EVALUATION OF PYTHIUM ROOT ROT AND DAMPING OFF RESISTANCE IN THE ANCESTRAL LINES OF NORTH AMERICAN SOYBEAN CULTIVARS AND CHEMICAL CONTROL OF THE ACTIVE INGREDIENT ETHABOXAM IN SEED TREATMENTS

BY

KATHERINE SUSAN MCLACHLAN

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Crop Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2016

Urbana, Illinois

Master's Committee:

Assistant Professor David Walker, Co-Chair
Associate Professor Carl Bradley, Co-Chair
Professor Brian Diers

ABSTRACT

Effects of *Pythium ultimum* var. *ultimum* and other *Pythium* species on the North American Ancestral Soybean Lines

A trend towards planting soybean (*Glycine max* (L.) Merrill) earlier in the growing season has made seedling diseases more prominent. A survey of biotic causes of yield loss between 2006 and 2009 rated seedling diseases second in only to soybean cyst nematode. *Pythium ultimum* var. *ultimum* is an oomycete that favors cool wet conditions in early spring and causes seed decay, root rot, and seedling damping off. Resistance to this pathogen has yet to be reported in soybean. The objective of this study was to evaluate the response of the North American ancestral soybean lines and their first progeny to determine if the genotypes had resistance. These lines contain approximately 99% of the genes of modern North American cultivars. An Illinois isolate of *P. ultimum* var. *ultimum* was used for the screen. Fourteen of the 90 ancestral and first progeny lines were found to have varying levels of partial resistance. A subset of five lines, four resistant and one susceptible, from the ancestral screen were then screened for resistance against isolates of three different species of *Pythium* that were collected in Illinois: *P. ultimum* var. *ultimum* from the ancestral screen, *P. irregulare*, and *P. sylvaticum*. The results showed that the partially resistant lines conferred resistance across the three *Pythium* species. The results also revealed that there were different levels of aggressiveness among the isolates of the *Pythium* species. *P. ultimum* var. *ultimum* showed to be the most aggressive, followed by *P. irregulare*, then *P. sylvaticum*, respectively. The lines identified in both studies could provide potential sources of resistance to Pythium damping-off and root rot for modern soybean breeding programs.

Effects of fungicide seed treatments specific to oomycetes pathogens on stand establishment and yield of soybean in Illinois

Seed treatments are a popular management tactic for seedling diseases. The active ingredient, metalaxyl, has been on the market for over 30 years to control oomycetes, especially *Pythium spp*. and *Phytophthora sojae*. A new active ingredient, ethaboxam, has recently come to the fungicide seed market as a new management tool against oomycetes. In order to understand the effects of the new active ingredient, non-inoculated field trials were established across the state of Illinois in 2014 and 2015. Trials were placed at the University of Illinois research stations near DeKalb, Urbana, and Dixon Springs. Six fungicide seed treatments consisted of an (1) untreated control, (2) metalaxyl (Sebring 2.65ST; Valent USA Corp., Walnut Creek, CA) at 4g a.i./100kg of seed, (3) ethaboxam (Intego Solo; Valent USA Corp., Walnut Creek, CA) at 7.5g a.i./100kg of seed, (4) ethaboxam + metalaxyl at 7.5g + 2g a.i./100kg of seed, (5) ethaboxam + metalaxyl at 7.5g + 4g a.i./100kg seed, and (6) ethaboxam + metalaxyl at 7.5g + 7.5g a.i./100kg of seed. A broad spectrum fungicide of Rizolex with the active ingredient of tolclofos-methyl at 5 g a.i./100 kg of seed (Valent USA Corp., Walnut Creek, CA) was applied to all of the seed treatments except the untreated control. Plant stands from each plot were taken within three weeks of emergence, R1 root weights, shoot weights, root rot severity were collected mid-growing season and seed yield was collected at harvest. Fungicide seed treatments had a significant effect on plant stand (P=.0002), but not on yield (P=0.7466), root weigh per plot (P=0.0823), shoot weight per plot (P=0.1873), and root rot per plot (P=0.4017). The untreated control had significantly lower plant stand that the other treatments. The treatments with the varying ratios of ethaboxam to metalaxyl were not significantly different from each other, but the treatment with 7.5 g + 7.5g a.i. of ethaboxam + metalaxyl had significantly higher plant stands than the ethaboxam only treatment. Yields from each of the treatments were not

significantly different from each other including the untreated control. The results show that seed treatments with ethaboxam and metalaxyl could help protect against stand loss associated with oomycetes seedling diseases.

ACKNOWLEDGEMENTS

I would like to thank my co-advisors Dr. David Walker and Dr. Carl Bradley for taking the chance on me, and for putting up with my endless questions. I would also like to extend thanks to my other committee member, Dr. Brian Diers for his thoughtful comments and criticisms. In addition, I would like to thank lab members who provided help in the greenhouse and field, especially Ursula Reuter-Carlson and Keith Ames. Special thanks to Sarah Schultz for the endless encouragement and advice throughout my time here. Lastly, I thank my family, friends, and Kyle for the constant support and love throughout my education.

TABLES OF CONTENTS

CHAPTER ONE
Literature Review

Soybean (*Glycine max* (L.) Merrill) is one of the most important legume crops in the world. According to the United States Department of Agriculture - World Agricultural Supply and Demand Estimates (2016), an estimated 283 million metric tons of soybeans are produced worldwide. The United States is the world's leading producer. In the U.S., 87% of the crop is produced in the north-central states, with Iowa, Illinois, Minnesota, and Indiana being the leading producers (Wilcox, 2004). Rincker et al. (2014) estimated the linear rate of genetic yield gain for soybean was 23 kg ha^{-1} yr^{-1} in maturity groups II and III, and 20 kg ha^{-1} yr^{-1} in maturity group IV. While the study did not reflect the entire soybean production area in the U.S., it does represent 75% of the total amount of production area. Soybean breeders have helped to increase seed yield for the past 80 years due to the genetic improvement and development of cultivars that have longer seed-filling periods, decreased lodging, and increased disease resistance (Egli, 2008; Rincker et al., 2014; Wilcox 2001).

Seedling diseases caused by species of *Pythium*, *Rhizoctonia*, *Fusarium*, and *Phomopsis* pathogens accounted for an estimated combined total of 12,539,000 metric tons of soybean yield losses from 1996-2009 (Wrather and Koenning, 2009; Koenning and Wrather, 2010). Seedling diseases occur in both the northern and southern U.S. (Koenning and Wrather, 2010; Rizvi and Yang, 1996). Between 2006 and 2009, seedling diseases were estimated to account for the second most yield reduction of soybean of all diseases/pathogens behind soybean cyst nematode (*Heterodera glycines*) (Koenning and Wrather, 2010). Cool, wet soils favor most seedling disease pathogens that reduce stands and cause root rot, especially *Pythium* spp. (Broders et al., 2007; Ellis et al., 2011). A study in Iowa showed that *Pythium* spp. and *Phytophthora sojae*

seemed to be the most important components of the seedling disease complex based on the frequency with which these pathogens were isolated from diseased soybean seedlings (Rizvi and Yang, 1996).

Pythium spp. are soilborne oomycetes that affect seedlings of crops grown in the Midwest, especially soybean and maize (*Zea mays*) (Zhang and Yang, 2000). Affected seedlings show symptoms of seed rot, root rot, and damping-off that can lead to poor emergence and reduced plant stands (Brown and Kennedy, 1965; Yang et al., 1999). Seed rot occurs when a pathogen attacks the radicle of a seed just after its eruption from the seed coat. Damping-off occurs when the hypocotyl arch is infected by the pathogen during emergence, and the cells die from infection (Brown and Kennedy, 1965). Root rot symptoms vary depending on the stage of the plant when it becomes infected and the severity of infection. Mild infection causes discoloration and small necrotic lesions on the root tips, while more severe infection results in a diminished tap root and secondary roots (Yang, 1999).

Oomycetes, which includes the genus *Pythium*, belong to the kingdom Straminipila and are no longer part of the kingdom Mycota (fungi). Oomycetes differ from true fungi in sexual reproduction, the nuclear state of vegetative mycelium, cell wall composition, and the type of flagella on the zoospore (Rossman and Palm, 2006). Oomycetes also include the highly important plant pathogens in the genus *Phytophthora*, including *Phytophthora sojae*, one of the most economically important pathogens of soybean. Species of *Pythium* have the ability to be saprophytic or plant-parasitic, depending on their life stage. In unfavorable conditions, the plant-pathogenic *Pythium* spp. may be saprophytic in the soil, while in favorable environments they may be parasitic on plants, with different species having different levels of pathogenicity (van der Plaats-Niterink, 1981). The severity of the disease is determined primarily by the initial

amount of inoculum, host age, and environmental conditions at the time of infection (Yang, 1999).

The life cycle of plant-pathogenic species of *Pythium* on soybean is monocyclic, and secondary spread during the season is not usual (Yang, 1999). Oospores are the overwintering survival structures for *Pythium* spp. These have the ability to survive unfavorable environmental conditions while inhabiting the soil for multiple years. They can be thick-walled, but change into thin-walled oospores with adequate soil moisture, moderate temperatures (25^0C) and a pH near 7.0 (Fry and Grunwald, 2010; Lumsden and Ayers, 1975). *Pythium* spp. can reproduce either sexually or asexually (Fry and Grunwald, 2010). Sexual reproduction occurs when the antheridium, containing the male nucleus, fertilizes the oogonium, containing the female nucleus, producing a zygote that then forms a thick wall and becomes an oospore (Allen et al., 2004; van der Plaats-Niterink, 1981). The oospore can have one of two responses once it changes into a thin-walled oospore at germination: (1) it can form a germ tube that then develops into a mycelium, or (2) it produces the sporangium that subsequently produces a vesicle in which zoospores are formed (Agrios, 2005; Lumsden and Ayers, 1975; van der Plaats-Niterink, 1981). In *Pythium* spp., zoospores are part of the asexual reproduction process and are not formed on the sporangium itself, but in a vesicle outside of it, from which zoospores are released under wet conditions (van der Plaats-Niterink, 1981). Once the zoospores are freed, they use their flagella to swim in the free water of the soil towards a host plant. Once on the plant's surface the zoospore will encyst at the infection site, geminating and forming a hyphal germ tube that leads to an appressorium and then a penetration peg that will allow the pathogen to infect the germinated seed or emerging seedling (Allen et al., 2004; van der Plaats-Niterink, 1981). Mycelia colonize the plant tissue by growing inter-cellularly throughout the plant (Agrois, 2005).

The mycelium has the ability produce oogonia and antheridia that then goes through sexual reproduction to produce the overwintering oospore (Lumsden and Ayers, 1975; van der Plaats-Niterink, 1981).

The life cycle of *Pythium* spp. can continue as long as there is a favorable environment and susceptible young host plants. Young shoots and roots become infected when mycelia penetrate the epidermal and cortical cells and break down cells walls, causing the plant tissue to collapse (Agrios, 2005). The pathogenic capacity is largely determined by the availability of pectolytic and cellulolytic enzymes in the pathogen (van der Plaats-Niterink, 1981). Mature tissue has considerable physical resistance against the mechanical pressure of the mycelium, and thus has the ability to better withstand the pathogen's enzymes, helping it to survive the attack (Agrios, 2005). *Pythium* spp. that affect soybean generally infect seeds and the roots of young seedlings.

Pythium species that are pathogenic on soybean can be found wherever the crop is grown. *P. ultimum* is one of the most commonly found species in fields across the U.S. soybean production areas (Brown and Kennedy, 1965; Dorrance et al., 2004; Grau et al., 2004; Rizvi and Yang, 1996; Rupe et al., 2011; Zhang and Yang, 2000). It grows in temperatures from a minimum of 5^0C to a maximum of 35^0C, with optimal growth around 25^0C. Temperatures below 23^0C are most favorable for infection of roots by *P. ultimum* (van der Plaats-Niterink, 1981). In Minnesota, *P. ultimum* was reported as being one of the most prevalent pathogens that caused pre-emergence damping off of soybean (Brown and Kennedy, 1965). *P. ultimum* was also reported to be the main component of the pathogen complex associated with early seedling diseases in Virginia (Griffin, 1990). *P. ultimum* can be split into two different subspecies: *P. ultimum* var. *ultimum* and *P. ultimum* var. *sporangiiferum*. *P. ultimum* var. *sporangiiferum* is

rarely found in soils, while *P. ultimum* var. *ultimum* is found more commonly and has worldwide distribution (Levesque and de Cock, 2004). The physiological difference between the two sub-species is that *P. ultimum* var. *sporangiiferum* is able to produce sporangia and zoospores at 20^0C, while *P. ultimum* var. *ultimum* usually develops hyphal swellings (Levesque and de Cock, 2004; van der Plaats-Niterink, 1981). Broders et al. (2009) reported that, within a sequenced 226 base-pair region, there were 11 base pair differences as well as five insertions in *P. ultimum* var. *sporangiiferum* compared to *P. ultimum* var. *ultimum*.

Management of diseases caused by *Pythium* spp. and other soilborne oomycetes requires a combination of improved soil drainage, tillage, crop rotation, resistant host plants, and fungicide seed treatments (Grau et al., 2004). Fields that have had multiple occurrences of disease incidence should have tile installed to improve drainage, and the fields should be tilled in the spring to increase soil temperature (Yang, 1999). Crop rotation alone is not an effective management tactic, especially in corn-soybean rotations where *Pythium* spp. population levels are high (Grau et al, 2004; Zhang and Yang, 2000; Broders et al., 2007). The two current management practices that show the most promise are host plant resistance and fungicide seed treatments.

Host plant resistance in soybean has been used since Chinese farmers started saving their best seeds for the next year's planting. Soybean breeders have been working on developing disease resistant cultivars since the start of commercial breeding. The development of commercial cultivars with multiple disease resistance has had a major impact in reducing economic losses (Palmer et al., 2004). While it is rare, host resistance to diseases caused by *Pythium* spp. has been reported in soybean. The cultivar Archer (Cianzio et al., 1991) has been shown to have some resistance to *P. ultimum* and other *Pythium* species (Bates et al., 2008;

Kirkpatrick et al., 2006). Kirkpatrick et al. (2006) examined the impact that *P. ultimum* had on the soybean cultivars Archer and Hutcheson in flooded environments, and evaluated the relationship between the pathogen and flooding tolerance of these two cultivars. In this study, both cultivars were affected by *P. ultimum*, but Archer had decreased disease symptoms and appeared to be more resistant when compared to Hutcheson. Bates et al. (2008) also evaluated the reactions of Archer and Hutcheson to different species of *Pythium*. The results of those assays indicated that Archer had statistically greater stands and root weights and less root discoloration than Hutcheson across the inoculated species of *Pythium* and over a range of plant developmental stages. This study helped confirm that Archer has partial resistance against *P. ultimum*.

Rosso et al. (2008) investigated the inheritance of resistance to *P. aphanidermatum* in the cultivar Archer, by identifying SSR markers linked to the resistance gene and by mapping the resistance gene in the genome. A population from the cross of Archer × Hutcheson was used for the mapping experiment that contained 86 $F_{2:4}$ lines. Archer was confirmed to be more resistant than Hutcheson based on the inheritance of resistance to *P. aphanidermatum*. The mapping population segregated in a 1 (resistant):2 (segregating):1 (susceptible) ratio which suggested a single dominant gene for resistance. Two markers, Satt510 and Satt114, from molecular linkage group F, were found to be associated with the resistant and susceptible phenotypes. This was the first report of a single dominant gene conferring resistance to Pythium damping off and root rot in soybean caused by *P. aphanidermatum*, which was named *Rpa1*.

Ellis et al. (2013) evaluated a set of soybean germplasm for resistance to *P. irregulare*. Public cultivar and plant introductions (PI) that had known *Rps* genes for *Phytophthora sojae* resistance as well as resistance to other soybean pathogens were used in this study. Of the 65

soybean genotypes evaluated, approximately one-third were moderately to highly resistant to *P. irregulare* based on root weights and root rot scores. PI 424354 was the most resistant genotype, which showed a high level of partial resistance, which was likely due to several genes (quantitative resistance).

Identification of sources of resistance is a prerequisite to the development of resistant cultivars. Screening germplasm for resistance to soilborne pathogens is costly and time consuming; therefore, screening a subset of germplasm accessions that represents a larger set of germplasm would be ideal. One germplasm subset that could be used for this is the ancestral lines of modern North American soybean cultivars (Carter et al., 2004). Gizlice et al. (1994) identified groups of plant introductions (ancestors) and progeny lines and cultivars derived from them (first progeny) which contributed 99% of the genes found in North American public cultivars released between 1947 and 1988. The North American ancestral soybean lines include both ancestors with unknown pedigrees and their first progeny. Gizlice et al. (1994) defined first progeny as cultivars and breeding lines that resulted from controlled hybridization of the original plant introductions, some of which were genetically heterogeneous at the time they were brought to the U.S. The so-called first progeny are more homogeneous and often are recorded more accurately as parents in pedigrees (Gizlice et al, 1994; Hymowitz and Bernard, 1991).

