DISTRIBUTION AND MANAGEMENT OF *DIAPORTHE* SPP. AND PLANT PARASITIC NEMATODES AFFECTING SOYBEANS IN DELAWARE AND MARYLAND

by

Alexandra C. Kessler

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Plant and Soil Sciences

Spring 2022

DISTRIBUTION AND MANAGEMENT OF

***DIAPORTHE* SPP. AND PLANT PARASITIC**

NEMATODES AFFECTING SOYBEANS

IN DELAWARE AND MARYLAND

by

Alexandra C. Kessler

Approved: ____
Alyssa Koehler, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: ____
Erik Ervin, Ph.D.
Chair of the Department of Plant and Soil Sciences

Approved: ____
Calvin Keeler, Ph.D.
Interim Dean of the College of Agriculture and Natural Resources

Approved: ____
Louis F. Rossi, Ph.D.
Vice Provost for Graduate and Professional Education and
Dean of the Graduate College

ACKNOWLEDGMENTS

First and foremost, I want to thank my advisor, Dr. Alyssa Koehler, for introducing me to plant pathology and her kindness, grace, and guidance throughout my research.

I want to thank my committee, Jill Pollok and Dr. Nicole Donofrio, for their compassion, thoughtfulness, and encouragement throughout this process.

I want to thank my funding sources, Delaware Soybean Board, Maryland Soybean Board, Atlantic Soybean Council, and the SCN Coalition, for providing me with funds to accomplish my research. I hope this research will make a positive impact on our community.

I want to thank Koehler Lab, Andy Kness, and Jake Jones for their support over the past three years. Without their help, I would not have recovered nearly as many *Diaporthe* or nematode soil samples or found a secondary location for my trial. Their help indefinitely impacted my work.

I want to thank my family and friends for their support and understanding during the past three years. They kept me anchored in these unprecedented times.

Finally, I want to thank my boyfriend, without whom I would have never accomplished this. He encouraged me to pursue a degree I had never dreamed of receiving, and his unwavering faith in my ability saw me through the end.

Thank you.

TABLE OF CONTENTS

Appendix

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Soybean (*Glycine max* L. Merrill*)* is a major agronomic commodity for the Mid-Atlantic region, with over 190,500 and 571,500 metric tons (MT, 1000kg) harvested in Delaware (DE) and Maryland (MD), respectively in 2020 (USDA NASS 2020). In annual disease loss estimates, *Diaporthe* species and plant parasitic nematodes cause over 168,736 MT (6.2 million bu) of yield suppression in soybean across both states. The *Diaporthe* complex includes multiple *Diaporthe* species causing diseases such as stem canker, pod and stem blight, and Diaporthe/Phomopsis seed decay. Due to similarities in pathogen signs and disease symptoms, misdiagnosis is common, and no molecular characterization of species distribution or abundance has been conducted in the Mid-Atlantic region. In this thesis, a two-year fungal survey was conducted to characterize *Diaporthe* species present within stem and root tissue of soybeans in DE and MD. This survey identified four species *D. longicolla, D. aspalathi, D. sojae,* and a novel *Diaporthe* pathogen, *D. ueckerae,* not yet reported within the region. *D. longicolla* accounted for 97% of samples. Koch's Postulates confirmed the pathogenicity of *D. ueckerae* and the association of zone lines with *D. longicolla*. Greenhouse aggressiveness trials were conducted to compare *D. longicolla, D. aspalathi,* and *D. ueckerae* symptom development on three soybean hybrids. *D. longicolla* had the highest disease severity score within each hybrid and *D. aspalathi* had the lowest. In addition to fungal pressure, nematodes are of great concern to the region. Soybean cyst nematode consistently ranks at the top yield-limiting pathogen of soybeans within the region and the US. In this thesis, a three-year nematode soil survey was completed to update data on the distribution and population levels of SCN while also monitoring for other nematodes of concern. Across the 311 samples, nine nematode genera were recovered. Of economic concern, 53.4% of samples had SCN, 18.3% root-knot nematode, and 57.2% lesion nematode. In addition to survey work, two seed treatment trials were conducted to evaluate the efficacy of chemical and biological seed treatments for management of SCN. Soil samples were collected at planting and prior to harvest, with mid-season evaluation of female SCN on roots. Differences in percent emergence, initial SCN soil counts, on-root SCN females, and yield were observed across both trials. Findings from this project expand characterization of regional soilborne pathogens of soybean and provide a foundation to develop improved management approaches.

Chapter 1

REVIEW OF SOILBORNE DISEASES IN MID-ATLANTIC SOYBEAN PRODUCTION

Introduction to Soybean Diseases

Soybeans (*Glycine max* (L.) Merrill) comprise a 46-billion-dollar industry in the United States (U.S.) (USDA ERS 2022). Soybeans are also an important crop within the Mid-Atlantic region, consistently ranking as a top crop by acre grown in Delaware (DE) and Maryland (MD), second only to maize, with 253,946 metric tons (MT) (9,331,000 bu) produced in DE and 709,832.1 MT (26,082,070 bu) in MD during the 2017 census (USDA, 2017 Census of Agriculture Delaware; Maryland). Soybean yield averages in DE and MD typically range from 2690.04 – 3429.8 kg per ha (40 – 51 bushels per acre) (USDA NASS and DE Department of Agriculture, 2019-2020; USDA NASS and MD Department of Agriculture, 2019-2020). Unfortunately, there are several diseases and pests that suppress yield of soybeans within both states. Foliar diseases such as Septoria brown spot (*Septoria glycines* Hemmi) and Downy Mildew (*Peronspora manshurica* (Naum.) Syd. ex Gaum) are frequently observed, but primary yield impacts are observed from soilborne diseases. In 2018, approximately 20,683.6 MT (760,000 bu) of soybeans were lost in DE due to soilborne diseases like Charcoal Rot (*Macrophomina phaseolina* (Tassi) Goid), *Diaporthe* associated diseases

