alterations were observed for *Rps*1b, *Rps*6, and *Rps*7 resistance, but slight shifts in the pathogenic frequency were observed for *Rps*1a and *Rps*1c. Overall, virulence to *Rps*7 was still widespread with more than 90% of isolates recovered in both studies being able to defeat this resistance gene, whereas, virulence to *Rps*1b and *Rps*6 remained relatively constant at 35 and 18% of isolates, respectively (Table 1.2 and 1.3). For *Rps*1c, efficacy dropped slightly from 72 to 67% and *Rps*1a virulence frequency increased moderately from 54 to 73%, despite the limited number of isolates recovered. A similar trend was observed for *Rps*1d, which had superior efficacy against *P. sojae* in previous assessments (Table 1.2 and 1.3). *Rps*1k and *Rps*8 were not screened during a survey conducted by White et al. (1983). More recently between 2012 and 2013, a third survey was conducted in Nebraska and

Table 1. 3. Proportion (%) of *Phytophthora sojae* isolates collected in a survey[x] between 2001 and 2002 in Nebraska by Schimelfenig et al. (2005) with virulent towards a particular *Rps* gene.



Pathotype[y]	Race[z]	Number of isolates	Isolate (%)
7	1	1	5
1a, 7	3	6	27
1a, 1c, 6, 7	5	1	5
1a, 1d, 6, 7	8	1	5
6, 7	13	1	5
1a, 1b, 1c, 1k, 7	25	3	14
1a, 1b, 1c, 1d, 1k, 7	33	4	18
1a, 1c, 1d, 7	43	2	9
1a, 1d, 7	44	2	9
1a, 1b, 1d, 1k, 3a	Undefined	1	5

[x] Isolates were obtained from diseased seedlings in a soil bioassay using 'Sloan' (*rps*) as baiting cultivar.
[y] Pathotype identified by susceptible reaction using hypocotyl technique and *P. sojae* differentials: 'Harosoy 13xx' (*Rps*1b), 'L75-3735' (*Rps*1c), 'HARO16' (*Rps*1d), 'Willians82' (*Rps*1k), 'PI 171.442' (*Rps*3a), 'L89-1581' (*Rps*6), and 'Harosoy' (*Rps*7) according to Schmitthenner et al. (1994).
[z] According to Sugimoto et al. (2012).

other states in the North Central region of U.S. (Dorrance et al. 2016). Among the 870 isolates of *P. sojae* recovered, more than 50% of them were virulent on *Rps*1k or *Rps*7, while more than 40% of isolates were virulent on *Rps*1a, *Rps*1b, or *Rps*1c. The same study demonstrated that in Nebraska, *P. sojae* populations maintained high virulence (>95%) to *Rps*1a, moderate to *Rps*1b, *Rps*1c, and *Rps*1k, and low (<25%) virulence towards *Rps*3a, *Rps*6, and *Rps*8 (Dorrance et al. 2016). Interestingly, roughly 30% of isolates were virulent to *Rps*6, a less commonly deployed resistance gene in soybean cultivars utilized in the state (Table 1.1). Based on all three surveys, *Rps*3a and its pyramided forms (*Rps*3a+1c and *Rps*3a+1k) should be considered for management of PSRR in problematic areas of the state.

Populations of *P. sojae* are not only macro- and micro-regionally structured, within-field variation has also been shown to exist (Robertson et al. 2009; Stewart et al. 2016). Robertson et al. (2009) found nine distinct in addition to nine undescribed *P. sojae* pathotypes in a commercial field in Iowa. Collectively, these isolates were able to defeat *Rps*1a, 1b, 1c, 1d, 1k, 3a, 3c, 4, 5, 6, and *Rps*7 genes through hypocotyl inoculation technique.

The high level of pathogenic diversity in the pathogen population makes PSRR management complex, requiring more than one type of resistance approach for proper disease control. Quantitative trait loci (QTLs) resistance (a.k.a. field tolerance, partial resistance) manifests as incomplete resistance with lower levels of root rot and the absence of stem rot in highly resistant cultivars (Schmitthenner 1985). QTL is controlled by several genes, with moderate to high genetic heritability, and interacts with pathotypes non-specifically by delaying infection, colonization and reducing oospores production in host

tissue (Glover and Scott 1998; Mideros Mora et al. 2007). The usefulness of cultivar tolerance for PSRR control and associated yield benefit has been demonstrated in field trials. Dorrance et al. (2003a) reported that high levels of tolerance combined with the presence of *Rps* genes provided greater yield stability compared to cultivars having either moderate or low levels tolerance and *Rps* genes. PSRR-tolerance evaluated from recombinant inbred lines (RILs) derived from the cross 'Conrad' and 'Sloan' demonstrated that resistant RILs outperformed susceptible RILs by 800.7 and 1062.5 kg ha^{-1}, depending on disease pressure (Wang et al. 2012). In the U.S., most soybean seed companies supply information about *Rps* genes and PSRR tolerance scores in seed catalogs, but subjectivity on scale systems offers practical challenges for producers wanting to analytically compare soybean cultivars within each brand or across seed companies.

1.1.3. Soil-applied residual pre-emergence herbicides and seedling diseases

The overreliance on single herbicide active ingredients and simplified weed management plan has put unprecedented selection pressure on the weed community and led to an exponential increase of herbicide-resistant weed cases worldwide (Green 2016). For example, glyphosate-resistance has been confirmed in 42 weed species worldwide, including 17 in the United States, many of which are commonly found in the soybean-corn cropping systems of North Central U.S. (Heap 2018). Effective weed management practices include the rotation of disparate herbicide sites of action, adoption of crop rotation, tillage, cover crops, and preventing weed establishment. In soybeans, some of these practices can be achieved through the use of pre-emergence (PRE) herbicides (Oliveira et al. 2017; Sarangi et al. 2017; Soltani et al. 2009).

In recent years, the use of PRE herbicides belonging to protoporphyrinogen oxidase inhibitors (PPO, WSSA group 14) and photosynthetic system II (PSII, WSSA group 5) inhibitors have increased in the U.S. (USDA 2018). However, some PRE herbicides can cause severe injury to sensitive seedlings, particularly when wet and cool soil conditions prevail after herbicide application (Hager 2014; Miller et al. 2012). Under these conditions, seedlings increase herbicide uptake and decreased plant metabolism, resulting in greater injury (Grey et al. 1997; Niekamp and Johnson 2001; Wise et al. 2015). Jhala (2017) stated that herbicide phytotoxicity risk can be reduced if (i) application is performed within three days of soybean planting; (ii) applications are not made to poorly drained soils under cool, wet conditions; (iii) seeds are completely covered by soil prior to herbicide applications; and, (iv) flumioxazin-based herbicides are applied before soybeans begun to crack through the soil.

Evidence of synergistic interaction between PRE herbicides and seedling diseases have been well documented in the literature. Carson et al. (1991) observed greater soybean root rot caused by *Fusarium oxysporum* resulting from trifluralin at PRE, with disease levels augmented under cool soil temperatures (10-15°C). Hypocotyl and root rot caused by *Rhizoctonia solani* can also be affected by pre-emergence herbicides. Chandler and Santelmann (1968) reported that in the presence of *R. solani*, trifluralin enhanced injury to cotton in controlled and field conditions. Findings by Pinckard and Standifer (1966) and Neubauer and Avizohar-Hershenson (1973) also support that trifluralin can increase cotton seedling blight caused by *R. solani*, but contradicts findings by Heydari and Misaghi (1998). In another study, Harikrishnan and Yang (2002) observed that pendimethalin at PRE resulted in higher soybean root rot incidence in soil infested with *R. solani* than non-

infested, non-treated control. In this context, Bradley et al. (2002) reported synergism between pendimethalin, acifluorfen, and imazethapyr at PRE and Rhizoctonia root and hypocotyl rot in soybeans. Similarly, Bowman and Sinclair (1989) reported reduced seedling vigor in *R. solani* infested-soil treated with alachlor, choramben, dinoseb, fluchloralin, or naptalam in greenhouse settings. Duncan and Paxton (1981) observed an additive effect of trifluralin incorporated in the soil on stand reduction caused by *Phytophthora sojae*.

While many studies have documented synergistic interactions between seedling diseases and PRE herbicides, particularly those belonging to WSSA group 3, one a few have evaluated how newer herbicide molecules may influence disease occurrence in soybeans (Barlow et al. 2018; Kandel et al. 2018). Results by Barlow et al. (2018) indicate a larger interaction between PRE herbicides and varieties than between PRE herbicides and seed treatments for soybean stand and yield. In other pathosystems, Daugrois et al. (2005) studied the effect of sulfentrazone and flumioxazin at PRE on Pythium root rot of sugarcane and observed no consistent effect on disease parameters, although some herbicide treatments affected the relative isolation frequency of *Pythium* spp. from roots and altered colonization by the pathogenic species *P. arrhenomanes*. It has also been shown that lactofen, a PPO-inhibiting herbicide, applied at post-emergence but prior to reproductive stages can suppress white mold incidence in soybeans caused by *Sclerotinia sclerotiorum* (Dann et al. 1999).

1.1.4. Seed treatments in soybean production

As result of immediate commodity price decline from international trade disruptions, change in soybean production practices towards earlier planting dates

(Bastidas et al. 2008), reduced seeding rates given the compensatory ability of modern cultivars (Suhre et al. 2014), and increased seed price (Schnitkey 2018); soybean seed treatments use has increased sharply in the U.S. Between 1996 and 2008, seed treatment adoption increased from 8 to 30%, and is currently estimated at >75% of total seed volume marketed (Gaspar et al. 2017; Munkvold 2009).

Seed treatments are important because of their efficacy against multiple seed and soilborne pathogens, insects, and nematodes. Seed treatments containing oomycides are effective towards *Pythium* and *Phytophthora* species (Radmer et al. 2017; Vargas et al. 2017), while seed treatment containing fungicides are generally more effective against pathogens such as *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium* spp. (Bradley 2008; Kandel et al. 2016; Xue et al. 2007). In soybean production, seed treatments are available in a variety of formulations (slurry- and mist-type applied on-farm or industrially) and combinations of actives (Giesler and Miller 2017). These actives are generally classified based on their mode of action (FRAC codes), which refers to the specific enzyme in the cellular process being targeted in the organism. In soybeans, commonly adopted fungicides with systemic activity include ipconazole, prothioconazole (DMI - FRAC code 3), fludioxonil (FRAC code 12), thiabendazole (FRAC code 1), trifloxystrobin, pyraclostrobin, azoxystrobin (QoI - FRAC code 11), and fluopyram, carboxin, sedaxane, penflufen (SDHI - FRAC code 7) (FRAC 2018).

Metalaxyl and its isomer mefenoxam are phenylamides oomycides that inhibit RNA synthesis (FRAC code 4) (FRAC 2018). In soybeans, these compounds have been utilized as seed treatments because of their systemic activity, chemical stability over a range of pH and temperatures, and more importantly, superior efficacy towards *Pythium*

and *Phytophthora* (Dorrance et al. 2009; Sukul and Spiteller 2000). Despite differences in nomenclature and application rate, metalaxyl and mefenoxam only vary by the proportion of the biologically active *R*-isomer (Nuninger et al. 1996), which upon continuous use, can lead to the selection of metalaxyl-insensitive populations. In *vitro* assays have detected variation in metalaxyl (and mefenoxam) sensitivity amongst isolates of multiple pathogens, including *Pseudoperonospora cubensis*, *Phytophthora infestans*, *P. erythroseptica*, and some oomycete soybean seedling pathogens (Broders et al. 2007a; Dorrance et al. 2004; Matson et al. 2015; Olson et al. 2013; Taylor et al. 2006). Nevertheless, metalaxyl and mefenoxam have still demonstrated satisfactory efficacy in field trials, especially when complemented with fungicides belonging to FRAC code 3 and 11 groups (Bradley 2008; Dorrance et al. 2009; Gaspar et al. 2015; Grau and Gaska 2000; Poag et al. 2005). For PSRR management, higher rates of metalaxyl (>15.5 g a.i. 100 kg^{-1} seed) and mefenoxam (>7.5 g a.i. 100 kg^{-1} seed) have been recommended in disease conducive environments (Dorrance 2013). In Nebraska, metalaxyl and mefenoxam can be purchased singly at rates varying from 1.9 to 30 g a.i. 100 kg^{-1} seed or in commercial formulations containing other partner mixes at rates varying from 3.75 to 15 g a.i. 100 kg^{-1} seed (Jackson-Ziems et al. 2017).

More recently, ethaboxam (FRAC code 22) was also registered as oomycide seed treatment in soybeans. Ethaboxam is an aminothiazole carboxamide compound discovered in Korea and registered initially for horticultural crops (Kim et al. 1999; Ra et al. 1995). In *vitro* assays, ethaboxam inhibited mycelial growth of *Pythium ultimum*, *P. irregulare*, *P. sylvaticum* (Radmer et al. 2017) but showed poor efficacy against *Rhizoctonia solani* and *Glomerella glycines* (Kim et al. 2004). In controlled conditions, ethaboxam persisted

systemically for 14 days in tomato seedlings (Kim et al. 2004). In Nebraska, ethaboxam is currently available as a commercial seed treatment formulation at a rate of 7.5 g a.i. 100 kg^{-1} seed (Intego Suite, Valent U.S.A. Corporation, Walnut Creek, CA).

1.1.5. Field efficacy of seed treatments

Each year, public and private initiatives conduct several field trials across the Midwest U.S. to determine the efficacy of seed treatments for seedling disease control in soybeans. In a multi-location study in North Dakota, Bradley (2008) reported no yield differences between seed treatments and untreated check in 6 of 14 locations studied. However, yield increases as high as 78% were observed from seed treatment use, depending on environmental conditions and the profile of pathogens active in a particular location (Bradley 2008). Gaspar et al. (2015) evaluated the benefit of broad-spectrum fungicide seed treatment and observed an increase of 8,000 plants ha^{-1} in plant stand and 21 kg ha^{-1} in yield across multiple locations in 2013 but not in 2011–2012.

In Nebraska, small-plot research trials have been inconsistent to the benefit of seed treatments in soybeans (Dorrance et al. 2009; Giesler and Gustafon 2009). Despite increases in plant population, mefenoxam had no effect on yield in fields with PSRR history (Giesler and Ziems 2007). Across two growing seasons, Dorrance et al. (2009) found no yield benefit with the use of mefenoxam + fludioxonil when compared to untreated check.

Discerning confounding factors that interact with fungicide seed treatment response is key to determine whether these inputs should be used in soybean production. Specifically, the knowledge of the (i) profile of seedling pathogens active in a particular

location (a.k.a. field history); (ii) genetic resistance of a desired cultivar; (iii) soil type and permeability; (iv) biological selectivity of seed treatment utilized; and (v) cost and expected economic return from seed treatment adoption is fundamental to determine the necessity these components in soybean production.

1.2. Research objectives

The two major objectives of this research were:

1. To determine the efficacy of seed treatment in combination to genetic resistance to *Phytophthora sojae* on soybean population density, canopy coverage, and yield in fields with disease history;

2. To investigate possible synergism between pre-emergence herbicides and seedling diseases in late-planted soybeans.

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CHAPTER 2. Integrated management of Phytophthora stem and root rot of soybean in Nebraska and Iowa

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Abstract

Integrating disease control strategies has been the foundation for effective management of Phytophthora stem and root rot (PSRR; caused by *Phytophthora sojae*) in soybean (*Glycine max*) in the North Central U.S. In order to determine the efficacy of seed treatment formulation (clothianidin + ethaboxam + ipconazole + metalaxyl) and host resistance (*Rps*1k or *Rps*1c and moderately resistant [MR] or moderately susceptible [MS]) in commercial cultivars, 6 environments were evaluated in Nebraska and Iowa in 2017 and 2018. Symptoms of *P. sojae* stem lesions were detected in 4 out of 6 environments. Compared to untreated control, seed treatment increased soybean emergence by 11,600 to 53,700 plants ha^{-1} and early-season canopy coverage (CC) by 0.7 to 1.2%. The efficacy of seed treatment ranged from 230.9 to 331.6 kg ha^{-1}, depending on the environment. While management programs with MR cultivars had greater yields (538.9 to 747.5 kg ha^{-1}) than MS cultivars, there were negligible yield differences between *Rps*1k and *Rps*1c genotypes, except in one environment. A weak to moderate (ρ = -0.32 to -0.45; $P \leq 0.001$) correlation was observed between CC and the number of plants with *P. sojae* stem lesions. Moderate to strong association between CC and yield was found (ρ = 0.32 to 0.82; $P < 0.001$).

Outcomes from this study demonstrate the benefits of combining genetic resistance and seed treatment to manage PSRR in disease conducive environments.

2.1. Introduction

Phytophthora stem and root rot (PSRR) is a yield-limiting disease in soybeans (*Glycine max* L. Merr.) caused by the soilborne oomycete *Phytophthora sojae* Kauffm. & Gerd. Yearly, soybean losses due to the disease are estimated at 9.4 MMT in North America (Allen et al. 2017). Disease symptoms include early-season damping-off and premature death of plants (Hartman et al. 2015). The stunting resulting from infection compromises yield and creates additional management problems, such as reduced crop competitiveness for weed control (Bussan et al. 1997).

Even though infection can occur at any stage of plant development, most of the damage is believed to occur at emergence (Workneh et al. 1998), which may justify the use of oomycides (e.g. fungicides) at planting for seedling protection. Historically, two active ingredients, metalaxyl and mefenoxam, have been applied to seeds, banded in granular form, or sprayed in-furrow for *P. sojae* root rot and damping-off management (Anderson and Buzzell 1982; Ryley et al. 1989). More recently, another seed treatment with a novel mode of action was registered in the U.S. Ethaboxam is classified as an inhibitor of ß-tubulin assembly during mitosis (FRAC group 22) and showed excellent control of foliar diseases in horticultural crops (Kim et al. 1999) and *in vitro* efficacy against common soybean oomycete pathogens (Matthiesen et al. 2016; Radmer et al. 2017). Information about the field efficacy of seed treatments containing ethaboxam for PSRR control and soybean yield effect is limited.

In addition to oomycides, PSRR has also been managed with host resistance (McBlain et al. 1991; Schmitthenner 1985). Host resistance occurs in two primary ways, race-specific through *Rps* resistance genes and non-race specific through polygenic resistance, commonly referred as cultivar tolerance or partial resistance (Anderson and Buzzell 1992; McBlain et al. 1991). In North Central U.S., common resistance genes deployed in commercial soybean lines are *Rps*1a, 1b, 1c, 1k and to a less extent, *Rps*3a and *Rps*6 (Robertson et al. 2009; Slaminko et al. 2010). However, the continuous use of a few, single *Rps* genes has led to increased selection pressure on pathogen populations (Dorrance et al. 2016; Schmitthenner et al. 1994) which combined with the natural in-field pathogen variability makes PSRR management complex (Stewart et al. 2016). On the other hand, cultivar tolerance is effective against multiple pathotypes by limiting the infection rate and lesion expansion (Thomas et al. 2007) and preventing yield losses in conducive environments (Rehm and Stienstra 1993; Tooley and Grau 1984). Information about the presence or absence of *Rps* genes and PSRR tolerance levels is available in soybean seed catalogs of companies operating in the U.S., which may assist growers to establish a disease management plan.

Many factors including pathogen inoculum, tillage, drainage, compaction, soil texture, and environmental conditions influence PSRR development (Duniway 1983; Gray and Pope 1986). Fine-textured soils cultivated in minimum or no-tillage tend to favor disease incidence, as propagules accumulate on the soil surface (Workneh et al. 1998, 1999), whereas, warm and saturated soil conditions following a brief drop in temperature increase PSRR occurrence by providing optimum conditions for propagule development and dispersal (Schmitthenner and Bhat 1994).

In field trials, PSRR severity is assessed on the basis of early-season damping-off, incidence of plants with characteristic stem lesions, plant height, root lesion length, and yield reduction of susceptible cultivars compared to resistant lines (Dorrance et al. 2003; Gray and Pope 1986; Guy et al. 1989; Rehm and Stienstra 1993). However, because highly tolerant cultivars do not always develop stem lesions but may still exhibit permanent aboveground stunting (Meyer and Sinclair 1972; Schmitthenner 1985), additional screening approaches are needed to support severity assessments in field trials. Proximal remote sensing is an alternative method to non-destructively configure plant health status (Bock et al. 2010; Mahlein 2016). User-friendly, rapid data collection has been originated using handheld, open-source, phenotyping/phytopathometric mobile platforms (Patrignani and Ochsner 2015; Pethybridge and Nelson 2015, 2018), which may be used to quantify plant architectural changes associated with PSRR occurrence and aid in future management decisions.

Despite the increasing PSRR occurrence in production areas of Nebraska and Iowa (Tachibana et al. 1975; White et al. 1983), previous studies were inconclusive in determining the effects of seed treatments and genetic resistance as part of an integrated disease management program in these states (Cerra 2007; Dorrance et al. 2009; Giesler and Gustafon 2009). Herein, we synthesize the field efficacy of a seed treatment formulation and quantitatively estimate the benefit of cultivar selection using soybean commercial lines, simulating a producer's approach for disease management. More specifically, we quantified differences in soybean population density, canopy coverage, and yield resulting from (i) the use of a seed treatment formulation with clothianidin + ethaboxam + ipconazole + metalaxyl *vs.* untreated control; (ii) the selection of PSRR moderately

resistant *vs.* moderately susceptible cultivars; (iii) the selection of cultivars carrying *Rps*1k and *Rps*1c; and, (iv) a combination of the above strategies.

2.2. Material and methods

A total of 6 experiments were conducted in Nebraska and Iowa during 2017 and 2018 (Table 2.1). All experiments were established in fields with PSRR history and corn (*Zea mays* L.) as the previous crop. In Nebraska, field trials were located near Tekamah (41.7079089, -96.1081753) in 2017, and at four locations near Tekamah (41.755558, -96.176062), Arizona (41.792885, -96.139346), Mead (41.182523, -96.459948), and Bruno (41.293432, -96.916723) in 2018 in collaboration with local producers. In Iowa, a single field trial was established near Boone (42.012612, -93.784207) in 2018 at the Iowa State University Field Extension and Education Laboratory. Site-specific information such as planting date, soil type and texture, and the chronological program of activities is provided in Table 2.1.

The experimental design consisted of a split-plot arranged in a randomized complete block design with four replications. In Nebraska, experimental units were four-row plots, 5.18-m long by 3.04-m wide, planted at 0.76-m row spacing and sown at a density of 308.881 seeds ha^{-1}, whereas, in Iowa, plots were 10.6-m long by 3.04-m wide and sown at a density of 296,526 seeds ha^{-1}. All locations were sown at 4-cm depth. Cultivars were randomly assigned to whole-plot units, and thereafter, seed treatments were randomly assigned to the subplot units (Mead 1990). Soybean cultivars of maturity groups II and III with commonly deployed *Rps* genes were obtained from private soybean seed companies (Table 2.2). Although genotypes varied across some locations, at least two with the same *Rps* resistance but distinct PSRR tolerance scores were selected from each

company to represent moderately resistant [MR] or moderately susceptible [MS] classes, based on company-supplied PSRR susceptibility information. At the subplot level, treatments consisted of (i) untreated control and (ii) clothianidin + ethaboxam + ipconazole + metalaxyl (Intego Suite Soybeans, Valent U.S.A., Walnut Creek, CA) applied at 50 g + 7.5 g + 2.4 g + 1.9 g a.i. 100 kg^{-1} seed. For seed treatment procedure, 1.76 ml of fungicide was added to water for a total mix volume of 2.6 ml, poured into a plastic bag with 800 g of seeds, and mixed until seeds were treated uniformly. Seeds were then allowed to air-dry and stored until planting. Soybean production practices related to nutrient management, pre- and post-emergence herbicide applications followed the university extension service recommendations in each state.