Statistical analysis of pedigree information is difficult to put into a numerical value. A coefficient of parentage value is used commonly to measure the degree of genetic relatedness of cultivars developed through hybridization. Coefficient of parentage is the probability that two cultivars are identical by descent at a random locus (Carter et al., 2004; Gizlice et al., 1994). Using coefficients of parentage, one can quantify (1) patterns of relatedness among cultivars, (2) the magnitude and importance of genetic drift, and (3) the genetic base for crop breeding (Gizlice

et al, 1994). A larger coefficient of parentage value indicates a closer relationship (St. Martin, 1982). For example, if the coefficient of parentage is 0, there is no relationship between the cultivars, and they do not have an ancestor in common. A value of 0.25 represents a half-sibling, 0.5 signifies a full sibling, and a value of 1 means that two individuals are identical (Carter et al., 2004). However, there are some limitations to using coefficient of parentage estimates, including (1) the lack of information on genetic relationships among the founding stock and (2) the inability to estimate breeder selection effects on relatedness of cultivars (Gizlice et al., 1993). Despite these limitations, however, coefficients of parentage are still useful to help determine relatedness between cultivars. When using coefficient of parentage in soybean, it is generally assumed that (1) ancestral lines are equally unrelated and (2) selection has no effect on allelic frequencies (Gizlice et al., 1993). The coefficient of parentage between elite lines and each originating ancestor is indicative of the relative contribution of the ancestors to the parentage of elite soybean lines (Sneller, 1994).

The coefficient of parentage values show that the genetic base of North American soybean breeding is not large. Fewer than 20 soybean ancestors accounted for 84% of the genetic base of the soybean cultivars that had been released prior to 1989, while more than half of the base originates from just six ancestors: Mandarin (Ottawa), CNS, Richland, S-100, and the presumed two unknown parents of Lincoln (Gizlice et al., 1994). Lincoln, CNS, S-100, Mandarin (Ottawa), Richland, and Dunfield combined accounted for approximately 61.2%, 59.7%, and 73.2% of the overall, northern, and southern elite parentage respectively (Sneller, 1994). Despite this small genetic base of North American soybean cultivars, there still remains a large amount of diversity in morphological and biochemical traits that has the possibility to increase soybean disease resistance while increasing yield (Gizlice et al., 1993). Kisha et al.

(1998) investigated the diversity among five gene pools consisting of ancestral lines, elite lines of northern cultivars, elite southern cultivars, northern plant introductions, and southern plant introductions. They found that the average diversity among land races (0.37) was greater than that for the ancestral cultivars (0.26), which was still greater than that of the cultivars (0.16). Based upon the average percent of heterozygosity across all loci for each pool of cluster analysis, the ancestral pool was determined to be the most diverse, while the southern elite cultivars were the least diverse. While the ancestral lines are diverse, they fall into two distinct groups: the Northern and Southern gene pools. These pools are separated by maturity, with the northern pool consisting of maturity groups 000 to IV and the southern pool having maturity groups V to IX (Hymowitz and Bernard, 1991). Within the two groups, there is more diversity among the northern lines than among the southern lines, even though both pools derive much of their parentage from just a handful of ancestral lines (Sneller, 1994; Gizlice et al., 1993; Gizlice et al., 1994). Inspection of the pedigrees shows that the coefficients of parentage between the southern and northern cultivars are low, suggesting that breeders have been maintaining these two distinct gene pools since 1947 (Gizlice et al., 1993). Delannay et al. (1983) evaluated the relative genetic contributions of ancestral lines of the northern and southern U.S. and Canadian soybean cultivars released in four successive time periods (before 1951, 1951-1960, 1961-1970, and 1971-1981). They examined trends in germplasm usage leading to the soybean gene pool in 1983, and identified three main trends with the data: (1) the number of ancestral lines increased over time as new sources of germplasm were introduced into their respective gene polls, (2) more introductions contributed to the northern gene pool than to the southern gene pool, and (3) a few plant introductions became increasingly predominant over time in their relative contributions to the gene pools. From 1971 to 1981, four introductions contributed to more than 50% of the

germplasm and ten introductions contributed more than 80% to the northern gene pool, whereas CNS and S-100 contributed more than 50% of the genes and seven introductions contributed to 80% of the genes in the southern pool (Delannay et al., 1983). Even though only a few plant introductions have contributed greatly to the gene pools, there is still diversity within and between the pools. The diversity between gene pools is primarily due to the gene frequency difference and not from the presence or absence of unique alleles. This pattern of diversity in the elite populations can be explained by molecular diversity among a few major ancestors (Kisha, 1998).

Gizlice et al. (1994) identified a core set of soybean cultivars for evaluating the presence, absence, and distribution of traits in North American cultivars. This set consisted of 91 first progeny and ancestors that contributed more than 99% of the genes found in modern cultivars. These consisted of five breeding lines, eight older cultivars, and 78 recently developed cultivars (Gizlice et al., 1994). These 91 lines are available from the USDA germplasm collection and have been used in screens for resistance against *Macrophomina phaseolina* (Pawlowski et al., 2015), *Fusarium virguliforme* (Mueller et al., 2003), *Rhizoctonia solani* (Bradley et al., 2001), and multiple soybean viruses (Wang et al., 2005).

Seed treatments have become a popular crop management practice recently (Esker and Conley, 2012; Douglas and Tooker, 2015). Despite the fact that species of *Pythium* are oomycetes and not fungi, they are sensitive to some fungicides used to treat seeds prior to planting. Crops are being planted increasingly earlier each spring, when soils are not yet warm enough to support quick seed germination, thereby enhancing opportunities for pathogens to infect seeds and seedlings (Broders et al., 2007; Esker and Conley, 2012). According to industry estimates, only 8% of the soybean seeds planted in 1996 were treated with fungicides, while in

2008 at least 30% of the seeds were treated (Munkvold, 2009). The trend increased since Monsanto and Pioneer Hi-Bred began to routinely use seed treatments on their major soybean product lines in 2009 (Munkvold, 2009). Fungicide seed treatments are commonly used to protect seeds and seedlings from soilborne pathogens. Fungicide seed treatments in soybean have been shown to prevent stand and yield loss, especially under cool and moist soil conditions (Bradley, 2008). There are two types of fungicides that are applied: those that affect a specific pathogen group and those that have broad spectrum activity (Urrea et al., 2013). Two fungicide active ingredients commonly used as seed treatments on soybean that have site-specific modes of action that affect only oomycetes are metalaxyl and mefenoxam (Broders et al., 2009). Fungicides such as azoxystrobin and trifloxystrobin have broad spectrum activity against several species of fungi and oomycetes (Urrea et al., 2013). Metalaxyl and mefenoxam have been used for years as part of a management strategy against species of *Pythium* and *Phytophthora,* but with continual use of these active ingredients, a decrease in sensitivity towards these fungicides has been observed in some *Pythium* species (Broders et al., 2007; Dorrance et al., 2004). Dorrance et al. (2004) indicated that the repeated use of metalaxyl and mefenoxam alone for seed treatment may have selected for insensitive strains of *Pythium* species. Combining metalaxyl or mefenoxam with broad spectrum fungicides has been shown to improve plant stands compared to non-treated seed (Urrea et al., 2013).

Although seed treatments are not considered to be at high-risk for fungicide resistance development, applying the same seed treatment or similar active ingredients year after year can lead to reduced sensitivity (Munkvold, 2009; Dorrance et al., 2004). A new chemistry on the market to protect seeds and seedlings from oomycete pathogens is ethaboxam (Kim et al., 2004). While metalaxyl (Allegiance, Bayer Crop Science) and mefenoxam (Apron XL, Syngenta) have

been commercially available for a relatively long time, ethaboxam has been approved recently for use on soybean in the U.S., and is available in the product known as Intego Suite (Valent USA Corp., Walnut Creek, CA).

Fungicides can be classified by their mode of action (MOA). A fungicide's MOA refers to how it disrupts a pathogen's biosynthetic pathway. Metalaxyl and mefenoxam both belong to the phenylamide MOA group. Fungicides in the phenylamide group inhibit nucleic acid synthesis at a target site in the RNA polymerase I reaction, which has an effect on the mitosis of the pathogen cells (Fungicide Resistance Action Committee, 2015; Fisher and Hayes, 1982). Ethaboxam belongs to the thiazole carboxamide MOA group which affects mitosis and cell division, with its target site in the beta-tubulin assembly in mitosis (Fungicide Resistance Action Committee, 2015). This different MOA has the potential to protect seeds and seedlings from strains of oomycete pathogens with reduced sensitivity to phenylamide fungicides such as mefenoxam and metalaxyl.

Ethaboxam was originally developed and commercialized in Korea in 1999 to be used on vegetable crops for protection against diseases caused by oomycete pathogens (Kim et al., 1999; Kim et al., 2004). Kim et al. (1999) were the first to report on the fungicidal activity of ethaboxam under field conditions in Korea on cucumber, potato, and pepper. They reported that ethaboxam controlled diseases caused by *Pseudoperonospora cubensis*, *Phytophthora infestans* and *Phytophthora capsici* better than metalaxyl. They also noted that new fungicides were needed with different MOAs to control *P. infestans* and *P. capsici* in Korea. Zhang et al. (2005) tested ethaboxam in potato field trials in Korea against *P. infestans.* Many field populations of *P. infestans* in Korea were moderately resistant or resistant to metalaxyl. Of 687 *P.infestans* isolates tested in 2003 and 2004, only 3% were sensitive to metalaxyl. In contrast, ethaboxam

had a control efficacy of 80.4% and 81.9% in 2003 and 2004, respectively. In controlled environment studies conducted in the laboratory and greenhouse, Kim et al. (2004) discovered that ethaboxam was more persistent on seedlings, which led to a higher suppression of disease development compared to fluazinam at all levels of active ingredient concentration. They also reported that ethaboxam inhibited the growth of nine *P. infestans* isolates, and that out of those nine, eight were less sensitive or resistant to metalaxyl.

Seedling diseases can be difficult to manage because multiple pathogen species are often part of the disease complex (Broders et al., 2007). Fungicides are a good option for managing soilborne pathogens, but if used continuously, they impose selection pressure on pathogen species, and reliance solely on seed treatments is unlikely to be a sustainable management method. Finding plant host resistance will offer more options to farmers to prevent stand and yield losses from seedling diseases.

LITERATURE CITED

Agrios, G. 2005. Plant pathology (Fifth ed.) San Diego: Academic Press.

Allen, T.W., A. Martinez, and L.L. Burpee. 2004. Pythium blight on turfgrass. The Plant Health Instructor. doi: 10.1094/PHP-I-2004-0929-01

Bates, G.D., C.S. Rothrock, and J.C. Rupe. 2008. Resistance of the soybean cultivar Archer to Pythium damping-off and root rot caused by several *Pythium* spp. Plant Dis. 92:763-766.

Bradley, C.A. 2008. Effect of fungicide seed treatments on stand establishment, seedling disease, and yield of soybean in North Dakota. Plant Dis. 92: 120-125.

Bradley, C. A., Hartman, G. L., Nelson, R. L., Mueller, D. S., and Pedersen, W. L. 2001. Response of ancestral soybean lines and commercial cultivars to Rhizoctonia root and hypocotyl rot. Plant Dis. 85:1091-1095.

Broders, K.D., P.E. Lipps, P.A. Paul, and A.E. Dorrance. 2007. Characterization of *Pythium* spp. associated with corn and soybean seed and seedling diseases in Ohio. Plant Dis. 91:727-735.

Broders, K. D., M.W. Wallhead, G.D. Austin, P.E. Lipps, P.A. Paul, R.W. Mullen, and A.E. Dorrance. 2009. Association of soil chemical and physical properties with *Pythium* species diversity, community, composition, and disease incidence. Phytopathology 99:957-967.

Brown, G.E. and B.W. Kennedy. 1965. Pythium pre-emergence damping-off in soybean in Minnesota. Plant Dis. 49:646-647.

Carter, T.E., R.L. Nelson, C.H. Sneller, and Z. Cui. 2004. Genetic diversity of soybean. In H.R. Boerma and J.E. Specht, Soybean: Improvement, Production, and Uses (Third Ed. pg. 303-416) Madison, WI: American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of American, Inc.

Cianzio, S.R., S.P. Shultz, W.R. Fehr, and H. Tachibana. 1991. Registration of 'Archer' soybean. Crop Sci. 31:1707.

Delannay, X., D. M. Rodgers, and R.G. Palmer. 1983. Relative genetic contributions among ancestral lines to North American soybean cultivars. Crop Sci. 23:944-949.

Dorrance, A. E., S.A. Berry, P. Bowen, and P.E. Lipps. 2004. Characterization of *Pythium* spp. from three Ohio fields for pathogenicity on corn and soybean and metalaxyl sensitivity. Online. Plant Health Progress. doi: 10.1094/PHP-2004-0309-01-RS

Douglas, M.R. and J.F. Tooker. 2015. Large scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. Environ. Sci. Technol. 49:5088-5097.

Egli, D.B. 2008. Comparison of corn and soybean yields in the United States: historical trends and future prospects. Agron. J. 100:S-79-S-88.

Ellis, M. L., K.D. Broders, P.A. Paul, and A.E. Dorrance. 2011. Infection of soybean seed by *Fusarium graminearum* and effect of seed treatments on disease under controlled conditions. Plant Dis. 95:401-407.

Ellis, M.L., L.K. McHale, P.A. Paul, S.K. St. Martin, and A.E. Dorrance. 2013. Soybean germplasm resistant to *Pythium irregulare* and molecular mapping of resistance quantitative trail loci derived from the soybean accession PI 424354. Crop Sci. 53:1008-1021.

Esker, P.D. and S.P. Conley. 2012. Probability of yield response and breaking even for soybean seed treatments. Crop Sci. 52:351-359.

Fisher, D.J. and A.L. Hayes., 1982. Mode of action of the systemic fungicides furalaxyl, metalaxyl, and ofurace. Pestic. Sci. 13:330-339.

Fungicide Resistance Action Committee. 2015. FRAC code list 2015: Fungicides sorted by mode of action (including FRAC code numbering). January 2015. http://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2016.pdf?sfvrsn=2

Fry, W.E. and N.J. Grunwald. 2010. Introduction to oomycetes. The Plant Health Instructor. doi: 10.1094/PHI-I-2010-1207-01

Gizlice, Z., T.E. Carter, and J.W. Burton. 1993. Genetic diversity in North American soybean: I. multivariate analysis of founding stock and relation to coefficient of parentage. Crop Sci. 33:614-620.

Gizlice, Z., T.E. Carter, and J.W. Burton. 1994. Genetic base for North American public soybean cultivars released between 1947-1988. Crop Sci. 34:1143-1151.

Grau, C.R, A.E. Dorrance, J. Bond, and J.S. Russin. 2004. Fungal Diseases. in H.R. Boerma and J.E. Specht, Soybean: Improvement, Production, and Uses (Third Ed. pg. 679-764) Madison, WI: American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of American, Inc.

Griffin, G.J. 1990. Importance of *Pythium ultimum* in a disease syndrome of cv. Essex soybean. Can. J. of Plant Pathol. 12:135-140.

Hymowitz, T. and R.L Bernard. 1991. Origin of the soybean and germplasm introduction and development in North America. Use of Plant Introduction in Cultivar Development, Part 1, Crop Science Society of America, Special Publication No. 17. Pg 147-164.

Kim, D.S., S.J. Chun, J.J. Jeon, S.W. Lee., and G.H. Joe. 2004. Synthesis and fungicidal activity of ethaboxam against oomycetes. Pest Manag. Sci. 60:1007-1012.

Kim, D.S., H.C. Park,S.J. Chun, S.H. Yu, K.J. Choi, J.H. Oh., K.H. Shin, Y.J. Koh, B.S. Kim, Y.I. Ham, and B.K. Chung. 1999. Field performance of a new fungicide ethaboxam against cucumber downy mildew, potato late blight, and pepper Phytophthora blight in Korea. Plant Pathol. J. 15:48-52.

Kirkpatrick, M. T., C.S. Rothrock, J.C. Rupe, and E.E. Gbur. 2006. The effect of *Pythium ultimum* and soil flooding on two soybean cultivars. Plant Dis. 90:597-602.

Kisha, T.J., B.W. Diers, J.M. Hoyt, and C.H. Sneller. 1998. Genetic diversity among soybean plant introductions and North American Germplasm. Crop Sci. 38:1669-1680.

Koenning, S.R. and J.A. Wrather. 2010. Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. Online. Plant Health Progress. doi:10.1094/PHP-2010-1122-01-RS

Levesque, C.A. and A.W. de Cock. 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. Mycol. Res. 108:1363-1383.

Lumsden, R.D. and W.A. Ayers. 1975. Influence of soil environment on the germinability of constitutively dormant oopsores of *Pythium ultimum*. Phytopathology 65:1101-1107.

Mueller, D.S., R.L Nelson, G.L. Hartman, and W.L. Pedersen. 2003. Response of commercially developed soybean cultivars and the ancestral soybean lines to *Fusarium solani* f. sp. *glycines*. Plant Dis. 87:827-831.