(Pod and Stem Blight, Phomopsis Seed Decay, and Stem Canker), and nematode infection by soybean cyst (*Heterodera glycines* Ichinohe) and southern root-knot (*Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949) nematodes (Allen et al. 2019). Other soilborne diseases, like damping off caused by various species of *Pythium* and *Rhizoctonia*, Phytophthora root and crown rot (*Phytophthora sojae* Kaufmann & Gerdemann), soybean sudden death syndrome (SDS, caused by *Fusarium virguliforme* O'Donnell & T. Aoki), and brown stem rot (BSR, caused by *Phialophora gregata* f. sp. *sojae* (Allington and Chamberlain 1948)) can also contribute to yield loss in the Mid-Atlantic. Foliar symptoms of SDS and BSR can appear very similar to symptoms caused by various *Diaporthe* species and it is possible to have coinfection of multiple fungi within the same plant. Nematode activity can also correlate with increased fungal disease issues (Mai and Abawi 1987). Many of these pathogens persist in the soil and level of disease observed is driven by environmental conditions, particularly wet spring conditions. Proper identification of the causal agent is an important first step to disease management.

***Diaporthe* species found in Soybean Production**

The genus *Diaporthe* Nitschke [syn. *Phomopsis* (Sacc.) Bubák] includes over 1,000 species described as endophytes, saprophytes, or pathogens on a broad host range worldwide (Gomes et al. 2013). *Diaporthe* are ascomycete fungi that fall under the class of Sordariomycetes, order Diaporthales, and family Diaporthaceae. They produce both sexually and asexually. Sexual ascospores are produced from perithecia that overwinter on debris and spread through rain splash or wind and asexual pycnidia produce conidia spread through seed or by wind and rain (Backman et al. 1985).

Yearly, thousands of metric tons of soybean are lost to diseases caused by multiple species of *Diaporthe.* To date, the U.S. has five *Diaporthe*-associated diseases including pod and stem blight, northern stem canker, southern stem canker, stem disease, and Diaporthe/Phomopsis seed decay. Northern stem canker (*D. caulivora* (Athow & Caldwell) Santos, Vrandecic & Phillips), southern stem canker (*D. aspalathi* Jansen, Castlebury & Crous), and stem disease (*D. gulyae* Shivas, Thompson and Young) are typically associated with only single species (Ghimire et al. 2019). Pod and stem blight can be caused by two species, *Diaporthe sojae* Lehman (Lehman 1923; Udayanga et al. 2015) or *D. longicolla* (Hobbs) Santos, Vrandecic & Phillips (Cui et al. 2009; Hobbs et al. 1985; Mathew et al. 2015; Santos et al. 2011; Udayanga et al. 2015). Diaporthe/Phomopsis seed decay was long associated with *D. longicolla* (Udayanga et al. 2015; Ghimire et al. 2019), but Petovic et al. 2021 recently showed that over ten species may be causal agents of seed decay. One of the species identified included a newly described species, *D. flavescens* Petrović, Skaltsas & Mathew, which was isolated from Kent County, Delaware.

The complex of pathogens associated with *Diaporthe* diseases account for more yield loss than any other fungal pathogen of soybean (Bradley et al. 2021). In 2019, 33,203 MT (1.22 million bu) were lost to Diaporthe/Phomopsis seed decay and 29,937 MT (1.10 million bu) to stem canker (southern) across the southern U.S., despite dry conditions and lack of rainfall (Allen et al. 2020). Comparatively, 619,150 MT (22.75

million bu) and 63,680 MT (2.34 million bu) were lost to Diaporthe/Phomopsis seed decay and stem canker, respectively, in the southern US in 2018 (Allen et al. 2019). That year was marked by above-average temperatures and abundant rainfall that delayed harvests and lead to yield loss projected at 2, 367700 MT (87 million bu) from Diaporthe/Phomopsis seed decay across the U.S. (Bradley et al. 2021). Similarities in morphology among *Diaporthe* species challenge accurate diagnosis of disease. However, recent improvements in molecular technologies have allowed for clearer genetic resolution of species. The use of the Elongation factor 1 alpha (EF1α), calmodulin (CAL), and beta-tubulin (TUB) primer sets, along with the internal transcribed spacers (ITS) for DNA sequencing has created species boundaries for *Diaporthe* complex, improving classification and accuracy of diagnosis (Udayanga et al. 2015). Within the Mid-Atlantic, stem canker, pod and stem blight (Mulrooney1985), and seed decay are all assumed to be present, but there have been no studies to document or molecularly confirm species distribution and abundance within the region, particularly in isolates from stem and root tissue.

Northern and Southern Stem Canker

Stem canker was first described in Maryland in 1943 (Petty 1943). During the 1950s, cultivars with susceptibility to stem canker were widely planted and yield losses of up to 50% were reported, until a shift away from the susceptible cultivars Hawkeye and Blackhawk (Backman 1985). The first report of stem canker in the southern United States came from Mississippi in 1975, and by the mid-1980s, stem canker was reported across much of the southern U.S. (Keeling 1982; Mulrooney 1985). Originally believed to be a single pathogen, differences in symptomology, cultural characteristics, and increased aggressiveness of southern isolates led to the proposition to divide stem canker into two diseases, northern and southern stem canker. It was first suggested to recognize the causal organisms as separate forma speciales of *D. phaseolorum (*f.sp. *caulivora* for northern and f. sp. *meridionalis* for southern) and random amplified polymorphic DNA (RAPD) analysis later supported sorting each organism as a variety of *D. phaseolorum* (Fernandez and Hanlin 1996). However, further molecular and phylogenetic analysis raised naming to species status, *D. aspalathi* (van Rensburg et al. 2006) and *D. caulivora* (Santos et al. 2011).