Disease development. Plots were examined periodically to determine the number of plants with symptoms of *Phytophthora sojae* stem lesions. All four rows of each plot were inspected and the total number of symptomatic plants (nPSR) was recorded throughout the season. Symptomatic plants were isolated and *P. sojae* was confirmed based on morphological characteristics and culture growth pattern in PDA (Dorrance et al. 2008).

Plant population. Plant population densities were collected at emergence (VE-VC) (Fehr et al. 1971), first to second trifoliate stage (V1-V2), sixth trifoliate to full bloom (V6-R2), and prior to harvest (R8). Plant population assessments were performed by counting the number of emerged plants in the center two rows of each experimental unit. Row segments were 3.05-m and 10.6-m in length in Nebraska and Iowa, respectively.

Canopy coverage. Early- (V1-V2) and mid-season (V6-R2) canopy coverage (CC) was estimated using the smartphone application *Canopeo* (Oklahoma State University, Stillwater, OK). For scale reference, a PVC tube frame with dimensions of 1.05-m long by

0.76-m wide was arbitrarily placed within each row in order to consistently delineate the section of 0.798 m^2 during data collection. Each harvestable row section was systematically photographed using an iPhone 7 with a screen size 4.7" and 12MP embedded camera with *f*/1.8 aperture positioned horizontally above the canopy and approximately 1.2-m from the soil line. No camera flash was used, and a minimal reflective dark velvet cloth was fixed below the canopy prior to imaging. CC assessments were performed on sunny, clear days between 8:00 am and 3:00 pm and usually lasted 90 s per experimental unit. The procedure specified above was repeated for all environments in Nebraska, with exception of Tekamah in 2017, where no frame and a single CC assessment depicting two rows at a time was performed. No CC assessments were collected in Iowa.

Yield. Prior to harvest, the experimental units were trimmed to 4.5-m and 5.3-m in length in Nebraska and Iowa, respectively, and the center two rows from experimental unit were harvested with a small plot combine (Almaco SPC20, Almaco, Nevada, IA) equipped with HarvestMaster grain gauge and handheld computer Allegro MX (Juniper Systems, Logan, UT) for data collection. Total seed weight and seed moisture content were measured per experimental unit. The seed weight was then adjusted to 13% moisture and yield expressed as kilograms per hectare.

Data analysis. Data analysis were performed in R (version 3.5.2, R Foundation for Statistical Computing, Vienna, Austria) using the R Studio (version 1.1.463, RStudio Inc.). A mixed linear model was fitted using *lme4* package (version 1.1.17) with soybean cultivars and seed treatments as fixed effects and blocks, whole-, subplot, and subsampling errors as random effects. Analysis of variance was conducted using *lmerTest* package

(version 3.0.1) for each environment. Degrees of freedom for the denominator were estimated with Kenward-Roger's method and variance components were obtained with restricted maximum likelihood (REML) method. Because CC was initially expressed in percentage, data were arcsine square root transformed (arcCC) prior to analysis to improve variance homogeneity.

A model to describe analysis of response variables at each environment is following,

$$y_{ijkl} = \mu + b_j + \alpha_i + (\alpha b)_{ij} + \beta_k + \alpha\beta_{ik} + \beta(\alpha b)_{ijk} + \varepsilon_{ijkl} \quad (1)$$

where y_{ijkl} = observed response variable; μ = overall experimental mean; b_j = random effect of j^{th} block, which is assumed to be distributed $N\sim(0,\sigma^2_b)$; α_i = effect of i^{th} cultivar; $(\alpha b)_{ij}$ = random whole plot error which is assumed to be distributed $N\sim(0,\sigma^2_{\alpha b})$; β_k = the k^{th} seed treatment effect; $\alpha\beta_{ik}$ = interaction effect of the i^{th} cultivar and k^{th} seed treatment; $\beta(\alpha b)_{ijk}$ = random subplot error that it is assumed to be distributed $N\sim(0,\sigma^2_{\beta(\alpha b)})$; and ε_{ijkl} = random subsampling error, assumed to be distributed $N\sim(0,\sigma^2_{\varepsilon})$. Grain yield data were evaluated with a similar model with the exception of subsampling error term (ε_{ijkl}). Least-squares means were obtained using *emmeans* package (version 1.3.1) and single-degree-of-freedom contrast statements were used to make treatment comparisons. Probability values were adjusted with Benjamini-Hochberg procedure to control for false discovery rate due to the lack of orthogonality between cultivar contrasts. In Mead and Bruno, some experimental units (totaling 6 and 12, respectively) were severely damaged by flooding, and therefore, were removed from the analysis. arcCC means were back-transformed to 0-100% scale to improve variable meaningfulness. Covariation between the PSRR disease

parameters and yield was determined using Spearman's rank correlation in a two-sided hypothesis test (Madden et al. 2007).

Weather data. Soil temperature at planting and cumulative seasonal precipitation were obtained from weather stations operated by public weather service websites (https://www.ncdc.noaa.gov/cdo-web/) located within a 10-km radius from trials. In addition to natural precipitation, irrigation was supplemented through overhead irrigation delivered by a center-pivot at some locations.

2.3. Results

Disease development. *Phytophthora sojae* was isolated from symptomatic plants in 4 out of 6 environments. The number of PSRR-positive plots was roughly 12, 8, 17 and 14% of total experimental units in Tekamah, Arizona, Mead, and Bruno in 2018, respectively. In addition to stem lesions, symptoms of seedling damping-off caused by oomycetes were observed in Tekamah in 2018. Poor crop establishment occurred in Arizona and contrasted trial conditions at Mead, where seedling damping-off incidence was low, despite later development of *P. sojae* stem lesions. Sentinel-border plots planted with PSRR-susceptible cultivar 'Sloan' also developed disease symptoms in all Nebraska locations, except at Tekamah in 2017. PSRR stem lesions did not develop in Boone, IA.

Plant population. Seed treatment had a significant effect on soybean emergence in 4 of the 6 locations with increases as low as 11,500 plants and as high as 53,000 plants ha^{-1}, depending on the environment (Table 2.4). Although seed treatment showed superior efficacy on early-season plant populations, negligible differences between MR and MS, less than 10,500 plants ha^{-1}, were quantified in Arizona and Boone. MR cultivars had

greater ($P \leq 0.10$) emergence than MS by 10.9 and 18.2% at Tekamah and Bruno in 2018, respectively (Table 2.4). While soybean cultivars significantly differed in emergence in 5 of the 6 environments, no differences were associated with the selection of *Rps*1c or *Rps*1k genes. In Tekamah, V1-V2 plant population assessment indicated that among cultivars carrying *Rps*1c, MS had fewer plants than MR but an opposite effect was observed for *Rps*1k cultivars (Table 2.5). For the integration of seed treatment and PSRR cultivar tolerance, effects were variable across environments. MR cultivars experienced an increment varying from 10.3 to 21.5% in population densities, whereas, MS cultivars had 23.4 to 46.1% increase upon seed treatment use at Tekamah and Arizona in 2018, respectively. However, at Boone, seed treatment significantly increased stand of MR cultivars (15,607 plants ha^{-1} on average; $P<0.10$) but not for MS cultivars (Table 2.5).

In agreement with early-season assessments, seed treatment effects were also identified during mid-season and final plant population evaluations with an average increase of 32,500 plants ha^{-1} observed across all Nebraska locations in 2018. However, even though cultivars differed substantially on the number of plants per hectare, effects were not clearly associated with the selection *Rps*1k or *Rps*1c genes (Table 2.6 and 2.7). Conversely, MS cultivars had lower ($P \leq 0.10$) final population densities than MR at Bruno and Tekamah in 2018. Exclusively among cultivars with *Rps*1c gene, MS cultivars had lower population densities than MR, but the opposite was observed for *Rps*1k at Tekamah in 2017 (Table 2.7). No interaction between cultivar, seed treatment, and environment was statistically significant in Nebraska in 2018 (Table 2.3). Relatively, Boone had the lowest final population density mean with 109,869 plants ha^{-1} and no differences were associated

to PSRR genetic resistance (*Rps* and tolerance), despite of significant cultivar effect during ANOVA (Table 2.3).

Canopy coverage. In total, 992 unique sampling measurements were recorded at two distinct phenological stages. At subplot unit, early- and mid-season CC ranged from 0.4 to 10.1% and 3.9 to 63.4 %, respectively (Figure 2.1). Seed treatment consistently increased CC (arcsine square root transformed) in 3 environments, but not at Tekamah in 2017 and Mead in 2018. Back-transformed mean CC increases as low as 0.7% and as high as 1.2% were quantified resulting from seed treatment use during early-season and developed to greater CC discrepancies (5.2-8.3%) during later assessments (Figure 2.2-A, D). MR cultivars had significantly greater CC compared to MS cultivars at Bruno and Tekamah in 2018, but differences were stage-dependent (Figure 2.2-B, E). In this study, planting MS cultivars did not result in increased CC mean values in any environment. Cultivars with *Rps*1c had lower early-CC than *Rps*1k by 1.8% on average at Mead, despite the lack of significant effect with seed treatment adoption. *Rps* resistance had negligible effects on CC during the mid-season assessment (Figure 2.2-C, F).

Yield. Yield ranged from 1,384.4 to 5,767.6 kg ha^{-1} and averaged 3,642 kg ha^{-1} in this study. Lower quantile and upper quantile at 0.25 and 0.75 of the values were 2,930.2 and 4,436.9 kg ha^{-1}, respectively. Grain yield varied greatly across environments and the efficacy of seed treatment averaged 259.9 kg ha^{-1} (CI_L: 151.3 and CI_U: 368.5 kg ha^{-1}) relative to the untreated control in environments which PSRR symptomatic plants were detected. For analysis performed individually at each environment, seed treatment had a significant effect ($P \leq 0.05$) on yield in nearly half of the trials, with increments ranging from 230.9 kg ha^{-1} (CI_L: 105.4 and CI_U: 356.4 kg ha^{-1}) to 331.6 kg ha^{-1} (CI_L: 121.1 and

CI_U: 542.2 kg ha^{-1}) depending on the environment (Table 2.8). For PSRR genetic resistance, monogenic *Rps* resistance and PSRR tolerance effects were only detected in environments where seed treatment effect co-existed. MR cultivars yielded in average more, between 538.9 kg ha^{-1} (CI_L: 262.7 and CI_U: 815.3 kg ha^{-1}) to 747.5 kg ha^{-1} (CI_L: 361.7 and CI_U: 1,133.2 kg ha^{-1}), than MS cultivars in Bruno and Tekamah in 2018, respectively. In relative terms, cultivar resistance in the form of tolerance had a greater absolute yield size effect than seed treatment alone. In relation to *Rps* genes, an average yield increase of 12.5% was detected for cultivars carrying *Rps*1c when contrasted to *Rps*1k at Tekamah in 2018 but no significant differences were observed at other environments. Exceptionally at Boone, although a significant cultivar effect was detected, it was not attributed to any of the two forms of genetic resistance to PSRR (Table 2.3 and Table 2.8). Seed treatment increased average yields by 231 kg ha^{-1} in Boone. No significant cultivar-seed treatment interaction was detected in this study. Accounting for interaction factors, seed treatment effectiveness seemed to be more dependent on the environment than with the examined PSRR genetic resistance from commercial soybean lines.

Correlations between disease components. The association between PSRR disease components varied across environments. Moderate Spearman's rank correlation coefficients (ρ) were observed between nPSR and yield (ρ = -0.50, n = 60) at Tekamah in 2018, but not at all in Mead (ρ = -0.01, n = 52) or Bruno (ρ = -0.02, n = 58) (Table 2.9). While early-season CC had a weak relationship (data not shown), mid-season CC was moderately associated with nPSR in Tekamah (ρ = -0.45, n = 56) and Arizona (ρ = -0.32, n = 64) in 2018. Correlation between CC and grain yield was always positive and significant

($P \leq 0.005$) and ranged from 0.32 to 0.82. In most instances, CC seemed to be equally or more closely associated to yield than population density estimated at maturity (Table 2.9). Correlation between yield and final plant population densities ranged from 0.10 to 0.79 across environments (Table 2.9). The number of observations (*n*) to correlate yield to plant population were 71 and 59 at Tekamah in 2017 and 2018, respectively, 52 at Mead, 58 at Bruno, and 55 at Boone.

2.4. Discussion

The present investigation examined the integration of genetic resistance and seed treatment in an effort to improve PSRR management in poorly drained, *Phytophthora sojae* infested areas in Nebraska and Iowa. The benefit of commercial seed treatment formulation with ethaboxam and metalaxyl was variable across locations, despite PSRR field history. Genetic resistance (*Rps* and tolerance) was most valuable in environments where the seed treatment effect co-existed. However, the combination of these management strategies was non-additive, indicating that in high disease pressure scenarios, all cultivars benefited from seed treatment adoption.

Precipitation pattern varied greatly across locations but in general, soil temperatures were relatively warm (>20°C) at planting during mid-May and early-June. In 2017, only 38.1 mm of precipitation was recorded during soybean emergence, which did not favor disease development (Table 2.10). In contrast, more precipitation ranging from 57.4 to 223.3 mm during the 15 days after planting were favorable for disease epidemics across locations in 2018. Under such conducive conditions, seed treatment increased yields by 231 to 331.6 kg ha^{-1} on average. Based on quantitative synthesis of data from integrated disease management trials, similar to those established in this study, Dorrance et al. (2009)

observed yield increases in the order of 215.0 to 416.6 kg ha^{-1} in Ohio and an average increase of 289.0 kg ha^{-1} in South Dakota from the addition of mefenoxam and metalaxyl as seed treatment in *Phytophthora*-infested soils. Overall, our results corroborate with findings by Dorrance et al. (2012) and Scott (2018) for the use of ethaboxam and metalaxyl to manage seedling diseases in PSRR endemic areas.

These results also support that in addition to chemical control, cultivar selection is an effective management tool for PSRR control (Anderson and Buzzell 1982; Dorrance et al. 2003; Guy et al. 1989; Tooley and Grau 1984). Notably, company-supplied PSRR tolerance scores were coherent with the level of disease suppression observed in the field, with MR cultivars having superior plant populations than MS cultivars from emergence to final stand assessments. In scenarios predisposed to damping-off and PSRR development, MR cultivars averaged around 538.9 to 747.5 kg ha^{-1} more than MS cultivars and no yield penalty was associated with the selection of moderately higher resistance in environments with lower disease pressure. These findings substantiate Dorrance et al. (2003) that showed an additive yield effect of 669 kg^{-1} through the use of MR compared to MS cultivars, both with *Rps*1k resistance, under severe PSRR outbreaks in Ohio. Alternatively, results are not supportive of the hypothesis that *Rps* genes differ substantially in terms of field efficacy, particularly when a comprehensive characterization of in-field *Phytophthora sojae* virulence composition is lacking, as is the case here. It is worthy to note though that at Tekamah in 2018, namely where early-season damping-off caused by oomycetes was the highest, comparable yield differences existed for *Rps*1c over *Rps*1k genotypes, even though such advantage was not accompanied by significant differences in stand or aboveground plant development. For *Rps* resistance examined in this study, little

disagreement between company-supplied and publicly evaluated resistance has been reported (Slaminko et al. 2010), suggesting that other factors, perhaps agronomic adaptability of genotypes, may have influenced this response. It also could be speculated that *P. sansomeana*, which is considered race non-specific (Reeser et al. 1991) and occurs in Nebraska and Iowa (Rojas-Flechas et al. 2017), was active in that particular field and affected *Rps*1k and *Rps*1c cultivars equally. This may be a reasonable assumption given that MR cultivars outperformed MS cultivars for nearly all parameters evaluated in that environment. Considering that quantitative disease resistance is polygenic (Glover and Scott 1998; Schneider et al. 2016) and coordinates the expression of physical barriers in the plant (Thomas et al. 2007), it may be worth examining the effects of PSRR tolerance on *P. sansomeana* infection and colonization rate, as to date, little is known about the host resistance mechanisms to this pathogen (Phibbs et al. 2014).

Acknowledging the numerous sources of variation that occur under natural conditions, including inoculum density (Miller et al. 1997), diversity (Robertson et al. 2009; Stewart et al. 2016), and environmental conditions (Dorrance et al. 2009), results from this study were unconvincing for the efficacy of seed treatment at reducing the incidence of *P. sojae* stem lesions solely. The relatively low frequency of disease-positive plots and low incidence of mature plants with stem lesions generated poor estimates for hypothesis testing, despite attempts to fit the count data with zero-inflated generalized linear mixed models using Poisson or negative binomial distributions (Madden et al. 2017; Stroup 2015), and thus, results are not presented here. It is possible that the number of PSRR stem lesions was low in part due to the superior levels of tolerance, even for moderately susceptible lines, found in commercial soybean cultivars. By contrast, studies

evaluating treatment efficacy on the basis of the number of PSRR symptomatic plants employed partially to highly susceptible materials (Dorrance et al. 2003). Other limitations encountered during the course of the study were an off-target growth regulator herbicide movement shortly prior to reproductive stages in Tekamah in 2017 and hail damage during a vegetative stage in Boone, IA in 2018. In Bruno, in addition to soil crusting that limited uniform emergence, stem blight caused by *Diaporthe* spp. was observed causing premature plant death for some genotypes, but disease incidence at the subplot level was low (<5%) and likely had minimum influence on averaged cultivar responses. The activity of bean leaf beetle (*Cerotoma* spp.) and other secondary pests including soybean orange gall midge (*Resseliella* spp.), which damage may resemble PSRR wilting/stem discoloration symptoms, were not observed in the study.

Yield losses resulting from PSRR damage are not exclusively related to damping-off and premature plant death (Wilcox and St. Martin 1998). Results from this study indicate that canopy coverage is a valid criterion to determine plant health status and constitute an important yield component for late-planted soybeans. Greater canopy development influences the plant's ability to intercept light and produce biomass (Board and Harville 1996; Purcell 2000), adequate transpiration rates (Monteith 1977), suppress weed emergence (Bussan et al. 1997), and counterbalance for plant production under suboptimal population densities (Gaspar and Conley 2015). This study confirms enhanced soybean canopy development upon oomycide use in *Phytophthora* spp. infested soils (Rehm and Stienstra 1993; Ryley et al. 1989) and present an innovative, standardized protocol to estimate seedling development using an open-source smartphone application, which potentially could replace traditional vigor ratings performed in field trials. Overall,

the association between canopy coverage and yield seemed to be slightly more robust in environments PSRR occurred than the opposite. There was also a noticeable improvement in the strength of the relationship between the number of *P. sojae* stem lesions and canopy coverage at assessments performed during more advanced growth stages (V6-R2) than earlier in the season (VC-V1), likely because PSRR onset (wilting/stem lesions) usually manifests after the development of trifoliate leaves (V5 and through reproductive stages), as noted by Dorrance et al. (2003). Variations of the remote sensing techniques have shown applicability in the study of root stress associated with biotic disorders in several crops (Reynolds et al. 2012; Steddom et al. 2003), including those caused by *Phytophthora* in cranberry (Pozdnyakova et al. 2002) and avocado root rot (Salgadoe et al. 2018), as well as other soybean diseases (Wang et al. 2004; Yang et al. 2016). Here, proximal remote sensing was well-fitted for quantifying architectural changes in soybean canopy coverage associated with PSRR occurrence most likely because moderately resistant cultivars do not always develop stem lesions but may still exhibit permanent aboveground stunting as a result of *P. sojae* infection (Meyer and Sinclair 1972; Schmitthenner 1985). In addition, despite the confounding lack of seed treatment effect, a significant increase in canopy coverage were observed for *Rps*1k compared to *Rps*1c cultivars at Mead. Considering that plant architecture is highly influenced by environmental and cultivar-specific factors (Tucker et al. 1978; Wells et al. 1982), we hypothesize that factors other than disease occurrence may have influenced that response.

This study documents the usefulness of cultivar selection using commercial soybean lines and emphasizes the importance of adopting effective seed treatments as part of an integrated PSRR management program in Nebraska and Iowa. Such information may

be valuable for producers and crop consultants wanting to develop an effective PSRR management program in the U.S. North Central region. Genetic resistance provided an overall better yield advantage than using seed treatment alone; however, seed treatment was more consistent across environments, possibly because of the broad-spectrum activity of active ingredients in the seed treatment commercial formulation. At this point in time, seed treatment combining metalaxyl + ethaboxam + clothianidin + ipconazole is highly effective against soilborne seedling diseases of soybeans. The selection of MR soybean cultivars carrying either *Rps*1k or *Rps*1c should be considered in PSRR endemic areas. Although not evaluated in this study, producers may also find beneficial to employ cultivars with *Rps*3a resistance and its pyramided forms (e.g. *Rps*3a+1c, *Rps*3a+1k), given its superior efficacy against *P. sojae* in Nebraska and Iowa (Dorrance et al. 2016; Schimelfenig et al. 2005; Yang et al. 1996). Cultural practices that encourage soil drainage are also recommended to reduce disease severity (Gray and Pope 1986) and increase the durability of genetic resistance genes and oomycide seed treatments.

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Table 2. 1. Description of experimental sites and activities performed[x] in Nebraska and Iowa in 2017 and 2018.

		Soil parameters							Execution date							
			Sand	Silt	Clay	O.M.				CC[z]		Plant population				Harvest
Year	Environment	Type[y]	(%)			g kg^{-1}	pH	Tillage	Planting	V1-V2	V6-R2	VE-VC	V1-V2	V6-R2	R8	
2017	Tekamah, NE	Onawa silty clay	17	41	42	1.3	7.9	No-till	2 Jun	-	5 Jul	13 Jun	21 Jun	5 Jul	28 Oct	6 Nov
2018	Tekamah, NE	Luton silty clay	17	16	67	5.4	6.2	Disked	18 May	5 Jun	6 Jul	31 May	5 Jun	6 Jul	12 Oct	1 Nov
	Arizona, NE	Haynie silt loam	19	36	46	3.4	7.6	No-till	18 May	5 Jun	9 Jul	31 May	5 Jun	9 Jul	-	-
	Mead, NE	Filbert silt loam	17	48	35	4.7	6.8	No-till	6 Jun	29 Jun	16 Jul	13 Jun	29 Jun	16 Jul	19 Oct	29 Oct
	Bruno, NE	Zook silty clay loam	14	53	33	3.2	6.8	No-till	6 Jun	29 Jun	16 Jul	-	29 Jun	16 Jul	22 Oct	29 Oct
	Boone, IA	Clarion Loam	45	34	21	3.5	6.2	Disked	5 Jun	-	-	12 Jun	22 Jun	6 Jul	22 Oct	31 Oct

[x] "-" indicates assessments were not performed. VE-VC (emergence to unifoliate), V1-V2 (first to second trifoliate), V6-R2 (sixth trifoliate to full bloom), and R8 (full maturity) growth stages according to Fehr et al. (1971).
[y] Soil data was obtained from Web Soil Survey of USDA Natural Resources Conservation Service (https://websoilsurvey.sc.egov.usda.gov).
[z] CC: canopy coverage.

Table 2. 2. Description of soybean cultivars evaluated in Nebraska and Iowa in 2017 and 2018.