Munkvold, G. 2009. Seed pathology in academia and industry. Ann. Rev. Phytopathol. 47:285-311.

Palmer, R.G., T.W. Pfeiffer, G.R. Buss, and T.C. Kilen. 2004. Qualitative Genetics. in H.R. Boerma and J.E. Specht, Soybean: Improvement, Production, and Uses (Third Ed. pg. 1-14) Madison, WI: American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of American, Inc.

Pawlowski, M.L., C.B. Hill, and G.L. Hartman. 2015. Resistance to charcoal rot identified in ancestral soybean germplasm. Crop Sci. 55:1230-1235.

Rizvi, S.S.A. and X.B. Yang. 1996. Fungi associated with soybean seedling disease in Iowa. Plant Dis. 80:57-60.

Rincker, K., R. Nelson, J. Specht, D. Sleper, T. Cary, S.R. Cianzio, S. Casteel, S. Conley, P. Chen, V. Davis, C. Fox, G. Graef, C. Godsey, D. Holshouser, G.L. Jiang, S.K. Kantartzi, W. Kenworthy, C. Lee, R. Mian, L. McHale, S. Naeve, J. Orf, V. Poysa, W. Schapaugh, G. Shannon, R. Uniatowski, D. Wang, and B. Diers. 2014. Genetic improvement of U.S. soybean in maturity groups II, III, and IV. Crop Sci. 54:1419-1432.

Rossman, A. and M. Palm. 2006. Why are *Phythothora* and other oomycota not true fungi? Outlooks on Pest Management. 17:217-219.

Rooso, M.L., J.C. Rupe., P. Chen, and L.A. Mozzoni. 2008. Inheritance and genetic mapping of resistance to *Pythium* damping-off caused by *Pythium aphanidermatum* in 'Archer' soybean. Crop Sci. 48:2215-2222.

Rupe, J.C., Rothrock, C.S., Bates, G., Rosso, M. L.,Avanzato, M. V. and Chen, P. 2011. Resistance to Pythium seedling disease in soybean. in (Ed) A. Sudaric, Soybean-Molecular Aspects of Breeding. InTech. Croatia.

Sneller, C.H. 1994. Pedigree analysis of elite soybean lines. Crop Sci. 34:1515-1522.

St. Martin, S.K. 1982. Effective population size for the soybean improvement program in maturity groups 00 to IV. Crop Sci. 22:151-152.

United States Department of Agriculture - World Agricultural Supply and Demand Estimates (USDA-WASDE). WASDE – 549. 12 January 2016. USDA, WASDE, Washington D.C. http://www.usda.gov/oce/commodity/wasde/latest.pdf

Urrea, K., J.C. Rupe, and C.S. Rothrock. 2013. Effect of fungicide seed treatments, cultivar, and soils on soybean stand establishment. Plant. Dis. 97:807-812.

van der Plaats-Niterink, J. 1981. Monograph of the genus *Pythium*. Stud. Mycol. 21:1-244.

Wang, Y., H.A. Hobbs, C.B. Hill, L.L. Domier, G.L. Hartman, and R.L. Nelson. 2005. Evaluation of ancestral lines of U.S. soybean cultivars for resistance to four soybean viruses. Crop Sci. 45:639-644.

Wilcox, J.R. 2001. Sixty years of improvement in publicly developed elite soybean lines. Crop. Sci. 49:1711-1716.

Wilcox, J.R. 2004. World distribution and trade of soybean. in H.R. Boerma and J.E. Specht, Soybean: Improvement, Production, and Uses (Third Ed. pg. 1-14) Madison, WI: American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of American, Inc.

Wrather, J.A., and S.R. Koenning, 2009. Effects of diseases on soybean yields in the United States 1996-2007. Online. Plant Health Progress. doi:10.1094/PHP-2009-0401-01-RS.

Yang, X. 1999. Pythium damping off and root rot. In Eds. G.L. Hartman, J.B. Sinclair, and J.C. Rupe. Compendium of Soybean Diseases (Fourth ed. pg. 42-44). St. Paul, MN: The American Phytopathological Society.

Zhang, B.Q. and X.B. Yang. 2000. Pathogenicity of *Pythium* populations from corn-soybean rotation fields. Plant Dis. 84:94-99.

Zhang, X.Z., K.Y. Ryu, J.S. Kim, J.U. Cheon, and B.S. Kim. 2005. Changes in the sensitivity to metalaxyl, dimethomorph, and ethaboxam of *Phytophthora infestans* in Korea. Plant Pathol. J. 21:33-38.

CHAPTER TWO

Effects of *Pythium ultimum* var. *ultimum* and other *Pythium* species on the North American Ancestral Soybean Lines

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the most important legume crops in the world. In the United States, 87% is produced in the north-central states, with Iowa, Illinois, Minnesota, and Indiana being the leading producers (Wilcox, 2004). Seedling diseases caused by *Pythium*, *Rhizoctonia*, *Fusarium*, and *Phomopsis* pathogens accounted for an estimated combined total of 12,539,000 metric tons of soybean yield losses from 1996-2009 (Wrather and Koenning, 2009; Koenning and Wrather, 2010). Between 2006 and 2009, seedling diseases were estimated to account for the second most yield reduction of soybean of all diseases/pathogens behind soybean cyst nematode (*Heterodera glycines*) (Koenning and Wrather, 2010). A study in Iowa showed that *Pythium spp*. and *Phytophora sojae* are among the most important components of the seedling disease complex based on the frequency with which these pathogens were isolated from diseased soybean seedlings (Rizvi and Yang, 1996).

Pythium spp. are soilborne oomycetes, and species that generally are most damaging to soybean are favored by cool and wet soils. Symptoms caused by *Pythium* spp. that infect soybean include seed rot, root rot, and damping-off, which can lead to poor emergence and reduced plant stands (Broders et al., 2007; Brown and Kennedy, 1965; Yang et al., 1999). The severity of disease is determined primarily by the initial amount of inoculum, host age, and environmental conditions at the time of infection (Yang, 1999). Species of *Pythium* have worldwide distribution and several *Pythium* species and isolates can be found in the soil or on

diseased root tissue (van der Plaats-Niterink, 1981). Broders et al. (2007) collected 105 *Pythium* isolates from diseased soybean seedlings from 30 locations in Ohio, which represented 11 different species of *Pythium*. In a different study from Ohio, researchers recovered over 7,000 isolates of *Pythium,* which represented 21 different species from 88 locations in 2006 and 2007 (Broders et al., 2009). Jiang et al. (2012) recovered 186 isolates from 12 corn-soybean rotation fields in Illinois which represented 27 different species of *Pythium*. In all of the collections, isolates were identified to species using digestion circularization polymerase chain reaction (DC-PCR), with internal transcribed spacers sequence of nuclear ribosomal DNA, and morphological traits (Broders et al., 2007; Broders et al., 2009; Jiang et al., 2012). With a vast amount of species diversity, it is often hard to establish which *Pythium* species are the causal agents of Pythium root and seed rot and damping off at a particular time (Broders et al., 2007).

Pythium ultimum var. *ultimum, Pythium irregulare,* and *Pythium sylvaticum* are pathogenic on soybean and other field crops that are commonly found in fields across U.S. soybean production regions (Broders et al, 2007; Broders et al., 2009; Dorrance et al., 2004; Grau et al., 2004; Griffin et al., 1990; Rupe et al., 2011). *P. ultimum* var. *ultimum* grows best in temperatures ranging from 5^0C to 35^0C, with temperatures below 23^0C most favorable for infection. *P. irregulare* grows in temperatures between 1^0C and 35^0C, with 30^0C being the optimal temperature, whereas *P. sylvaticum* grows optimally at 25^0C, but can grow at a minimum of 5^0C to a maximum of 35^0C to 40^0C (van der Plaats-Niterink, 1981). *P. ultimum* is one of the most common species associated with seedling diseases across the major U.S. soybean production areas (Broders et al., 2007; Brown and Kennedy, 1965; Dorrance et al., 2004; Griffin, 1990; Rizvi and Yang 2000; Zhang and Yang, 2000).

Management of diseases caused by *Pythium* spp. and other soilborne oomycetes requires a combination of improved soil drainage, tillage, crop rotation, resistant host plants and fungicide seed treatments (Grau et al., 2004). Host plant resistance and fungicide seed treatments show the most promise as long term management tactics. While it is rare, host resistance to *Pythium* spp. has been reported in soybean. The cultivar Archer (Cianzio et al., 1991) has been shown to have some resistance to *P. ultimum* (Bates et al., 2008; Kirkpatrick et al., 2006). Cultivars Archer and Hutcheson, were both affected by *P. ultimum*, but Archer had fewer disease symptoms and appeared to be more resistant than Hutcheson (Kirkpatrick et al., 2006). Bates et al. (2008) also evaluated the reactions of Archer and Hutcheson to different species of *Pythium*. They found that Archer had significantly greater stands and root weights, and less root discoloration than Hutcheson across the inoculated species of *Pythium* and over a range of plant developmental stages. Rosso et al. (2008) investigated the inheritance of *P. aphanidermatum* resistance from the cultivar Archer, identified SSR markers linked to the resistance gene, and mapped the resistance gene in the genome to molecular linkage group F (chromosome 13). Ellis et al. (2013) evaluated a subset of soybean germplasm that had partial resistance to *Phytophthora sojae*, to find resistance to *P. irregulare*. Plant Introduction (PI) 424354 was the most resistant genotype, which showed a high level of partial resistance likely due to several genes (quantitative resistance). This land race from South Korea also has resistance to *Fusarium graminearum* and at least 12 races of *Phytophthora sojae* (Dorrance and Schmitthenner, 2000; Ellis et al., 2013).

Identification of sources of resistance is a prerequisite to the development of resistant cultivars. Although high levels of resistance to *Pythium* spp. have not been reported in soybean it is possible that some modern North American cultivars other than Archer have inherited a

moderate level of resistance from one or more of their ancestors. In the case of resistance to *P. ultimum* var. *ultimum* this possibility has not been investigated.

Gizlice et al. (1994) identified a core set of soybean ancestors and older cultivars for the purpose of evaluating the presence, absence, or distribution of traits in North American cultivars. This set consisted of 91 ancestor and first progeny lines that contributed more than 99% of the genes found in 258 public cultivars released between 1947 and 1988. These consisted of five breeding lines, eight older cultivars, and 78 cultivars released before 1988 (Gizlice et al., 1994). These 91 lines are available from the United States Department of Agriculture (USDA) Soybean Germplasm Collection (Dr. Randall Nelson, USDA-ARS, Urbana, IL) and have been evaluated for resistance against *Macrophomina phoseolina* (Pawlowski et al., 2015), *Fusarium virguliforme* (Mueller et al., 2003), *Rhizoctonia solani* (Bradley et al., 2001), and multiple soybean viruses (Wang et al., 2005).

The objectives of this study were to evaluate major contributors to the genetic base of North American soybean cultivars for resistance to *P. ultimum* var. *ultimum* and evaluate lines identified with resistance to *P. ultimum* var. *ultimum* for resistance against *P. irregulare* and *P. sylvaticum*.

MATERIALS AND METHODS

Preliminary Isolate Aggressiveness Testing

Different isolates of a species can have different levels of aggressiveness (Broders et al., 2007). Six isolates of *P. ultimum* var. *ultimum* originally collected from soybean fields in Illinois in 2011 and 2012 by Dr. Carl Bradley's laboratory (University of Illinois, Urbana, IL) were tested for aggressiveness on soybean in a greenhouse assay. These isolates had been identified to

species level by Dr. Martin Chilvers laboratory (Michigan State University, East Lansing, MI) as part of a different research project that was funded by a USDA-NIFA grant. For this project, aggressiveness was defined as the relative ability of a pathogen to cause disease.

Before an aggressiveness study could be done, the appropriate amount of inoculum to be used per pot to achieve disease levels that discriminated between resistance and susceptibility of soybean genotypes needed to be determined. The six *P. ultimum* var. *ultimum* isolates were grown on sterilized millet (*Panicum miliaceum*) seeds to produce inoculum for the experiment. The millet seeds were soaked overnight in tap water and drained the next day. The millet was separated into aliquots for each isolate. All of the millet was then autoclaved at 121^0C (0.1034 MPa), for one hour, allowed to cool to room temperature, and then autoclaved a second time. Once cooled, two cultures of five day old isolates of *P. ultimum* var. *ultimum* that were grown on potato dextrose agar (PDA) in 89 mm diameter petri dishes were added to each autoclaved millet bag respectively, under a laminar flow hood. The inoculated millet seeds were incubated for one week at room temperature (21-25^0C) to allow the pathogen to colonize the seeds. The inoculum was then dried with forced air at 25^0C. Once dried, the infested millet seeds were ground using a Model 60GM Grinding Mills grinder (The C.S. Bell Co., Tiffin OH) to ensure the inoculum was a uniform size (1-2 mm in diameter). The inoculum was stored at 4^0C until used.

A factorial experiment comprised of two inoculation methods and five different inoculation rates was designed to determine which method and amount would best allow discrimination between resistant and susceptible soybean genotypes. Five levels of inoculum were tested: 0.5 g, 1 g, 2 g, 5 g, and 10 g. Each level was placed into a pot that contained approximately 1.6 liters of potting soil. The effect of inoculum distribution in the pots was also tested by comparing a layer method and a mixed method. The layer method was done as

described by Kirkpatrick et al. (2006) and consisted of filling the pots approximately three-fourths of the way full with a sandy loam mix of approximately two parts torpedo sand and one part soil. The inoculum was then added in an even layer, followed by another layer of soil until the pot was full. Seeds were planted into the top layer of soil. The inoculum layer was approximately 5 cm from the top of the soil line and was at least 2.5 cm below the seeds. The mixed method consisted of incorporating the inoculum throughout the pot by thoroughly mixing the soil and desired level of inoculum in a bag to evenly distribute the inoculum before it was placed in the pot. Both inoculation methods were done at each level of the inoculum with each isolate. The soybean cultivar Dwight (Nickell, 1998) was selected to be used in the concentration study. Dwight was derived from the cultivar Jack (Nickell, 1990), which is moderately susceptible to *P. irregulare* (Ellis et al., 2013). Five seeds were planted per pot and allowed to grow for three weeks. Data were collected for plant stand, plant height (from soil level to terminal node), root rot severity, and dry plant weights. Plant stands were collected on a per pot basis, and plant height and root rot severity data were collected per plant and then averaged across the plants in the pot. Dried plant weight data were collected by carefully removing the entire plant from the soil, washing off the roots with water, drying in a dryer with forced air at 25^0C for three days, and weighing plants from the same pot together using an electronic balance. Dried plant weight was then divided by the plant stand of the pot to calculate weight per plant. Root rot severity was rated using a 1 to 5 scale that was reported by Ellis et al. (2013), in which 1 = a healthy root system with no symptoms of lesions or root rot; 2 = the presence of small lesions on the lateral roots, with approximately 1 to 20% of roots showing visible symptoms; 3 = rot on lateral roots and visible symptoms of rot beginning on the main tap root, with approximately 21 to 75% of the roots displaying visible symptoms; 4 = both lateral

roots and the main tap root developed visible symptoms of root rot and approximately 76 to 100% of the roots displaying visible symptoms; and 5 = no germination. From the results of the inoculum level study, 5 g of *P. ultimum* var. *ultimum* colonized millet inoculum was found to be the optimal amount to produce symptoms (Appendix Table 1A). Also from this study, it was determined that the layered method of inoculum placement was more effective for obtaining the desired symptoms and would reduce the chance for escapes (Appendix Table 2A).

Once optimal inoculum amount and placement were determined, a factorial experiment to test the aggressiveness of the isolates was performed with six isolates and three different cultivars of varying susceptibility. Each isolate by cultivar combination had three replications, and the experiment was repeated twice. The cultivar Archer was chosen to be the resistant check (Bates et al., 2008; Kirkpatrick et al., 2006). Jack was included as a moderately susceptible cultivar and Sloan (Bahrenfus and Fehr, 1980) was the susceptible check (Dorrance et al., 2004; Ellis et al., 2013). The same size pots that were used in the concentration assay were used. Pots were inoculated with 5 g of millet inoculum in a layered placement. Five seeds were planted per pot and were allowed to grow for three weeks in a greenhouse room with temperature ranges of 20-26°C in saturated soil. Plant stand per pot, whole plant dry weights per plant, plant height per plant, and root rot score per plant averaged across the plants in the pot were collected. Root rot was scored using the same scale from Ellis et al. (2013) that was used in the inoculum concentration assay. From the results of the isolate aggressiveness assay (Appendix Table 3A), it was determined that isolate 12Py391 consistently caused disease levels that would be adequate for discriminating levels of susceptibility and resistance in soybean genotypes. This isolate was chosen to be used for screening the set of ancestral PIs and first progeny lines representing most of the genetic base for North American public cultivars released between 1947 and 1988.