The disease cycle of both species begins with the overwintering of fungal structures on and in soybean debris left in the field. When proper temperature and rainfall conditions are met early in the growing season – at least 20ºC with high humidity (85-100%) for 4-5 days (Balducchi and McGee 1987), the pathogen germinates to produce perithecia and pycnidia. Later, ascospores and conidia are released, which can be rain splashed onto surrounding plants, infecting the rest of the field through leaves and wounded stems (Backman et al. 1985). Infection of the soybean occurs in the early vegetative stage, before pod formation and fill, and remains latent until late reproductive stages. Once the plant's vasculature is colonized, several signs and symptoms distinct to each species become visible. *Diaporthe caulivora* produces perithecia along the stem of the soybean in clustered groups embedded within the tissue. Additionally, the stems become dark and brown, with the formation of lesions along the outside length of the stem. These lesions are brown and vary in size, contributing to leaf withering, and in some cases, leaf lesions (Mena et al. 2020). These lesions may result in whole plant death. *D. aspalathi* is known for its "brick red lesions" that elongate and become sunken over time. The cankers often turn necrotic with age. On some occasions, these cankers will girdle the stem. Interveinal chlorosis, wilting, and whole plant death with retained leaves are also associated with southern stem canker (Backman et al. 1985). The similarities in both species' signs and symptoms make field diagnosis difficult in transition zones across the U.S. When stem canker is found in the field, it is often assumed to be a species based on geographic location. This is likely due to environmental conditions. Research suggests that *D. caulivora* is rendered non-pathogenic at 30 ºC, whereas *D. aspalathi* is still pathogenic at higher temperatures (Keeling, 1988). While temperature certainly plays a role in species limitation, there are isolated incidences when *D. aspalathi* has been found to affect soybeans as far north as Wisconsin (Li et al. 2004). This suggests that *D. aspalathi* may be more temperature tolerant and virulent than its northern counterpart. In transition zones like the Mid-Atlantic, it is assumed that both species are present, but there has been no molecular confirmation of species distribution or abundance in this region. Proper identification of causal organism is an important first step in disease management approaches to minimize yield loss.

Management options to reduce yield loss due to *D. aspalathi* or *D. caulivora* infection, include crop rotation, tillage, foliar and systemic fungicides, and genetic resistance. Since both species survive on debris in the soil, tillage is an effective management option, but the large-scale adoption of no till practices limits feasibility. This has also been observed in other *Diaporthe* species with other hosts (Mancebo et al. 2019). Rotating to a non-host crop is another option to reduce inoculum, especially in no-till fields. Most commercial fungicides target the *Diaporthe* complex, rather than *D. aspalathi* or *D. caulivora*. Therefore, labels may include language for *D. phaseolorum* or "Pod and Stem Blight". With the reoccurrence of *D. caulivora* and *D. aspalathi* across the U.S., the need for management tools is increasing. A 2019 study in Kentucky evaluated stem canker control by comparing management of *Diaporthe aspalathi* using foliar fungicides or foliar fungicides in combination with genetic resistance in two different counties. In the susceptible cultivar, foliar fungicides alone were not enough to control disease with incidence ranging from 16.7% to 98.8%. In fields with resistant cultivars and foliar fungicide applications, incidence ranged from 0 to 1.7% and yields from 3846.8 kg/ha (57.2 bu/a) to 5649.1 kg/ha (84 bu/a). (Bradley et al. 2019) There are several known resistance genes for *D. aspalathi* (Rdm1, 2, 3, 4) and *D. caulivora* (Rdc 1) (Peruzzo et al. 2019; Mena et al. 2020), as well as several tolerant cultivars that can handle disease without significant yield reduction. Experimental resistance breeding can be traced back to Keeling's work in 1982 when ten-day old greenhouse seedlings were screened for resistance (Keeling 1982). The single genes responsible for this resistance were determined in 1987 to be Rdc1 and Rdc2 (Kilen and Hartwig 1987). After updates in classification, the southern stem canker resistance gene became known as Rdm and could be found in the Crockett, Dowling, Hutcherson, and Tracy M cultivars (Pioli et al. 2003; Chiesa et al.

2009). It is important to note that the gene conferring resistance against *D. caulivora* does not confer resistance to *D. aspalathi*, or vice versa (Pioli et al. 2003). Additionally, it is essential to understand that these tolerant or resistant cultivars do not confer total genetic resistance against *Diaporthe caulivora* or *Diaporthe aspalathi* and therefore cannot be relied on for total disease management.

Pod and Stem Blight

Pod and stem blight can be caused by two species. Pod and stem blight caused by what is now known as *D. sojae* was first discovered in North Carolina in 1920 (Wolf and Lehman 1920). Symptoms were present on pods, stems, and leaves, with the lower stem covered in linear lines of pycnidia (Athow 1951). *D. sojae* is capable of infecting pods; the pycnidia may also develop on pods that turn brown in color, while the seed inside becomes shrunken, wrinkled and incapable of germination (Lehman 1923; Kmetz et al. 1978). Recent studies have shown that *D. longicolla,* more often associated with Diaporthe/Phomopsis Seed Decay may also cause pod and stem blight. Zhang et al. 1998 confirmed that *D. longicolla* could be isolated from stem tissues. This was further supported with the isolation of *D. longicolla* from red, elongated stem lesions resembling stem canker on dry edible beans in North Dakota (Mathew et al. 2015). A similar case occurred in western Canada in 2021 when *D. longicolla* was isolated from red elongated stem lesion on a soybean plant with linear pycnidia rows and shriveled seed (Abdelmagid et al. 2022). Udayanga et al. 2015 confirmed that *D. sojae* and *D. longicolla* were not conspecific and that it is likely that Lehman collected a sample with co-occurring infection. This coinfection skewed the description of the disease Lehman called “Pod and Stem Blight”, when those disease symptoms were caused by two separate species *D. sojae* and *D. longicolla*, with the *D. longicolla* pathogen not being isolated.