Year	Environment	Soybean cultivars[z]
2017	Tekamah, NE	AG3432 (*Rps*1c, 7), AG3034 (*Rps*1c, 5), H3230NR (*Rps*1k, 5), H2913NR (*Rps*1k, 3), C3070R2 (*Rps*1k, 9), C3171R2 (*Rps*1k, 7), C3010RX (*rps*, 8), C2890R2 (*Rps*1c, 9), C3026RX (*Rps*1c, 8).
2018	Tekamah, NE Arizona, NE Bruno, NE Mead, NE	AG28x7 (*Rps*1c, 6), AG27x8 (*Rps*1c, 5), H2862NX (*Rps*1k, 5), H2512NX (*Rps*1k, 4), NK3195X (*Rps*1c, 3), NK2788X (*Rps*1c, 4), C2888RX (*Rps*1c, 8), C3140RX (*Rps*1c, 7).
	Boone, IA	AG28x7 (*Rps*1c, 6), H2862NX (*Rps*1k, 5), H2512NX (*Rps*1k, 4), NK3195X (*Rps*1c, 3), NK2788X (*Rps*1c, 4), C2888RX (*Rps*1c, 8), C3140RX (*Rps*1c, 7).

[z] Cultivars and Phytophthora stem and root rot resistance. Resistance gene and tolerance (in parentheses) were provided by the respective companies: Asgrow (AG) and Golden Harvest (NK) on a 1-to-9 scale, where 1 = most resistant and 9= most susceptible; and Hoegemeyer (H) and LG seeds (C) on a 1-to-9 scale, where 9= most resistant and 1= most susceptible.

Table 2. 3. Probability values[x] from analysis of variance using combined data by year for plant population, arcsine square root canopy coverage (arcCC), and yield in Nebraska and Iowa.

State	Year	Sources[z]	Plant population[y]				arcCC[y]		Yield
			VE-VC	V1-V2	V6-R2	R8	V1-V2	V6-R2	
Nebraska	2017	Cultivars (C)	0.0861	**0.0072**	**0.0044**	**0.0002**	-	**0.0166**	0.0656
		Seed treatment (ST)	0.6613	0.5065	**0.0132**	0.3846	-	0.7682	0.5817
		C x ST	0.0928	0.1321	0.0716	0.6641	-	0.1839	0.7060
Nebraska	2018	Cultivars (C)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
		Seed treatment (ST)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
		Environment (E)	**0.0130**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0004**	**0.0020**	**0.0043**
		C x ST	**0.0130**	**<0.0001**	**0.0216**	0.4848	0.3520	0.7577	0.1208
		C x E	**0.0005**	**0.0153**	**0.0009**	**0.0032**	**<0.0001**	**0.0022**	**<0.0001**
		ST x E	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.5650	0.1468	0.3733
		C x E x ST	**0.0084**	**0.0012**	0.1200	0.8240	0.6707	0.7001	0.5790
Iowa	2018	Cultivars (C)	0.5720	0.4253	0.3016	0.5719	-	-	**0.0067**
		Seed treatment (ST)	**0.0099**	**0.0257**	**0.0050**	**0.0026**	-	-	**0.0010**
		C x ST	0.6059	0.7327	0.4581	0.7505	-	-	0.2352

[x] Bold indicates statistical significance ($P \leq 0.05$) and "-" indicates assessment was not performed.
[y] VE-VC (emergence to unifoliate), V1-V2 (first to second trifoliate), V6-R2 (sixth trifoliate to full bloom), and R8 (full maturity) growth stages to Fehr et al. (1971).
[z] Cultivars: AG3432, AG3034, H3230NR, H2913NR, C3070R2, C3171R2, C3010RX, C2890R2, C3026RX in Nebraska in 2017, and AG28x7, AG27x8, H2862NX, H2512NX, NK3195X, NK2788X, C2888RX, C3140RX in Nebraska in 2018. In Iowa, soybean cultivars were the same as in Nebraska in 2018, with the exception of AG27x8 that was not planted. Seed treatment: clothianidin, ethaboxam, ipconazole, and metalaxyl was applied at rate of 50 + 7.5 + 2.5 + 2 g a.i. 100 kg^{-1} seed. Environments: Tekamah, Arizona, Mead, and Bruno in Nebraska in 2018.

Table 2. 4. Least-square means and probabilities values[x] of seed treatment and cultivar resistance to *Phytophthora sojae* on soybean population density estimated at VE-VC growth stages at environments in Nebraska and Iowa.

	VE-VC population (plants ha^{-1})					
	2017	**2018**				
	Tekamah-NE	**Tekamah-NE**	**Arizona-NE**	**Mead-NE**	**Bruno-NE**	**Boone-IA**
Seed treatment[y] (ST)						
Treated	217,311	244,851	183,255	172,294	-	114,481
Untreated control	214,739	191,109	152,510	181,639	-	102,795
Diff. (%)	1.2	28.1	20.2	-5.1		11.4
P>F	0.6613	**<0.0001**	**<0.0001**	0.1620		**0.0099**
Cultivars[z] (C)						
Tolerance						
MR	214,504	228,823	170,675	171,481	-	111,495
MS	208,886	206,138	165,091	182,452	-	101,050
Diff. (%)	2.7	11.0	3.4	-6.0		10.3
P>F	0.7375	0.0506	0.4295	0.1436		0.2930
Rps resistance						
*Rps*1c	217,565	214,445	169,979	173,753	-	110,868
*Rps*1k	212,519	226,557	161,592	186,607	-	103,064
Diff. (%)	2.4	-5.3	5.2	6.9		7.6
P>F	0.7375	0.2033	0.4087	0.1436		0.2930
Tolerance - *Rps*						
MR - *Rps*1c	217,565	225,998	176,617	163,968	-	116,250
MS - *Rps*1c	217,565	202,912	163,342	183,538	-	104,006
Diff. (%)	0	11.4	8.1	-10.7		11.8
P>F	>0.9999	0.0556	0.4087	0.0765		0.2930
MR - *Rps*1k	204,783	237,298	152,847	194,019	-	102,795
MS - *Rps*1k	220,256	215,816	170,338	179,195	-	113,828
Diff. (%)	-7.0	10.0	-10.3	8.3		-9.7
P>F	0.7375	0.2033	0.4087	0.2491		0.2930
C x ST						
MR x Treated	213,232	249,130	182,717	170,719	-	118,223
MR x Untreated	214,201	208,515	158,632	172,243	-	104,768
Diff. (%)	-0.5	19.5	15.2	-0.9		12.8
P>F	0.9019	**0.0025**	**0.0037**	0.8699		0.0593
MS x Treated	177,927	240,573	183,793	173,869	-	113,329
MS x Untreated	172,329	171,703	146,388	191,035	-	97,772
Diff. (%)	3.2	40.1	25.6	-9.0		15.9
P>F	0.8574	**<0.0001**	**<0.0001**	0.1431		0.0593
Mean	216,025	217,198	167,883	177,461	-	108,638

[x] Bold indicates statistical significance ($P \leq 0.05$) and "-" indicates assessment was not performed.
[y] Seed treatment: clothianidin + ethaboxam + ipconazole + metalaxyl applied at 50 + 7.5 + 2.4 + 1.9 g a.i. 100 kg^{-1} seed.
[z] *Rps* genes and tolerance information listed in Table 2.2. MS= moderately susceptible and MR= moderately resistant cultivars to Phytophthora stem and root rot.

Table 2. 5. Least-square means and probabilities values[x] of seed treatment and cultivar resistance to *Phytophthora sojae* on soybean population density estimated at V1-V2 growth stages at environments in Nebraska and Iowa.

	V1-V2 population (plants ha^{-1})					
	2017	2018				
	Tekamah-NE	Tekamah-NE	Arizona-NE	Mead-NE	Bruno-NE	Boone-IA
Seed treatment[y] (ST)						
Treated	246,792	257,035	178,075	157,398	115,173	117,864
Untreated control	243,443	193,999	152,174	158,603	98,853	107,331
Diff. (%)	1.4	32.5	17.0	-0.8	16.5	9.8
P>F	0.5065	**<0.0001**	**<0.0001**	0.8253	**0.0478**	**0.0257**
Cultivars[z] (C)						
Tolerance						
MR	241,548	237,181	166,974	155,837	115,964	116,877
MS	238,258	213,853	163,274	160,164	98,062	106,293
Diff. (%)	1.4	10.9	2.3	-2.7	18.3	10.0
P>F	0.8481	**0.0185**	0.5428	0.7194	0.0735	0.1582
Rps resistance						
*Rps*1c	249,991	223,074	168,230	153,933	107,020	114,581
*Rps*1k	236,671	232,846	155,807	170,204	106,993	107,638
Diff. (%)	5.6	-4.2	8.0	-9.6	0.0	6.4
P>F	0.2903	0.3754	0.1317	0.3277	0.9976	0.2490
Tolerance - *Rps*						
MR - *Rps*1c	248,780	236,537	174,195	145,621	116,958	120,824
MS - *Rps*1c	251,202	209,611	162,265	162,244	97,082	106,293
Diff. (%)	-1.0	12.8	7.4	-10.2	20.5	13.7
P>F	0.8481	**0.0185**	0.1317	0.3277	0.0735	0.1582
MR - *Rps*1k	222,677	239,112	145,312	186,484	112,983	105,486
MS - *Rps*1k	250,664	226,579	166,302	153,923	101,002	118,671
Diff. (%)	-11.2	5.5	-12.6	21.2	11.9	-11.1
P>F	0.1393	0.4116	0.1317	0.3277	0.6060	0.2490
C x ST						
MR x Treated	243,479	260,178	175,182	157,374	127,013	124,681
MR x Untreated	237,451	214,184	158,767	154,300	104,915	109,074
Diff. (%)	2.5	21.5	10.3	2.0	21.1	14.3
P>F	0.7487	**0.0006**	**0.0203**	0.6983	0.1129	0.0600
MS x Treated	200,746	253,893	180,967	157,422	103,333	109,971
MS x Untreated	200,746	173,814	145,581	162,906	92,792	102,615
Diff. (%)	0	46.1	24.3	-3.4	11.4	7.2
P>F	>0.9999	**<0.0001**	**<0.0001**	0.6983	0.3443	0.2851
Mean	245,117	224,477	165,124	158,176	107,211	112,598

[x] Bold indicates statistical significance ($P \leq 0.05$).
[y] Seed treatment: clothianidin + ethaboxam + ipconazole + metalaxyl applied at 50 + 7.5 + 2.4 + 1.9 g a.i. 100 kg^{-1} seed.
[z] *Rps* genes and tolerance information listed in Table 2.2. MS= moderately susceptible and MR= moderately resistant cultivars to Phytophthora stem and root rot.

Table 2. 6. Least-square means and probabilities values[x] of seed treatment and cultivar resistance to *Phytophthora sojae* on soybean population density estimated at V6-R2 growth stages at environments in Nebraska and Iowa.

	V6-R2 population (plants ha^{-1})					
	2017	2018				
	Tekamah-NE	Tekamah-NE	Arizona-NE	Mead-NE	Bruno-NE	Boone-IA
Seed treatment[y] (ST)						
Treated	252,473	212,773	182,313	146,504	116,250	120,940
Untreated control	240,034	163,536	155,403	145,606	90,820	108,100
Diff. (%)	5.2	30.1	17.3	0.6	28.0	11.9
P>F	**0.0133**	**<0.0001**	**0.0001**	0.8408	**0.0001**	**0.0050**
Cultivars[z] (C)						
Tolerance						
MR	246,694	197,132	176,796	141,366	115,173	119,120
MS	239,093	179,177	160,920	150,743	91,896	108,446
Diff. (%)	3.3	10.0	9.9	-6.2	25.3	9.8
P>F	0.2593	0.0530	0.2438	0.1443	**0.0007**	0.1441
Rps resistance						
*Rps*1c	250,260	186,145	172,850	143,163	104,738	117,487
*Rps*1k	240,573	194,183	156,883	154,732	99,924	107,100
Diff. (%)	4.0	-4.1	10.2	-7.5	4.8	9.7
P>F	0.2593	0.4612	0.2438	0.1443	0.4469	0.1441
Tolerance - *Rps*						
MR - *Rps*1c	255,507	196,894	182,717	135,028	117,924	125,264
MS - *Rps*1c	245,013	175,396	162,983	151,297	91,552	108,984
Diff. (%)	4.3	12.3	12.1	-10.8	28.8	14.9
P>F	0.2593	0.0530	0.2438	0.0881	**0.0007**	0.1441
MR - *Rps*1k	230,212	197,847	159,036	160,381	106,921	107,908
MS - *Rps*1k	250,933	190,520	154,730	149,082	92,928	118,941
Diff. (%)	-8.3	3.8	2.8	7.6	15.1	-9.3
P>F	0.1269	0.6086	0.8322	0.3095	0.2795	0.3021
C x ST						
MR x Treated	251,014	217,804	187,830	142,313	127,552	128,269
MR x Untreated	238,743	176,460	165,763	140,420	102,795	109,971
Diff. (%)	5.1	23.4	13.3	1.3	24.1	16.6
P>F	0.0836	**0.0009**	**0.0131**	0.9876	**0.0035**	**0.0163**
MS x Treated	203,437	207,742	176,796	150,695	104,947	112,662
MS x Untreated	193,319	150,612	145,043	150,792	78,845	104,230
Diff. (%)	5.2	37.9	21.9	-0.1	33.1	8.1
P>F	0.0836	**<0.0001**	**0.0015**	0.9876	**0.0035**	0.1926
Mean	246,253	187,750	168,858	146,348	103,253	114,520

[x] Bold indicates statistical significance ($P \leq 0.05$).
[y] Seed treatment: clothianidin + ethaboxam + ipconazole + metalaxyl applied at 50 + 7.5 + 2.4 + 1.9 g a.i. 100 kg^{-1} seed.
[z] *Rps* genes and tolerance information listed in Table 2.2. MS= moderately susceptible and MR= moderately resistant cultivars to Phytophthora stem and root rot.

Table 2. 7. Least-square means and probabilities values[x] of seed treatment and cultivar resistance to *Phytophthora sojae* on soybean population density estimated at R8 growth stage at environments in Nebraska and Iowa.

	R8 population (plants ha^{-1})					
	2017	**2018**				
	Tekamah-NE	**Tekamah-NE**	**Arizona-NE**	**Mead-NE**	**Bruno-NE**	**Boone-IA**
Seed treatment[y] (ST)						
Treated	214,380	215,991	-	148,210	125,601	116,788
Untreated control	210,493	162,023	-	143,554	95,238	102,948
Diff. (%)	1.8	33.3		3.2	31.9	13.4
P>F	0.3846	**<0.0001**		0.5092	**0.0002**	**0.0026**
Cultivars[z] (C)						
Tolerance						
MR	216,892	202,035	-	140,987	117,505	112,482
MS	199,771	175,979	-	150,777	103,333	105,575
Diff. (%)	8.6	14.8		-6.5	13.7	6.5
P>F	**0.0158**	0.0648		0.4263	0.1928	0.3497
Rps resistance						
*Rps*1c	207,877	192,673	-	144,599	110,509	112,536
*Rps*1k	212,048	178,007	-	149,733	110,150	103,198
Diff. (%)	-2.0	8.2		-3.4	0.3	9.0
P>F	0.4740	0.2117		0.6389	0.9641	0.2883
Tolerance - *Rps*						
MR - *Rps*1c	223,216	205,065	-	133,087	116,549	117,595
MS - *Rps*1c	192,539	180,281	-	156,110	104,469	106,293
Diff. (%)	15.9	13.7		-14.7	11.6	10.6
P>F	**0.0036**	0.0878		0.2180	0.2010	0.2883
MR - *Rps*1k	202,630	192,942	-	164,687	120,376	106,562
MS - *Rps*1k	221,467	163,072	-	134,779	99,924	114,904
Diff. (%)	-8.5	18.3		22.2	20.5	7.3
P>F	**0.0387**	0.1903		0.2526	0.2010	0.4302
Mean	212,437	188,883	-	145,568	110,134	109.869

[x] Bold indicates statistical significance ($P \leq 0.05$) and "-" indicates assessment was not performed.
[y] Seed treatment: clothianidin + ethaboxam + ipconazole + metalaxyl applied at 50 + 7.5 + 2.4 + 1.9 g a.i. 100 kg^{-1} seed.
[z] *Rps* genes and tolerance information listed in Table 2.2. MS= moderately susceptible and MR= moderately resistant cultivars to Phytophthora stem and root rot.

Table 2. 8. Least-square means and probabilities values[x] of seed treatment and cultivar resistance to *Phytophthora sojae* on soybean yield at environments in Nebraska and Iowa.

	Yield (kg ha^{-1})					
	2017	**2018**				
	Tekamah-NE	**Tekamah-NE**	**Arizona-NE**	**Mead-NE**	**Bruno-NE**	**Boone-IA**
Seed treatment[y] (ST)						
Treated	5,051.2	3,475.7	-	3,581.3	2,925.9	3,191.0
Untreated control	5,011.9	3,217.8	-	3,418.9	2,594.2	2,960.0
Difference	39.3	257.9		162.4	331.6	230.9
P>F	0.5818	**0.0010**		0.1674	**0.0035**	**0.0010**
Cultivars[z] (C)						
Tolerance						
MR	5,030.3	3,720.5	-	3,484.1	3,029.5	3,009.2
MS	5,028.8	2,973.0	-	3,516.2	2,490.6	3,027.0
Diff.	1.5	747.5		-32.1	538.9	-17.7
P>F	0.9845	**0.0001**		0.8411	**0.0001**	0.8674
Rps resistance						
*Rps*1c	5,104.8	3,443.5	-	3,391.1	2,752.3	3,124.8
*Rps*1k	4,971.2	3,056.5	-	3,827.2	2,783.4	2,962.2
Diff.	133.6	387.0		-436.1	-31.0	172.6
P>F	0.3494	**0.0263**		0.1042	0.7940	0.2492
Tolerance - *Rps*						
MR - *Rps*1c	5,107.2	3,819.9	-	3,321.1	3,014.2	3,028.9
MS - *Rps*1c	5,102.5	3,067.1	-	3,461.2	2,490.3	3.073,2
Diff.	4.7	752.8		-140.1	523.9	-44.2
P>F	0.9845	**0.0003**		0.6100	**0.0004**	0.8674
MR - *Rps*1k	4,951.6	3,422.4	-	3,973.0	3,075.4	3,079.2
MS - *Rps*1k	4,990.7	2,690.6	-	3,681.3	2,491.3	3.419.9
Diff.	-39.1	731.8		291.7	584.0	-340.7
P>F	0.9845	**0.0214**		0.6100	**0.0131**	0.2492
Mean	5,031.6	3,346.8	-	3,500.1	2,760.1	3,075.5

[x] Bold indicates statistical significance ($P \leq 0.05$) and "-" indicates assessment was not performed.
[y] Seed treatment: clothianidin + ethaboxam + ipconazole + metalaxyl applied at 50 + 7.5 + 2.4 + 1.9 g a.i. 100 kg^{-1} seed.
[z] *Rps* genes and tolerance information listed in Table 2.2. MS= moderately susceptible and MR= moderately resistant cultivars to Phytophthora stem and root rot.

Table 2. 9. Spearman's rank correlation[x] coefficient (ρ) for the relationship between the number of plants with *Phytophthora sojae* stem lesions (nPSR), mid-season canopy coverage (CC), plant population, and yield across environments in Nebraska and Iowa.

	2017	2018				
Association	**Tekamah, NE**	**Tekamah, NE**	**Arizona, NE**	**Mead, NE**	**Bruno, NE**	**Boone, IA**
nPSR - Yield	*[y]	-0.50 (**<0.0001**)	-[z]	-0.01 (0.9269)	-0.02 (0.8388)	*
nPSR - CC	*	-0.45 (**0.0003**)	-0.32 (**0.0089**)	0.08 (0.5691)	-0.03 (0.7900)	*
Pop. (V6-R2) - CC	0.24 (0.0376)	0.34 (**0.0096**)	0.41 (**0.0006**)	0.50 (**0.0001**)	0.69 (**<0.0001**)	-
Yield - CC	0.32 (**0.0054**)	0.82 (**<0.0001**)	-	0.69 (**<0.0001**)	0.63 (**<0.0001**)	-
Yield - Pop. (R8)	0.23 (0.0514)	0.43 (**0.0005**)	-	0.24 (0.0856)	0.79 (**<0.0001**)	0.10 (0.4243)

[x] *P*-values in parenthesis. Bold indicates statistical significance ($P \leq 0.01$).
[y] Phytophthora stem and root rot not detected.
[z] At least one of the assessments was not performed.

Table 2. 10. Soil temperature at planting and accumulated precipitation at the experimental sites in Nebraska and Iowa.

				DAP precip.[z] (mm)					Monthly precip. (mm)						15-year average monthly precip. (mm)					
Year	**City, State**	**Tm[x]**	**Irrig.[y]**	**0-15**	**16-30**	**31-45**	**46-60**	**61-75**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**
2017	Tekamah, NE	23.7	Yes	38.1	19.3	36.8	52.3	103.4	125.9	54.8	91.7	146.5	82.3	81.0	94.6	112.3	66.8	96.6	78.9	46.4
2018	Tekamah, NE	20.3	Yes	57.4	23.3	97.0	32.2	37.3	137.9	114.8	100.8	154.1	149.1	55.6	96.3	113.3	70.3	94.8	83.5	47.1
	Arizona, NE	20.3	Yes	73.4	41.6	202.7	57.6	33.7	223.0	221.5	114.7	159.7	168.4	94.4	134.5	152.6	103.0	127.3	116.1	70.3
	Mead, NE	26.1	Yes	67.0	109.2	19.5	61.7	90.4	76.4	158.7	91.4	141.4	194.5	61.4	132.1	128.8	70.2	109.0	85.6	59.5
	Bruno, NE	26.1	No	74.9	123.4	13.9	20.8	91.7	45.7	176.0	76.7	113.8	115.5	67.3	122.6	125.2	66.1	117.4	82.7	62.8
	Boone, IA	24.7	No	223.3	132.1	4.3	43.1	78.7	101.1	281.9	106.9	213.6	171.4	123.2	127.9	122.9	104.9	134.4	93.1	67.3

[x] Tm: soil temperature (°C) during planting at a depth of 10-cm.
[y] Irrig.: irrigation system.
[z] Accumulated precipitation based on days after planting (DAP), including irrigation.