Ancestral North American Soybean Screen

Before the ancestral screen, another concentration study was done with just 12Py391, to confirm the fresh inoculum that was made for this study was active. The cultivars used for this study were Archer, Jack, and Sloan. Five levels of inoculum were used: 0.25 g, 0.5 g, 1 g, 2 g, and 5 g. Each combination of cultivar by inoculum level had six inoculated replications and one non-inoculated control. Ten seeds were planted per 15.2 cm in diameter pot that held approximately 1.6 liters of soil, and allowed to grow for two weeks before data were collected. 0.25 g, 0.5 g, and 1 g, levels were completed in the trial one. Levels 1 g, 2 g, and 5 g were grown in trial two. The 1 g inoculation dosage was included in both trials to provide an indication of consistency between the trials. Trial one and trial two were planted within one week of each other in the same greenhouse room. Plant stand per pot, dried shoot weight per pot, and dried root weight per pot data were collected. Plant stand was measured as the number of plants per pot that emerged from the soil. To measure root weights, soil was washed away from the roots and then plants were cut at the soil line. Shoots and roots were dried with forced air at 25^0C for three days before being weighed. Total weigh per root or shoot was divided by plant stand to calculate root or shoot weight per plant. Results of this experiment can be found in Appendix Table 4A. On the basis of the results, it was determined that 5 g of millet inoculum should be used to get desired symptoms.

Plant material for the North American Ancestral Screen experiment consisted of 90 ancestral PIs and first progeny lines (referred to from here on out as ancestral lines); Archer and its predecessors, totaling in 10 lines; as well as the two susceptible checks Jack and Sloan (Table 2.1). The ancestral line, Sioux was not used in the screen because of unviability of seed from the USDA Soybean Germplasm Collection. In total, 102 lines were screened. The ancestral

soybean lines were obtained from the USDA Soybean Germplasm Collection, in Urbana, Illinois. Each of the lines had three inoculated replications and one non-inoculated control. Pots were arranged in a completely randomized design, and the experiment was repeated. Trial one was planted on 4 May 2015, and trial two was planted on 11 May 2015. Both trials were allowed to grow for two weeks. Millet inoculum infested with the 12Py391 isolate was used. Pots contained approximately 1.6 liters of soil. The 5 g layer method that was described in the concentration assay was used in each of the inoculated pots. The non-inoculated control pots did not contain any inoculum. Ten seeds were planted per pot. Two weeks after planting, plant stand per pot, dried shoot weight per plant, and dried root weight per plant data were collected as described for the previous concentration study.

Pythium Species Screen

Five soybean lines that showed varying levels of resistance to *P. ultimum* var. *ultimum* in the ancestral screen that was performed in May 2015 were screened with isolates from three different species of *Pythium*. *P. irregulare*, and *P. sylvaticum* isolates were used in addition to the *P. ultimum* var. *ultimum* isolate from the ancestral screen. *P. irregulare* and *P. sylvaticum* were isolates collected from Illinois soybean fields by Dr. Carl Bradley's Laboratory. These three species of *Pythium* are all pathogenic to soybean (Dorrance et al., 2004; Ellis et al., 2013; Griffin, 1990; Jiang et al., 2012).

The soybean lines selected for the species screen were based on the results from the ancestral screen. Lines PI 084637, Maple Isle, and Fiskeby III were selected as the resistant lines because they consistently had higher plant stand and dried shoot and root weights in the ancestral screen experiment. Fiskeby 840-7-3 was selected as the moderately resistant line because it was close to the median of the cultivars that were significantly different from the

lowest mean. All four of the resistant lines are maturity group II or earlier. Kanro was selected as the susceptible cultivar because of consistently having significantly low relative means for each of the data types collected and because it is a maturity group II soybean line. This was done so the susceptible line was closely matched in maturity group to help diminish any unknown variation that might be present due to large distances between maturity groups between the lines.

The species screen was conducted as a factorial experiment comprised of the three different *Pythium* species and the five different soybean lines. The experiment was set up in a completely randomized design and was repeated twice over time. Trial one was planted on 14 September 2015 and trial two was planted on 17 September 2015. Each species by cultivar combination had six inoculated replications and one non-inoculated control. Inoculum (5 g) was layered as described previously in each 15.2 cm pot that held approximately 1.6 liters of soil and ten seeds were planted per pot. The experiment was conducted in a greenhouse room that was kept at 20^0C-23^0C during the day and 16^0C-19^0C at night. After two weeks of growth, plant stand per pot, dried shoot weight per pot, and dried root weight per pot were collected. Shoot and roots of inoculated replications were dried on a whole pot basis, weighed, and divided by the number of plants per pot to calculate weights on a per plant basis.

Statistical Analysis

The ancestral screen and species screen data were analyzed using SAS v9.4 (SAS Institute Inc., Cary, NC). Plant stand from inoculated pots were compared to their non-inoculated control and expressed as a percent compared to the control. The average weight per plant of either the shoot or root was then compared to the average weight per plant part of the non-inoculated control, and was expressed as a percent compared to the control. In all replicated experiments, analysis of variance (ANOVA) was conducted using the mixed model procedure

(PROC MIXED). For the stages of each screening, the data within each stage were pooled and analyzed together because there was not a significant ($P \leq 0.05$) cultivar by trial interaction. Means from the lines in the ancestral screen were compared using Fisher's protected least significant difference (LSD) with the PDMIX800 macro (Saxton, 1998) where $\alpha = 0.05$. In the species screen, the pathogen by cultivar interaction was significant ($P \leq 0.05$), and means from the species and cultivars were compared using Fisher's protected LSD with the PDMIX800 macro (Saxton, 1998) where $\alpha = 0.05$. Correlations between plant stand, root weight, and shoot weight were determined using the Pearson correlation procedure (PROC CORR) in SAS v9.4 (SAS Institute Inc., Carry, NC).

RESULTS

Ancestral Screen

There were no significant ($P \leq 0.05$) cultivar by trial interactions; therefore, data from each trial could be pooled and analyzed together. Out of the 102 lines planted in the screen, 16 lines' inoculated replications did not emerge in either of the two planting. These 16 lines are part of the ancestral lines and were from varying maturity groups. Fisher's protected LSD was conducted to compare inoculated replication means relative to their non-inoculated control in all of the genotypes. All of the genotypes had their own non-inoculated controls to be compared with to account for genotypic variation. The highest mean stand count achieved by an ancestral line was from PI 548595 (Maple Isle) with a mean of 0.57 (Table 2.2). The greatest root weight mean relative to the control came from PI 084637 with a mean of 0.92 compared to the PI 084637 non-inoculated control. PI 548352 (Jogun), one of the ancestral lines, had the greatest shoot weight with a mean of 0.95 relative to its non-inoculated control.

From the North American ancestral lines, 14 lines appeared to be partially resistant to *P. ultimum* var. *ultimum* based primarily on their plant stand and higher root weights (Table 2.2). Shoot weights were considered to some degree, but more emphasis was placed on plant stand and root weights. All of the lines selected were significantly different from the worst lines, though most were not significantly different from each other for dried shoot and root weights.

Pythium Species Screen

There were no significant ($P \leq 0.05$) trial by genotpye or trial by *Pythium* species interactions; therefore, the data from the trials were combined for analysis. There was a significant interaction between soybean lines and *Pythium* species for plant stand ($P < 0.0001$), dried shoot weights ($P < 0.0001$), and dried root weights ($P < 0.0001$). All genotypes were compared to their own non-inoculated control. Therefore, the same genotypes could be compared to each other to standardize for genotype variation. The resistant lines, PI 084637, Maple Isle, Fiskeby III, and the moderately resistant line, Fiskey 840-7-3, appeared to be resistant against the isolates of *P. ultimum* var. *ultimum*, *P. irregulare*, and *P. sylvaticum*. Plant stand had a significant positive correlation with dried shoot weight and dried root weights. Dried shoot weight and dried root weight also had significant positive correlation with each other (Table 2.3).

The isolates of the three *Pythium* species used in the screen had varying levels of aggressiveness. On the four partially-resistant soybean lines, *P. ultimum* var. *ultimum* appeared to be the most aggressive, causing a relative mean plant stand of 0.67, relative mean root weight of 0.54, and a relative mean shoot weight of 0.76 (Table 2.4). The *P. irregulare* isolate appeared to be the second most aggressive pathogen out of the three, causing a relative mean plant stand of 0.78, a relative mean root weight of 0.67, and a relative mean shoot weight of 0.74 (Table 2.5).

P. sylvaticum observed to be the least aggressive out of the three. *P. sylvaticum* caused a relative mean stand count of 0.93, a relative mean root weight of 0.97, and a relative mean shoot weight of 0.94 (Table 2.6). The susceptible soybean line Kanro was significantly different than the partially resistant and moderately resistant lines inoculated with the *P. ultimum* var. *ultimum* isolate in plant stand and shoot weight. Kanro was significantly different than the partially resistant and moderately resistant lines inoculated with the *P. irregulare* isolate for plant stand. When challenged with the *P. sylvaticum* isolate, the mean stand counts and the dried root and shoot weights of Kanro were above 0.90 relative to the non-inoculated control.

DISCUSSION

Ancestral Screen

This is the first study to report on the reactions of the North American ancestral soybean lines to *P. ultimum* var. *ultimum.* Most of the lines were susceptible to the isolate used; approximately 63% were not significantly different from the worst lines screened. Of the ninety ancestral lines screened, 14 lines appeared to be partially resistant, but none appeared to be highly resistant. More emphasis was given to a higher root weight and plant stand than shoot weight, because *P. ultimum* var. *ultimum* is a soilborne pathogen that causes seed decay, root rot, and damping off, symptoms that lower plant populations and reduced root weights. These 14 lines range from maturity groups 00 to IV and originate from across the globe (Table 2.7). All of the latitudes of the originating countries are 37.55^0N or greater. It is not surprising that these cultivars originated from locations with cooler climates, since *P. ultimum* var. *ultimum* is adapted to those conditions and can be found across the world (van der Plaats-Niterink, 1981). It is also

interesting that the most resistant (PI 084637) line originates from South Korea just like the most resistant line (PI 424354) line from Ellis et al. (2013) whose screen used *P. irregulare.* PI 424354 line has resistance to *Fusarium graminearum* and at least 12 races of *Phytophthora sojae* (Dorrance and Schmitthenner, 2000; Ellis et al., 2013). These two resistant lines could point to South Korea as a potential germplasm location source for resistance to Pythium root rot and damping off.

Out of the 14 partially resistant lines, five lines are in a group of 35 ancestors and first progeny constituting 95% of the genes in the soybean genetic base of public soybean cultivars released between 1947 and 1988 (Gizlice et al., 1994). Fiskeby 840-7-3, Fiskeby III, PI 080837 (Mejior), and Jogun, each contribute less than 1% to the total genes, while Richland contributes 8.2% of the genes to the public cultivars (Gizlice et al., 1994). Richland was a major contributor to the northern gene pool, contributing approximately 11.3% of the genes (Gizlice et al., 1994).

The other nine lines, PI 084637, Novosodksa Bela, T204, Aoda, Maple Isle, PI 091110-1, Delmar, PI 054615-1, and Chico, showed partial resistance and are part of a group of lines that contributed the final 4% of genes to total 99% of the genes that the North American ancestral lines donated to the public cultivars released between 1947 and 1988. It is interesting that the lines that showed the most resistance to *P. ultimum* var. *ultimum* have very little contribution to the 99% of genes in the modern public cultivars. Nearly 75% of the genes in the public cultivars released from 1947 to 1988, are present in 17 lines released before 1960 (Gizlice et al., 1994), but none of the nine partially resistant lines were among those. The 14 resistant lines found in the present screening, made only limited genetic contributions to the public cultivars prior to 1989, and could be potential sources of resistance to Pythium root rot and damping off for

breeders to incorporate into their germplasm. It is not known whether *Pythium* resistance genes are among the genes that were introgressed from these donor lines into improved cultivars.

Seed age was a variable source for this screen. Seed sources ranged from 2003 to 2014, an 11 year gap. Seed viability tends to decline with older seed age (Fabrizius et al., 1999) and that can be slightly seen in this screen. The 14 partially resistant lines, came from seed five years or newer. The 16 lines that did not emerge, had older seed sources. These seed sources ranged from 2003 to 2008, with most of these seed sources being from 2003, 2004, and 2006. While these seed sources were older, there were no visible cracks on the seed planted, however that does not mean that there were not minuscule cracks on the seed. Oomycetes have the ability to move in the soil towards a seed because of the exudates that the seed releases (Keeling, 1974). This could be a reason that these inoculated lines did not emerge and that other lines with seed sources were susceptible. It would be interesting to see if the results would vary, if there were a newer seed source for more of the lines, but this might be difficult to achieve because of the USDA Soybean Germplasm Collection seed increase schedule.

This is not the first time the ancestral soybean lines of North American cultivars have been used in a germplasm resistance screen. Mueller et al. (2003) evaluated the ancestral lines for host resistance against *Fusarium virguliforme*. They found nine lines that were identified as being moderately resistant. Two of those lines, PI 54615-1 and Aoda, had been recognized as partially resistant to *P. ultimum* var. *ultimum* in this study. These two lines could have the potential to have resistance to general root rot diseases. Bradley et al. (2001) screened the ancestral lines for host resistance to *Rhizoctonia solani*, another soilborne seedling disease. They found 21 ancestral lines that were not significantly different from their partially resistant check. Out of their partially resistant lines, Novosodksa Bela and Aoda were also partially resistant to *P.*

ultimum var. *ultimum* in our study. Pawlowski et al. (2015) also screened the ancestral lines for resistance to *Macrophomina phaseolina*, a soilborne disease that commonly appears late in the season causing wilt or premature senescence in soybean. Their most resistant lines, Bansei, Sioux, and T145, did not express partial resistance to *P. ultimum* var. *ultimum*. It is possible that the partial resistance found towards seedling soilborne diseases is different than the partial resistance that is associated with late season soilborne diseases.

The USDA Soybean Germplasm Collection screened some of their accession in 1964 and 1966 for multiple morphological traits. Accessions screened were between FC 01547 and PI 266807 and that were maturity group 000 to IV. One of the traits that they looked at was resistance to *P. ultimum*. Artificial inoculations were done at Purdue University in 1964 and 1966. They were able to identify 60 accessions that were resistant to *P. ultimum* (Bernard et al., 1998). Out of our 14 partially resistant lines, only four were included in the 1960s screens, and they were classified as susceptible. Little is known about the *P. ultimum* isolate used and the inoculation method of the USDA screens. It would be interesting to see the reactions of the soybean lines used in the 1960s screens, to our isolate of *P. ultimum* var. *ultimum*. This could be another set of germplasm that could be screened in the future for possible resistance to Pythium root rot and damping off.

The cultivar Archer did not show the resistance that was reported out of Arkansas in this screen (Bates et al., 2008; Kirkpatrick et al., 2006). In both of these studies Archer had shown very good resistance to an Arkansas isolate of *P. ultimum*, however against our Illinois isolate of *P. ultimum* var. *ultimum*, it did not make it into the top 14 partially resistant lines. This could be because of the different levels of aggressiveness that isolates of the same species have (Broders et al., 2007). It would be interesting to see how the ancestral lines would react to the Arkansas

isolate. If the results differ from that of the Illinois isolate, this would be a good example of a genotype by environmental effect that breeders work with every day and how germplasm should be screen with isolates from that particulate growing region.

Pythium Species Screen

PI 084637, Maple Isle, and Fiskeby III, which were the most resistant lines from the ancestral screen, and the moderately resistant line of Fiskeby 840-7-3 showed resistance to all three *Pythium* species that were used. Kanro remained susceptible to all three species. PI 084637 appeared to have the highest level of resistance of the five lines that were selected for the species screen. This PI contributed less than 10% of its genes to each of its progeny and has contributed less than 0.01% to North American cultivars in total (Gizlice et al., 1994). Fiskeby III and Fiskeby 840-7-3 are related to each other but are not full siblings. The breeding line Fiskeby 840-7-3 is a plant introduction with a known pedigree. It is from a cross of 201-14-20 × 680+993+994, and 201-14-20 is a full sib to Fiskeby III (Gizlice et al., 1994). Interestingly, they were not significantly different from each other in any of the pathogen screens, suggesting that they might carry the same resistance gene(s). Maple Isle is a first progeny maturity group 00 cultivar developed by Harvey Voldeng of Agriculture Canada in the 1980s (Germplasm Resources Information Network). It has contributed approximately 0.1% of the genes to the 258 public cultivars developed between 1947 and 1988 and contributes approximately 0.14% of the genes to the northern gene pool cultivars (Gizlice et al., 1994). Maple Isle is derived from a cross to Holmberg 744-2 (PI 194641), a line from Sweden presumably developed by Sven Holmberg, the same breeder who developed Fiskeby III and Fiskeby 840-7-3 (Shurtleff and Aoyagi, 2010). Fiskeby soybean lines have been introgressed into Canadian germplasm as a source of cold tolerance resulting from their development in Sweden (Shurtleff and Aoyagi,

2010). Fiskeby III and Fiskeby 840-7-3 have been shown to exhibited foliar resistance to ozone injury in both greenhouse and field settings (Burkey and Carter, 2009). This is of interest because of the resistance the Fiskeby lines have shown to both biotic and abiotic stresses. With the cold tolerance from Sweden, these Fiskeby lines may have a better chance to germinate quicker in cool wet soils and outcompete the seedling disease pathogens present in the soil, helping the lines to be partially resistant to *Pythium* species. PI 084637, Maple Isle, Fiskeby III, and Fiskeby 840-7-3 could potentially be used in modern soybean breeding programs as sources of resistance.