Diaporthe/Phomopsis Seed Decay

Phomopsis seed decay is the seed-borne soybean disease caused by the pathogen known as either *Phomopsis longicolla* or *Diaporthe longicolla*. It is an endemic disease to soybean that results in thousands of MT lost every year. It is an integral part of the *Diaporthe* complex sharing similar morphologies, disease signs, disease cycle, and environmental conditions.

The disease cycle for *D. longicolla* begins with the overwintering of pycnidia on soybean residue or the seed. When environmental conditions are conducive, typically high temperatures and rainfall, the pathogen germinates and can be rain-splashed from the soil and debris onto nearby seedlings. While *D. longicolla* infection tends to occur early in the growing season, the pathogen remains latent until the R5 and R6 stage, when it begins to colonize the pod, though *D. longicolla* can be isolated from the plant as early as late vegetative stages (Kmetz et al. 1978). After colonization of the pod, then seed becomes infected. At R7, as the plant reaches maturity and starts drying down, zone lines may also be visible within the stem (Olson et al. 2015; Mueller et al. 2016). Zone lines were previously attributed to multiple soybean pathogens, but completion of Koch's Postulates along with a comparison of morphology and DNA sequencing (ITS, EF-1, ACT) confirmed *D. longicolla* as the causal agent of zone lines in Illinois, Indiana, Iowa, Michigan, and South Dakota (Olson et al. 2015). Other signs and symptoms of *D. longicolla* infection include black pycnidia on infected seed, small lesions, cotyledon streaks, and the colonization of pycnidia along the stem. The presence of pycnidia and stem lesions during later reproductive stages can easily lead to a diagnosis of stem canker pathogen rather than *D. longicolla*. As described above, recent surveys have found that *D. longicolla* may be more prevalent than initially believed, warranting further research and observations to help growers correctly identify causal agent. While *D. longicolla* is widely associated with seed decay, Petrović et al. 2021 identified over ten species involved in seed decay. These species included *D. caulivora, D. aspalathi*, *D. longicolla*., *D. sojae, D. kongii*, *D. ueckerae, D. unshiuensis, D. bacilloides, D. flaviscens,* and *D. insulistroma*.

The *Diaporthe* organism colonizes the pod and seed during the late reproductive stages, affecting yield and pod fill. This can lead to reduction of seed quality, a primary concern of Diaporthe/Phomopsis seed decay. When used for processing, infected seeds are not typically associated with reduced oil quality; however, the severity of the infection and environmental conditions may play a role in this (Wrather et al. 2004). The larger issues arise in seed fields where infected seed have decreased germination or emergence and can become a source of inoculum. As *D. longicolla* infection increases, greater reduction in germination rate is observed, along with stunted emergence and damping-off (Zorrilla et al. 1994). In severe cases, germination rates as low as 10% have been observed (Li et al. 2017).

Management strategies for *Diaporthe longicolla* are limited. Tillage and crop rotation can be used to break up inoculum. Single mode of action fungicide azoxystrobin, labeled to treat pod and stem blight, was shown to increase *D. longicolla* incidence in a susceptible soybean line when disease pressure was low (Wrather et al. 2004). This trend was later confirmed in in 2020 (Batzer and Mueller 2020). Multiple plant introductions have been found to confer genetic resistance of *Diaporthe longicolla* over the years; PI 417479 (different genes) in 1993, PI 80837 (single gene) and PI 562694 in 2005, and PI 360841 in 2008 (Zimmerman and Minor 1993; Jackson et al. 2005; Smith et al. 2008). More recently, these include PI 158765, PI 235335, PI 346308, and PI 416779 (Li et al. 2017). While these lines, and others, may reduce incidence of *D. longicolla,* they do not confer total resistance (Li 2018). The lack of efficacious fungicides combined with partial genetic resistance leaves management of *D. longicolla* nuanced.

Nematode Genera Associated with Soybean Disease

In 2019, 1,389,000 MT (51.04 million bu) of soybeans were lost across the southern U.S. Of that loss, nearly half was caused by nematode infection and subsequent disease. Plant parasitic nematodes are non-segmented, typically microscopic and transparent worms that reside within the soil (Ingham, USDA NRCS). The most devastating soybean losses in 2019 came from *Heterodera glycines* (soybean cyst nematode, SCN, 346,180 MT), and *Meloidogyne* spp. (root-knot nematodes, 6,368 MT) (Allen et al. 2020).

Heterodera glycines

Soybean cyst nematode was first identified in North Carolina in 1954 and can now be found in at least 31 soybean-producing states (Winstead et al. 1955; Niblack, 2005; Tylka and Marett 2021). The first detection of SCN in DE was made in the fall of 1979 in Sussex County. This was followed by detection in Kent County in the 80s and New Castle County in 1991 (Mulrooney 2011). Various legume species, including soybeans, vetches, clovers, and sennas are hosts for SCN (Davis and Tylka 2021; Rocha et al. 2021).