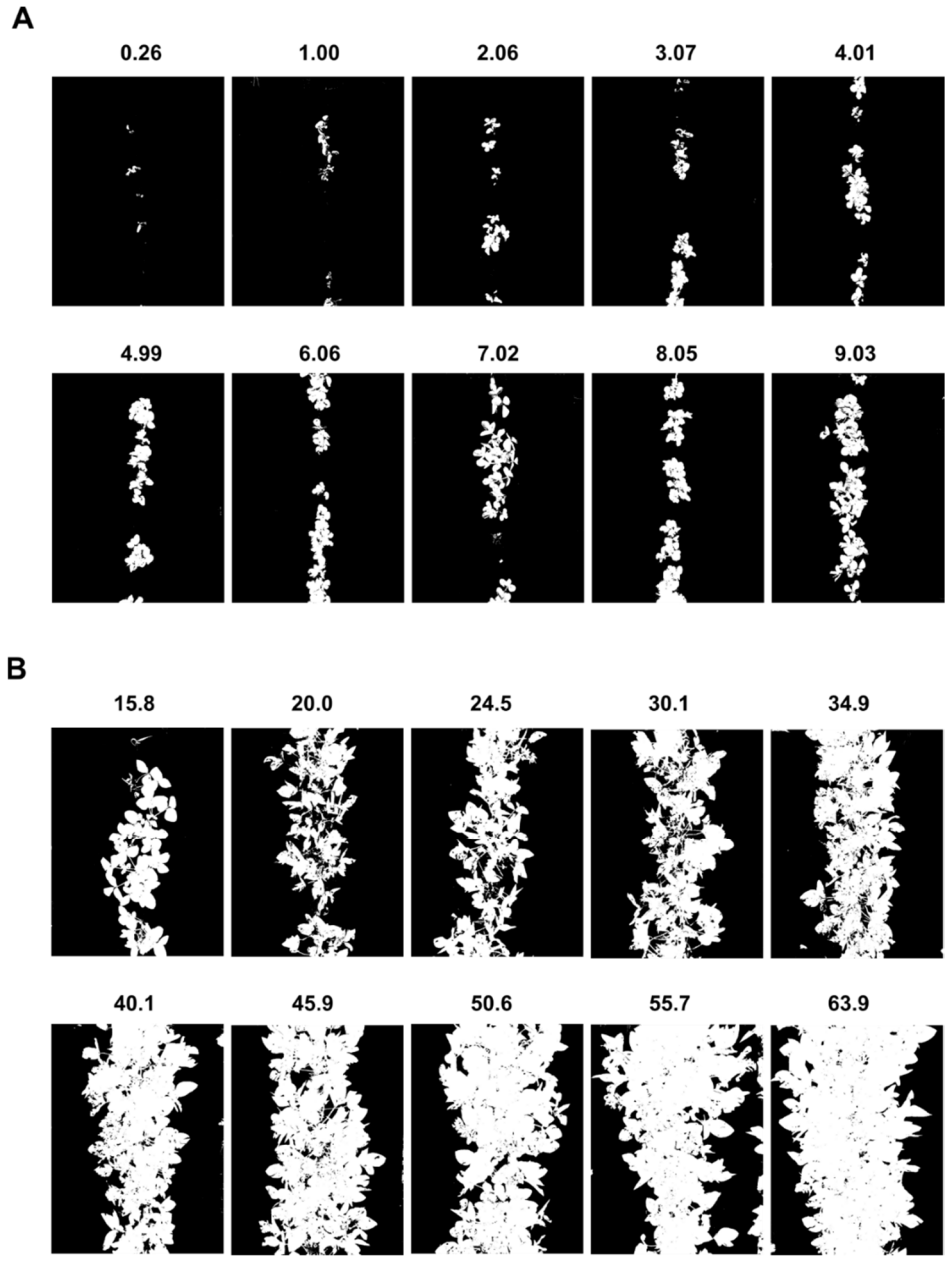

Figure 2. 1. Representative soybean canopy coverage values (%) estimated with *Canopeo* smartphone application at **A,** V1-V2 and **B,** V6-R2 growth stages in Nebraska in 2018.

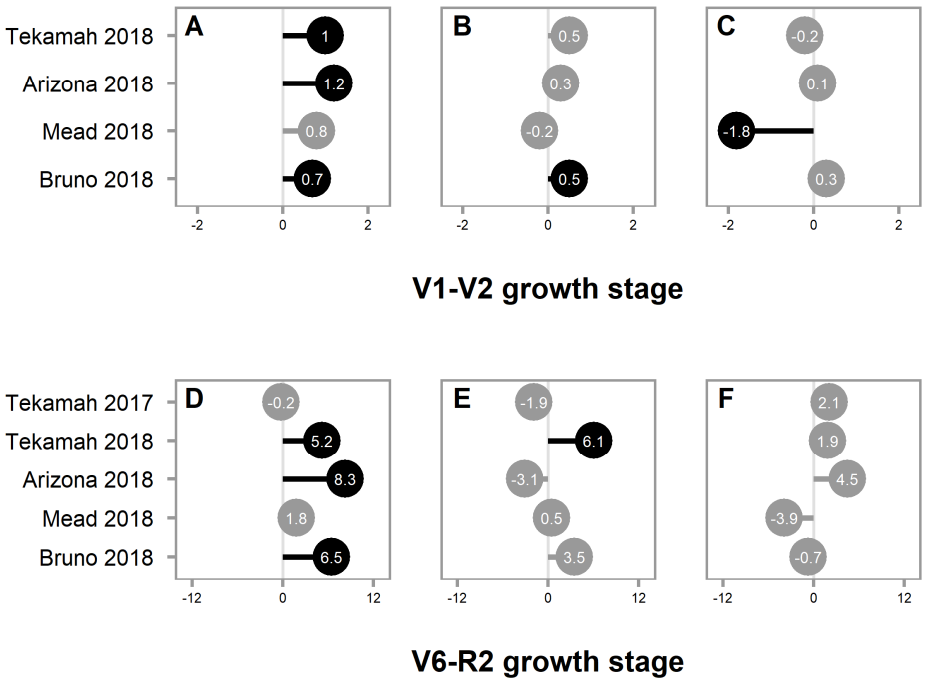

Figure 2. 2. Mean differences between seed treatment and cultivar resistance to *Phytophthora sojae* on early- and mid-season soybean canopy coverage (CC) in Nebraska. Positive significant differences ($P \leq 0.05$, black circles) indicate increasing CC associated with **A,** and **D,** seed treatment *vs.* untreated control; **B,** and **E,** moderately resistant *vs.* moderately susceptible cultivars; and **C,** and **F,** *Rps*1k *vs. Rps*1c cultivars. Contrasts were performed on arcsine square root transformed data and mean differences were calculated on back-transformed data.

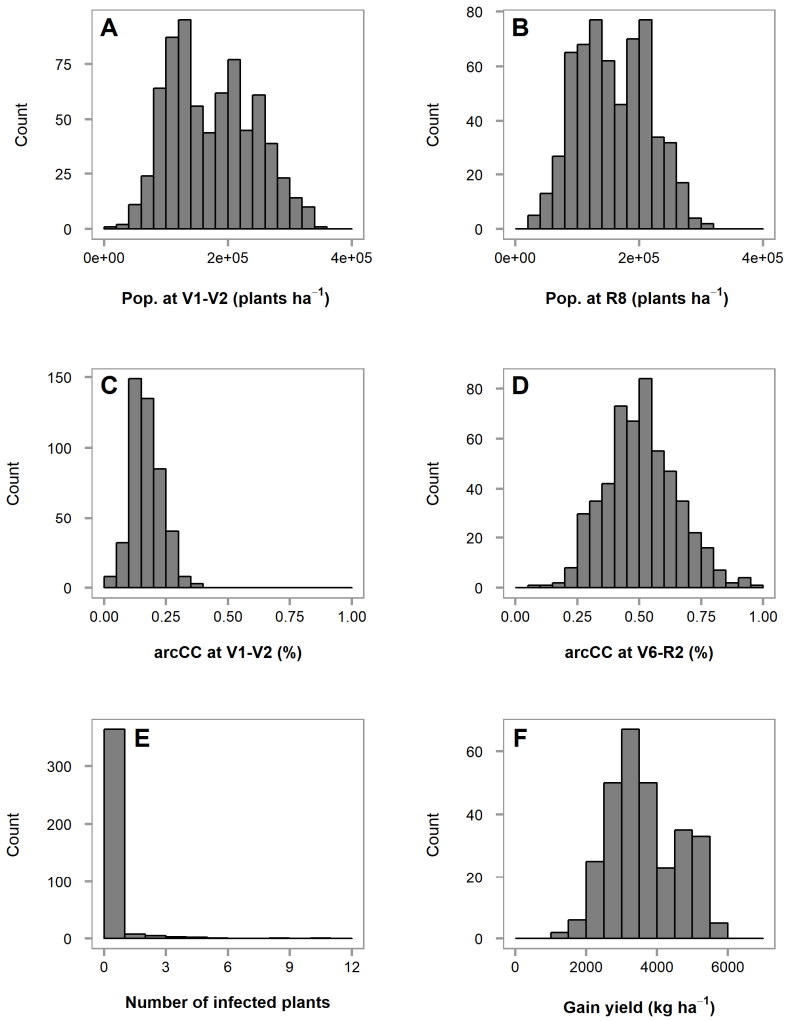

Figure S. 2. 1. Histograms for the distribution of responses collected in the study. **A** and **B,** soybean population densities at V1-V2 and R8 growth stages; **C** and **D,** arcsine square root transformed canopy coverage (arcCC) at V1-V2 and V6-R2 growth stages; **E,** incidence of plants with *Phytophthora sojae* stem lesions; and **F,** soybean yield.

CHAPTER 3. Effect of soil-applied pre-emergence herbicides on severity of soybean seedling diseases in alluvial naturally-infested soils

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Abstract

Six field studies were conducted during 2017 and 2018 in Nebraska to investigate potential interactions between pre-emergence (PRE) herbicides on soybean (*Glycine max* (L.) Merr.) root rot severity, herbicide injury, plant height and population, and yield in fields with history of stand establishment problems. Chlorimuron-ethyl, metribuzin, saflufenacil, sulfentrazone, and flumioxazin did not impact root rot disease severity index (DSI) compared to non-treated control ($P \leq 0.05$). At one of the environments, the application of PPO-inhibiting herbicides seemed to have a detrimental effect on root health status when contrasted to non-PPO-inhibiting herbicides. Herbicide injury was minimum during the study and no significant differences between PRE herbicides were detected on plant height, population, or yield; however, significant differences existed across environments. Systematic isolations from symptomatic root material indicated a rich diversity of filamentous organisms from fine-textured, poorly drained agroecosystems. Among isolates, the soilborne genera *Fusarium*, *Pythium*, *Phytophthora*, *Rhizoctonia*, *Trichoderma*, *Alternaria*, *Mortierella* were recovered. Community composition depicting

primary pathogenic genera *Fusarium*, *Phytophthora*, *Pythium,* and *Rhizoctonia* did not occur at random but rather varied across environments ($P<0.0001$) and DSI classes ($P=0.002$). In two of the three habitats, *Phytophthora* species structured approximately 22% of primary pathogenic genera, whereas, *Rhizoctonia* species recovery was low (<5.5%) and sporadic. Results from this study demonstrate the compatibility of single PRE herbicides programs in mid-to-late planted soybeans in fields with seedling disease history.

3.1. Introduction

Soybean seedling diseases are an important yield-limiting factor in soybean (*Glycine max* (L.) Merr.) production (Hartman et al. 2015; Koenning and Wrather 2010). Annual losses due to this malady are estimated at 1.3 MMT in North America (Allen et al. 2017). Symptoms include seed decay, pre- and post-emergence damping-off, and root rot leading to stunted plant development and stand variability. Surveys conducted throughout major soybean-producing regions of U.S indicate a rich composition of filamentous organisms associated with symptomatic seedlings, including *Fusarium* spp., *Pythium* spp., *Phytophthora sojae*, and *Rhizoctonia solani* (Ajayi-Oyetunde and Bradley 2017; Radmer et al. 2017; Rizvi and Yang 1996; Zitnick-Anderson and Nelson Jr. 2015). Edaphic and climatic factors also play a role in disease incidence, with moist soil conditions being favorable to epidemics (Martin and Loper 1999; Schmitthenner 1999). Understanding the combined effects of abiotic and biotic stresses are prerequisite towards effective management of seedling diseases.

Severe weed infestation also results in loss of soybean yield and quality (Hager et al. 2002). Effective weed management practices include the rotation of disparate herbicide

sites of action, crop rotation, tillage, adoption of cover crops, and preventing weed establishment (Norsworthy et al. 2012). Pre-emergence (PRE) and/or post-emergence (POST) herbicides are primary tools for weed management in soybean (Riar et al. 2013). However, with the introduction of glyphosate-tolerant soybean in 1996, management programs combining the application of PRE herbicide with one-or-two POST passes of distinct sites of action herbicides were substituted by burndown and multiple in-season glyphosate applications (Shaner 2014). While the benefits of glyphosate-resistant crops have been extensively reviewed (Dill 2005; Gianessi 2005), intensification on glyphosate use across vast areas has resulted in an increased selection of glyphosate-resistant (GR) weed biotypes (Beres et al. 2018). In Nebraska, GR weeds from Amaranthaceae and Asteraceae families have been reported as a result of continuous use of glyphosate for weed management in GR crops (Chahal et al. 2017; Vieira et al. 2018). The adoption of residual PRE herbicides provide early-season weed control and allow rotation of herbicide sites of action, which may mitigate selection pressure towards GR biotypes while improving the efficacy of POST treatments (Jhala et al. 2017).

Soil-applied PRE herbicides belonging to protoporphyrinogen oxidase inhibitors (PPO, WSSA group 14) and photosynthetic system II (PSII, WSSA group 5) inhibitors have increased across soybean-producing regions of the U.S. in recent years (Owen 2017). For example, while consumption of herbicide trifluralin (WSSA group 3) was stagnated at approximately 0.57 million kg between 2012 and 2017, sulfentrazone, a PPO-inhibiting soybean PRE herbicide, consumption increased from 0.49 million kg to 1.5 million kg (USDA 2012, 2018). Similarly, during the same period, saflufenacil, a PPO inhibitor, and metribuzin, a PS II inhibitor, had their use increased by 130% and 452%, respectively

(USDA 2012, 2018). The increased adoption of these herbicide programs may be related to their superior effectiveness on key GR weeds (Krausz and Young 2003; Sarangi et al. 2017). Sarangi et al. (2017) observed reduced density of GR common waterhemp (*Amaranthus rudis*) from 107 to 13 plants m^{-2} upon application of flumioxazin + chlorimuron at PRE followed by fomesafen + glyphosate at POST when compared to glyphosate at POST alone. Occasionally, however, soil-applied PRE herbicides cause adverse side-effects to sensitive soybean seedlings, including height reduction, leaf burn, desiccation, chlorosis, stand reduction, and yield losses (Miller et al. 2012; Vidrine et al. 1996; Zhaohu et al. 1999). PRE herbicide injury risk increases under cool and prolonged moist conditions (Hager 2014; Poston et al. 2008), which also characterize favorable conditions for some soilborne seedling diseases to thrive (Kirkpatrick et al. 2006; Martin and Loper 1999).

Herbicides exert profound physiological and developmental changes in plants, which may alter plant susceptibility to soilborne pathogens (Altman and Campbell 1977b; Grinstein et al. 1976; Hale et al. 1981). Herbicide phytotoxicity can adversely affect disease-resistance mechanisms and predispose plants to root infection (Keen et al. 1982; Levésque and Rahe 1992). Herbicide-stressed plants liberate more root exudates that change the chemical nature of leaked components and stimulate or inhibit pathogen propagule germination (Brown and Curl 1987; Lai and Semeniuk 1970; Lee and Lockwood 1977). There are studies showing no synergism between PRE herbicides and seedling diseases, particularly in cotton and soybean (Agamalian 1964; Bauske and Kirby 1992; Heydari et al. 2007), but controversial evidence has also been documented in a handful of pathosystems tested under field and controlled conditions (Bowman and

Sinclair 1989; Bradley et al. 2002; Carson et al. 1991; Chandler and Santelmann 1968; El-Khadem et al. 1979; Espinoza et al. 1968; Harikrishnan and Yang 2002; Heydari and Misaghi 1998; Montazeri and Hamdollah-Zadeh 2005; Neubauer and Avizohar-Hershenson 1973; Pankey et al. 2005; Pinckard and Standifer 1966; Wiley and Ross 1974). Particularly with PPO-inhibiting herbicides, it has been shown that flumioxazin and sulfentrazone can alter the recovery of *Pythium arrhenomanes* from sugarcane roots (Daugrois et al. 2005).

Inquiries from soybean growers and crop consultants regarding the potential interactions between seedling diseases and PRE herbicides, primarily commercial formulations containing PPO inhibitors herbicides, have increased over the last years (Adesemoye et al. 2016; Dorrance and Loux 2017; Giesler 2017; Jhala 2017). However, studies providing updated information about the topic are few and lack a comprehensive assessment of the microbial community associated with symptomatic plant root tissue and its influence on herbicide-induced disease susceptibility (Barlow et al. 2018; Kandel et al. 2018). Therefore, the objectives of this research were to (i) evaluate the effect of single active PRE herbicide application on early-season soybean disease development, and (ii) determine the frequency of primary root pathogenic genera (*Fusarium*, *Phytophthora*, *Pythium*, and *Rhizoctonia*) associated to symptomatic soybean roots at each environment for the understanding of potential herbicide interactions.

3.2. Material and methods

Field trials were conducted at two locations near Mead and Lincoln (East Central Nebraska) in 2017 and at four locations near Tekamah and Arizona (Northeast Nebraska), and Mead and Bruno (East Central Nebraska) in 2018. All six locations were previously

planted with corn and were chosen based on the history of oomycete-related soybean seedling diseases. Information regarding the soil characteristics, site GPS coordinates, tillage, data collection, and harvest dates are presented in Table 3.1. Weather data were obtained from public weather service websites (https://hprcc.unl.edu/) and (https://www.ncdc.noaa.gov/cdo-web/), using the nearest automated weather station located within a 10-km radius from trials. Soil temperature at 10-cm depth was obtained from the nearest weather station which provided soil temperature readings.

The experimental units were 5.18-m long by 3.04-m wide plots consisting of four rows planted 0.76-m apart and at a density of 308.881 seeds ha^{-1} and at a depth of 4-cm. In both years, the experimental design was a randomized complete block design with four replications, but the treatment design differed across growing seasons. In 2017, a two-way factorial between two soybean cultivars P28T08R (*Rps*1k) and P22T41R2 (*Rps*1k) and five PRE herbicide treatments was used. In 2018, the same five PRE herbicide treatments were evaluated but only one cultivar AG27x8 (*Rps*1c) was planted at all four locations. Herbicide treatments were applied at the labelled rate and consisted of (i) chlorimuron-ethyl (Classic 25DF, DuPont, Wilmington, DE) at 44 g a.i. ha^{-1}; (ii) metribuzin (Sencor 75 DF, Bayer CropScience, Research Triangle Park, NC) at 560 g a.i. ha^{-1}; (iii) saflufenacil (Sharpen, BASF, Research Triangle Park, NC) at 22 g a.i. ha^{-1}; (iv) sulfentrazone (Spartan 4F, FMC, Philadelphia, PA) at 290 g a.i. ha^{-1}; and (v) flumioxazin (Valor SX, Valent U.S.A., Walnut Creek, CA) at 90 g a.i. ha^{-1}. A non-treated herbicide control was included for comparison. No seed treatment was applied to seeds prior to planting. Soybeans were planted between mid-May to early-June and all herbicide treatments were applied between one to two days after planting (DAP) but prior to soybean emergence.

Herbicide treatments were applied using a handheld CO_2–pressurized backpack sprayer equipped with a five-nozzle boom fitted with XR8002VS flat-fan nozzles (TeeJet Technologies, Spraying Systems Co., Wheaton, IL) and spaced 50 cm apart. The system was calibrated to deliver herbicide treatments at a rate of 140.3 L ha^{-1} at 275 kPa at a constant speed of 6.4 km h^{-1}. Approximately, 1 L of water was rinsed through the sprayer system between herbicide treatment applications. Plots were maintained weed-free with either one or two POST applications of glyphosate (Roundup Power Max, Monsanto, St. Louis, MI) at 1140 g a.i. ha^{-1} + ammonium sulfate (N-Rich, American Plant Food Co., Galena Park, TX) at 2% by weight shortly after planting and between the fourth to sixth-trifoliate stages (V4-V6) (Fehr et al. 1971) over the entire experimental area and by hand-hoeing as needed throughout the season.

Root rot severity index **(DSI)**. Seedling root rot was assessed at the first to second trifoliate (V1-V2; approx. 18 to 21 DAP) stages on six plants randomly collected from the outer non-harvested rows of each experimental unit. Root systems and adhered soil were dug with a shovel, then soaked in water for approximately 20 min and gently washed until soil particles were removed. Water in the bucket was frequently replaced as samples were processed. For evaluation, the individual entire root systems were rated for root rot using a graded rating board on a 0-to-10 scale adapted from Bates et al. (2008), where 0= symptomless; 1= few small reddish to brown lesions, 0.1–0.2 cm in length, at base of hypocotyl or root tips; 2= progressing lesions, discoloration evident but many healthy roots present; 3= taproot intact but increasing color intensity, minor reduction in root mass; 4=10-20% root mass reduction and discoloration and coalescing of localized root lesions; 5= root system discolored with increasingly lesions with 20-40% root mass reduction and

hypocotyl lesions; 6= intensely reddish-brown discoloration and compromised mass reduction affecting roughly ½ of root volume; 7= mass reduction affecting roughly ¾ of root volume, taproot compromised; 8= further damage; and 9= remaining entire root blackened; and 10=dead seedling (Figure 3.1). For statistical analysis, a disease severity index (DSI) was calculated for each experimental unit with the following formula DSI= ∑(*severity rating x plants per rating*)/(*total plants* x 10), similar to Harveson et al. (2005). Additionally, the total fresh root and shoot biomass for the six plants were also recorded by cutting previously rated plants at the cotyledonal node and weighing plant parts. The ratio between root and shoot was calculated by dividing fresh root by shoot weight.

Composition of root-associated organisms. To determine the composition of filamentous organisms associated with symptomatic root tissue, rated soybean roots were brought to the laboratory in coolers and washed with liquid detergent (Dawn, Procter and Gamble) until soil particles were removed, then rinsed thoroughly with tap water. Roots were surface disinfested in a 0.5% sodium hypochlorite solution for approximately 1.5 min, left under tap water at constant flow for approximately 10 min, and air dried in a sterile laminar flow cabinet for 20-30 min. For isolations, one-to-two lateral and taproot fragments of 2-cm long per plant displaying tan to brown, dark to reddish discoloration were excised with a sterile scalpel and placed onto 10-cm diameter Petri dishes containing the following isolating media: (i) water agar at 20 g L^{-1}; (ii) water agar at 20 g L^{-1} + streptomycin (Sigma-Aldrich, St. Louis, MO) at 0.03 g L^{-1}; (iii) corn meal agar (Difco, Sparks, MD) at 20 g L^{-1} + pentachloronitrobenzene (Sigma-Aldrich, St. Louis, MO) at 0.054 g L^{-1} + benomyl (Sigma-Aldrich, St. Louis, MO) at 0.01 g L^{-1} + spiramycin (Fisher Scientific, Pittsburgh, PA) at 0.005 g L^{-1}; and (iv) PBNIC V8 agar with rifampicin (Fisher

Scientific, Pittsburgh, PA) added at 0.01 g L^{-1} (Dorrance et al. 2008). Rifampicin, spiramycin, and hymexazole were added after autoclaving. All roots fragments were processed and plated the same day of collection. For each environment, twelve Petri plates of each media and four pieces of symptomatic root tissue per plate were used, resulting in 192 total possible isolates. Culture plates were incubated for 3 to 12 days at 20ºC, and checked daily for hyphal growth. Single pure isolates were obtained by sub-culturing marginal hyphal growth onto a fresh Petri dish with the same media. Isolates were then transferred to potato dextrose agar (PDA, Difco, Sparks, MD) at 39 g L^{-1} for storage until identification.

Each sub-cultured isolate was examined microscopically and identified to the genus level following Watanabe (2010) soil fungi key. Briefly, isolates of *Fusarium* species were tentatively identified based on cultural appearances, mycelial growth with pale- to dark-violet color on PDA, as well as microscopic characteristics such as the presence of fusiform hyaline, septate, curved macroconidia and microconidia. *Phytophthora* species were identified by the slow growth of dense white mycelium in PBNIC, exhibiting coenocytic, right-branched hyphae, and presence of oospores around 40-50 µm in diameter. *Pythium* spp. were identified based on its reduced growth on media amended with hymexazole, by forming a mycelial rosette pattern growth on PDA with filamentous, coenocytic hyphae. *Rhizoctonia* species were examined for colony color, sclerotia formation, lack of asexual spores, and characteristic homogeneous septate hyphae branching at right angles with a slight constriction at the branch origin.