A result that was interesting was the varying levels of aggressiveness among *P. ultimum* var. *ultimum*, *P. irregulare*, and *P. sylvaticum*; however, this could be explained by ability of the pathogen species to have different levels of aggressiveness among its isolates (Broders et al., 2007). The varying aggressiveness levels could be because of the environmental conditions under which the experiment was conducted. During the day, the greenhouse room was kept between 20-23^{0}C, and during the night, the room was kept between 16-18^{0}C. These conditions are optimal temperatures for *P. ultimum* var. *ultimum*. However, these conditions may not have been optimal for all three of the *Pythium* species. Out of the species screened, *P. ultimum* var. *ultimum* grows better under cooler environments at 23^{0}C while *P. sylvaticum* grows better conditions such as 25-30^{0}C (van der Plaats-Biterink, 1981). It is possible that some other *P. sylvaticum* isolates could be more aggressive than the one used in this experiment. Jiang et al. (2012) saw similar results in their petri plate assay, where *P. ultimum* had a higher disease severity rating than *P. irregulare* and *P. sylvaticum*. However, in their study, *P. sylvaticum* had just as high of disease severity ratings as did *P. irregulare*.

The resistant and moderately resistant lines that were advanced to the species screen continued to express their resistance against the *P. ultimum* var. *ultimum*, *P. irregulare*, and *P. sylvaticum* isolates used. This is the first time that this has been seen in *Pythium* spp. In Canada, Zhang et al. (2013), screened 70 Canadian soybean cultivars and found a few cultivars that were partially resistant to *Fusarium graminearum, Fusarium oxysporum, Fusarium solani* and *Rhizoctonia solani* found in fields. Some of the lines were resistant to at least two of the four soilborne pathogens, however, none of the accessions were resistant to all four species. While not in soybean, Bilgi et al. (2008) looked at different genotypes of dry bean for resistance against *Fusarium solani* f. sp. *phaseoli*, a soilborne root rot in North Dakota. They were able to identify two different dry bean genotypes that had resistance to this disease. VAX 3, a small red bean cultivar, expressed low root rot severity to *F. solani* f. sp. *phaseoli* and has known resistance to *Xanthomonas campestris* pv. *phaseoli*. T-39, a black bean cultivar, also expressed low root rot severity to *F. solani* f. sp. *phaseoli* in the study. These two dry bean genotypes were also used in a screen against *Fusarium graminearum* in North Dakota. They displayed the lowest root rot severity compared to the other dry bean genotypes used in the screen (Bilgi et al., 2011). Considering that both VAX 3 and T-39 showed resistance to different *Fusarium* species, it is likely that the genotypes that have resistance to *F. graminearum* will have resistance to *F. solani* f. sp. *phaseoli* and vice versa. This coincides with the four partially resistant soybean lines that we found in our study that show partial resistance across *P. ultimum* var. *ultimum, P. irregulare*, and *P. sylvaticum*. It could be possible for PI 084637, Maple Isle, Fiskeby III, and Fiskeby 840-7-3 to have partial resistance to other *Pythium* species.

Typically, host resistance genes tend to be pathogen species-specific instead of across species, however this *Pythium* spp. screen, the *Fusarium* spp. screen by Zhang et al. (2013), the

Fusarium solani f. sp. *phaseoli* screen by Bilgi et al. (2008), and the *Fusarium graminearum* screen of Bilgi et al. (2011) show that there is a possibility that some legume genotypes could be at least partially resistant to pathogenic species of the same genus. Further studies would need to be done with the lines PI 084637, Maple Isle, Fiskeby III, and Fiskeby 840-7-3 to see if this resistance holds up across other *Pythium* species and other oomycetes and fungal seedling pathogens.

From these experiments, we were able to conclude that potential sources for resistance to *P. ultimum* var. *ultimum* exist in the genetic base of North American soybean cultivars released prior to 1989, especially from the 14 lines that were least affected by infection with the moderately aggressive isolate used. Of the lines that were advanced to the species screen, the resistant and moderately resistant lines continued to express their resistance against the *P. ultimum* var. *ultimum*, *P. irregulare*, and *P. sylvaticum* isolates used. In the conditions that were used in the experiment, we were able to see that the *Pythium* species had varying levels of aggressiveness, with *P. ultimum* var. *ultimum* being the most aggressive and *P. sylvaticum* being the least aggressive. From this study, future work could investigate whether the partial resistance that was detected is caused by host resistance genes or if the partial resistance is effective against different species of *Pythium*. Identification of these resistant accessions has the potential to be of use to breeders for development of soybean cultivars with resistance against Pythium root rot and damping off.

LITERATURE CITED

Bahrenfus, J.B. and W.R. Fehr. 1980. Registration of 'Sloan' soybean. Crop Sci. 20:673.

Bates, G.D., C.S. Rothrock, and J.C. Rupe. 2008. Resistance of the soybean cultivar Archer to Pythium damping-off and root rot caused by several *Pythium* spp. Plant Dis. 92:763-766.

Bernard, R.L. C.R Cremeens, R.L. Cooper, F.I. Collins, O.A. Krober, K.L. Athow, F.A. Laviolette, C.J. Coble, and R.L. Nelson. 1998. Evaluation of the USDA Soybean Germplasm Collection: maturity groups 000-IV (FC 01.547-PI 266.807) Techn. Bull. USDA 1844:30-85.

Bilgi, V.N., C.A. Bradley, S.D. Khot, K.F. Grafton, and J.B. Rasmussen. 2008. Response of dry bean genotypes to Fusarium root rot, caused by *Fusarium solani* f. sp. *phaseoli*, under field and controlled conditions. Plant Dis. 92:1197-1200.

Bilgi, V. N., C.A. Bradley, F.M. Mathew, S. Ali, and J.B. Rasmussen. 2011. Root rot of dry edible bean caused by *Fusarium graminearum*. Online. Plant Health Progress. dio: 10.1094/PHP-2011-0425-01-RS.

Bradley, C. A., Hartman, G. L., Nelson, R. L., Mueller, D. S., and Pedersen, W. L. 2001. Response of ancestral soybean lines and commercial cultivars to Rhizoctonia root and hypocotyl rot. Plant Dis. 85:1091-1095.

Broders, K.D., P.E. Lipps, P.A. Paul, and A.E. Dorrance. 2007. Characterization of *Pythium* spp. associated with corn and soybean seed and seedling diseases in Ohio. Plant Dis. 91:727-735.

Broders, K. D., M.W. Wallhead, G.D. Austin, P.E. Lipps, P.A. Paul, R.W. Mullen, and A.E. Dorrance. 2009. Association of soil chemical and physical properties with *Pythium* species diversity, community, composition, and disease incidence. Phytopathology 99:957-967.

Brown, G.E. and B.W. Kennedy. 1965. Pythium pre-emergence damping-off in soybean in Minnesota. Plant Dis. 49:646-647.

Burkey, K.O., and T.E Carter. 2008. Foliar resistance to ozone injury in the genetic base of U.S. and Canadian soybean and prediction of resistance in descendent cultivars using coefficient of parentage. Field Crops Res. 111:207-217.

Carter, T.E., R.L. Nelson, C.H. Sneller, and Z. Cui. 2004. Genetic diversity of soybean. In H.R. Boerma and J.E. Specht, Soybean: Improvement, Production, and Uses (Third Ed. pg. 303-416) Madison, WI: American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of American, Inc.

Cianzio, S.R., S.P. Shultz, W.R. Fehr, and H. Tachibana. 1991. Registration of 'Archer' soybean. Crop Sci. 31:1707.

Delannay, X., D. M. Rodgers, and R.G. Palmer. 1983. Relative genetic contributions among ancestral lines to North American soybean cultivars. Crop Sci. 23:944-949.

Dorrance, A.E., and A.F. Schmitthenner. 2000. New sources of resistance to *Phytophthora sojae* in the soybean plant introductions. Plant Dis. 84:1303-1308.

Dorrance, A. E., S.A. Berry, P. Bowen, and P.E. Lipps. 2004. Characterization of *Pythium* spp. from three Ohio fields for pathogenicity on corn and soybean and metalaxyl sensitivity. Online. Plant Health Progress. doi: 10.1094/PHP-2004-0309-01-RS

Ellis, M.L., L.K. McHale, P.A. Paul, S.K. St. Martin, and A.E. Dorrance. 2013. Soybean germplasm resistant to *Pythium irregulare* and molecular mapping of resistance quantitative trail loci derived from the soybean accession PI 424354. Crop Sci. 53:1008-1021.

Fabrizius, E., D. TeKrony, D.B. Egli, and M. Rucker. 1999. Evaluation of a viability model for predicting soybean seed germination during warehouse storage. Crop Sci. 39:194-201.

Gizlice, Z., T.E. Carter, and J.W. Burton. 1994. Genetic base for North American public soybean cultivars released between 1947-1988. Crop Sci. 34:1143-1151.

Grau, C.R, A.E. Dorrance, J. Bond, and J.S. Russin. 2004. Fungal Diseases. in H.R. Boerma and J.E. Specht, Soybean: Improvement, Production, and Uses (Third Ed. pg. 679-764) Madison, WI: American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of American, Inc.

Griffin, G.J. 1990. Importance of *Pythium ultimum* in a disease syndrome of cv. Essex soybean. Can. J. of Plant Pathol. 12:135-140.

Jiang, Y.N., J.S. Haudenshield, and G.L. Hartman. 2012. Characterization of *Pythium* spp. from soil samples in Illinois. Can. J. of Plant Pathol. 34:448-454.

Keeling, B.L. 1974. Soybean seed rot and relation of seed exudate to host susceptibility. Phytopathology 64:1445-1447.

Kirkpatrick, M. T., C.S. Rothrock, J.C. Rupe, and E.E. Gbur. 2006. The effect of *Pythium ultimum* and soil flooding on two soybean cultivars. Plant Dis. 90:597-602.

Koenning, S.R. and J.A. Wrather. 2010. Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. Online. Plant Health Progress. doi:10.1094/PHP-2010-1122-01-RS

Nickell, C.D., G.R. Noel, D.J. Thomas, and R. Waller. 1990. Registration of 'Jack' soybean. Crop Sci 30:1365

Nickell, C.D., G.R. Noel, T.R. Cary, and D.J. Thomas. 1998. Registration of 'Dwight' soybean. Crop Sci 38:1398

Mueller, D.S., R.L Nelson, G.L. Hartman, and W.L. Pedersen. 2003. Response of commercially developed soybean cultivars and the ancestral soybean lines to *Fusarium solani* f. sp. *glycines*. Plant Dis. 87:827-831.

Pawlowski, M.L., C.B. Hill, and G.L. Hartman. 2015. Resistance to charcoal rot identified in ancestral soybean germplasm. Crop Sci. 55:1230-1235.

Rizvi, S.S.A. and X.B. Yang. 1996. Fungi associated with soybean seedling disease in Iowa. Plant Dis. 80:57-60.

Rooso, M.L., J.C. Rupe., P. Chen, and L.A. Mozzoni. 2008. Inheritance and genetic mapping of resistance to *Pythium* damping-off caused by *Pythium aphanidermatum* in 'Archer' soybean. Crop Sci. 48:2215-2222.

Rupe, J.C., Rothrock, C.S., Bates, G., Rosso, M. L.,Avanzato, M. V. and Chen, P. 2011. Resistance to Pythium seedling disease in soybean. in (Ed) A. Sudaric, Soybean-Molecular Aspects of Breeding. InTech. Croatia.

Saxton, A.M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. Pages 1243-1246 in Proc. 23rd SAS Users Group International.

Shurtleff, W. and A. Aoyagi. 2010. History of soybeans and soy foods in Canada (1831-2010). Lafayette, CA: Soyinfo Center.

St. Martin, S.K. 1982. Effective population size for the soybean improvement program in maturity groups 00 to IV. Crop Sci. 22:151-152.

van der Plaats-Niterink, J. 1981. Monograph of the genus *Pythium*. Stud. Mycol. 21:1-244.

Wang, Y., H.A. Hobbs, C.B. Hill, L.L. Domier, G.L. Hartman, and R.L. Nelson. 2005. Evaluation of ancestral lines of U.S. soybean cultivars for resistance to four soybean viruses. Crop Sci. 45:639-644.

Wilcox, J.R. 2004. World distribution and trade of soybean. in H.R. Boerma and J.E. Specht, Soybean: Improvement, Production, and Uses (Third Ed. pg. 1-14) Madison, WI: American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of American, Inc.

Wrather, J.A., and S.R. Koenning, 2009. Effects of diseases on soybean yields in the United States 1996-2007. Online. Plant Health Progress. doi:10.1094/PHP-2009-0401-01-RS.

Yang, X. 1999. Pythium damping off and root rot. In Eds. G.L. Hartman, J.B. Sinclair, and J.C. Rupe. Compendium of Soybean Diseases (Fourth ed. pg. 42-44). St. Paul, MN: The American Phytopathological Society.

Zhang, J.X., A.G. Xue, E.R. Cober, M.J. Morrison, H.J Zhang, S.Z. Zhang, and E. Gregorich. 2013. Prevalence, pathogenicity and cultivar resistance of Fusarium and Rhizoctonia species causing soybean root rot. Can. J. Plant Sci. 93:221-236.

Table 2.1. Material screened in North American ancestral screen.

Accession	Cultivar Name	Maturity Group	Province	Country	Year	% class	Identifier
FC031745	-	VI	unknown	unknown	1948	95	True Ancestor
FC033243	Anderson	IV	unknown	unknown	1954	95	True Ancestor
PI054615-1	No. 55	III	Jilin	China	1921	99	True Ancestor
PI065338	Botanical Garden No. 4	II	Heilongjiang	China	1925	99	True Ancestor
PI071506	No. 94	IV	Jiangsu	China	1927	95	True Ancestor
PI080837	Mejiro	IV	unknown	Japan	1929	95	True Ancestor
PI084631	S-56	III	Kyonggi	South Korea	1930	99	True Ancestor
PI084637	S-62	II	Kyonggi	South Korea	1930	99	True Ancestor
PI084946-2	(Kandokon)	IV	Pusan	South Korea	1930	99	True Ancestor
PI086972-1	Pakute	II	Cholla Puk	South Korea	1930	99	True Ancestor
PI088788	-	III	Liaoning	China	1930	95	True Ancestor
PI088811	Pakute	IV	Pyongan Puk	North Korea	1930	99	True Ancestor
PI091110-1	-	I	Heilongjiang	China	1931	99	True Ancestor
PI096983	-	V	Hwanghae Puk	North Korea	1932	99	True Ancestor
PI159925	Glycine H	VIII	Lima	Peru	1947	99	True Ancestor
PI171450	Kisaya	III	Kagoshima	Japan	1948	99	True Ancestor
PI171451	Kosamame	VII	Kanagawa	Japan	1948	99	True Ancestor
PI180501	Strain No. 18	0	unknown	Germany	1949	95	True Ancestor
PI200492	Komata	VII	Shikoku	Japan	1952	99	True Ancestor
PI240664	Bilomi #3	X	unknown	Philippines	1957	95	True Ancestor
PI248404	Novosadska Bela	0	Serbia	Yugoslavia	1958	99	True Ancestor
PI317335	Koganejiro	I	Hokkaido	Japan	1966	99	True Ancestor
PI360955A	Fiskeby V	000	Östergotland	Sweden	1971	95	First Progeny
PI360955B	(Fiskeby V)	000	Östergotland	Sweden	1971	95	First Progeny
PI438471	Fiskeby III	00	Östergotland	Sweden	1980	95	First Progeny

Table 2.1 (cont.)

PI438477	Fiskeby 840-7-3	00	Östergotland	Sweden	1980	95	First Progeny
PI508269	Stafford	IV	Virginia	United States	1986	99	First Progeny
PI513382	Glenwood	0	Minnesota	United States	1987	99	First Progeny
PI535807	Crockett	VIII	Texas	United States	1988	99	First Progeny
PI542402	Chico	00	Minnesota	United States	1983	99	First Progeny
PI548169	T117	IV	Illinois	United States	1954	99	True Ancestor
PI548178	T145	III	Illinois	United States	1954	99	True Ancestor
PI548193	T201	IV	Iowa	United States	1957	99	True Ancestor
PI548195	T204	IV	Illinois	United States	1957	99	True Ancestor
PI548237	T260H	VII	North Carolina	United States	1976	99	True Ancestor
PI548298	A.K. (Harrow)	III	Northeast China	China	by 1939	95	True Ancestor
PI548301	Aoda	IV	Hokkaido	Japan	1939	99	True Ancestor
PI548302	Bansei	II	Hokkaido	Japan	1936	95	True Ancestor
PI548307	Blackeye	0	Heilongjiang	China	1940	99	First Progeny
PI548311	Capital	0	Northeast China	China	1944	95	First Progeny
PI548318	Dunfield	III	Jilin	China	1923	95	True Ancestor
PI548325	Flambeau	00	unknown	Russia	1944	95	True Ancestor
PI548336	Habaro	I	Khabarovsk	Russia	1910	99	True Ancestor
PI548342	Higan	IV	Tokyo	Japan	1936	99	True Ancestor
PI548348	Illini	IV	Heilongjiang	China	1927	95	True Ancestor
PI548352	Jogun	III	Hamgyong Puk	North Korea	1936	95	True Ancestor
PI548356	Kanro	II	Pyongyang	North Korea	1936	95	True Ancestor
PI548359	Kingwa	IV	Beijing	China	1931	99	True Ancestor
PI548360	Korean	II	unknown	North Korea	by 1928	95	True Ancestor
PI548362	Lincoln	III	unknown	China	1943	95	First Progeny
PI548379	Mandarin (Ottawa)	0	Heilongjiang	China	1934	95	True Ancestor
PI548382	Manitoba Brown	00	unknown	unknown	by 1939	95	True Ancestor

Table 2.1 (cont.)