Infection begins when eggs hatch in the soil at juvenile molt 2 (J2s). Once hatched, the motile J2s migrate to find a root food source. Once in contact with the root, SCN use enzymatic secretions and a stylet mouthpart to puncture root cells and feed. The J2 will then penetrate the root completely, leaving a zone of necrosis behind as it moves intracellularly to its feeding site. There, the J2 will experience two more molts. After the final molt, sexually

Figure 1.1: Soybean cyst nematode females on a soybean root system. (Arrows denote SCN females.)

differentiated males will remove themselves from the root, remaining vermiform, while the females remain sedentary and continue to swell, eventually breaking through the root surface (Figure 1.1). The motile males will then move to mate with sedentary females, though they do not feed on the root surface (Davis and Tylka 2021). Female SCN stay on the root and swell with eggs to a lemon-shape at maturity. The females may deposit a few eggs outside their gelatinous matrix, but most remain within the female's body. Female coloration changes from yellow to white at female maturity and to brown once the female dies. A dead female is then referred to as a cyst, which serves as an overwintering structure for eggs. Eventually, the eggs will hatch, break open the cyst and find a new root. At ideal moisture conditions and a constant temperature of 25 ºC or higher, this entire process takes approximately 22 days to complete (Niblack 2005). The damage to the roots caused by SCN often leads to plant stunting or reduced vigor that is not readily noticed from aboveground, earning SCN the anecdotal moniker of "silent yield robber". There are relatively few visual indicators of SCN in a field and they are not easily distinguished from other diseases, abiotic disorders, or herbicide injury (Mueller et al. 2016). When present, these symptoms can include stunting, low vigor, and chlorosis. Even without visible symptoms, yield losses of up to 40 percent could be occurring (Wang et al. 2003). The best method for understanding SCN populations, especially in fields with a history of SCN pressure, is periodic soil sampling throughout the field (Niblack 2005).

SCN management has historically relied on nematicides for control. However, a reduction in available options, associated costs, toxicity to humans (typically low

LD50 values), and the general lack of control has prompted research into alternative control measures (Wrather et al. 1984; Gowen 1997). Although many products may be broadly defined as a nematicide (chemical and biological agents used to stun, deter, or kill plant-parasitic nematodes), most products available today are considered nemastatic. Nematistat is a term for chemical control measures that disrupt the life cycle at a critical point, but typically without killing the nematode compared to nematicides, which kill nematodes. Nematicides/nematistats are available as fumigants or non-fumigants. The active ingredients of fumigants are dispersed by injecting into the soil as gas not taken up by plants; non-fumigants contain active ingredients formulated as liquids or granulars and move through the soil via water with a narrower spectrum of activity. Fumigants are injected into the soil, and non-fumigants can be injected or applied with spray (Grabau 2016). There are several nematicides labeled for SCN. These include soil fumigants, Vapam HL (Sodium methyldithiocarbamate; AVMC; Newport Beach, CA), K-Pam HL (Potassium N- methyldithiocarbamate; AVMC; Newport Beach, CA) and non-fumigant products, Vydate (Oxamyl; Corteva Agriscience; Wilmington, DE), and AgLogic15 (aldicarb; AgLogic; Chapel Hill, NC). Oxamyl, aldicarb, and other carbamates affect the nervous and muscular systems of the nematode (Group N-1A: Acetylcholinesterase Inhibitors). Soil applied nematicides do not guarantee a yield benefit or population suppression and tend to be highly toxic to humans (Davis and Tylka 2021). There is a movement away from nematicide application by injection or fumigation. Current chemical management is moving towards seed treatments as an alternative.

There are several promising seed treatments on the market, including Poncho/VOTiVO (*Bacillus firmus*, BASF, Ludwigshafen, Germany), ILEVO (fluopyram, BASF), and Saltro (pydiflumetofen, Syngenta, Basel, Switzerland). *Bacillus firmus* is a nematicidal bacteria which targets the hatching and motility of J2s, making this product a biological in the N-UNB group. Fluopyram is a succinate dehydrogenase inhibitor (SDHI) fungicide with nemastatic properties in the N-3 group. Pydiflumetofen is also an SDHI fungicide with nemastatic properties. VoTiVO was registered in 2010, ILEVO in 2014, and Saltro in 2020. In greenhouse and in vitro conditions, ILEVO was more effective at inhibiting the motility of J2 SCN as well as root penetration of J2s when close to the seed (Beeman et al. 2019). A similar study conducted in 2018 found that ILEVO succeeded in reducing root penetration in greenhouse systems but failed to have consistent results regarding motility and hatching inhibition (Beeman and Tylka 2018). In a small plot and strip trial study in Iowa, ILEVO significantly reduced SCN reproduction factors (RF) by 50% in one strip trial and 36-60% in the four small plot experiments, but yield was not increased by ILEVO in those trials. In three other small plot experiments, ILEVO improved yield, but RF was not reduced (Bissonnette et al. 2020). Saltro has been found to reduce SDS severity and increase yield in areas of low SCN pressure with a history of SDS (Adee 2020). Saltro has also been shown to reduce SCN hatching in a lab setting by 55.4% compared to a double distilled water control. It also was found to reduce the number of cysts per plant in a greenhouse setting by 90.5% at 30 days after planting and 80.3% at 60 days after planting compared to the control (Dhital 2020). Limited work has been completed directly comparing ILEVO and Saltro, and no data exists screening these two seed treatments in Delaware.

While seed treatments can offer slight yield improvements, genetic resistance has historically been the most cost-effective and efficacious way to manage SCN. Commercial cultivars with varying levels of partial resistance are widely available. Currently, PI88788 is the most common source of resistance found in greater than 95% of available germplasm. Its resistance is derived for the Rhg1-b allele (Brucker et al. 2005). For the first decades of use, reproduction levels stayed below 10% on varieties with PI88788. However, since the early 2000s, increasing levels of reproduction have been reported across the Mid-West (McCarville et al. 2017). Peking is another resistant plant introduction. Its resistance is derived from the Rhg1-a and Rhg4 genes (Bayless et al. 2019). The newest resistance source is PI 89772, currently available as NK S23-G5X/GH2329X. Flowering and maturity of soybeans depend on the day length of where they are grown, complicating soybean breeding. Thirteen maturity group designations ranging from 000 to 10 are used by soybean breeders. Maturity groups planted in Delaware typically range from mid 3s to mid 4s. Currently, PI89772 is only available as a 2.3 maturity group. Until this source is incorporated into other cultivars, PI 89772 remains unavailable to large portions of the US.