Plant population. Population density was estimated at emergence to unifoliate (VE-VC; 7 to 13 DAP), first to second trifoliate (V1-V2; 19 to 24 DAP) growth stages, and prior to

harvest (R8). Plant stand was estimated by counting the number of emerged plants in a 3.05-m section of each two center rows, averaged, and converted to per hectare basis.

Seedling vigor and plant height. Aboveground plant vigor was rated at VE-VC growth stages on a 0-to-10 scale, where 0= seedling death and 10= no visual injury. Vigor was estimated on the basis of combined symptomology including stunting, necrotic areas on cotyledon and hypocotyl, deformation of cotyledons, and yellowing of unifoliate leaves. In addition, plant heights were also collected during at V1-V2 and sixth trifoliate to full bloom (V6-R2; 31 to 55 DAP) growth stages on six random plants within each experimental unit.

Yield. At maturity, experimental units were trimmed to 4.5-m in length and soybeans were harvested from the center two rows using a plot combine Almaco SPC20 (Almaco, Nevada, IA) equipped with grain gauge and handheld computer Allegro MX (Juniper Systems, Logan, UT) for data collection. Grain yield was adjusted to 13% moisture and expressed in kilograms per hectare.

Data analysis. Data analysis was performed in R (version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria) using the R Studio graphical user interface (version 1.1.463, RStudio Inc.). For treatment effects, analysis of variance (ANOVA) was performed separately for two years due to differences in treatment design using type III sum of squares with the *car* package (version 3.0.0). In the linear model, all factors being environment, block, cultivar, and PRE herbicide were considered fixed effects.

The treatment means were estimated using least-squares procedure from *emmeans* package (version 1.2.3) and multiple pairwise comparisons were performed for statistically

significant factors ($P \leq 0.05$) using Bonferroni's adjustment. Single-degree-of-freedom orthogonal contrasts statements were applied to test pre-planned comparisons between (i) PRE herbicides (chlorimuron-ethyl + metribuzin + saflufenacil + sulfentrazone + flumioxazin) *vs.* non-treated herbicide control, (ii) PPO-inhibiting herbicides (saflufenacil + sulfentrazone + flumioxazin) *vs.* non-PPO herbicides (chlorimuron-ethyl + metribuzin), and (iii) PS II (metribuzin) *vs.* ALS (chlorimuron-ethyl) at each environment. At Tekamah in 2018, 5 out of 24 experimental units were removed from analysis due to varietal misplacement at planting.

To evaluate the relative abundance of filamentous organisms recovered from symptomatic root tissue in relation to categorical factors studied, a log-linear model was fit to the isolate collection 6 x 5 contingency table depicting the environments and four primary soybean root pathogenic genera (*Fusarium*, *Pythium*, *Phytophthora*, *Rhizoctonia*) plus the category *Others*. The category *Others* represented here is composed of all secondary pathogenic, non-pathogenic and/or contaminants recovered in the study. In the composition analysis, if the numbers of isolates in the cells of the contingency table occur at random, then no statistical linkage between the two categorical factors is determined, and thus H_0, the null hypothesis of independence fails to reject. However, if the null is rejected at pre-specified likelihood ($P \leq 0.05$), an indication of dependency between categorical factors may exist (Everitt 1992). The log-linear model was fitted with the *loglm* function from the package *MASS* (version 7.3.50) and both Pearson's and Likelihood Ratio (RL) chi-square χ^2 coefficients were calculated for the global contingency table and subsequently for orthogonal, structured sub-tables depicting the partitioning of degrees-of-freedom and previously calculated chi-square coefficients.

3.3. Results

Weather data. In 2017, no considerable rain events were observed within 12 days of planting in either environment, and soil temperatures increased from 21.6 to 25.4°C and from 23.3 to 28.5°C at Mead and Lincoln, respectively. In 2017, weather conditions varied considerably with accumulated precipitation of 31.2, 42.9, 7.4, and 19.1 mm at Tekamah, Arizona, Mead, and Bruno, respectively. Meanwhile, soil temperatures averaged 21.6°C at Tekamah and Arizona, and 26.4°C at Mead and Bruno. Shortly after planting, a brief drop in soil temperature was recorded in Tekamah and Arizona (Figure 3.2).

Root rot severity index **(DSI).** Seedling root rot epidemics developed naturally in the environments. Discoloration on lateral root systems was more common but lesions extending externally on the epidermis and internally in cortical tissue of taproots were also present. In 2017, DSI ranged from 21.6 to 46.6% across experimental units but PRE herbicides did not significantly affect DSI ($F_{5, 68}$=0.850, P=0.5186). Similarly, cultivars had no effect on DSI ($F_{1, 68}$=0.834, P=0.3640), regardless of the environment that they were grown (Table 3.2). No statistical differences were observed between environments and DSI averaged 34 to 35.6% Lincoln and Mead, respectively. In 2018, significant DSI differences were detected between environments ($F_{3, 63}$=21.357, P<0.0001) with the highest DSI mean at Tekamah (41.7%), followed by intermediate values at Bruno and Arizona (34.6% and 32.9%, respectively), and the lowest mean at Mead (27.7%). In addition to significant DSI differences across environments, the herbicide-environment interaction was found significant ($F_{15, 63}$=2.355, P=0.009; Table 3.2), therefore analysis was performed separately for each environment in 2018. At Tekamah, DSI varied from 26.6 to 61.6% across experimental units but no effect of PRE herbicides was statistically

significant ($F_{5, 10}$=2.305, *P*=0.1223). However, when PRE herbicides groups were contrasted, the application of PPO-inhibiting herbicides suggested (t-value$_{10}$=2.695, *P*=0.0225) slightly increased DSI when compared to non-PPO herbicides (Figure 3.3C; Table 3.3). Alternatively, a comparison between PRE herbicides and non-treated control was not significant (t-value$_{10}$=-0.063, *P*=0.9509) in that environment. In Arizona, DSI varied from 26.6 to 45% but no consistent effect of PRE herbicide application on DSI was detected. At Mead and Bruno, DSI values ranged from 18.3 to 43.3%, and 26.6 to 48.3%, respectively, but the application of PRE herbicides had no significant effect on DSI (Table 3.3).

Composition of root-associated organisms. In conjunction, 417 isolates representing groups of pathogenic, non-pathogenic and antagonistic organisms were isolated from symptomatic soybean root tissues. In 2017, 61 isolates (38%) represented primary root pathogenic genera (*Fusarium*, *Phytophthora*, *Pythium*, and *Rhizoctonia*), whereas, in 2018, 170 isolates (66%) composed that group (Table 3.4).

Pearson's and LR chi-square (χ^2) analysis of factors depicting *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, and *Others* indicated community composition was highly different across environments (Pearson's χ^2_{20}= 110.14, *P*<0.0001; LR=124.01, *P*<0.0001). The community composition corresponding exclusively to primary pathogenic genera also differed across sampled environments (Pearson's χ^2_{15}= 60.95, *P*<0.0001; LR=73.41, *P*<0.0001, Figure 3.4A), with *Fusarium* as the dominant genus representing 91.2 and 70.4% of relative estimated frequency at Lincoln and Mead in 2017, respectively. *Fusarium* spp. structured 54.4% of isolates obtained from Tekamah 2018, whereas the remaining composition was represented by *Phytophthora* and *Pythium* species with 27.8

and 17.7% of primary pathogenic isolates, respectively. There was also strong evidence that the abundance of primary pathogenic genera and *Others* was influenced by environment (Pearson's χ^2_{15}= 48.830, P<0.0001; LR=50.601, P<0.0001) (Figure 3.4E), but no comprehensive identification and quantification of members of the group *Others* was performed.

To further evaluate the composition of pathogenic genera collection, all six environments were grouped into low (<30%), intermediate (≥30 to <40%) and high (≥40%) DSI classes based on pairwise mean separations. Both Pearson's and LR chi-square χ^2 tests showed that the variation of primary pathogenic groups and DSI incidence did not occur at random (Pearson's χ^2_4= 18, P=0.001; LR=16.88, P=0.002), but it was rather highly associated, using the field-specific data. Within high DSI habitats, oomycete isolates represented 45.5%, as opposite of 54.5% of *Fusarium* isolates (Figure 3.4B). Conversely, at intermediate DSI environments, oomycetes structured 25.2%, while the majority of remaining isolates, more precisely 72.3% of the total pathogenic genera, corresponded to *Fusarium* species. Relatively, the lowest number of primary pathogenic isolates was recovered from the lowest DSI habitat (Figure 3.4B). Across intermediate and low DSI classes, it was not evident that the corresponding frequencies between oomycete and *Fusarium* groups were considerably different (25.2 and 72.3% *versus* 24.1 and 65.5%, for oomycete and *Fusarium* at intermediate and low DSI, respectively).

The decomposition of oomycete group between DSI classes demonstrated (Pearson's χ^2_2= 0.28, P=0.8683; LR=0.29, P=0.8638) that frequencies of *Phytophthora* spp. and *Pythium* spp. were at least its core not associated with discrepancies in seedling root rot (Figure 3.4C). Also, the relative frequency contemplating *Pythium* spp. seemed to

be low (<4%) across environments, except in Mead 2017 and Tekamah 2018, where it structured 7.3 and 13.7% of the recovered collection. Accounting exclusively for primary pathogenic genera, *Pythium* spp. represented 8.8, 22.2, 17.7, 5.2, 6.8, and 4.6% of isolates collected at Lincoln, Mead, Tekamah, Arizona, Mead, and Bruno, respectively. Contrastingly, *Phytophthora* was more geographically confined with presence detected in only half of the environments. However, when *Phytophthora* spp. were present, they constituted a relatively larger portion of isolated with 17.2, 27.8, and 44.1% with the primary pathogenic genera at Mead, Tekamah, and Bruno in 2018, respectively.

Despite similarities on DSI mean values, the variation on recovery within intermediate environments (Lincoln and Mead in 2017, and Arizona and Bruno in 2018) was significantly different for *Fusarium*, *Phytophthora* and *Pythium* (Pearson's χ^2_6= 45.95, P<0.0001; LR=50.12, P<0.0001). The partitioning of calculated Likelihood ratio coefficient suggested that community composition was unique at Bruno amongst other intermediate DSI environments for the variation of *Fusarium* and oomycete frequencies (Pearson's χ^2_1= 18.50, P<0.0001, LR=18.06, P<0.0001), and among the primary pathogenic oomycete group (Pearson's χ^2_1= 23.37, P<0.0001; LR=28.17, P<0.0001). Exclusively for Lincoln and Mead in 2017 and Arizona in 2018, weak evidence of community richness was found amongst major root rot soybean pathogenic genera (Pearson's χ^2_2= 4.08, P=0.1297; LR=3.89, P=0.1427) (Figure 3.4D). *Rhizoctonia* was the least predominant pathogenic genus recovered from symptomatic roots in this multi-environment study, comprising 1.5% of the total collection (Table 3.4).

Plant population. Across all experimental units, plant population estimates taken at emergence varied between 101,180 to 299,236 plants ha^{-1} and 53,819 to 273,403 plants ha^{-}

[1] in 2017 and 2018, respectively. No effect of the PRE herbicides was detected on any of the three developmental growth stages evaluated (Table 3.2). In 2017, P22T41R2 had consistently higher stands ($F_{1, 69}$=48.878, P<0.0001) compared to P28T08R, which performed the poorest in Lincoln at emergence. Similarly, PRE herbicide had no significant effect on plant population assessed at V1-V2 growth stages. Significant differences were detected ($F_{3, 63}$=66.851, P<0.0001) between environments where Arizona had the highest stands (214,134 plants ha^{-1}), followed by Mead (184,511 plants ha^{-1}), and Tekamah and Bruno with the lowest mean population (92,749 and 119,184 plants ha^{-1}, respectively). Final plant population estimates varied from 146,389 to 290,625 plants ha^{-1}, and 49,513 to 230,347 plants ha^{-1} in 2017 and 2018, respectively. In 2017, the cultivar P22T41R2 had statistically superior final stands than P28T08R ($F_{1, 69}$=74.454, P<0.0001). No significant differences between PRE herbicides or modes of action contrasts were detected in the study (Table 3.2 and 3.7). Factors that accounted for the greatest manageable variability on population estimates were cultivars and environments in 2017, and environments in 2018. Due to unforeseen circumstances, R8 stand count was not collected in Arizona.

Fresh root and shoot weight and ratio. No interactions were present (P≤0.05) between PRE herbicides, cultivars, and environments. With the exception of Mead and Bruno in 2018, no significant differences were observed between PRE herbicides on soybean fresh root weight across environments (Table 3.8). At Mead in 2018, sulfentrazone reduced fresh root weight by 1.75g on average when compared to metribuzin sprayed treatment. At Bruno, PRE herbicide application reduced fresh shoot and root weight by 1.95 and 1.15 g on average, respectively, when compared to non-treated control (Table 3.8). No significant

differences between treatments were detected for the ratio between shoot and root fresh weight.

Seedling vigor and plant height. Soybean injury was minimal in the study and there were no statistically significant differences between PRE herbicide treatments on seedling vigor (Table 3.9). In 2017, the cultivar P22T41R2 had superior seedling vigor scores than P28T08R ($F_{1, 69}$=5.621, *P*=0.0205). In 2018, a significant effect of environment was detected ($F_{3, 63}$=21.836, *P*<0.0001) with Mead and Arizona having the highest and Tekamah and Bruno the poorest aboveground seedling development.

Plant heights collected at two distinct phenological stages (V1-V2 and V6-R2) indicated no significant differences between PRE herbicides in the study (Table 3.9). Alternatively, the cultivar P28T08R had significantly higher plant heights than P22T41R2 in both early- and mid-season plant height assessments (Table 3.9).

Yield. In 2017, grain yield ranged from 3,409.1 to 5,685.8 kg ha^{-1} but no differences were attributed to the effect of PRE herbicides ($F_{5, 69}$=1.409, *P*=0.2317; Table 3.10). A positive yield difference of 678.5 kg ha^{-1} occurred for Mead in comparison to Lincoln ($F_{1, 69}$=133.360, *P*<0.0001). In 2018, grain yield varied between 1372.8 to 5,058.3 kg ha^{-1} across experimental units but no yield penalty was detected as result of PRE herbicide application (Table 3.2 and 3.10). Soybeans planted in Mead had greater yields than in Tekamah and Bruno by 1,656.1 and 1465.1 kg ha^{-1} on average. At Bruno, PPO-inhibiting herbicides yielded more than non-PPO herbicides by 322.2 kg ha^{-1} (t-$value_{15}$=2.240, *P*=0.0406). Unforeseen circumstances impeded yield data to be collected in Arizona.

3.4. Discussion

The present multi-environment field study provides additional insights into the effect of PRE herbicides on early-season disease development in late-planted soybeans. For most of the components evaluated, results are not supportive of the hypothesis that PRE herbicides consistently interact with the occurrence of seedling diseases under field conditions. Noticeable differences in seedling root rot severity existed between environments and were related to field-specific elements including the biological profile of organisms associated with symptomatic roots. Despite variation on growing conditions across environments, PRE herbicides did not result in higher root rot when compared to the non-treated control. These results corroborate with findings by Bauske and Kirby (1992) and Barlow et al. (2018) but contradict Bradley et al. (2002), Harikrishnan and Yang (2002), and Carson et al. (1991) which showed enhanced seedling root rot severity as result of the application of PRE herbicides on soybean under field conditions.

Minimal PRE herbicide injury was observed on newly emerging seedlings throughout the study. Differences in seedling vigor, height, and population density were rather attributed to cultivars and environments. There is sufficient evidence in the literature that herbicide injury can vary depending upon cultivar sensitivity and site characteristics including soil granulometry, organic matter, herbicide adsorptive behavior, and available moisture (Gannon et al. 2014; Miller et al. 2012; Stewart et al. 2012; Taylor-Lovell et al. 2001). We assume that the deleterious effects often associated to PRE herbicide application may have been minimized with to the use of compatible herbicide rates and the prevalence of warmer temperatures that offered satisfactory conditions for seedling emergence and development during the study (Johnson et al. 1989; Poston et al. 2008). In

2017, limited moisture during emergence may have alleviated herbicide injury but empirical evidence of differing weed emergence among experimental plots suggested PRE herbicides were somewhat active in the soil in that year. Contrastingly, in 2018, environments experienced moderate to high levels of precipitation at emergence (Figure 3.2) but the majority of PRE injury consisted of mild stunting rather than characteristic cotyledon and hypocotyl necrosis, occasionally associated with PPO-inhibiting herbicides (Hager 2014). The influence of prolonged cooler soil temperatures was not investigated due to unexpected warmer conditions following planting at all locations during this 2-year field study. Aside from these factors, we also observed substantial variability on plant population estimates taken at the Tekamah site due to widespread seed rot and damping-off related to seedling pathogens and at Bruno site due to soil crusting that limited uniform seedling emergence until 15 days after planting.

Results from the present investigation provided evidence about the role of community composition on the development of early-season disease epidemics in soybeans and confirms the predominance of *Fusarium*, *Pythium*, and *Phytophthora* species as important regional contributors to seedling root rot in alluvial soils of eastern Nebraska. A range of organisms representing antagonistic (e.g. *Trichoderma* spp.), secondary pathogenic (e.g. *Alternaria* spp.), and other ecologic groups (e.g. *Aspergillus*, *Mortierella*) were isolated from soybean symptomatic roots. In this study, however, efforts were directed towards identification of *Fusarium*, *Pythium*, *Phytophthora*, and *Rhizoctonia* which have historically occupied a position of epidemiological concern among root-rooting seedling pathogens in Nebraska (Giesler et al. 2012; Parikh et al. 2018). Qualitatively, surveys accessing species diversity associated with diseased soybean

seedlings throughout regions of North Central U.S. corroborate with results of genera composition in this study, although, major quantitative differences exist. Rizvi and Yang (1996) reported that species of *Pythium* and *Phytophthora* were cumulatively the predominant pathogens with 60 and 56% of the diseased seedlings in Iowa in 1993 and 1994, respectively, while *Fusarium* spp. represented only 12 and 14% of the total number. In the present study, *Fusarium* spp. were the dominant genus among common pathogenic genera with frequency varying between 23 to 43% of isolates, whereas *Pythium* spp., although recovered from all surveyed environments, represented only 2.5 to 13.7% of the total isolates. Given the ubiquitous presence of *Fusarium* in soil and ability to survive as plant rhizosphere inhabitants and secondary invaders, some overrepresentation could be anticipated (Summerell et al. 2003). However, given the field history and despite regular occurrence, it is unknown why the incidence of *Pythium* spp. was relatively low in the study. It is possible that higher temperatures prevailing at soybean emergence might have reduced the fitness of some *Pythium* spp. and in contrast, favored the occurrence of *Phytophthora* spp. (Rojas-Flechas et al. 2017; Thomson et al. 1971). In this study *Phytophthora* spp. occurrence was geographically clustered with presence detected in only half of the environments. However, when present, *Phytophthora* spp. structured a large percentage (>20%) of total primary group isolates, with the exception of one location with the lowest mean root rot. Overall, results from the present investigation suggest increased problems during seedling establishment and development in alluvial fields with the predominance of *Pythium* and *Phytophthora* species as opposite of *Fusarium* and *Rhizoctonia*. As far as other common seedling pathogens, Rizvi and Yang (1996) showed that *R. solani* recovery reached nearly a quarter of isolates collected from diseased

seedlings in Iowa during 1993 and 1994. Conversely, in the present study, the isolation of *Rhizoctonia* was low relative to other common seedling pathogens, possibly because the targeted selection of environments for oomycete damping-off did not overlap with areas where Rhizoctonia root and hypocotyl rot was endemic.

Attempts to determine the pathogenicity of isolates collected from this study were not performed, although evidence exists that not all isolates belonging to pathogenic genera necessarily cause disease (Coffua et al. 2016; Kirkpatrick et al. 2006). We also do not eliminate that selection of root material processed, isolation technique, isolate-media adaptability, and the presence of fast-growing species outcompeting others, amongst numerous other variants could have influenced overall community structure recorded this study. Future studies should take advantage of more robust detection techniques, such as molecular identification, as it easily allows for species identification, quantification, and detection of non-culturable organisms (McCartney et al. 2003) that may be related to herbicide-induced disease susceptibility.

Results from this study suggest that chlorimuron-ethyl, metribuzin, saflufenacil, sulfentrazone, and flumioxazin applied pre-emergently do not consistently interact with seedling disease incidence in late-planted soybeans. These findings, albeit speculatively, suggest minimum to no significant interaction between PRE herbicide mixes and the occurrence soybean seedling diseases under field conditions. This observation is important because PRE herbicide mixes, particularly those containing ALS- and PPO-inhibiting herbicides, are common in weed management programs in Nebraska (Sarangi and Jhala 2018). It is important to emphasize, however, that the majority of the isolates collected in this study were composed of species of *Fusarium*, *Phytophthora*, and to some extent

Pythium, as opposed to *Rhizoctonia*, which presumably limited the comprehension of the synergistic effects between PRE herbicides and this pathogen, despite conflicting literature (Bauske and Kirby 1992; Bradley et al. 2002; Espinoza et al. 1968; Harikrishnan and Yang 2002; Montazeri and Hamdollah-Zadeh 2005; Wiley and Ross 1974). Moreover, there is evidence that synergism between soil-applied PRE herbicides and seedling diseases is inoculum-dependent (Altman and Campbell 1977a; Carson et al. 1991; Chandler and Santelmann 1968; Harikrishnan and Yang 2002), but in this study experiments were only carried out in naturally-infested soil and no attempts to quantify pathogen inoculum in soil were performed. Notably, the effect of PPO-inhibiting *vs.* non-PPO herbicides on DSI was only detected in the highest seedling disease incidence environment, but inherited difficulties related to the variability of environmental conditions in which field trials are conducted did not allow for further ratification. Nevertheless, PRE herbicides did not result in higher root rot or damping-off when compared to a non-treated control, despite the amplitude of edaphic, climatic, and biological conditions existing in environments studied.

In Nebraska, significant soybean acreage occurs in the stream valleys formed in alluvium or a mixture of alluvium and colluvium. These soils, usually fertile, have dark surface horizons in which clay and silt compose a large percentage of soil granulometry. Under extended soil saturation, these fine-textured soils provide adequate conditions for the development of seedling diseases in soybeans (Broders et al. 2009; Duniway 1983; Workneh et al. 1999). The lack of a clear relationship between PRE herbicides and seedling diseases is favorable, given the widely adoption of PRE herbicides and limited curative treatment options for seedling disease control. These results are inconclusive about potential herbicide interactions under cool soil temperatures which also increase

PRE herbicide injury risk (Hager 2014; Jhala 2017). In the Midwest U.S., as early-plantings become more common, the incidence of seedling diseases such as Pythium seed and root rot may increase (Rod et al. 2018), therefore, further studies would be necessary to characterize responses in these scenarios and finalize management recommendations. Overall, results from this study are support the use of PRE herbicides as part of the integrated weed management program in alluvial soils of eastern Nebraska and minimize concerns regarding potential herbicide interactions in late-planted soybeans.

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Table 3. 1. Description of experimental sites and activities performed[x] in Nebraska in 2017 and 2018.