PI548383	Mansoy	III	Heilongjiang	China	by 1928	99	True Ancestor
PI548391	Mukden	IV	Liaoning	China	1932	95	True Ancestor
PI548402	Peking	III	Beijing	China	1910	95	True Ancestor
PI548406	Richland	II	Jilin	China	1938	95	True Ancestor
PI548409	Sato	IV	Hokkaido	Japan	1936	99	True Ancestor
PI548411	Seneca	II	Northeast China	China	1939	99	True Ancestor
PI548414	Sioux†	000	Hokkaido	Japan	1939	99	True Ancestor
PI548438	Arksoy	VI	Pyongyang	North Korea	1937	95	True Ancestor
PI548444	Biloxi	VIII	Zhejiang	China	1918	99	True Ancestor
PI548445	CNS	IX	Jiangsu	China	1943	95	True Ancestor
PI548456	Haberlandt	V	Pyongyang	North Korea	1907	95	True Ancestor
PI548457	Hahto	VI	Fukushima	Japan	1918	99	True Ancestor
PI548461	Improved Pelican	VIII	unknown	China	1950	95	First Progeny
PI548463	Laredo	IV	Shaanxi	China	by 1923	99	True Ancestor
PI548469	Mammoth Yellow	VII	unknown	Japan	by 1895	99	True Ancestor
PI548477	Ogden	VI	unknown	unknown	1940	95	First Progeny
PI548484	Ralsoy	VI	Pyongyang	North Korea	1940	95	True Ancestor
PI548485	Roanoke	VII	Jiangsu	China	1946	95	True Ancestor
PI548488	S-100	V	Heilongjiang	China	1945	95	True Ancestor
PI548493	Tokyo	III	Kanagawa	Japan	1907	99	True Ancestor
PI548494	Volstate	VII	unknown	unknown	1942	99	First Progeny
PI548528	Protana	II	Indiana	United States	1969	99	First Progeny
PI548548	Delmar	IV	Delaware	United States	1963	99	First Progeny
PI548559	Emerald	IV	Delaware	United States	1975	99	First Progeny
PI548561	Hodgson	I	Minnesota	United States	1974	99	First Progeny
PI548587	Kim	III	Iowa	United States	1956	99	First Progeny
PI548595	Maple Isle	00	Ontario	Canada	1984	99	First Progeny

Table 2.1 (cont.)

PI548599	Monroe	I	Ohio	United States	1948	99	First Progeny
PI548603	Perry	IV	Indiana	United States	1952	95	First Progeny
PI548604	Pershing	IV	Missouri	United States	1984	99	First Progeny
PI548623	Vansoy	0	Ontario	Canada	1970	99	First Progeny
PI548626	Wabash	IV	Indiana	United States	1948	99	First Progeny
PI548633	Wye	IV	Maryland	United States	1971	99	First Progeny
PI548657	Jackson	VII	North Carolina	United States	1953	95	First Progeny
PI548663	Dowling	VIII	Texas	United States	1978	99	First Progeny
PI548697	Majos	VIII	South Carolina	United States	1990	99	First Progeny
PI548983	Tracy	VI	Mississippi	United States	1973	99	First Progeny
PI553048	Vance	V	Virginia	United States	1986	99	First Progeny
PI567790	Curtis	VI	Louisiana	United States	1958	99	First Progeny
PI 546487	Archer	I	Iowa	United States	1991		Resistant check
PI548519	BSR-101	I	Iowa	United States	1986		Archer pedigree
PI548506	Amsoy	II	Iowa	United States	1962		Archer pedigree
PI548527	Calland	III	Indiana	United States	1966		Archer pedigree
PI548502	Adams	III	Iowa	United States	1956		Archer pedigree
PI548573	Harosoy	II	Ontario	Canada	1956		Archer pedigree
PI548504	Altona	00	Manitoba	Canada	1966		Archer pedigree
PI548516	Blackhawk	I	Iowa	United States	1956		Archer pedigree
PI548533	Clark	IV	Illinois	United States	1952		Archer pedigree
PI548628	Wayne	III	Illinois	United States	1962		Archer pedigree
PI540556	Jack	II	Illinois	United States	1988		Susceptible check
PI548616	Sloan	II	Iowa	United States	1978		Susceptible check

† Sioux was not screened in the ancestral screen because it was not able to be obtained from the USDA Germplasm Collection.

Table 2.2. Relative means of the 14 most resistant lines. These lines are part of the North American ancestor collection and were screened against *P. ultimum* var. *ultimum*. Each inoculated replication was compared to its non-inoculated control. The non-inoculated controls have a value of 1 and were included in the LSD analysis. LSD analysis was ran on all 102 lines screened; only the 14 partially resistant lines are shown.

PI Accession	Line	Identifier[†]	Plant stand[*]	Dried root weight[*‡]	Dried shoot weight[*‡]
PI 084637	PI 086437	True Ancestor	0.47 ABC	0.93 A	0.87 ABC
PI 548595	Maple Isle	First Progeny	0.57 A	0.63 ABCDEFG	0.68 ABCDEFGHIK
PI 438471	Fiskeby III	First Progeny	0.37 BCDEFG	0.67 ABCD	0.94 AB
PI 548548	Delmar	First Progeny	0.49 AB	0.57 ABCDEFGHIJK	0.69 ABCDEFGHIJ
PI 248404	Novosodksa Bela	True Ancestor	0.35 BCDEFGH	0.72 AB	0.71 ABCDEFGHI
PI 438477	Fiskeby 840-7-3	First Progeny	0.28 DEFGHIJKLM	0.58 ABCDEFGHIJ	0.76 ABCDE
PI 548352	Jogun	True Ancestor	0.23 DEFGHIJKLMNOPQ	0.71 AB	0.95 A
PI 542402	Chico	First Progeny	0.40 ABCDE	0.52 ABCDEFGHIJLKM	0.69 ABCDEFGHIJ

Table 2.2 (cont.)

PI 091110-1	PI 091110-1	True Ancestor	0.35 BCDEFGHI	0.60 ABCDEFGH	0.71 ABCDEFGHI
PI 548406	Richland	True Ancestor	0.37 BCDEFG	0.61 ABCDEFG	0.70 ABCDEFGHIJ
PI 080837	Mejiro	True Ancestor	0.25 DEFGHIJKLMNO	0.69 ABC	0.86 ABCD
PI 548301	Aoda	True Ancestor	0.30 CDEFGHIJ	0.64 ABCDE	0.72 ABCDEFGHI
PI 054615-1	PI 054615-1	True Ancestor	0.37 BCDEFG	0.53 BCDEFGHIJKLM	0.63 ABCDEFGHIJKL
PI 548195	T204	True Ancestor	0.30 CDEFGHIJ	0.65 ABCDE	0.84 ABCD

† First progeny – a cultivar or breeding line derived through hybridization for which there are no available intermediates between them and the ancestors.

* Means followed by the same letter within a column are not significantly different ($P \leq 0.05$).

‡ Weights used were one a per plant basis

Table 2.3. Correlations from species screen. Correlation between stand counts, dried root weight, and dried shoot weight in the species screen. All of the correlations are significant.

	Plant stand	**Dried root weight†**	**Dried shoot weight†**
Plant stand	1	0.59*	0.34*
Dried Root Weight†	0.59*	1	0.59*
Dried Shoot Weight†	0.34*	0.59*	1

* Significant at $P \leq 0.05$

†Weights used were on a per plant basis

Table 2.4. Relative means of the genotypes against *P. ultimum* var. *ultimum*. All means are relative to the genotype's non-inoculated control

	Pythium ultimum* var. *ultimum		
Genotypes	**Plant stand**[†]	**Dried root weight**[*†]	**Dried shoot weight**[*†]
PI 084637	0.82 A	0.62 A	0.83 A
Maple Isle	0.61 B	0.58 AB	0.76 A
Fiskeby III	0.60 B	0.56 AB	0.76 A
Fiskeby 840-7-3	0.67 AB	0.42 BC	0.71 A
Kanro	0.13 C	0.27 C	0.41 B

[*] Weights used were on a per plant basis.

[†]Means followed by the same letter within a column are not significantly different ($P \leq 0.05$).

Table 2.5. Relative means of the genotypes against *P. irregulare***.** All means are relative to the genotype's non-inoculated control.

	Pythium irregulare		
Genotypes	**Plant stand**[†]	**Dried root weight**[*†]	**Dried shoot weight**[*†]
PI 084637	0.96 A	0.94 A	0.82 A
Maple Isle	0.73 B	0.64 B	0.66 A
Fiskeby III	0.75 B	0.59 B	0.80 A
Fiskeby 840-7-3	0.70 B	0.50 B	0.69 A
Kanro	0.33 C	0.55 B	0.74 A

[*] Weights used were on a per plant basis.

[†]Means followed by the same letter within a column are not significantly different ($P \leq 0.05$).

Table 2.6. Relative means of the genotypes against *P. sylvaticum.* All means are relative to the genotype's non-inoculated control

	Pythium sylvaticum		
Genotypes	**Plant stand**[†]	**Dried root weight**[*†]	**Dried shoot weight**[*†]
PI 084637	0.97 A	0.96 AB	0.94 AB
Maple Isle	0.94 A	0.99 A	0.91 B
Fiskeby III	0.94 A	0.98 AB	0.96 A
Fiskeby 840-7-3	0.84 B	0.94 AB	0.95 AB
Kanro	0.93 A	0.92 B	0.98 A

[*] Weights used were on a per plant basis.

[†]Means followed by the same letter within a column are not significantly different ($P \leq 0.05$).

Table 2.7. Contributions consist of how much each genotype contributed to the modern North American cultivars and to each gene pool respectively. Contributions of germplasm adapted from Gizlice et al. (1994)

PI Accession	Line	Identifier†	Maturity Group	Origin	Latitude	Contribution to North American cultivars	Contribution to northern cultivars	Contribution to southern cultivars
PI 084637	PI 086437	True Ancestor	II	South Korea	37.55^{0}N	0.0016	0.0022	0
PI 548595	Maple Isle	First Progeny	00	Canada	50.00^{0}N	0.1014*	0.1437*	0*
PI 438471	Fiskeby III	First Progeny	00	Sweden	58.52^{0}N	0.5087*	0.7212*	0*
PI 548548	Delmar	First Progeny	IV	United States	39.00^{0}N	0.5814*	0.4121*	0.9869*
PI 248404	Novosodksa Bela	True Ancestor	0	Yugoslavia	44.80^{0}N	0.0485	0.0687	0
PI 438477	Fiskeby 840-7-3	First Progeny	00	Sweden	58.52^{0}N	0.7752*	1.0989*	0*
PI 548352	Jogun	True Ancestor	III	North Korea	40.00^{0}N	0.5329	0.7555	0
PI 542402	Chico	First Progeny	0	United States	46.00^{0}N	<0.01‡	<0.01‡	<0.01‡
PI 091110-1	PI 091110-1	True Ancestor	I	China	48.00^{0}N	0.0485	0.0687	0
PI 548406	Richland	True Ancestor	II	China	43.70^{0}N	8.2124	11.3055	0.0853
PI 080837	Mejiro	True Ancestor	IV	Japan	43.00^{0}N	0.6783	0	2.3026
PI 548301	Aoda	True Ancestor	IV	Japan	43.00^{0}N	0.3876	0.5495	0

Table 2.7 (cont.)

PI 054615-1	PI 054615-1	True Ancestor	III	China	43.70^{0}N	0.0031	0.0044	0
PI 548195	T204	True Ancestor	IV	United States	40.00^{0}N	0.0045	0.0066	0

† Frist progeny – a cultivar or breeding line derived through hybridization for which there are no available intermediates between them and the ancestors.

* Contribution to 258 North American public cultivars released between 1947 and 1988.

‡ Part of a group of 48 first progeny that contribute less than 1.0% to the overall genes found in the 258 North American public cultivars that were released between 1947 and 1988.

CHAPTER THREE

Effects of fungicide seed treatments specific to oomycete pathogens on stand establishment and yield of soybean in Illinois

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is the second most widely planted field crop in the United States after corn (*Zea mays*), with an estimated 34.4 million hectares planted in 2015 (USDA NASS, 2015). In the U.S., 87% of the crop is produced in the north-central states, with Illinois and Iowa leading in production in 2014 and 2015 (USDA NASS, 2015; Wilcox, 2004). Since the early 2000s, there has been a shift towards earlier planting dates in an effort to maximize yields (Robinson et al., 2009). With the earlier planting dates, soybean seeds are being planted in cooler and wetter soils, which increase the probability of slowed seed germination and seedling emergence, and the risk of soybeans being affected by seedling diseases (Dorrance et al., 2009). Between 2006 and 2009, seedling diseases caused by pathogenic species of *Pythium*, *Rhizoctonia*, *Fusarium*, and *Phomopsis* were estimated to be the second most important biotic cause of yield reduction in soybean (Koening and Wrather, 2010). Cool wet soils favor most seedling disease pathogens that reduce stands and cause root rot, especially *Pythium* spp. (Broders et al., 2007; Ellis et al., 2011). A study in Iowa showed that *Pythium* spp. and *Phytophthora sojae* appeared to be the most important components of the seedling disease complex based on the frequency with which these pathogens were isolated from diseased soybean seedlings (Rizvi and Yang, 1996). The severity of Pythium root rot and

damping off disease is determined primarily by the initial amount of inoculum, host age, and environmental conditions at the time of infection (Yang, 1999).

Pythium species that are pathogenic on soybean can be found wherever the crop is grown. Affected seedlings show symptoms of seed rot, root rot, and damping-off that can lead to poor emergence and reduced plant stands (Brown and Kennedy, 1965; Yang et al., 1999). The typical life cycles of pathogenic species of *Pythium* are monocyclic (Yang, 1999). Management of pathogenic species of *Pythium* and other soilborne oomycetes, including *Phytophthora sojae*, requires a combination of improved soil drainage in low wet locations, tillage, crop rotation, resistant host plants, and fungicide seed treatments (Grau et al., 2004). Fields that have had multiple occurrences of disease caused by soilborne pathogens should have tile installed to improve drainage, and the fields should be tilled in the spring to increase soil temperatures (Yang, 1999). Crop rotation alone is not an effective management practice, especially in corn-soybean rotations where *Pythium* spp. population levels may be high (Grau et al., 2004; Zhang and Yang, 2000; Broders et al., 2007). Two current management practices that show the most promise are host plant resistance and fungicide seed treatments. Plant host resistance to diseases caused by *Pythium* spp. has been reported in soybean, but the level of resistance is typically not complete (Bates et al., 2008; Ellis et al., 2013; Kirkpatrick et al., 2006; Rosso et al., 2008).

Since the early 2000s, seed treatments have become a popular component of disease management (Esker and Conley, 2012; Douglas and Tooker, 2015). Despite the fact that species of *Pythium* are oomycetes and not true fungi, they are still sensitive to some fungicides used to treat seeds prior to planting. According to industry estimates, only 8% of the soybeans planted in 1996 were treated with fungicides, while in 2008 at least 30% of the seeds were treated and by 2012, 50% of seeds sold were treated (Munkvold, 2009; Esker and Conley 2012). Fungicide

seed treatmentshave been shown to prevent soybean plant stand and yield loss, especially under cool and moist soil conditions (Bradley, 2008). Two fungicide active ingredients, metalaxyl and mefenoxam, have been used for years to help reduce losses to species of *Pythium* and *Phytophthora*, but with continuous use, a decrease in sensitivity to these fungicides has been observed in some *Pythium* species (Broders et al., 2007; Dorrance et al., 2004). Combining metalaxyl or mefenoxam with broad spectrum fungicides has been shown to improve plant stands compared to untreated seed (Urrea et al., 2013). Metalaxyl and mefenoxam both belong to the phenylamide mode of action (MOA) group and are enantiomers of each other. Enantiomers have identical chemical and physical properties, but differ in their configuration (R or S) at the stereogenic center. The S-metalaxyl enantiomer is the active ingredient metalaxyl. The R-metalaxyl enantiomer is commonly known as mefenoxam and is slower degrading in soils (Monkiedje and Spiteller, 2005). Fungicides in the phenylamide group inhibit nucleic acid synthesis at a target site in the RNA polymerase I reaction, which has an inhibitory effect on mitosis of cells (Fungicide Resistance Action Committee, 2015; Fisher and Hayes, 1982).