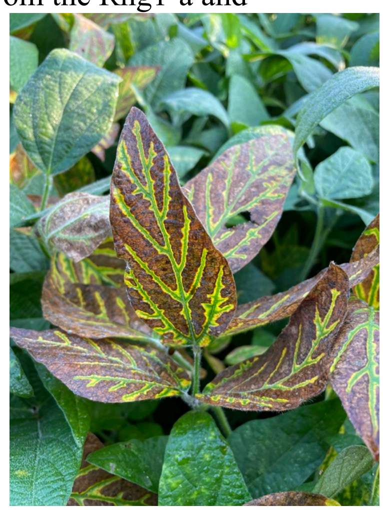

Figure 1.2: Interveinal chlorosis in a soybean plant with sudden death syndrome (SDS)

In addition to causing damage, nematodes can also predispose soybean plants to more severe fungal and bacterial infections. This is the case for soybean sudden death syndrome (SDS), caused by the pathogen *Fusarium viruliforme*. Elevated incidence of this fungal pathogen is associated with the presence of SCN. Higher disease levels are also observed in fields with earlier planting times (March and April) and cool soil temperatures. This disease was first reported in Arkansas in 1971 and is now common across many soybean growing areas of the United States (Hartman et al. 2015). The disease cycle begins with the colonization of soybean roots in cool, wet conditions (Gongora-Canul and Leandro 2011). *F. virguliforme,* which exists as mycelium in soybean cyst nematode cysts or within the soil, colonizes the root.

Figure 1.3: root necrosis caused by *F. virguliforme*

Figure 1.4: Stem discoloration from SDS

Colonization causes root rot, which affects water uptake. *F. virguliforme* also produces a toxin that is translocated through the xylem to the leaves, resulting in chlorosis (Pudake et al. 2013). Symptoms of interveinal chlorosis develop shortly after flowering, resulting in leaf drop and defoliation (Figure 1.2). These symptoms can result in the abortion of flowers and pods. External root symptoms of necrosis may or may not be present (Figure 1.3). Internally, gray to red discoloration spreads throughout the vascular tissue, while the pith remains white (Figure 1.4). Blue spores on the taproot of the infected plant are a notable sign of *F. virguliforme* (Roy et al. 1997). Management of SDS is limited. Currently, planting date, crop rotation, and tillage are the primary approaches. ILEVO (fluopyram) seed treatments have also been shown to reduce foliar and root symptoms of SDS while increasing yield (Kandel et al. 2019).

Meloidogyne incognita

Meloidogyne spp., known as root-knot nematodes (RKN), are a collection of over 100 species affecting a wide range of host plants. In soybean production, RKN typically ranks as the second most destructive pathogen, trailing behind SCN. In 2019, the south saw a loss of 235,140 MT (8.64 million bu) of soybeans due to RKN infection and destruction, while 2018 saw an even higher loss of 324,400 MT (11.92 million bu) in the south to RKN (Allen et al. 2020; Allen et al. 2019). The primary root-knot species of concern for soybeans across the south and in DE and MD is southern root-knot nematode, *Meloidogyne incognita*.

In the RKN life cycle, mature females lay eggs in large gelatinous masses within a galled root or plant tissue. These egg masses are capable of overwintering in the soil. The eggs hatch and release J2s into the soil. Newly hatched J2s are motile and begin moving towards a suitable food, root tips (Mitowski and Abawi 2011). Once a root is found, the J2s migrate to vascular tissue and use their stylet to establish a permanent feeding site. J2s are not sexually differentiated until they molt into J4s. At

J4, sexual differentiation into male or female is based on environmental conditions, sex hormones, parasitism, and temperature. In favorable conditions, J4s develop into globose and sedentary females, while vermiform males develop in unfavorable conditions. They are capable of sexual differentiation reversal with a change in conditions during this time (Triantaphyllou 1973). Many species are parthenogenetic or facultatively parthenogenetic: viable eggs can be produced in the absence of fertilization. Due to this, males are often rare and may only be observed when the population is under environmental stress (Mitkowski and Abawi 2011). The enzymatic secretions of the females at the feeding site cause swelling of the cells in the area, creating a symptomatic gall (Figure 1.5). The gall is made of a "giant cell", a large nutrient sink in which the RKN digest nutrients directly. Neighboring cells will also become enlarged and divide rapidly, resulting in the signature gall (Mitkowski & Abawi, 2011). Adult females deposit their eggs in the surrounding soil in gelatinous masses, restarting the life cycle. The entire life cycle occurs over 20-30 days and each female can produce hundreds of eggs (Perry et al. 2009). The continuous formation and enlargement of galls leaves the plant with limited ability to absorb and transport vital nutrients and water, leading to stunted growth or wilting (Mitokowski & Abawi, 2011). RKN is characterized by the

Figure 1.5: Soybean root system with galling from root-knot nematode

formation of galls on the roots, and several above-ground symptoms can also indicate infection. Symptoms are often noticed in patches or clusters throughout the field. Within these clusters, plants may appear wilted and or stunted, especially compared to other parts of the field (Mitokowski & Abawi 2011) (Figure 1.6). These symptoms can resemble other diseases, nutrient deficiencies, or other abiotic disorders. Management approaches for RKN aim to disrupt the life cycle and prevent gall formation using either cultural or chemical practices. Current chemical applications for RKN include all previously listed nematicides for SCN. Additionally, fluensulfone (NIMITZ; ADAMA; Tel Aviv, Israel) and fluazaindolizine, have been found effective.