			Soil parameters								Execution date					
				Sand	Silt	Clay	O.M.					Root rot	Stand counts			
Year	Environment	GPS coordinates	Type[y]	(%)			g kg^{-1}	pH	NWS[z]	Tillage	Planting	V1-V2	VE-VC	V1-V2	R8	Harvest
2017	Lincoln	40.861614, -96.595594	Kennebec silt loam 0-1% slope	17	51	32	3	6.9	1-km	No-till	31 May	20 Jun	10 Jun	20 Jun	26 Sep	1 Nov
	Mead	41.155339, -96.422101	Filbert silt loam 0-1% slope	15	47	38	2.4	5.9	9.4-km	Disked	1 Jun	20 Jun	10 Jun	20 Jun	21 Sep	6 Nov
2018	Tekamah	41.755558, -96.176062	Luton silty clay 0-1% slopes	17	16	67	5.4	6.2	1-km	No-till	18 May	5 Jun	31 May	5 Jun	12 Oct	1 Nov
	Arizona	41.792885, -96.139346	Haynie silt loam 0-2% slopes	19	36	46	3.4	7.6	7.8-km	No-till	18 May	5 Jun	31 May	5 Jun	-	-
	Mead	41.182523, -96.459948	Filbert silt loam, 0-1% slopes	17	48	35	4.7	6.8	5.7-km	No-till	6 Jun	22 Jun	13 Jun	29 Jun	19 Oct	Oct 29
	Bruno	41.293432, -96.916723	Zook silty clay loam 0-2% slopes	14	53	33	3.2	6.8	10-km	No-till	6 Jun	27 Jun	-	29 Jun	22 Oct	Oct 29

[x] "-" indicates assessments were not performed. VE-VC (emergence to unifoliate), V1-V2 (first to second trifoliate), and R8 (full maturity) growth stages according to Fehr et al. (1971).
[y] Soil data was obtained from Soil Survey of USDA Natural Resources Conservation Service (https://websoilsurvey.sc.egov.usda.gov).
[z] NWS: nearest public weather station.

Table 3. 2. Probability values[x] from analysis of variance using combined data by year for disease severity index (DSI), population density, and grain yield in Nebraska.

					Plant population[y]										
		DSI			VE-VC			V1-V2			R8			Yield		
Year	**Sources[z]**	**df**	***F* value**	***P*>F**	**df**	***F* value**	***P*>F**	**df**	***F* value**	***P*>F**	**df**	***F* value**	***P*>F**	**df**	***F* value**	***P*>F**
2017	Herbicides (H)	5	0.850	0.5186	5	0.445	0.8148	5	0.377	0.8626	5	1.559	0.1831	5	1.409	0.2317
	Cultivars (C)	1	0.834	0.3640	1	48.878	**<0.0001**	1	63.336	**<0.0001**	1	74.454	**<0.0001**	1	2.715	0.1039
	Environments (E)	1	2.039	0.1577	1	18.663	**<0.0001**	1	17.681	**<0.0001**	1	1.635	0.2053	1	133.360	**<0.0001**
	H x C	5	0.827	0.5344	1	0.399	0.8477	1	0.484	0.7864	1	0.385	0.8570	1	0.532	0.7510
	H x E	5	0.255	0.9357	5	0.387	0.8555	5	0.546	0.7403	5	0.619	0.6852	5	0.351	0.8795
	C x E	1	0.011	0.9165	1	4.754	**0.0326**	1	5.606	**0.0207**	1	6.134	**0.0157**	1	0.749	0.3898
	H x C x E	5	1.456	0.2156	5	1.318	0.2666	5	0.928	0.4677	5	0.633	0.6748	5	0.405	0.8437
2018	Herbicides	5	1.262	0.2910	5	1.089	0.3793	5	0.788	0.5617	5	0.803	0.5529	5	0.759	0.5836
	Environments	3	21.357	**<0.0001**	2	43.271	**<0.0001**	3	66.851	**<0.0001**	2	73.973	**<0.0001**	2	121.079	**<0.0001**
	H x E	15	2.355	**0.009**	10	1.669	0.1180	15	1.177	0.3127	10	1.949	0.0622	10	1.338	0.2392

[x] Bold indicates statistical significance ($P \leq 0.05$).

[y] VE-VC (emergence to unifoliate growth stage), V1-V2 (first to second trifoliate), and R8 (full maturity) growth stages according to Fehr et al. (1971).

[z] Herbicides: Chlorimuron-ethyl at 44 g a.i. ha^{-1}, metribuzin at 560 g a.i. ha^{-1}, saflufenacil at 22 g a.i. ha^{-1}, sulfentrazone at 290 g a.i. ha^{-1}, and flumioxazin at 90 g a.i. ha^{-1}. Cultivars: P28T08R and P22T41R2 in 2017, and AG27x8 in 2018. Environments: Lincoln and Mead in 2017 and Tekamah, Arizona, Mead, and Bruno in 2018.

Table 3. 3. Least-square means and probability values[x] of soil-applied pre-emergence herbicides on soybean disease severity index (DSI) estimated at V1-V2 growth stages at six environments in Nebraska.

	DSI (%)					
	2017		2018			
PRE herbicide program[y]	**Lincoln**	**Mead**	**Tekamah**	**Arizona**	**Mead**	**Bruno**
Non-treated control	31.8	35.0	41.3	31.6	27.9	30.4
Chlorimuron-ethyl	34.7	36.6	38.0	31.6	30.0	35.8
Metribuzin	34.8	36.6	31.2	37.0	27.9	33.7
Saflufenacil	31.6	34.8	52.6	32.9	22.9	31.6
Sulfentrazone	24.2	34.6	40.4	30.4	25.8	38.3
Flumioxazin	36.7	36.1	42.9	33.9	32.0	37.9
Herbicide *P*>F	0.5749	0.9268	0.1223	0.4475	0.3284	0.3549
Cultivar *P*>F	0.4895	0.5342	-	-	-	-
H x C *P*>F	0.5250	0.2176	-	-	-	-
A *priori* contrasts[z]						
PRE *vs.* Control	2.6	0.8	-0.3	1.5	-0.1	5.0
P>F	0.2794	0.6936	0.9509	0.5395	0.9579	0.1399
PPO *vs.* non-PPO	-0.5	-1.4	10.6	-1.9	-2.0	1.1
P>F	0.7764	0.3907	**0.0225**	0.3733	0.4487	0.6702
PSII *vs.* ALS	<0.1	<0.1	-6.7	5.4	-2.0	-2.0
P>F	0.9891	0.999	0.2739	0.1094	0.6110	0.6279

[x] Bold indicates statistical significance ($P \leq 0.05$) and "-" indicates non-existing factors.

[y] Chlorimuron-ethyl at 44 g a.i. ha^{-1}, metribuzin at 560 g a.i. ha^{-1}, saflufenacil at 22 g a.i. ha^{-1}, sulfentrazone at 290 g a.i. ha^{-1}, and flumioxazin at 90 g a.i. ha^{-1}. Cultivars: P28T08R and P22T41R2 in 2017, and AG27x8 in 2018.

[z] A *priori* orthogonal contrasts: PRE *vs*. Control (difference between all pre-emergence herbicides and non-treated control), PPO *vs*. non-PPO (diff. between saflufenacil + sulfentrazone + flumioxazin and chlorimuron-ethyl + metribuzin), and PSII *vs*. ALS (diff. between metribuzin and chlorimuron-ethyl).

Table 3. 4. Data summary of disease severity index (DSI) and the number of isolates obtained from symptomatic soybean roots at six environments in Nebraska.

	2017		2018			
Disease (%), collection (n)	**Lincoln**	**Mead**	**Tekamah**	**Arizona**	**Mead**	**Bruno**
Parameter by environment						
DSI[y]	34 ±6.5	35.6 ±5.5	41.7 ±8.5	32.9 ±4.4	27.7 ±5.9	34.6 ±5.8
Isolates/collection[z]						
Fusarium spp.	31	19	43	17	19	22
Phytophthora spp.	0	0	22	0	5	19
Pythium spp.	3	6	14	1	2	2
Rhizoctonia spp.	0	2	0	1	3	0
Others	44	55	23	21	26	17

[y] Least-square means ± standard deviation for disease severity index based on a 0-to-10 scale. DSI= ∑(*severity rating* x *roots per rating*) x 100/ (*Total roots* x 10).
[z] Sum of the within-field collection of isolates obtained from symptomatic seedling root system plated onto four different media.

Table 3. 5. Least-square means and probability values[x] of soil-applied pre-emergence herbicides on soybean population density estimated at VE-VC growth stages at six environments in Nebraska.

	VE-VC population (plants ha^{-1})					
	2017		2018			
PRE herbicide program[y]	**Lincoln**	**Mead**	**Tekamah**	**Arizona**	**Mead**	**Bruno**
Non-treated control	207,474	223,620	143,302	211,511	208,281	…
Chlorimuron-ethyl	210,434	229,809	106,704	186,754	222,812	…
Metribuzin	191,328	223,081	147,465	190,521	212,587	…
Saflufenacil	192,405	236,806	107,009	207,205	223,351	…
Sulfentrazone	190,521	221,198	176,325	238,095	199,132	…
Flumioxazin	199,670	227,925	110,868	222,238	223,889	…
Herbicide *P*>F	0.8325	0.8451	0.1740	0.4329	0.7316	…
Cultivar *P*>F	**<0.0001**	**<0.0001**	-	-	-	-
H x C *P*>F	0.5193	0.5362	-	-	-	-
A *priori* contrasts[z]						
Control *vs.* PRE	-10,602	4,144	-13,627	-2,548	8,073	…
P>F	0.4757	0.6800	0.5560	0.8984	0.5951	…
Non-PPO *vs.* PPO	-6,682	2,197	4,316	33,875	-2,242	…
P>F	0.5890	0.7928	0.8189	0.0609	0.8588	…
ALS *vs.* PSII	-19,105	-6,727	40,760	3,767	-10,225	…
P>F	0.3212	0.6042	0.1628	0.8830	0.6020	…

[x] Bold indicates statistical significance ($P \leq 0.05$), "-" indicates non-existing factors elements, and "…" indicates data was not collected. Seeding rate: 308,881 plants ha^{-1}.

[y] Herbicide program: chlorimuron-ethyl at 44 g a.i. ha^{-1}, metribuzin at 560 g a.i. ha^{-1}, saflufenacil at 22 g a.i. ha^{-1}, sulfentrazone at 290 g a.i. ha^{-1}, and flumioxazin at 90 g a.i. ha^{-1}. Cultivars: P28T08R and P22T41R2 in 2017, and AG27x8 in 2018.

[z] A *priori* orthogonal contrasts: PRE *vs*. Control (difference between all pre-emergence herbicides and non-treated control), PPO *vs*. non-PPO (diff. between saflufenacil + sulfentrazone + flumioxazin and chlorimuron-ethyl + metribuzin), and PSII *vs*. ALS (diff. between metribuzin and chlorimuron-ethyl).

Table 3. 6. Least-square means and probability values[x] of soil-applied pre-emergence herbicides on soybean population density estimated at V1-V2 growth stages at six environments in Nebraska.

	V1-V2 population (plants ha^{-1})					
	2017		2018			
PRE herbicide program[y]	**Lincoln**	**Mead**	**Tekamah**	**Arizona**	**Mead**	**Bruno**
Non-treated control	236,536	250,260	125,250	221,198	186,215	88,802
Chlorimuron-ethyl	245,955	261,832	98,699	196,441	184,601	90,955
Metribuzin	227,118	262,101	148,542	200,208	189,982	83,958
Saflufenacil	230,078	256,719	84,131	202,361	185,139	100,642
Sulfentrazone	225,773	262,101	152,573	223,180	183,524	75,885
Flumioxazin	234,922	250,260	104,410	238,879	177,604	116,250
Herbicide *P*>F	0.8369	0.7596	0.2867	0.8051	0.9910	0.2085
Cultivar *P*>F	**<0.0001**	**<0.0001**	-	-	-	-
H x C *P*>F	0.7092	0.5017	-	-	-	-
A *priori* contrasts[z]						
PRE *vs.* Control	-3,767	8,342	-7,579	-8,983	-2,045	4,736
P>F	0.7692	0.3478	0.7667	0.7413	0.8869	0.6998
PPO *vs.* non-PPO	-6,278	-5,606	-9,915	23,148	-5,202	10,136
P>F	0.5581	0.4478	0.6373	0.3238	0.6652	0.3287
PSII *vs.* ALS	-18,836	269	49,842	3,767	5,381	-6,996
P>F	0.2601	0.9812	0.1282	0.9139	0.7722	0.6593

[x] Bold indicates statistical significance ($P \leq 0.05$) and "-" indicates non-existing factors. Seeding rate: 308,881 plants ha^{-1}.

[y] Herbicide program: chlorimuron-ethyl at 44 g a.i. ha^{-1}, metribuzin at 560 g a.i. ha^{-1}, saflufenacil at 22 g a.i. ha^{-1}, sulfentrazone at 290 g a.i. ha^{-1}, and flumioxazin at 90 g a.i. ha^{-1}. Cultivars: P28T08R and P22T41R2 in 2017, and AG27x8 in 2018.

[z] A *priori* orthogonal contrasts: PRE *vs*. Control (difference between all pre-emergence herbicides and non-treated control), PPO *vs*. non-PPO (diff. between saflufenacil + sulfentrazone + flumioxazin and chlorimuron-ethyl + metribuzin), and PSII *vs*. ALS (diff. between metribuzin and chlorimuron-ethyl).

Table 3. 7. Least-square means and probability values[x] of soil-applied pre-emergence herbicides on soybean population density estimated at R8 growth stage at six environments in Nebraska.

	R8 population (plants ha^{-1})					
	2017		2018			
PRE herbicide program[y]	**Lincoln**	**Mead**	**Tekamah**	**Arizona**	**Mead**	**Bruno**
Non-treated control	211,241	232,231	135,825	…	208,281	94,184
Chlorimuron-ethyl	229,271	241,918	106,403	…	222,812	104,948
Metribuzin	219,314	223,351	139,931	…	212,587	111,944
Saflufenacil	224,427	222,543	107,933	…	223,351	99,028
Sulfentrazone	211,511	219,583	158,585	…	199,132	86,649
Flumioxazin	216,623	211,241	95,798	…	223,889	123,785
Herbicide *P*>F	0.3814	0.3877	0.2263	…	0.3575	0.4323
Cultivar *P*>F	**<0.0001**	**<0.0001**	-	-	-	-
H x C *P*>F	0.8529	0.7283	-	-	-	-
A *priori* contrasts[z]						
PRE *vs.* Control	8,987	-8,503	-14,094	…	12,486	11,087
P>F	0.2391	0.4530	0.5296	…	0.3449	0.4484
PPO *vs.* non-PPO	-6,772	-14,845	-2,394	…	-269	-5,292
P>F	0.2862	0.1211	0.8954	…	0.9802	0.6621
PSII *vs.* ALS	-9,956	-18,567	33,527	…	11,302	6,997
P>F	0.3111	0.2079	0.2291	…	0.5045	0.7089

[x] Bold indicates statistical significance ($P \leq 0.05$), "-" indicates non-existing factors elements, and "…" indicates data was not collected. Seeding rate: 308,881 plants ha^{-1}.

[y] Herbicide program: chlorimuron-ethyl at 44 g a.i. ha^{-1}, metribuzin at 560 g a.i. ha^{-1}, saflufenacil at 22 g a.i. ha^{-1}, sulfentrazone at 290 g a.i. ha^{-1}, and flumioxazin at 90 g a.i. ha^{-1}. Cultivars: P28T08R and P22T41R2 in 2017, and AG27x8 in 2018.

[z] A *priori* orthogonal contrasts: PRE *vs*. Control (difference between all pre-emergence herbicides and non-treated control), PPO *vs*. non-PPO (diff. between saflufenacil + sulfentrazone + flumioxazin and chlorimuron-ethyl + metribuzin), and PSII *vs*. ALS (diff. between metribuzin and chlorimuron-ethyl).

Table 3. 8. Least-square means and probability values[u] of soil-applied pre-emergence herbicides on soybean fresh root and shoot weight and ratio estimated from seedlings rated for root rot at six environments in Nebraska.

	2017						2018											
	Lincoln			Mead			Tekamah			Arizona			Mead			Bruno		
PRE herbicide program[v]	**RW[w]**	**SW[x]**	**RA[y]**	**RW**	**SW**	**RA**	**RW**	**SW**	**RA**	**RW**	**SW**	**RA**	**RW**	**SW**	**RA**	**RW**	**SW**	**RA**
Non-treated control	2.75	11.87	.23	4	12.37	.32	1.79	4.16	.50	3	7	.44	5.75 ab	…	…	8.75	10.25	.85
Chlorimuron	3.5	10.37	.33	4.37	13.12	.34	1.52	3.69	.41	3.25	7.5	.44	5.25 ab	…	…	8.25	8.25	1.02
Metribuzin	3	11	.27	4	12.12	.33	2.75	4.5	.62	3.5	7.25	.48	6.75 a	…	…	7.5	8.75	.87
Saflufenacil	3.25	10.62	.30	4	12.37	.34	2.21	4.32	.51	3.5	6	.59	6.25 ab	…	…	7.75	9.50	.82
Sulfentrazone	2.62	10	.28	4	12.87	.32	2.34	4.05	.57	2.96	7.33	.40	5 b	…	…	7.25	7.75	.94
Flumioxazin	2.62	11.25	.24	3.37	12	.28	1.75	4	.43	3.71	7.64	.52	5.25 ab	…	…	7.25	7.25	1.03
Herbicides *P*>F	0.5399	0.7583	0.2805	0.1848	0.9130	0.5644	0.6556	0.9236	0.8748	0.7219	0.6471	0.4587	**0.0115**	…	…	0.1514	0.0758	0.1023
Cultivar *P*>F	0.7979	0.9563	0.4678	0.2372	0.6599	0.8750	-	-	-	-	-	-	-	-	-	-	-	-
H x C *P*>F	0.9281	0.2957	0.3720	0.4527	0.9130	0.3391	-	-	-	-	-	-	-	-	-	-	-	-
A *priori* contrasts[z]																		
PRE *vs.* Control	0.25	-1.22	0.056	-0.05	0.12	<0.0001	0.32	-0.04	-0.008	0.38	0.14	0.040	-0.05	…	…	-1.15	-1.95	0.008
P>F	0.5676	0.2354	0.1341	0.8587	0.8881	0.9770	0.6333	0.9427	0.9611	0.3626	0.8555	0.5812	0.8898	…	…	**0.0287**	**0.0229**	0.2427
PPO *vs.* non-PPO	-0.41	-0.06	-0.029	-0.39	-0.20	-0.025	-0.03	0.02	-0.012	0.01	-0.38	0.044	-0.50	…	…	-0.45	-0.33	-0.017
P>F	0.2565	0.9415	0.3380	0.0977	0.7784	0.3214	0.9497	0.9580	0.9291	0.9588	0.5756	0.4779	0.1114	…	…	0.2652	0.6109	0.7505
PSII *vs.* ALS	-0.50	0.62	-0.056	-0.37	-1.00	-0.014	1.22	0.80	0.208	0.25	-0.25	0.042	1.50	…	…	-0.75	0.50	-0.156
P>F	0.3776	0.6361	0.2373	0.3048	0.3858	0.7244	0.1552	0.3184	0.3115	0.6421	0.8078	0.6551	**0.0051**	…	…	0.2404	0.6222	0.0864

[u] Bold indicates statistical significance ($P \leq 0.05$), "-" indicates non-existing factors elements, and "…" indicates data was not collected. Common letters in the same column were separated by pairwise comparisons using Bonferroni's adjustment.

[v] Herbicide program: chlorimuron-ethyl at 44 g a.i. ha^{-1}, metribuzin at 560 g a.i. ha^{-1}, saflufenacil at 22 g a.i. ha^{-1}, sulfentrazone at 290 g a.i. ha^{-1}, and flumioxazin at 90 g a.i. ha^{-1}. Cultivars: P28T08R and P22T41R2 in 2017, and AG27x8 in 2018.

[w] Root weight (g).

[x] Shoot weight (g).

[y] Ratio (Root/Shoot).

[z] A *priori* orthogonal contrasts: PRE *vs*. Control (difference between all pre-emergence herbicides and non-treated control), PPO *vs*. non-PPO (diff. between saflufenacil + sulfentrazone + flumioxazin and chlorimuron-ethyl + metribuzin), and PSII *vs*. ALS (diff. between metribuzin and chlorimuron-ethyl).

Table 3. 9. Least-square means[x] of soil-applied pre-emergence herbicides on seedling vigor and plant height at six environments in Nebraska in 2017 and 2018.

			Seedling vigor[z] (0-10)	Plant height (cm)	
Year	**Environment**	**PRE herbicide program[y]**	**VE-VC**	**V1-V2**	**V6-R2**
2017	Lincoln	Non-treated control	9.1	7.09	18.32
		Chlorimuron	8.9	6.76	17.35
		Metribuzin	8.9	7.36	19.57
		Saflufenacil	9	7.39	19.09
		Sulfentrazone	8.8	7.21	18.66
		Flumioxazin	9.3	7.45	18.99
		Herbicide *P*>F	0.8605	0.2845	0.2964
		Cultivar *P*>F	**0.0511**	0.0917	**<0.0001**
		H x C *P*>F	0.7250	0.4129	0.7551
	Mead	Non-treated control	9.4	6.8	20.60
		Chlorimuron	9.3	6.8	20.70
		Metribuzin	9.4	6.85	20
		Saflufenacil	9.1	6.72	19.59
		Sulfentrazone	9.2	6.58	20.21
		Flumioxazin	9	6.49	19.83
		Herbicide *P*>F	0.7440	0.3711	0.5017
		Cultivar *P*>F	0.2416	**<0.0001**	**<0.0001**
		H x C *P*>F	0.7608	**0.0297**	0.9277
2018	Tekamah	Non-treated control	7	5.64	17.55
		Chlorimuron	7	5.33	16.37
		Metribuzin	8	5.48	18.14
		Saflufenacil	5.2	4.73	16.62
		Sulfentrazone	7.8	5.52	18.48
		Flumioxazin	6.2	5.18	17.90
		Herbicide *P*>F	0.1918	0.3458	0.8406
	Arizona	Non-treated control	7.5	5.43	37.85
		Chlorimuron	7.5	5.71	33.44
		Metribuzin	8	5.69	39.36
		Saflufenacil	7.7	5.65	38.38
		Sulfentrazone	9	5.01	40.45
		Flumioxazin	8.2	5.77	38.80
		Herbicide *P*>F	0.6762	0.4016	0.3882
	Mead	Non-treated control	8.8	10.15	20.08
		Chlorimuron	9.4	10.57	21.03
		Metribuzin	8.7	10.44	20.41
		Saflufenacil	8.3	11.64	19.72
		Sulfentrazone	8.6	10.99	18.70
		Flumioxazin	8.7	10.46	21.05
		Herbicide *P*>F	0.3261	0.5946	0.6576
	Bruno	Non-treated control	6.8	7.93	15.52
		Chlorimuron	5.7	8.75	14.64
		Metribuzin	6.1	7.64	15.57
		Saflufenacil	6.6	8.08	15.49
		Sulfentrazone	6.3	8.26	14.22
		Flumioxazin	7.3	8.66	15.30
		Herbicide *P*>F	0.3086	0.4083	0.2572

[x] Bold indicates statistical significance ($P \leq 0.05$).

[y] Herbicide program: chlorimuron-ethyl at 44 g a.i. ha^{-1}, metribuzin at 560 g a.i. ha^{-1}, saflufenacil at 22 g a.i. ha^{-1}, sulfentrazone at 290 g a.i. ha^{-1}, and flumioxazin at 90 g a.i. ha^{-1}. Cultivars: P28T08R and P22T41R2 in 2017, and AG27x8 in 2018.

[z] Overall seedling vigor estimated on a 0-to-10 scale with 0 being the worst and 10 the best.

Table 3. 10. Least-square means and probability values[x] of soil-applied pre-emergence herbicides on soybean yield at six environments in Nebraska.