A new chemistry on the market to protect seeds and seedlings from oomycete pathogens is ethaboxam (Kim et al., 2004). While metalaxyl and mefenoxam have been commercially available for a relatively long time, ethaboxam has been approved recently for use on soybean in the U.S., and is available in the product known as Intego Suite (Valent USA Corp., Walnut Creek, CA). Ethaboxam belongs to the thiazole carboxamide MOA group which affects mitosis and cell division, with its target site in the beta-tubulin assembly in mitosis (Fungicide Resistance Action Committee, 2015). The different MOA of ethaboxam has the potential to protect seeds and seedlings from strains of oomycetes pathogens with reduced sensitivity to phenylamide fungicides such as mefenoxam and metalaxyl.

Ethaboxam was originally developed and commercialized in Korea in 1999 to be used on vegetable crops for protection against diseases caused by oomycetes (Kim et al., 1999; Kim et al., 2004). Ethaboxam has been reported to provide a better control of *Pseudoperonospora cubensis*, *Phytophthora infestans* and *Phytophthora capsici* on cucumber, potato, and pepper in field plots in Korea compared to metalaxyl (Kim et al., 1999). Zhang et al. (2005) also tested ethaboxam in potato field trials in Korea against *P. infestans*. Many field populations of *P. infestans* in Korea were moderately resistant to metalaxyl. Of 687 *P. infestans* isolates tested, ethaboxam had a control efficacy of 80.4% and 81.9% in 2003 and 2004, respectively.

The objectives of this study were to evaluate the active ingredient ethaboxam in comparison to metalaxyl and to evaluate differing ratios of ethaboxam with metalaxyl that are currently available on the market for prevention of stand loss and effects on yield.

MATERIALS AND METHODS

Non-inoculated field trials were established in three locations in 2014 and 2015 in Illinois, resulting in six different environments. The locations were near DeKalb in northern Illinois, near Urbana in east central Illinois, and near Dixon Springs in southern Illinois. The trials were conducted at University of Illinois research facilities at those locations. The DeKalb plots were placed on an El Paso silty clay loam soil, which is poorly drained, with a 0-2% slope (Soil Survey, NRCS, 2015) that had been continuously planted to soybean for several years. The Urbana plots were on Elburn silt loam soils with a 0-2% slope that are also poorly drained. These plots contained corn residue from the previous year (Soil Survey, NRCS, 2014). The Urbana plots moved to adjacent fields each year, depending on field rotation. The Dixon Springs

locations, were planted in a Grantsburg silt loam soil. This soil has a 2-5% slope usually, and is a moderately drained soil that is usually found in a wooded area (Soil Survey, NRCS, 2011). These plots were on a corn-soybean rotation, and were at different sites each year within the research farm. At Urbana and DeKalb, the Syngenta cultivar NK S34-Z1 cultivar was planted (relative maturity 3.4), while at Dixon Springs, Asgrow AG4034, (relative maturity 4.0) was planted. The two different soybean cultivars were used to match maturity group with the latitudes of the different sites and contained the same Phytophthora root rot resistant gene, *Rps1c* as well as similar resistance to other soil and foliar diseases.

Fungicide seed treatments consisted of (1) an untreated control, (2) metalaxyl (Sebring 2.65ST; Valent USA Corp., Walnut Creek, CA) at 4 g a.i./100 kg of seed, (3) ethaboxam (INTEGO Solo; Valent USA Corp., Walnut Creek, CA) at 7.5 g a.i./100 kg of seed, (4) ethaboxam + metalaxyl at 7.5 g + 2 g a.i./100 kg of seed, (5) ethaboxam + metalaxyl at 7.5 g + 4 g a.i./100 kg seed, and (6) ethaboxam + metalaxyl at 7.5 g + 7.5 g a.i./100 kg of seed (Table 3.2). Rizolex, a broad spectrum fungicide with the active ingredient tolclofos-methyl at 5 g a.i./100 kg of seed (Valent USA Corp., Walnut Creek, CA) was applied to all of the seed treatments except the untreated control. Rizolex was applied to help control Rhizoctonia damping off and Fusarium root rot that might be present in the soil. Slurries of the seed treatments were applied to the seed with a Seedburo batch lab seed treater (Seedburo Equipment Co, Des Plaines, IL) approximately two weeks before planting. Plots in 2014 were planted on 27 May in Urbana, 3 June in DeKalb, and 9 June in Dixon Springs. In 2015, plots were planted on 30 April in Urbana, 3 May in Dixon Springs, and 21 May in DeKalb. All plots were planted using a four row Almaco 360 research plot planter (Almaco Co; Nevada, IA). Plots were four rows wide, with a 76 cm row spacing and were 6.1 meters long. The planting population rate was

approximately 345,800 seeds per hectares. Plots were arranged in a randomized complete block design with four replications.

Plant stand counts were made within three weeks of emergence by counting the plants from the middle two rows of each plot that were within the length of a 3 m pole. Numbers were then converted into plant stand per hectare. Roots were collected at two time points during the growing season: (1) at approximately VC-V1 growth stages, and (2) at approximately R1 developmental stage (Fehr et al., 1971). Ten plants per plot were collected for the R1 root observations. Plants were retrieved by digging approximately 32 cm deep with a shovel to free the roots from the soil and washed until clean with tap water. Data for root rot, dried root weight, and dried shoot weight were taken. Roots were scored for root rot severity, from a scale adapted from Nelson et al. (1996). Roots were scored as follows: 0 = no lesions, 1 = less than 20% of root area containing lesions, 2 = between 20-40% of root area containing lesions, 3 = 40-60% of root area containing lesions, 4 = 60-80% of root area containing lesions, and 5 = plant death or greater than 80% of the root area covered with lesions. Once plants were rated, roots were separated from the tops at the soil line and all plant parts were dried at 25^0C with forced air for three days and then weighed. Plant parts were then divided by the number of plants collected from the plot to obtain the average root or shoot weights per plant. All plants collected were taken from the outer two rows, while the inner two rows remained untouched and were used for yield data. Yield data were collected on 23 October in Urbana, 27 October in DeKalb, and 5 November in Dixon Springs in 2014 and on 24 September in Urbana, 8 October in DeKalb, and 17 October in Dixon Springs in 2015. Seeds were harvested using a Kincaid 8-XP small plot combine (Kincaid Equipment Manufacturing, Haven, KS) equipped with a HarvestMaster grain

gauge (Juniper System, Inc, Logan, UT) to calculate grain moisture and weight for each plot. Individual plot weights were adjusted to 13% moisture and yields were calculated to kg/ha.

Statistical Analysis

Data were analyzed using the mixed linear model procedure (PROC MIXED) in SAS v9.4 (SAS Institute Inc., Cary, NC). Each year and location combination was considered to be a different environment. Seed treatment and environment were considered fixed, while block was considered a random effect. Least square means were compared using the PDMIX800 macro (Saxton, 1998) where $\alpha=0.05$. Correlations between plant stand, yield, root weight per plot, and root rot severity were determined using the Pearson correlation procedure (PROC CORR) in SAS v9.4 (SAS Institute Inc., Cary, NC).

RESULTS

The main effect of seed treatment was significant for plant stand (P=0.0002), but it was not significant for yield (P=0.7466) (Table 3.1). The main effect of environment was significant for plant stand (P<0.0001) and for yield (P<0.0001). The environment by seed treatment interaction was not significant for plant stand (P=0.0522) or yield (P=0.6176). The environment by seed treatment interactions were not significant, therefore, only the main effects of seed treatment and environment are presented.

Seed treatments did not significantly affect root weight (P=0.0823), shoot weight (P=0.1873), and root rot (P=0.4017) (Table 3.1). Environment, however, did have a significant effect on average root weight (P<0.0001), shoot weight (P<0.0001), and root rot (P<0.0001). The environment by seed treatment interaction was not significant for average root weight

(P=0.7878), shoot weight (P=0.3648), and root rot (P=0.0758). The 2014 shoot weight data from Dixon Springs were not included in the analysis because of severe deer damage to the plants prior to collection.

Seed treatments had a significant effect on plant stands. The untreated control had significantly fewer plants than all of the seed treatments (Table 3.2). The ethaboxam only treatment did not significantly differ from the metalaxyl only treatment or the treatments of the lower ratios of 7.5 g + 2 g a.i., and 7.5 g + 4 g a.i. of ethaboxam + metalaxyl respectively. Treatment 7.5 g + 7.5 g a.i. of ethaboxam + metalaxyl, was the only treatment that was significantly higher from the ethaboxam only treatment for plant stands. The three treatments with varying ratios of ethaboxam + metalaxyl were not significantly different from each other. Seed treatments did not have a significant effect on the yield data collected.

Pearson correlation analysis indicated positive significant correlations between both plant stand and yield and plant stand and root rot, but a significant negative correlation between plant stand and root weight (Table 3.3). Yield had a significant negative correlation with average root rot per plot, but showed no correlation with root weight. There was no significant correlation between root weight and root rot.

DISCUSSION

In this non-inoculated field study, all of the seed treatments had significantly higher plant stands than the untreated control. Oomycete seedling pathogens have the ability to reduce plant stands in fields, especially species of *Pythium* and *Phytopthora sojae*, which are important components of the seedling disease complex (Broders et al., 2007; Dorrance et al., 2009; Griffin,

1990; Rizvi and Yang, 1996). These results correspond with other seed treatment studies done, showing that seed treatments help prevent loss of plant stand compared to untreated seed (Bradley, 2008; Guy et al., 1989; Urrea et al., 2013).

The ethaboxam only treatment and the metalaxyl only treatment were not significantly different from each other. This is the first time that ethaboxam and metalaxyl have been tested against each other on soybean in the U. S. There have been few studies done comparing the two active ingredients against each other. In Korea, Kim et al. (1999) studied the effect of ethaboxam and metalaxyl in field trials to control the diseases caused by *Pseudoperonospora cubensis, Phytophthora infestans*, and *Phytophthora capsici*, in cucumber, potato, and pepper, respectively. They reported that ethaboxam controlled these diseases better than metalaxyl. Zhang et al. (2005) also tested ethaboxam and metalaxyl in potato fields in Korea against *Phytophthora infestans*. A majority of the *Phytophthora infestans* populations tested were resistant or moderately resistant to metalaxyl, however, ethaboxam had a control efficacy of approximately 81% compared to the 3% efficacy of metalaxyl. All of the field studies done in Korea, used foliar applied fungicides to control oomycete pathogens. No studies have been published yet about seed treatments with ethaboxam in a field setting. Application timing and placement could explain why seed treatments with ethaboxam and metalaxyl did not differ from each other when applied as seed treatments on soybean fields in the U.S. This also could be explained by absence of disease pressure from oomycete seedling diseases because this soybean seed treatment trial was non-inoculated.

The results showed that the treatments that contained the different ratios of ethaboxam to metalaxyl did not differ from each other. The treatment of 7.5 g + 7.5 g a.i. of ethaboxam + metalaxyl resulted in significantly higher plant stands than the ethabxoam only treatment. This

possibly shows that having chemistries with two different MOAs in a fungicide seed treatment might protect seed and seedlings better than just ethaboxam on its own. Urrea et al. (2013) reported that plant stands improved in soybeans when seeds were treated with metalaxyl and a broad spectrum fungicide with a different MOA. Bradley (2008) also observed higher plant stands with seed treatments that contain either mefenoxam or metalaxyl and broad spectrum fungicide. Metalaxyl has been used as a fungicide since the 1980s in fields across the U.S. soybean production areas, and there have been reports of *Pythium* spp. with reduced sensitivity to metalaxyl (Broders et al., 2007; Dorrance et al., 2004; Urrea et al., 2013). Ethaboxam has been shown in Korea to have a better efficacy towards metalaxyl resistant *Phytophthora infestans* compared to just metalaxyl (Zhang et al., 2005). Even though the Korean studies were not done using species of *Pythium* or *Phytophthora sojae*, in theory, using a combination of active ingredients with two different modes of action would be expected to prolong the usefulness of both chemistries by slowing down the selection of fungicide resistant isolates (Broders et al., 2007).

Environment had a significant effect in this study. The environment plays a key role in the development of seedling diseases that are caused by oomycetes pathogens. *Pythium* spp. and *Phytophthora sojae* prefer saturated soils in early season conditions for disease development (Dorrance et al., 2004; Dorrance et al., 2009). The 2015 Dixon Springs environment had lower stand counts compared to those grown in the other environments, although the yields from the 2015 Dixon Springs environment were not significantly different from those in the other environments. The lower stand counts could be attributed to the over 47 cm of rain that Dixon Springs received in March, April, and May of 2015, which kept soils saturated for longer periods of time compared to the 37 cm of rain that occurred in that time period for 2014 (Illinois State

Water Survey, 2016). The 2015 Dixon Springs seed and seed treatments were planted when soil temperatures were approximately 21^{0}C (Illinois State Water Survey, 2016), which is in the temperature range that oomycete seedling pathogens prefer (van der Plaats-Niterink, 1981; Dorrance et al., 2009). The amount of rain that Dixon Springs received in March to May in 2014 and 2015 is more than what the other locations received. The Urbana location in 2014 received 24 cm of rain during that time period, while in 2015, it received 29 cm of rain. The 2015 Urbana seed and seed treatments were planted into soil temperatures around 14°C (Illinois State Water Survey, 2016). The 2015 Urbana environment which was plated in late April, is a prime example of planting early in cool soil temperatures, yet it had some of the highest plant stands out of the six environments. The DeKalb location had on average the least amount of rain in March, April, and May in 2014 and 2015 compared to the two other locations across the state. In 2014, DeKalb received 18.5 cm of rain during the spring, while in 2015 the location received 25 cm of rain. In 2015, the DeKalb seed and seed treatments were planted in soil temperatures approximately 17°C (Illinois State Water Survey, 2016). Even with the cooler soil temperatures that *Pythium* spp. prefer, there might not have been enough moisture in the soil at the correct time for optimal seedling disease conditions.

The environment effects for this study also include the different soil types that the trials were planted on, as well as potential differences in the composition of soilborne pathogen communities in the fields used. Each trial location was on a different type of soil; the DeKalb plots were on an El Paso silty clay loam soil, the Urbana plots were on an Elburn silt loam, and the Dixon Springs trials were planted on a Grantsburg silt loam. The El Paso and the Elburn soils are poorly drained with low potential for surface run off, and the permeability is moderate in the loess (Soil Survey, NRCS, 2014; Soil Survey, NRCS, 2015). Unlike the El Paso and

Elburn soils, Grantsburg silt loam is moderately well drained soil and is usually found in wooded areas. This soil has medium to high potential for surface run off, and its water permeability is moderately slow (Soil Survey, NRCS, 2011). Even with the moderately well drained soils, the slow permeability leaves the Grantsburg silt loam soil saturated for longer, making conditions ideal for seedling diseases. Grantsburg soils are largely found only in southern Illinois, while the El Paso and Elburn soils are found throughout northern and central Illinois (Soil Survey, NRCS, 2011; Soil Survey, NRCS, 2014; Soil Survey, NRCS, 2015). These different types of soils were probably one of the larger effects on the environment, due to of the role that they play in seedling pathogen populations. Broders et al. (2009) reported that different soil classes, composed of soil chemical and physical properties, were correlated with the presence and distribution of *Pythium* spp. in Ohio. They also found that the probability of disease should be assessed on a field by field basis because soil types and properties vary over relatively small areas. Another larger effect that the environment accounts for is the differences of genotypes at each location. The Urbana and DeKalb locations had the same genotype, while the Dixon Springs location had a different genotype. Even with the similarities between the two genotypes, there is enough differences that this could be contributing a great deal to the significant environmental effect. Besides soil temperature at planting, rainfall, soil type, and seedling pathogen populations already present in the soil, the environmental effect takes into account the two different genotypes used at different locations, the moisture during early season crop development, air temperature, and other abiotic and biotic stresses that the crop could encounter throughout the growing season. Most of these factors can also contribute to the speed of seed germination and plant and root growth as well as the rate of pathogen growth and seedling infections. With

colder temperatures seed germination rate slows down and there could be a higher opportunity for seedling diseases to attack the slow germinating seed (Hatfield and Egli, 1974).

Pearson correlation analysis indicated a positive significant correlation between stand and yield, however the yields were not significantly different from each other. No matter how significant the correlation was between stand and yield, it still had a correlation coefficient of 0.29. The low correlation coefficient could be due to the ability of soybean plants to produce adequate yields in spite of lower plant population densities (Stivers and Swearingin, 1980). The negative correlation between yield and root rot, could be explained by the fact that plants with better root systems are able to absorb more nutrients and water to produce more or larger seeds.