Figure 1.6: Stunting in soybean due to varying level of root-knot nematode galling

Fluensulfone is a nematicide that falls into the N-UN (unknown mode of action) group and has been successfully used to manage southern RKN in greenhouse conditions, along with fluopyram (Jones et al. 2017). Fluazaindolizine is also a recently developed nematicide that falls into the same group as fluensulfone and provided excellent suppression of various *Meloidogyne* species (Lahm et al. 2017). While there are multiple options on the market to combat RKN, nematicides can be costly and difficult to apply.

As with SCN, nematicide seed treatments are a preferred application approach for RKN management. Current commercially available chemical seed treatments include Avicta (abamectin; Syngenta; Basel, Switzerland), Poncho VOTiVO (clothiandin, *Bacillus firmus*; Syngenta; Basel, Switzterland), ILEVO (fluopyram; BASF; Ludwigshafen, Germany), and Saltro (pydiflumetofan; Syngenta; Basel, Switzerland), Jackson et al. 2014 found that Avicta and VOTiVO reduced galling and had fewer eggs near or on roots. Further work in 2015 compared the use of ILEVO (in-furrow application), seed treatments Avicta, Poncho/VOTiVO, ILEVO+Poncho/VOTiVO, and a control against RKN in two soybean cultivars. They observed lower reproduction in the ILEVO+Poncho/VOTiVO, Avicta, and Poncho/VOTiVO seed treatments than the ILEVO in-furrow or control treatments. In this study, ILEVO performed similarly to the other seed treatments in terms of reproduction and yield (Jackson et al. 2015). In a recent study from Arkansas, seed applied fluopyram was able to suppress *M. incognita* root penetration when planted at a planting depth of 2.5 cm in a laboratory assay, echoing the results of abamectin-treated seed used as a comparison. Seed applied fluopyram did suppress *M. incognita* root entry at 7 days after planting. Seed applied fluopyram did not affect nematode maturity based on nematode developmental stages from 7 to 21 days after planting. Both fluopyram and abamectin seed treatments were more effective at suppressing nematode root entry than nematode maturity (Hawk and Faske 2020).

Breeding for genetic resistance is an important management method. In the Midwestern U.S., RKN resistance was found in cultivars ranging from maturity groups 2 – 4 in greenhouse studies (DE growers typically use plants from maturity groups 3 – 4.4). These cultivars were H444NRR, LNX97164-35-5, HF9665-2-15. Some even showed SCN resistance as well (Kruger et al. 2008). More recently, a study out of Arkansas identified four maturity group 4 and seven maturity group 5 cultivars that rated moderately resistant against southern root-knot nematode. The average gall ratings for the maturity group 4 and 5 cultivars were 2.0-2.1 and yield from metric conversion (42-46 bu/a). The maturity group 4 cultivars are: Delta Grow DG 4995 GLY, Delta Grow DG 4940, Pioneer P47T59R, and Terral REV48A46. The maturity group 5 cultivars are: Agventure 52M7R, Armor 53D31, NK S53-G5, Pioneer 52T86R, Pioneer 53T73SR, Stine 51D02, and Terral REV52A94 (Emerson et al. 2016). Resistance genes continue to be mapped and identified, with the goal of deploying resistance in soybeans. Nematode management relies on an integrated approach, combining all management methods available.

Overall, soilborne diseases continue to plague soybean production in DE and MD. There is a lack of understanding regarding species distribution of *Diaporthe* in the region along with species aggressiveness. This gap in knowledge continues to hinder *Diaporthe* management. Soybean cyst nematodes are well-known yield impacting pests in the region, but the distribution of this species has not been updated in over a decade. The distribution of other nematode taxa, like RKN, has not been widely evaluated for the region. There continues to be a push for increased use of seed treatments as a nematode management approach, but no regional data exists assessing these products.

REFERENCES

Abdelmagid, A., Hafez, M., Lawley, Y., Rehal, P.K., Daayf, F. 2022. First Report of Pod and Stem Blight and Seed Decay Caused by *Diaporthe longicolla* on Soybean in Western Canada. *Plant Disease.*

Adee, E.A. 2020. Effect of Saltro Soybean Seed Treatment on Sudden Death Soyndrome in Kansas in 2019. *Kansas Field Research, 6*(5): 93-94.

Allen, T. W., Bissonnette, K., Bradley, C. A., Damicone, J. P., Dufault, N. S., Faske, T. R., Isakeit, T., Kemerait, R.C., Koehler, A., Mehl, H.L.., Mueller, J.D., Padgett, G.B., Price, P.P., Sikora, E.J., Small, I.M., Thiessen, L., Young, H. 2019. Southern United States Soybean Disease Loss Estimates for 2018. *Proceedings of the Southern Soybean Disease Workers*.

Allen, T. W., Bissonnette, K., Bradley, C. A., Damicone, J. P., Dufault, N. S., Faske, T. R., Isakeit, T., Kemerait, R.C., Koehler, A., Mehl, H.L.., Mueller, J.D., Padgett, G.B., Price, P.P., Sikora, E.J., Small, I.M., Thiessen, L., Young, H. 2020. Southern United States Soybean Disease Loss Estimates for 2019. *Proceedings of the Southern Soybean Disease Workers*.

Athow, K.L. 1951. Comparative Study of Diaporthe Stem Canker and Pod and Stem Blight of Soybean (Accession No. 27714134) [Doctoral Dissertation, Purdue University]. ProQuest Dissertations Publishing.