	Yield (kg ha^{-1})					
	2017		2018			
PRE herbicide program[y]	**Lincoln**	**Mead**	**Tekamah**	**Arizona**	**Mead**	**Bruno**
Non-treated control	3,755.3	4,433.4	2,773.5	…	3,978.7	2,893.1
Chlorimuron-ethyl	3,850.6	4,590.2	2,208.2	…	4,233.4	2,401.1
Metribuzin	3,745.3	4,558.7	2,842.3	…	4,184.2	2,606.9
Saflufenacil	3,735.5	4,330.6	2,413.3	…	4,393.4	2,923.8
Sulfentrazone	3,960.9	4,558.6	2,826.3	…	4,302.7	2,598.0
Flumioxazin	3,771.3	4,418.2	2,430.0	…	4,078.0	2,957.1
Herbicide *P*>F	0.3640	0.6081	0.4071	…	0.7508	0.1270
Cultivar *P*>F	0.4993	0.1430	-	-	-	-
H x C *P*>F	0.6234	0.8860	-	-	-	-
A *priori* contrasts[z]						
PRE *vs.* Control	57.4	57.8	-229.4	…	259.6	-195.6
P>F	0.5289	0.6640	0.4384	…	0.2694	0.2748
PPO *vs.* non-PPO	24,6	-138.6	31.3	…	49.2	322.2
P>F	0.7457	0.2164	0.8959	…	0.7977	**0.0406**
PSII *vs.* ALS	-105.3	-31.4	634.0	…	-49.2	205.8
P>F	0.3728	0.8547	0.0949	…	0.8683	0.3704

[x] Bold indicates statistical significance ($P \leq 0.05$), "-" indicates non-existing factors elements, and "…" indicates data was not collected.

[y] Herbicide program: chlorimuron-ethyl at 44 g a.i. ha^{-1}, metribuzin at 560 g a.i. ha^{-1}, saflufenacil at 22 g a.i. ha^{-1}, sulfentrazone at 290 g a.i. ha^{-1}, and flumioxazin at 90 g a.i. ha^{-1}. Cultivars: P28T08R and P22T41R2 in 2017, and AG27x8 in 2018.

[z] A *priori* orthogonal contrasts: PRE *vs*. Control (difference between all pre-emergence herbicides and non-treated control), PPO *vs*. non-PPO (diff. between saflufenacil + sulfentrazone + flumioxazin and chlorimuron-ethyl + metribuzin), and PSII *vs*. ALS (diff. between metribuzin and chlorimuron-ethyl).

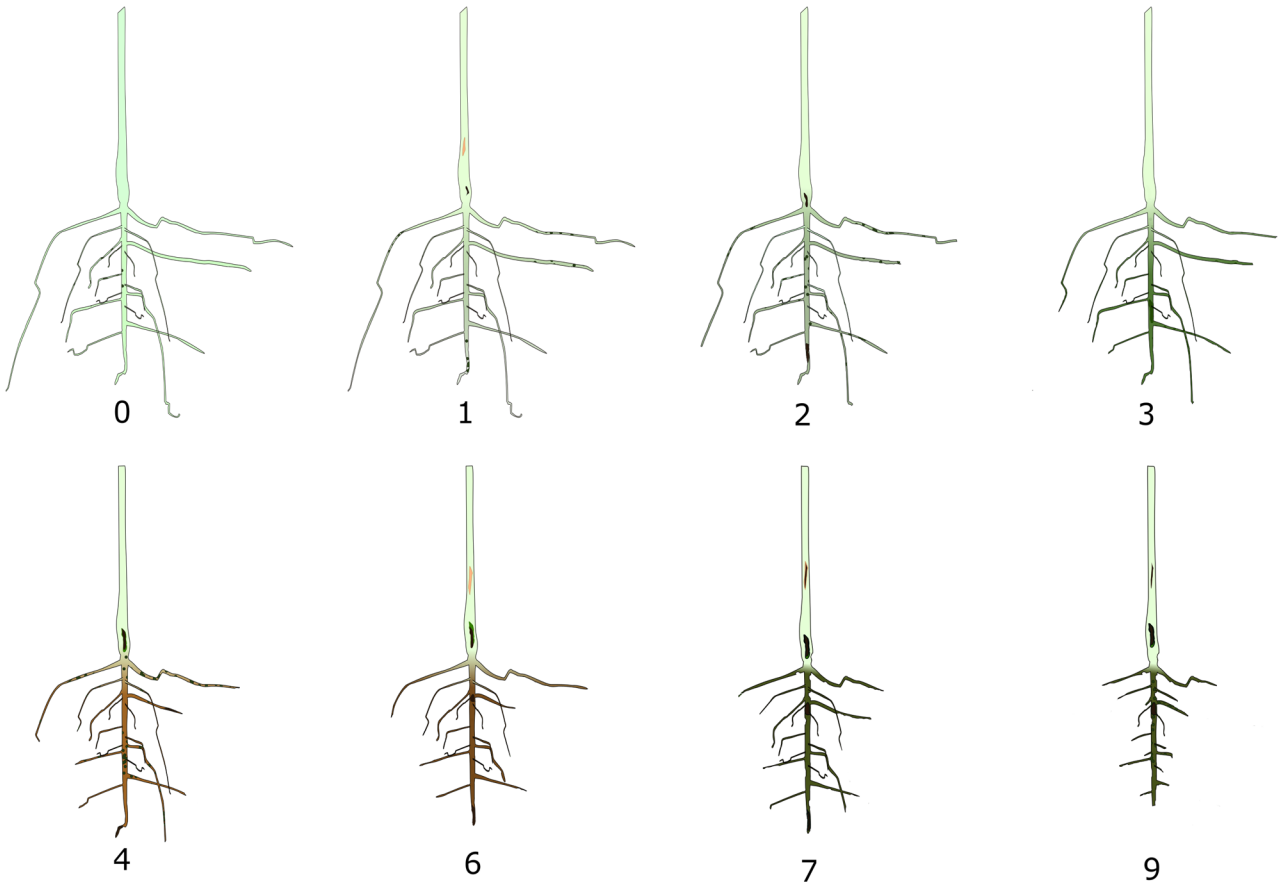


Figure 3. 1. Root rot severity scale used to rate soybean seedlings in field trials. Ratings range between 0-to-10, where 0= symptomless, 1= few small reddish to brown lesions, 0.1–0.2 cm in length, at base of hypocotyl or root tips, 2= progressing lesions, discoloration evident but many healthy roots present, 3= taproot intact but increasing color intensity, minor reduction in root mass, 4=10-20% root mass reduction and discoloration and coalescing of localized root lesions, 5= root system discolored with increasingly lesions with 20-40% root mass reduction and hypocotyl lesions, 6= intensely reddish-brown discoloration and compromised mass reduction affecting roughly ½ of root volume, 7= mass reduction affecting roughly ¾ of root volume, taproot compromised, 8= further damage (not illustrated), and 9= remaining entire root blackened, and 10=dead seedling (not illustrated).

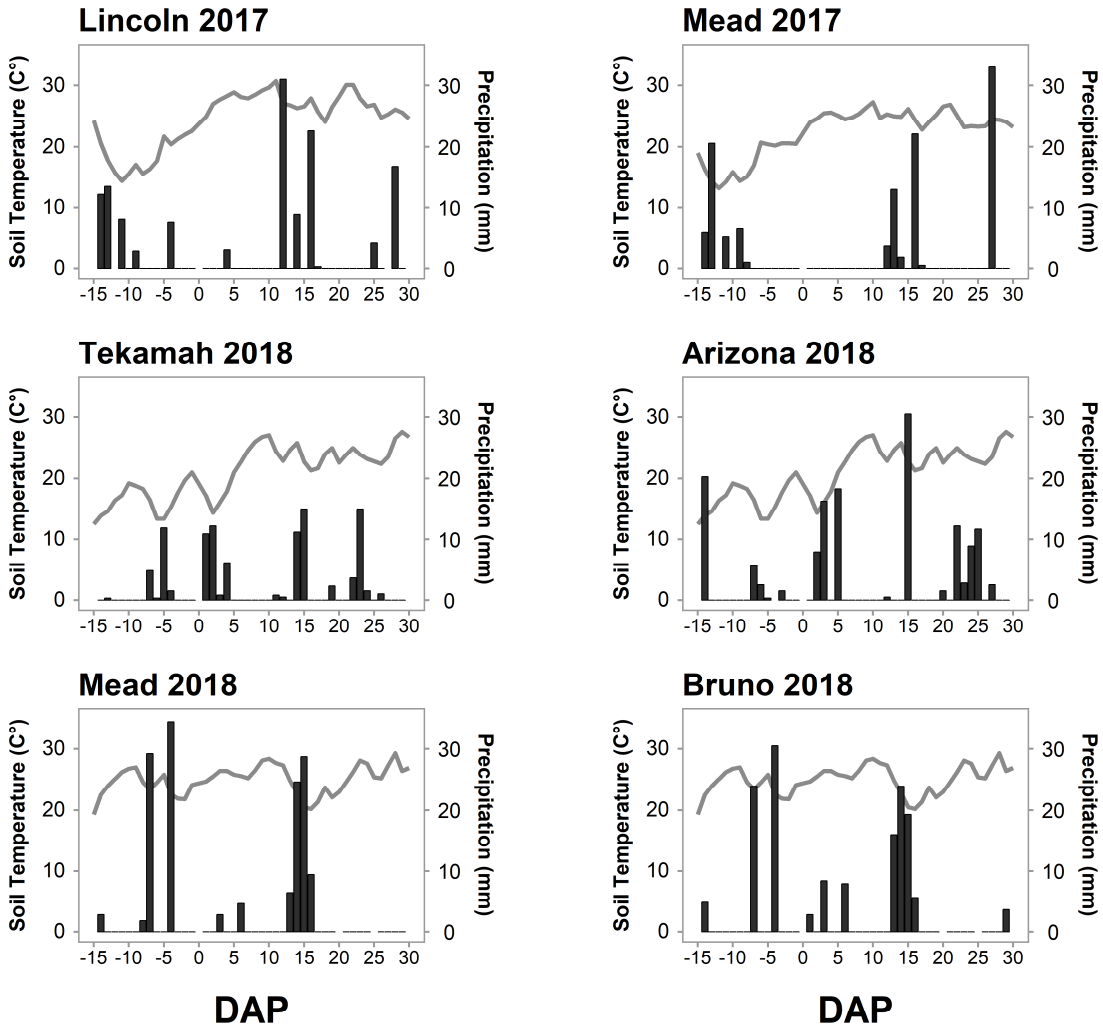


Figure 3. 2. Average soil temperature (lines) at 10-cm depth and daily accumulated precipitation (bars) from 15 days prior to planting to 30 days after planting (DAP) at six environments in Nebraska in 2017 and 2018.

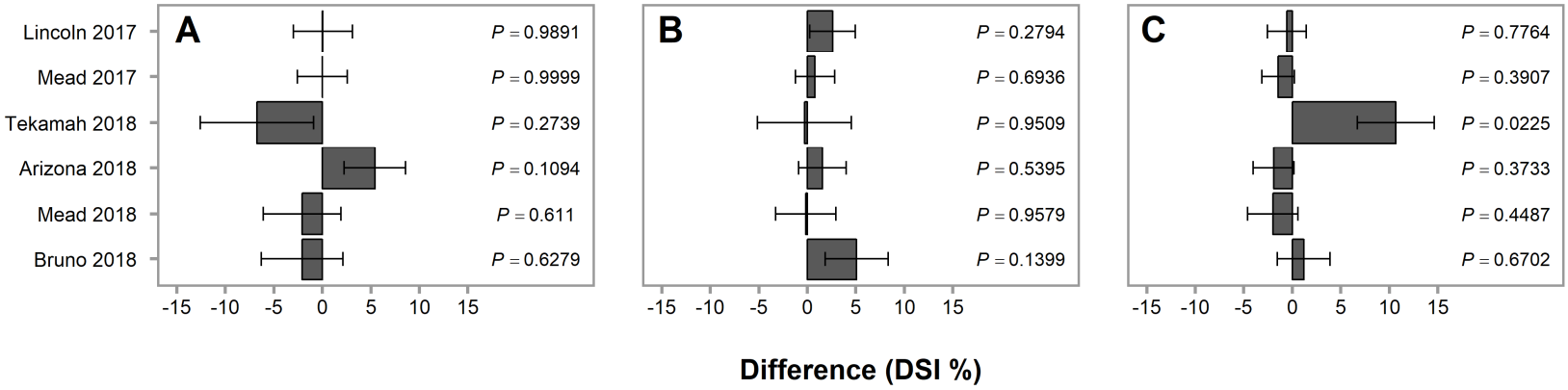


Figure 3. 3. Mean differences between soil-applied pre-emergence herbicides modes of action on disease severity index (DSI) in Nebraska. When statistically significant ($P \leq 0.05$), positive differences indicate increasing DSI associated with **A,** PS II *vs.* ALS; **B,** pre-emergence herbicides *vs.* non-treated control; and **C,** PPO-inhibiting herbicides *vs.* non-PPO herbicides. Error bars represent ± standard error of the contrast difference.

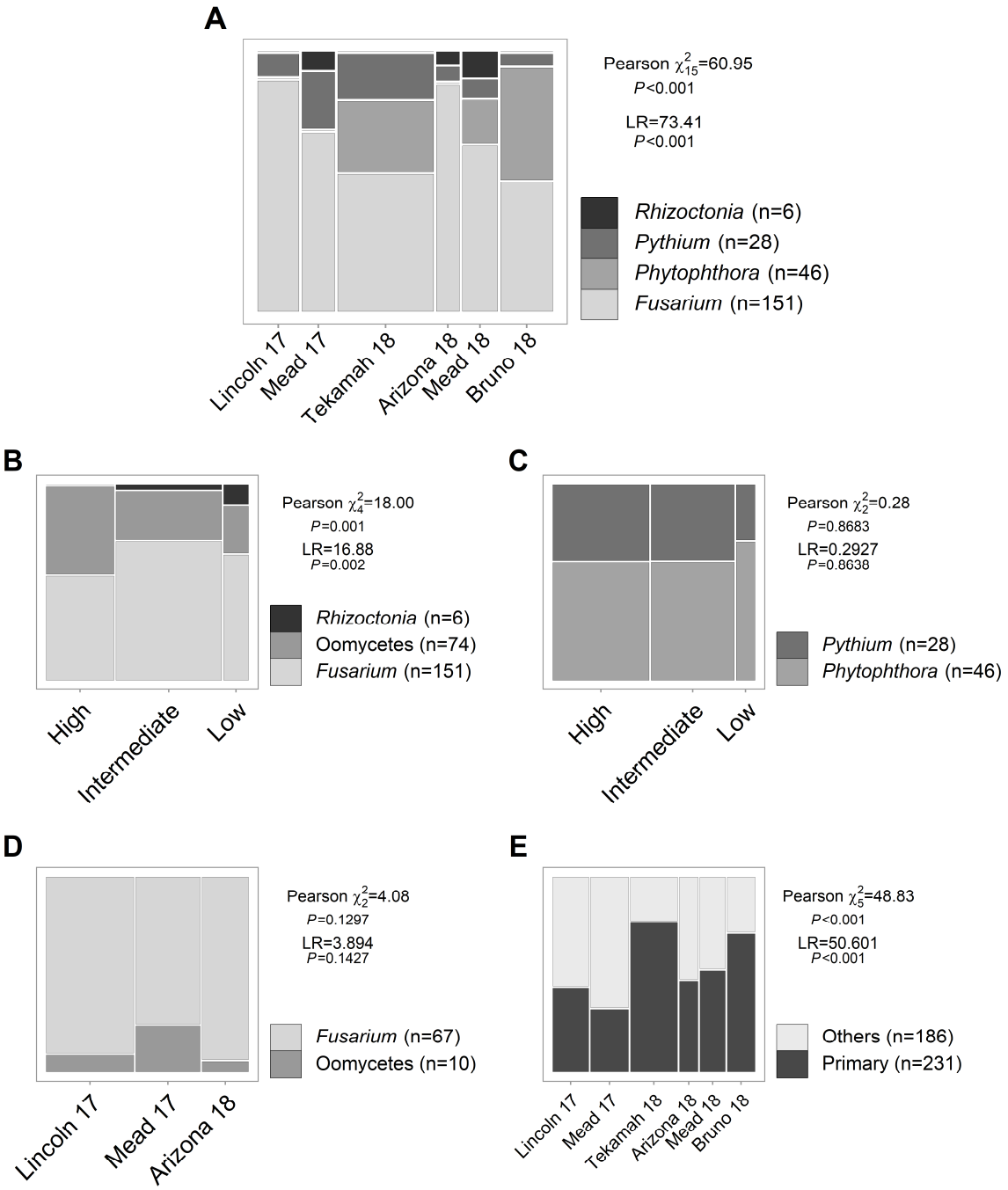


Figure 3. 4. Mosaic plots of the relative frequencies of isolates obtained from symptomatic seedling roots in Nebraska in 2017 and 2018. Pearson's and Likelihood Ratio (LR) χ^2 coefficients and *P*-values were obtained from loglinear model fit to the data and orthogonal contrasts. Categorical factors varied between **A,** primary pathogenic genera (*Fusarium*, *Phytophthora*, *Pythium* and *Rhizoctonia*) by environment; **B,** primary pathogenic groups and disease severity index (DSI) classes; **C,** primary pathogenic oomycetes and DSI classes; **D,** *Fusarium* and oomycetes across three intermediate DSI environments; and **E,** primary pathogenic genera and *Others* (secondary pathogens, antagonistic, and contaminants) by environments. In total, 417 isolates were obtained from the six environments combined.

Table S. 3. 1. The frequency of primary filamentous genera isolated from symptomatic soybean roots by semi-selective media.

	2017		2018			
Composite genera	**Lincoln**	**Mead**	**Tekamah**	**Arizona**	**Mead**	**Bruno**
Fusarium spp.						
WA[v]	12	11	17	8	13	9
WA+S[w]	9	6	19	7	6	13
CMA[x]	0	0	1	0	0	0
PBNIC[y]	10	2	6	2	0	0
Phytophthora spp.						
WA	0	0	7	0	1	0
WA+S	0	0	0	0	0	1
CMA	0	0	5	0	0	9
PBNIC	0	0	10	0	4	9
Pythium spp.						
WA	0	3	1	1	1	1
WA+S	0	0	1	0	0	0
CMA	3	2	3	0	1	0
PBNIC	0	1	9	0	0	1
Rhizoctonia spp.						
WA	0	2	0	1	0	0
WA+S	0	0	0	0	0	0
CMA	0	0	0	0	3	0
PBNIC	0	0	0	0	0	0
Others species[z]	44	55	23	21	26	17
Total	78	82	102	40	55	60

[v] Water agar 20 g L^{-1}.
[w] Water agar 20 g L^{-1} + streptomycin at 0.03 g L^{-1}.
[x] Corn meal agar 20 g L^{-1} + pentachloronitrobenzene 0.054 g L^{-1} + benomy 0.01 g L^{-1} + piramicin 0.005 g L^{-1}.
[y] PBNIC V8 agar containing benomyl 0.01 g L^{-1} + pentachloronitrobenzene 0.054 g. L^{-1} + neomycin sulfate 0.10 g L^{-1} + chloramphenicol 0.01 g L^{-1} + iprodione at 0.04 g. L^{-1} + rifampicin 0.01 g L^{-1} + hymexazole 0.02 g L^{-1}.
[z] Secondary pathogenic and non-pathogenic genera or contaminants.

CHAPTER 4. General conclusions

In soybean production, uniform crop establishment and optimum plant densities are key for obtaining high yields. In this thesis, field trials were conducted to determine how production inputs may affect the occurrence of soybean pathogens and plant performance in seedling disease and Phytophthora stem and root rot (PSRR) conducive environments.

Previous field research conducted in Nebraska and Iowa have been inconclusive regarding the efficacy of seed treatment on crop stand and yield. The work presented in this thesis demonstrates the benefit of seed treatments containing ethaboxam and metalaxyl to manage soybean seedling diseases in PSRR endemic areas. Similarly, management programs employing PSRR moderately resistant cultivars had a significant yield advantage over moderately susceptible lines under greater disease incidence. Following university management recommendations and results obtained in this study, soybean producers adopting seeding rates between 296.000 to 309.000 plants ha^{-1} (120.000 to 125.000 seeds a^{-1}) are encouraged to apply oomycides and select resistant cultivars in fields with PSRR history. Note, however, that soybean seed companies may adopt different disease susceptibility scales, and that for one company, moderately resistant cultivars may have a score of 1, whereas for a second company, a score of 9. Results from the present investigation do not support the hypothesis that *Rps*1c and *Rps*1k cultivars differ substantially in terms of field efficacy, although, in one environment *Rps*1c had superior yield than *Rps*1k cultivars. Overall, tolerance provided a greater absolute yield advantage than seed treatment alone. Alternatively, seed treatment response was more consistent across environments. These results reinforce previous field research in the North Central

region of the U.S. indicating the importance of integrating genetic resistance and seed treatment to reduce yield impact due to the incidence of seedling disease and PSRR in soybeans.

Canopy development is a relevant parameter associated with yield for soybeans planted in mid-May through early-June in Nebraska. As sunlight interception increases, yield potential also increases, particularly in late planting scenarios in which soybean plants are shorter and rely comparatively more on seed weight than the number of productive nodes and pods for yield component. However, enhanced plant aboveground development can also have its caveats. In fact, from a plant health perspective, denser canopies can increase the severity of foliar and stem diseases in soybeans, including Septoria brown spot (*Septoria glycines*), target spot (*Corynespora cassiicola*), and Sclerotinia white mold (*Sclerotinia sclerotiorum*). This is attributed to the extended period of leaf wetness and conducive microclimate resulting from limited air movement below soybean canopies. In contrast, for soilborne diseases, increased vegetative growth indicates less root colonization and more vigorous plants. It is also worthy to note that, although seed treatments can speed row closure in soybeans, minimum to no effect whatsoever is expected from this response on the incidence of foliar and stem diseases, mainly because vegetative increments are relatively small when compared to the total plant vegetative growth. This study contributes to the etiology of soilborne diseases in soybeans by providing a protocol to assess active vegetative land cover. Measurements collected with an open-source, user-friendly mobile application indicated significant and negative correlations between soybean aboveground development and the number of *P. sojae* symptomatic plants at 2 of the 4 locations where the disease was observed. Researchers are

encouraged to adopt and improve the protocol present in this thesis so more efficient canopy assessments can be performed while removing the background noise of bare soil, previous crop residue, and weeds.

The study also evaluated the effect of pre-emergence herbicides on seedling disease development in late-planted soybeans. Stressed soybean seedlings are more susceptible to infection by pathogens and environmental factors are very important in influencing the development of seedling diseases. Seedling diseases occur more frequently under cool, wet conditions and are more prevalent on fine-textured soils with high organic matter content. Other factors such as planting depth, compaction, nematode damage, and misapplication of soil-applied pre-emergence herbicides have been shown to interact with disease incidence. Results gathered in this thesis, however, indicate minimum interaction between pre-emergence herbicides and soybean seedling disease under uncontrolled field conditions, and suggests that other factors, such as the predominant pathogen group present in a field, play a more important role in disease severity. This study confirms the predominance of *Pythium*, *Phytophthora*, and *Fusarium* species as common organisms associated with soybean symptomatic seedlings in alluvial soils of eastern Nebraska. Comparatively, greater soybean seedling root rot and damping-off can be expected from oomycetes (*Pythium*, and *Phytophthora*) activity relative to other members in the primary pathogenic group commonly found in alluvial soils. Across multiple environments, *Fusarium* spp. were the dominant genus among common pathogenic genera and *Rhizoctonia* recovery was low compared to seedling pathogens. Overall, these results support the use of pre-emergence herbicides as part of integrated weed management program in late-planted

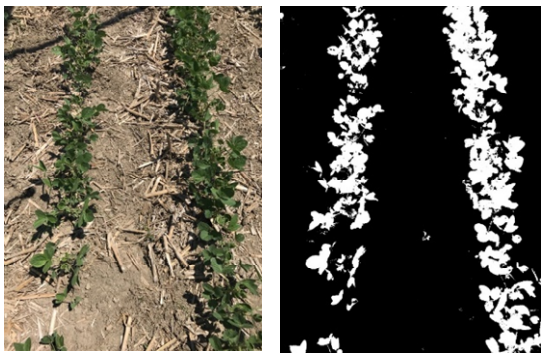
soybeans but also indicate more research is needed, particularly under increased herbicide injury risk and seedling disease development, to finalize management recommendations.