In this study, the yields were not affect by the seed treatments. With no change in yield, there was little evidence that the additional cost of seed treatments would be beneficial to soybean producers. However there are many studies that contradict our findings. Poag et al. (2005) found that high quality seed treated with metalaxyl maximized the profit received from using the seed treatment in Arkansas. Bradley (2008) found similar results in North Dakota, where the economic net return achieved from the use of seed treatments was $33/ha more than the net return achieved from the use of untreated seed. Esker and Conley (2012) indicated that seed treatments can be a cost effective component of soybean production although several factors must be considered, especially environments and cultivars. Our study had a limited number of environments, and cultivars compared to the other studies. Further studies with ethaboxam and metalaxyl seed treatments in more environments are needed to confirm if these seed treatments would be an economic benefit to soybean producers.

LITERATURE CITED

Bates, G.D., C.S. Rothrock, and J.C. Rupe. 2008. Resistance of the soybean cultivar Archer to Pythium damping-off and root rot caused by several *Pythium* spp. Plant Dis. 92:763-766.

Bradley, C.A. 2008. Effect of fungicide seed treatments on stand establishment, seedling disease, and yield of soybean in North Dakota. Plant Dis. 92: 120-125.

Broders, K.D., P.E. Lipps, P.A. Paul, and A.E. Dorrance. 2007. Characterization of *Pythium* spp. associated with corn and soybean seed and seedling diseases in Ohio. Plant Dis. 91:727-735.

Broders, K. D., M.W. Wallhead, G.D. Austin, P.E. Lipps, P.A. Paul, R.W. Mullen, and A.E. Dorrance. 2009. Association of soil chemical and physical properties with *Pythium* species diversity, community, composition, and disease incidence. Phytopathology 99:957-967.

Brown, G.E. and B.W. Kennedy. 1965. Pythium pre-emergence damping-off in soybean in Minnesota. Plant Dis. 49:646-647.

Dorrance, A. E., S.A. Berry, P. Bowen, and P.E. Lipps. 2004. Characterization of *Pythium* spp. from three Ohio fields for pathogenicity on corn and soybean and metalaxyl sensitivity. Online. Plant Health Progress. doi: 10.1094/PHP-2004-0309-01-RS

Dorrance, A.E., A.E. Robertson, S. Cianzo, L.J. Giesler, C.R. Grau, M.A. Draper, A.U. Tenuta, and T.R. Anderson. 2009. Integrated management strategies for *Phytophthora sojae* combining host resistance and seed treatments. Plant Dis. 93:875-882.

Douglas, M.R. and J.F. Tooker. 2015. Large scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. Environ. Sci. Technol. 49:5088-5097.

Ellis, M. L., K.D. Broders, P.A. Paul, and A.E. Dorrance. 2011. Infection of soybean seed by *Fusarium graminearum* and effect of seed treatments on disease under controlled conditions. Plant Dis. 95:401-407.

Ellis, M.L., L.K. McHale, P.A. Paul, S.K. St. Martin, and A.E. Dorrance. 2013. Soybean germplasm resistant to *Pythium irregulare* and molecular mapping of resistance quantitative trail loci derived from the soybean accession PI 424354. Crop Sci. 53:1008-1021.

Esker, P.D. and S.P. Conley. 2012. Probability of yield response and breaking even for soybean seed treatments. Crop Sci. 52:351-359.

Fisher, D.J. and A.L. Hayes., 1982. Mode of action of the systemic fungicides furalaxyl, metalaxyl, and ofurace. Pestic. Sci. 13:330-339.

Fungicide Resistance Action Committee. 2015. FRAC code list 2015: Fungicides sorted by mode of action (including FRAC code numbering). January 2015. Web.

Grau, C.R, A.E. Dorrance, J. Bond, and J.S. Russin. 2004. Fungal Diseases. In H.R. Boerma and J.E. Specht, Soybean: Improvement, Production, and Uses (Third Ed. pg. 679-764) Madison, WI: ASA, CSSA, and SSSA.

Griffin, G.J. 1990. Importance of *Pythium ultimum* in a disease syndrome of cv. Essex soybean. Can. J. of Plant Pathol. 12:135-140.

Guy, S.O., E.S. Oplinger, and C.R. Grau. 1989. Soybean cultivar response to metalaxyl applied in furrow and as a seed treatment. Agron. J. 81:529-532.

Hatfield, J.L., and D.B. Egli. 1974. Effect of temperature on the rate of soybean hypocotyl elongation and field emergence. Crop Sci. 14:423-426.

Kim, D.S., S.J. Chun, J.J. Jeon, S.W. Lee., and G.H. Joe. 2004. Synthesis and fungicidal activity of ethaboxam against oomycetes. Pest Manag. Sci. 60:1007-1012.

Kim, D.S., H.C. Park,S.J. Chun, S.H. Yu, K.J. Choi, J.H. Oh., K.H. Shin, Y.J. Koh, B.S. Kim, Y.I. Ham, and B.K. Chung. 1999. Field performance of a new fungicide ethaboxam against cucumber downy mildew, potato late blight, and pepper Phytophthora blight in Korea. Plant Pathol. J. 15:48-52.

Kirkpatrick, M. T., C.S. Rothrock, J.C. Rupe, and E.E. Gbur. 2006. The effect of *Pythium ultimum* and soil flooding on two soybean cultivars. Plant Dis. 90:597-602.

Koenning, S.R. and J.A. Wrather. 2010. Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. Online. Plant Health Progress. doi:10.1094/PHP-2010-1122-01-RS

Illinois State Water Survey - Water and Atmospheric Resources Monitoring Program. 2016. Illinois Climate Network: Monthly Data Archive. Web 15 March 2016. http://www.isws.illinois.edu/warm/dataarchive.asp?from=&site=

Monkiedje, A. and M. Spiteller. 2005. Degradation of metalaxyl and mefenoxam and effects on the microbiological properties of tropical and temperate soils. Int. J. Environ. Res Public Health 2:272-285.

Munkvold, G. 2009. Seed pathology in academia and industry. Ann. Rev. Phytopathol. 47:285-311.

National Climatic Data Center, National Oceanic and Atmospheric Administration. 2016. Annual Climatological Summary Report. Subset 2014-2015. Web 8 March 2016.

Poag, P.S., M. Popp, J. Rupe, B. Dixon, C. Rothrock, and C. Boger. 2005. Economic evaluation of soybean fungicide seed treatments. Agron. J. 97:1647-1657.

Rizvi, S.S.A. and X.B. Yang. 1996. Fungi associated with soybean seedling disease in Iowa. Plant Dis. 80:57-60.

Robinson, A.P., S.P. Conley, J.J. Volenec, and J.B. Santini. 2009. Analysis of high yielding, early-planted soybean in Indiana. Agron. J. 101:131-139.

Rooso, M.L., J.C. Rupe., P. Chen, and L.A. Mozzoni. 2008. Inheritance and genetic mapping of resistance to *Pythium* damping-off caused by *Pythium aphanidermatum* in 'Archer' soybean. Crop Sci. 48:2215-2222.

Rupe, J.C., Rothrock, C.S., Bates, G., Rosso, M. L.,Avanzato, M. V. and Chen, P. 2011. Resistance to Pythium seedling disease in soybean. In (Ed) A. Sudaric, Soybean-Molecular Aspects of Breeding. InTech. Croatia.

Saxton, A.M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. Pages 1243-1246 in Proc. 23rd SAS Users Group International.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. 2011. Official soil descriptions; Grantsburg Series. Web. 11 March 2016. https://soilseries.sc.egov.usda.gov/OSD_Docs/G/GRANTSBURG.html

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. 2014. Official soil descriptions; Elburn Series. Web. 11 March 2016. https://soilseries.sc.egov.usda.gov/OSD_Docs/E/ELBURN.html

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. 2015. Official soil descriptions; Elpaso Series. Web. 11 March 2016. https://soilseries.sc.egov.usda.gov/OSD_Docs/E/ELPASO.html

Stivers, R.K. and M.L. Swearingin. 1980. Soybean yield compensation with different populations and missing plant patterns. Agron. J. 72:98-102.

Urrea, K., J.C. Rupe, and C.S. Rothrock. 2013. Effect of fungicide seed treatments, cultivar, and soils on soybean stand establishment. Plant. Dis. 97:807-812.

United States Department of Agriculture-National Agricultural Statistics Service. 2015. Acreage Report 30 June 2015. Web 15 March 2016. http://www.usda.gov/nass/PUBS/TODAYRPT/acrg0615.pdf

United States Department of Agriculture-National Agricultural Statistics Service. 2015. Major soybean producing U.S. states in 2014 and 2015. Statista. Web 27 February 2016. http://www.statista.com/statistics/192076/top-10-soybean-producing-us-states/

Wilcox, J.R. 2001. Sixty years of improvement in publicly developed elite soybean lines. Crop. Sci. 49:1711-1716.

Wilcox, J.R. 2004. World distribution and trade of soybean. In H.R. Boerma and J.E. Specht, Soybean: Improvement, Production, and Uses (Third Ed. pg. 1-14) Madison, WI: ASA, CSSA, and SSSA.

Wrather, J.A., S.R. Koenning, 2009. Effects of diseases on soybean yields in the United States 1996-2007. Online. Plant Health Progress. doi:10.1094/PHP-2009-0401-01-RS.

Yang, X. 1999. Pythium damping off and root rot. In Eds. G. Hartman, J. Sinclair, and J. Rupe. Compendium of Soybean Diseases (Fourth ed. pg. 42-44). St. Paul, MN: The American Phytopathological Society.

Zhang, B.Q. and X.B. Yang. 2000. Pathogenicity of *Pythium* populations from corn-soybean rotation fields. Plant Dis. 84:94-99.

Zhang, X.Z., K.Y. Ryu, J.S. Kim, J.U. Cheon, and B.S. Kim. 2005. Changes in the sensitivity to metalaxyl, dimethomorph, and ethaboxam of *Phytophthora infestans* in Korea. Plant Pathol. J. 21:33-38.

Table 3.1. Analysis of variance for the dependent variables: soybean plant stand, seed yield, root weight per plot, shoot weight per plot, and root rot per plot for six fungicide seed treatments evaluated over six environments in Illinois in 2014 and 2015.

Dependent variable, source of variation	df	MS	Pr>F
Plant stand			
Seed treatment	5	465028032	0.0002
Environment	5	17988058375	<0.0001
Block (environment)	18	152841476	0.0406
Seed treatment x Environment	25	139278890	0.0522
Yield			
Seed treatment	5	88595	0.7466
Environment	5	10865765	<0.0001
Block (environment)	18	970850	<0.0001
Seed treatment x Environment	25	146370	0.6176
Root weight			
Seed treatment	5	0.099791	0.0823
Environment	5	8.686076	<0.0001
Block (environment)	18	0.092177	0.0284
Seed treatment x Environment	25	0.037067	0.7878
Shoot weight †			
Seed treatment	5	6.743944	0.1873
Environment	4	587.466821	<0.0001
Block (environment)	15	5.789722	0.2102
Seed treatment x Environment	20	4.826886	0.3648
Root rot			
Seed treatment	5	0.257251	0.1725
Environment	5	2.676975	<0.0001
Block (environment)	18	0.176231	0.3796
Seed treatment x Environment	25	0.248363	0.0758

† 2014 shoot weight data from Dixon Springs were not included in the analysis because of severe deer damage prior to collection.

Table 3.2. Effects of seed treatments across the six environments

Treatment	Active ingredient†	Plant stand plants/ha‡	Yield kg/ha‡
1	Untreated control	214283 C	3206 A
2	metalaxyl at 4 g a.i./100 kg	240873 AB	3366 A
3	Ethaboxam at 7.5 g a.i./100 kg	228200 B	3364 A
4	ethaboxam + metalaxyl at 7.5 g + 2 g a.i./100kg	236195 AB	3288 A
5	ethaboxam + metalaxyl at 7.5 g + 4 g a.i./100kg	238629 AB	3310 A
6	ethaboxam + metalaxyl at 7.5 g + 7.5 g a.i./100kg	243814 A	3345 A

† The active ingredient tolclofos-methyl, a broad spectrum fungicide, was applied to treatments 2-6 at 5 g a.i./100 kg of seed to control for non-oomycetes seedling diseases.

‡ Means followed by the same letter within a column are not significantly different ($P \leq 0.05$).

Table 3.3. Pearson correlation coefficients for relationships between plant stand, yield, average root weight per plant, and average root rot per plot.

	Plant stand (plants/ha)	**Yield (kg/ha)**	**Average root weight†**	**Average root rot**
Plant stand (plants/ha)	1	0.295*	-0.267*	0.235*
Yield (kg/ha)	0.295*	1	0.113^{ns}	-0.309*
Average root weight†	-0.267*	0.113^{ns}	1	-0.095^{ns}
Average root rot	0.235*	-0.309*	-0.095^{ns}	1

†Weights used were on a per plant basis

* Significant at P≤0.05

^{ns} Not significant

APPENDIX A

Table A.1. Inoculum level and placement experiment. An inoculum level and inoculum placement experiment was done to determine the amount of millet inoculum that would be needed per pot to achieve desired symptoms. Data collect was compared to its non-inoculated control, and data below is expressed as relative means to the control. The inoculum is of isolates of *P. ultimum* var. *ultimum*. From the results, it was determined that an inoculum level of 5 g of millet inoculum per pot gives the desired symptoms and would have a low chance of escapes. This level was not significantly different from the 2 g of inoculum per pot however, the 5 g of inoculum level per pot was still chosen to aggressively test the aggressiveness of the isolates and the resistance of the cultivars in later tests.

Inoculum amount (g)	**Plant stand**[†]	**Dried plant weight**[†]	**Average plant height**[†]	**Root rot score**[†]
0.5	0.51 A	0.51 A	0.67 A	1.92 C
1	0.43 A	0.37 AB	0.61 AB	2.26 BC
2	0.43 A	0.39 AB	0.55 AB	2.35 B
5	0.42 A	0.26 BC	0.49 BC	2.46 B
10	0.27 B	0.20 C	0.37 C	3.22 A

[†] Means followed by the same letter within a column are not significantly different ($P \leq 0.05$).

Table A.2. Inoculation placement experiment. Each concentration of inoculum of the *P. ultimum* var. *ultimum* isolates from Appendix Table 1A was placed into pots in two different methods; a layered placement or mixed throughout the pot. Neither method was significantly different from each other in plant stand per pot, plant weight per pot and root rot score. However the layered method was chosen because of the high probability that the seedlings would come into contact with the pathogen and for the lower chance of escapes.

Method	Plant stand[†]	Dried plant weight[†]	Average plant height[†]	Root rot score[†]
Layer	0.40 A	0.40 A	0.56 A	2.3 A
Mixed	0.43 A	0.30 B	0.51 A	2.5 A

[†] Means followed by the same letter within a column are not significantly different ($P \leq 0.05$).

Table A.3. Results of the Isolate Aggressiveness Assay**.** Data collected was stand counts per pot, plant height, whole plant weight per pot, and root rot score per pot. All isolates used were of *P. ultimum* var. *ultimum*. The data collected was taken against the non-inoculated controls and is expressed as a proportion relative of the control. The data was ran in SAS 9.4 with PROC MIXED. The isolate 12Py393 was determined to be the most aggressive because of its low plant stand compared to the control as well as having the lowest height and whole plant weight compared to the control and it had the highest root rot score compared to the control. Isolate 11Py719 showed to the be the least aggressive out of the six isolates tested because of its plant stand, average plant height and whole plant weight were very comparable to the control. 12Py391 was chosen to be used in further experiments because it was a middle isolate that would infect the host and still give symptoms.

Isolate	**Plant stand**[†]	**Dried plant weight**[†]	**Average plant height**[†]	**Root rot score**[†]
11Py719	1 A	0.91 A	0.90 A	1.96 C
11Py724	0.65 B	0.27 BC	0.36 CD	3.30 A
12Py726	0.74 B	0.36 B	0.52 B	2.80 B
12Py391	0.71 B	0.33 B	0.41 BC	3.16 AB
12Py392	0.69 B	0.28 BC	0.36 CD	3.15 AB
12Py393	0.47 C	0.16 C	0.24 D	3.51 A

[†] Means followed by the same letter within a column are not significantly different ($P \leq 0.05$).

Table A.4. Inoculum level for ancestral screen assay**.** Data shown in from trial two with the higher inoculum levels of *P. ultimum* var. *ultimum*. The data is expressed as the proportion relative to the non-inoculated control. The two trials were not significant different from each other in the 1g level where they overlapped. Upon visual inspection, it was determined to analyze the higher inoculum level trial. 5 g inoculation level was chosen to be used in the ancestral screen because of the low plant stand and low dried root weights as well as a higher root rot score for a scoring scale adapted from Ellis et al. (2013) for *Pythium*. The 5 g level also lowers the probability of possible escapes.

Inoculum amount (g)	**Plant stand**[†]	**Dried root weight**[†‡]	**Dried shoot weight**[†‡]	**Root rot score**[†]
1	0.75 A	0.69 A	0.86 A	3.36 C
2	0.72 A	0.58 B	0.79 A	3.76 B
5	0.53 B	0.47 C	0.87 A	4.16 A

[†] Means followed by the same letter within a column are not significantly different ($P \leq 0.05$).

[‡]Weights used were on a per plant basis.