Throughout this thesis, it was clear that seedling diseases and Phytophthora stem and root rot (PSRR) are important yield-limiting diseases in soybeans in Nebraska and Iowa. Accounting for all effects evaluated, the conjunction of biological, edaphic, and climatic factors that compose an environment was the primary factor driving disease incidence and yield. As we gain more knowledge about how multifactorial abiotic and biotic effects can affect disease severity, producers in North Central U.S. will be able to better manage seedling diseases and PSRR and reduce the negative impact that these maladies have on soybean production.

APPENDIX A: Canopy coverage protocol in chapter 2

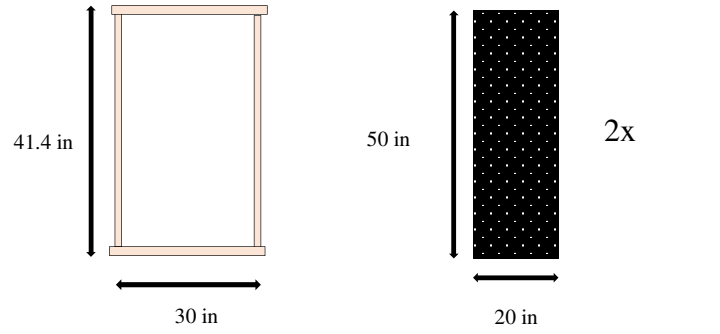
Option I

1. Hold the smartphone at waist-height and take a picture of the plot capturing two rows at a time for an area of approximately 19 ft square. Perform image collection systematically for other experimental units.



Option II

1. The frame was built with PVC tube, 1" diameter, and would be suited for iPhone 7 with 4.7" screen size. Adjustments may be necessary if other devices are used. In addition to materials shown below, 4 curves joints (2 in long) are needed to connect PVC tubes. Frame concept by UNL Weed Science Team.



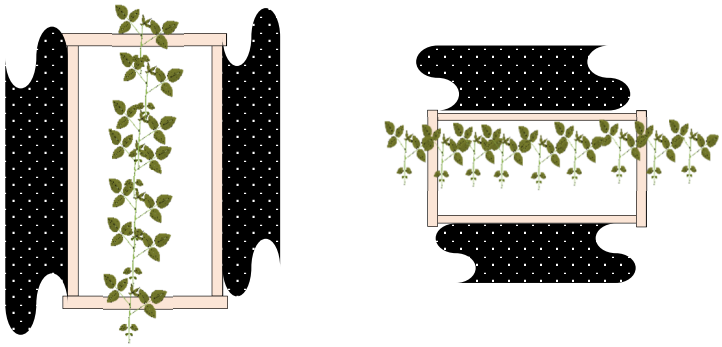
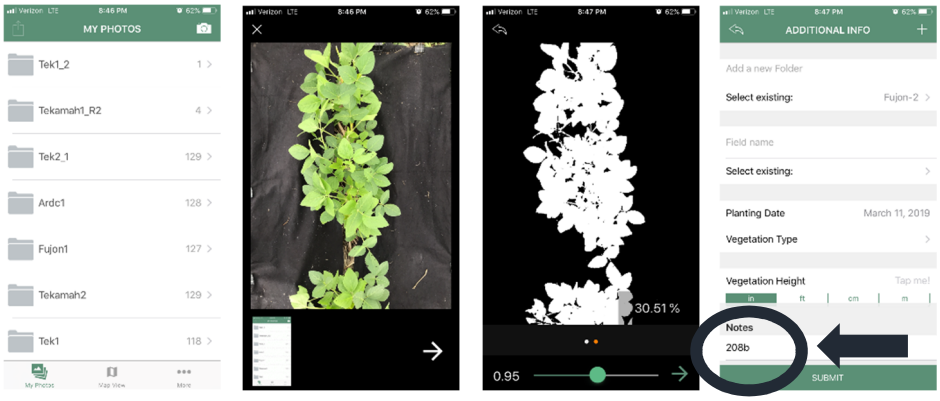


Figure A. 1. Example of **A,** non-ideal (bare soil, residue and weeds included in the image) and **B,** ideal camera placement (detail on clips delineating frame area).

Click on the camera icon on the top right. Adjust brightness according to the situation in the field. With phone camera facing downwards and covering the entire frame, take the picture and define black-white contrast. This value represents the sensitivity of the greenness measurement (more or less inclusive). As shown below, a 0.95 value was defined to be standard. Hit the arrow on the bottom right and create a new file under the additional info tab. Enter the plot number under notes. Submit and it is saved.



Reference

Patrignani, A., and Ochsner, T. E. 2015. *Canopeo*: A powerful new tool for measuring fractional green canopy cover. Agronomy Journal, 107:2312-2320. doi: 10.2134/agronj15.0150.

APPENDIX B: R codes for analysis in chapter 2

Field trial analysis

```
library(dplyr); library(lme4); library(lmerTest); library(MASS); library(emmeans); library(car); library(multcomp);
library(pbkrtest)

  data17$BLOCK=as.factor(data17$BLOCK)
  data17$TRT=as.factor(data17$TRT)
  data17$STI=factor(data17$STI, levels = c("Y","N"))
  data17$CULT=factor(data17$CULT, levels =
c("AG3432","AG3034","H3220NR","H2913NR","C3070R2","C3171R2","C3010RX","C2890R2","C3026RX"))
  data17$TRT=factor(data17$TRT)

  data18$BLOCK=as.factor(data18$BLOCK)
  data18$LOC=as.factor(data18$LOC)
  data18$STI=factor(data18$STI,levels = c("Y","N"))
  data18$TRT=factor(data18$TRT)
  data18$CULT=factor(data18$CULT,levels = c("C2888RX","C3140RX","AG27x8", "AG28x7","H2862NX","H2512NX",
"NK3195X","NK2788X"))


#Contrasts for 2017
  Rps1c.vs.Rps1k<-c(1,1,-1,-1,-1,-1,0,1,1)/4
  high.vs.low <-c(-1,1,1,-1,2,-3,2,2,-3)/8
  Rps1c_High.vs.low <-c(-1,1,0,0,0,0,0,1,-1)/2
  Rps1k_High.vs.low <-c(0,0,1,-1,1,-1,0,0,0)/2
  round(crossprod(cbind(Rps1c.vs.Rps1k,high.vs.low,Rps1c_High.vs.low,Rps1k_High.vs.low)),2)
  Contr<-cbind(Rps1c.vs.Rps1k,high.vs.low,Rps1c_High.vs.low,Rps1k_High.vs.low)
  high_sti.vs.control<-c(0,1,1,0,1,0,1,1,0,0,-1,-1,0,-1,0,-1,-1,0)/5
  low_sti.vs.control<-c(1,0,0,1,0,1,0,0,1,-1,0,0,-1,0,-1,0,0,-1)/5
  round(crossprod(cbind(high_sti.vs.control,low_sti.vs.control)),2)
  Contr1<-cbind(high_sti.vs.control,low_sti.vs.control)

#Analysis by environment (Tekamah) in 2017
  lmer1=lmerTest::lmer(YIELD_kg_ha~CULT*STI+(1|BLOCK/CULT),REML=TRUE,data= data17,contrasts=list(CULT =
contr.sum, STI=contr.sum))
  plot(lmer1)
  anova(lmer1,ddf="Kenward-Roger")
  Yield.CULT=emmeans::emmeans(lmer1,~CULT,lmer.df = "Kenward-Roger")
  Yield.STI=emmeans::emmeans(lmer1,~STI,lmer.df = "Kenward-Roger")
  Yield.CULT.STI=emmeans::emmeans(lmer1,~CULT*STI,lmer.df = "Kenward-Roger")
  emmeans::contrast(Yield.CULT, list(Contr), adjust="fdr")
  emmeans::contrast(Yield.CULT.STI, list(Contr1), adjust="fdr")
  pairs(Yield.STI)

#Contrats for 2018 (Nebraska)
  high.vs.low <-c(1,-1,1,-1,1,-1,1,-1)/4
  Rps1c.vs.Rps1k<-c(1,1,1,1,-3,-3,1,1)/6
  Rps1c_High.vs.low <-c(1,-1,1,-1,0,0,1,-1)/3
  Rps1k_High.vs.low <-c(0,0,0,0,1,-1,0,0)
  round(crossprod(cbind(high.vs.low,Rps1c.vs.Rps1k,Rps1c_High.vs.low,Rps1k_High.vs.low)),2)
  Contr<-cbind(high.vs.low,Rps1c.vs.Rps1k,Rps1c_High.vs.low,Rps1k_High.vs.low)
  high_sti.vs.control<-c(1,0,1,0,1,0,1,0,-1,0,-1,0,-1,0,-1,0)/4
  low_sti.vs.control<-c(0,1,0,1,0,1,0,1,0,-1,0,-1,0,-1,0,-1)/4
  round(crossprod(cbind(high_sti.vs.control,low_sti.vs.control)),2)
  Contr1<-cbind(high_sti.vs.control,low_sti.vs.control)

#Contrats for 2018 (Boone-IA)
  high.vs.low.IA <-c(1,-1,0,1,-1,1,-1)/3
  Rps1c.vs.Rps1k.IA<-c(2,2,2,-5,-5,2,2)/10
  Rps1c_High.vs.low.IA <-c(1,-1,0,0,0,1,-1)/2
```

```
  Rps1k_High.vs.low.IA <-c(0,1,-1,0,0,0,0)
  round(crossprod(cbind(high.vs.low.IA,Rps1c.vs.Rps1k.IA,Rps1c_High.vs.low.IA,Rps1k_High.vs.low.IA)),2)
  Contr.IA<-cbind(high.vs.low.IA,Rps1c.vs.Rps1k.IA,Rps1c_High.vs.low.IA,Rps1k_High.vs.low.IA)
  sum(Rps1k_High.vs.low.IA)
  high_sti.vs.control.IA<-c(1,0,0,1,0,1,0,-1,0,0,-1,0,-1,0)/3
  low_sti.vs.control.IA<-c(0,1,0,0,1,0,1,0,-1,0,0,-1,0,-1)/3
  round(crossprod(cbind(high_sti.vs.control.IA,low_sti.vs.control.IA)),2)
  Contr1.IA<-cbind(high_sti.vs.control.IA,low_sti.vs.control.IA)

#Combined analysis in 2018 (except Boone-IA)
  lmer2=lmerTest::lmer(YIELD_kg_ha~CULT*STI*LOC+(1|BLOCK/LOC/CULT),REML=TRUE,data= data18 %>%
filter(LOC!="boone"), contrasts=list(CULT = contr.sum, STI=contr.sum, LOC=contr.sum))
  plot(lmer2)
  print(VarCorr(lmer2),comp=c("Variance","Std.Dev."),digits=6)
  anova(lmer2,ddf="Kenward-Roger")

#Analysis by environment (Tekamah) in 2018
  lmer3=lmerTest::lmer(YIELD_kg_ha~CULT*STI+(1|BLOCK/CULT),REML=TRUE,data= data18 %>%
filter(LOC=="tek1"),contrasts=list(CULT = contr.sum, STI=contr.sum))
  plot(lmer3)
  print(VarCorr(lmer3),comp=c("Variance","Std.Dev."),digits=6)
  anova(lmer3,ddf="Kenward-Roger")
  yield.tek1.CULT=emmeans::emmeans(lmer3,~CULT,lmer.df = "Kenward-Roger");yield.tek1.CULT
  yield.tek1.STI=emmeans::emmeans(lmer3,~STI,lmer.df = "Kenward-Roger");yield.tek1.STI
  yield.tek1.CULT.STI=emmeans::emmeans(lmer3,~CULT*STI,lmer.df = "Kenward-Roger");yield.tek1.CULT.STI
  emmeans::contrast(yield.tek1.CULT, list(Contr), adjust="fdr")
  emmeans::contrast(yield.tek1.CULT.STI, list(Contr1), adjust="fdr")
  pairs(yield.tek1.STI)

#Correlations by environment
  tek1cor=data18 %>% filter(LOC=="tek1")
  cor.test(tek1cor$PHYTOPHTHORA, tek1cor$YIELD_kg_ha, method = "spearman",use="complete.obs")
```

APPENDIX C: Isolation protocol in chapter 3

1. Root rot evaluation (14 - 21 days after planting – VC-V1 growth stage)
   - Dig 6 plants per plot (3 from each non-harvestable rows)
   - Wash roots on site
     - Rate (0-10 severity scale) individual plants (6 plants, record root volume, total root area rotted, discolored) (Fig. C.1-A)
     - Cut each plant at cotyledon scar
       - Measure top fresh weight (total 6 plants)
       - Measure root fresh weight (total 6 plants)

2. Combine root material from experimental units into blocks (1, 2, 3, 4) of experimental design and then combine blocks 1+2 and 3+4 into two pools (pool 1 and 2). Place plants in coolers for transportation. Same day processing is preferred

3. In the lab
   - Wash pool 1 and 2 separately and thoroughly with detergent and tap water until soil is removed from root material (Fig. C.1-B)
   - Surface disinfest each pool in a beaker by keeping roots in 0.5% sodium hypochlorite solution for 1-1.5 min (Dorrance et al. 2008). Leave roots under tap water for 10 min with a cheesecloth cover to remove residual bleach
   - Aseptically cut taproot and root sections targeting symptomatic tissue (~2 cm long) (Fig. C.1-C, D). Avoid thinner roots because they desiccate over time
   - Leave pool in the laminar hood for 20-30 min to remove excessive moisture
   - Randomly select four root segments from processed pool to be plated on 6 Petri dishes of 4 types of media (24 total plates/pool) (Fig. C.1-E)
     - Suggested plate label: site, date, # pool, media type, and # plate (1-6)

4. Check plates daily for growth and characterize types of growth for pathogen ID (Fig. C.1-F)
   - Record frequency (# of root pieces that produced mycelial growth/total # of root pieces per pool)
   - Subculture types of growth to fresh plates (Fig. C.1-G). Label isolates (e.g. A, B, C, D) as they are transferred to fresh plates to avoid doubles
   - Take notes on types of growth (color, speed of growth, segmentation, any change in visual appearance)
   - Subculture isolates to potato dextrose agar for storage and future ID.

Reference

Dorrance, A. E., Berry, S. A., Anderson, T. R., and Meharg, C. 2008. Isolation, storage, pathotype characterization, and evaluation of resistance for *Phytophthora sojae* in soybean. Online. Plant Health Progress. doi: 10.1094/PHP-2008-0118-01-DG.

Figure C. 1. Procedure utilized for root isolation.

APPENDIX D: R codes for analysis and visuals in chapter 3

Field trial analysis

```
library(dplyr); library(MASS); library(car); library(emmeans); library(multcomp)

data17$BLOCK=as.factor(data17$BLOCK)
data17$TREAT=as.factor(data17$TREAT)
data17$HERB=factor(data17$HERB, levels = c("Control","Classic","Sencor","Sharpen","Spartan","Valor"))
data17$CULT=factor(data17$CULT, levels = c("P22T41R2", "P28T08R"))

data18$BLOCK=as.factor(data18$BLOCK)
data18$TREAT=as.factor(data18$TREAT)
data18$HERB=factor(data18$HERB, levels = c("Control","Classic","Sencor","Sharpen","Spartan","Valor"))

#Constrats
Non.treated.vs.treated<-c(-5,1,1,1,1,1)/5
NonPPO.vs.PPO<-c(0,-3,-3,2,2,2)/6
ALS.vs.PSII<-c(0,-1,1,0,0,0)
round(crossprod(cbind(Non.treated.vs.treated,NonPPO.vs.PPO,ALS.vs.PSII)),2)
Contr<-cbind(Non.treated.vs.treated,NonPPO.vs.PPO,ALS.vs.PSII)

#Combined analysis 2017
par(mfrow=c(1,2))
aov1=aov(DSI~BLOCK+HERB*CULT*LOC, data17,contrasts=list(HERB=contr.sum, CULT=contr.sum, LOC=contr.sum))
plot(aov1, which = c(2,3))
Anova(aov1, type="III", test="F")

#Analysis by environment (Lincoln) in 2017
par(mfrow=c(1,2))
aov2=aov(DSI~BLOCK+HERB*CULT, data17 %>% filter(LOC=="Lincoln"),contrasts=list(HERB=contr.sum,
CULT=contr.sum))
plot(aov2, which = c(2,3))
Anova(aov2, type="III", test="F")
rot.L.CULT=emmeans(aov2,~CULT)
rot.L.HERB=emmeans(aov2,~HERB)
rot.L.CULT.HERB=emmeans(aov2,~CULT*HERB)
emmeans::contrast(rot.L.HERB, list(Contr))

#Combined analysis 2018
par(mfrow=c(1,2))
aov1=aov(DSI~BLOCK+HERB*LOC, data18,contrasts=list(HERB=contr.sum, LOC=contr.sum))
plot(aov1, which = c(2,3))
Anova(aov1, type="III", test="F")

#Analysis by environment (Tekamah) in 2018
par(mfrow=c(1,2))
aov1=aov(DSI~BLOCK+HERB, data18 %>% filter(LOC=="tek1"),contrasts=list(HERB=contr.sum))
plot(aov1, which = c(2,3))
Anova(aov1, type="III", test="F")
Root.tek1.HERB=emmeans(aov1,~HERB);Root.tek1.HERB
emmeans::contrast(Root.tek1.HERB, list(Contr))
```

Genera frequency

```
library(ggmosaic); library(ggplot2); library(grid); library(gridExtra); library(ggplotify); library(MASS)
data=read.csv('C:/Users/Garnica/Box/Analysis Experiments/fungal frequency.csv',header=TRUE)
data$Location <- factor(data$Location, levels=c("Lincoln 17", "Mead 17", "Tekamah 18","Arizona 18", "Mead 18","Bruno
18"))

#Contingency table and orthogonal contrasts
```

```
  fungi<-xtabs(n~Location+Pathogen, data)
  ct1=fungi[,-2]
  ct2=as.table(matrix(c(margin.table(ct1,margin = 1),fungi[,2]), nrow = 2, byrow = T,
                  dimnames = list(Genera = c('Primary', 'Secondary'), Location= c('Lincoln 17', 'Mead 17' ,'Tekamah 18',
'Arizona 18', 'Mead 18', 'Bruno 18'))))
  c<- ct1[-3,]
  d=matrix(c(ct1[3,],margin.table(c[-4,],margin=2),ct1[5,]), nrow = 3,ncol = 4, byrow = T)
  ct3=as.table(matrix(c(d[,1],margin.table(d[,2:3], margin=1),d[,4]),nrow = 3,ncol = 3, byrow = F,
                  dimnames = list(DSI = c('High', 'Intermediate', 'Low'), Genera= c('Fusarium','Oomycete','Rhizoctonia'))))
  e<-fungi[-3,3:4]
  ct4=as.table(matrix(c(fungi[3,3:4],margin.table(e[-4,], margin=2), fungi[5,3:4]),nrow = 3,ncol = 2, byrow = T,
                  dimnames = list(DSI = c('High', 'Intermediate', 'Low'), Genera= c('Phytophthora','Pythium'))))
  ct5=c[-4,]
  ct6=as.table(matrix(c(margin.table(ct5[,-4], margin = 1), ct5[,4]),nrow = 4,ncol = 2, byrow = F,
                  dimnames = list(Site = c('Lincoln 17', 'Mead 17', 'Arizona 18', 'Bruno 18'), Genera=
c('Fusarium+Phytophthora+Pythium','Rhizoctonia'))))
  f<-ct5[,-4]
  g<-matrix(c(margin.table(f[-4,], margin=2),f[4,]),nrow = 2,ncol = 3, byrow = T)
  ct7=as.table(matrix(c(margin.table(g[,2:3], margin=1),g[,1]),nrow = 2,ncol = 2, byrow = F,
                  dimnames = list(Site = c('Lincoln+Mead17+Arizona', 'Bruno 18'), Genera= c('Oomycete','Fusarium'))))
  ct8=as.table(matrix(g[,2:3],nrow = 2,ncol = 2, byrow = F,dimnames = list(Site = c('Lincoln+Mead17+Arizona', 'Bruno 18'),
Genera= c('Phythophthora','Pythium'))))
  ct9=f[1:3,2:3]
  ct10=as.table(matrix(c(margin.table(ct9,margin = 1),f[1:3,1]),nrow = 3,ncol = 2, byrow = F,
                   dimnames = list(Site = c('Lincoln','Mead17','Arizona'), Genera= c('Oomycete','Fusarium'))))

#Loglinear models
  total<-loglm(~Location+Pathogen, fungi)
  pathogenic<-loglm(~Location+Pathogen, ct1)
  others<-loglm(~Location+Genera, ct2)
round(pathogenic$lrt + others$lrt,12) == round(total$lrt,12)
  DSI<-loglm(~DSI+Genera, ct3)
  DSI1<-loglm(~DSI+Genera, ct4)
  interm<-loglm(~Location+Pathogen, ct5)
round(DSI$lrt + DSI1$lrt + interm$lrt,12) == round(pathogenic$lrt,12)
  rhizocinter<-loglm(~Site+Genera, ct6)
  phypyfusinter<-loglm(~Location+Pathogen, f)
round(rhizocinter$lrt + phypyfusinter$lrt,12) == round(interm$lrt,12)
  brun<-loglm(~Site+Genera, ct7)
  brunoo<-loglm(~Site+Genera, ct8)
  LMA<-loglm(~Location+Pathogen, ct9)
  oofusa<-loglm(~Genera+Site, ct10)
round(brun$lrt + brunoo$lrt + LMA$lrt + oofusa$lrt ,12) == round(phypyfusinter$lrt,12)

#Mosaic plot
ggplot(data = as.data.frame(prop.table(ct1))) +
  geom_mosaic(aes(weight= Freq, x = product(Pathogen, Location), fill=Pathogen), color="black", na.rm=TRUE,
show.legend = TRUE)+
  scale_fill_manual('Primary Pathogenic Genera',values=c("gray80", "gray55","grey30","black"),
                labels = c(substitute(paste(italic('Fusarium')," (n=151)")), substitute(paste(italic('Phytophthora')," (n=46)")),
                        substitute(paste(italic('Pythium')," (n=28)")),substitute(paste(italic('Rhizoctonia')),"
(n=6)")),guide=guide_legend(override.aes=aes(color="black"),reverse=T))+
  scale_y_productlist(labels = c(substitute(paste(italic('Fusarium'))), substitute(paste(italic('Phytophthora'))),
                          substitute(paste(italic('Pythium'))),substitute(paste(italic('Rhizoctonia')))), position="left")+
  scale_x_productlist(labels = c("Lincoln 17","Mead 17", "Tekamah 18", "Arizona 18", "Mead 18", "Bruno 18"))
```