Backman, P.A., Weaver, D.B., and Morgan-Jones, G. 1985. Soybean Stem Canker: An Emerging Disease Problem. *Plant Disease, 69*(8): 641 – 647.

Balducchi, A.J., McGee, D.C. 1987. Environmental Factors Influencing Infection of Soybean Seeds by *Phomopsis* and *Diaporthe* Species During Seed Maturation. Plant Disease, *79*(3): 209–212.

Batzer, J.C., Mueller, D.S. 2020. Soybean Fungal Endophytes *Alternaria* and *Diaporthe* spp. are Differentially Impacted by Fungicide Application. *Plant Disease, 104*(1): 52-59

Bayless, A.M., Zapotocny, R.W., Han, S. Grunwald, D.J., Amundson, K.K., Bent, A.F. 2019. The rhg1-a (Rhg1 low-copy) nematode resistance source harbors copia-family retrotransposon within the Rhg1-encoded α-SNAP gene. *Plant Direct, 3*(8).

Beeman, A.Q., Njus, Z.L., Pandey, S., Tylka, G.L. 2019. The Effects of ILEVO and VOTiVO on Root Penetration and Behavior of the Soybean Cyst Nematode, *Heterodera glycines.* (2019). *Plant Disease, 103*(3), 392-397. doi:10.1094/PDIS-02-18-0222-RE.

Beeman, A.Q., Tylka, G.L. 2018. Assessing the Effects of ILEVO and VOTiVO Seed Treatments on Reproduction, Hatching, Motility, and Root Penetration of the Soybean Cyst Nematode, *Heterodera glycines*. *Plant Disease*, *102(*1), 107-113. DOI: 10.1094/pdis-04-17-0585-re.

Bissonnette, K., Marrett, C., Mullaney, M.P., Gebhart, G.D., Kyveryga, P.M., Mueller, T.A., Tylka, G. L. 2020. Effects of ILEVO Seed Treatment on *Heterodera glycines* Reproudction and Soybean Yield in Small-Plot and Strip-Trial Experiments in Iowa. *Plant Disease, 104*(11): 2914-2920.

Bradley, C.A., Allen, T.W., Sisson, A.J., Bergstrom, G.C., Bissonnette, K.M., Bond, J., Byamukama, E., Chilvers, M.I., Collins, A.A., Damicone, J.P. and Dorrance, A.E., Default N.S., Esker P.D., Faske T.R., Fiorellino N.M., Giesler L.J., Hartman G.L., Hollier C.A., Isakeit T.I., Jackson-Ziems T.A., Jardine D.J., Kelly H.M., Kemerait R.C., Kleczaeski N.M., Koehler A.M., Kratochvil R.J., Kurle J.E., Malvic D.K., Markell S.G., Mathew F.M., Mehl H.L., Mehl K.M., Mueller D.S., Mueller J.D., Nelson B.D., Overstreet C., Padgett G.B., Price P.P., Sikora E.J., Smith D.L., Spurlock T.N., Tande C.A., Telenko D.E.P., Tenuta A.U., Thiessen L.D., Warner F., Wiebold W.J., Wise K.A. 2021. Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2015 to 2019. *Plant Health Progress*, *22* (4).

Bradley, C.A., Mehl, K., Walsh, J. 2019. Effect of Soybean Cultivar and Foliar Fungicide Application on Southern Stem Canker of Soybean. University of Kentucky Soybean Science Research Report.

Brucker, E., Carlson, S., Wright, E., Niblack, T., Diers, B. 2005. Rhg1 alleles from soybean PI437654 and PI88788 respond differentially to isolates of *Heterodera glycines* in the greenhouse. Theor Appl Genet. 2005 Jun;111(1):44-9.

Chiesa, M.A., Pioli, R.N., Morandi, E.N. 2009. Specific resistance to soybean stem canker conferred by the *Rdm4* locus. *Plant Pathology, 58*(69), 1032–1039.

Cui, Y.L., Duan, C.X., Wang, X.M., Li, H.J., Zhu, Z.D., 2009. First report of *Phomopsis longicolla* causing soybean stem blight in China. *Plant Pathology*, *58*(4): 799-799.

Davis, E.L., G.L. Tylka. 2021. Soybean cyst nematode disease. *The Plant Health Instructor*.

Dhital, B.P. 2020. Determining the Effects of Plant Extracts and Saltro Nematicide on Hatching, Mortality, and Reproduction of the Soybean Cyst Nematode (*Heterodera glycines*) (Accession No. 4082) [Master's Thesis, South Dakota State University]. Electronic Theses and Dissertations.

Emerson, M., Brown, K., Faske, T.R., Kirkpatrick, T.L. 2016. Field Performance of Several Glyphosate-Resistant Maturity Groups 4 and 5 Soybean Cultivars in a Root-Knot Nematode Infested Field. *Arkansas Soybean Research Studies 2016*.

Fernández, F.A., Hanlin, R.T., 1996. Morphological and RAPD analyses of *Diaporthe phaseolorum* from soybean. *Mycologia*, *88*(3): 425-440.

Ghimire, K., Petrović, K., Kontz, B.J., Bradley, C.A., Chilvers, M.I., Mueller, D.S., Smith, D.L., Wise, K.A., Mathew, F.M., 2019. Inoculation method impacts symptom development associated with *Diaporthe aspalathi*, *D. caulivora*, and *D. longicolla* on soybean (*Glycine max*). *Plant Disease*, *103*(4): 677-684.

Gomes, R.R., Glienke, C., Videira, S.I.R., Lombard, L., Groenewald, J.Z., Crous, P.W., 2013. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, *31*(1): 1-41.

Gongora-Canul, C.C., Leandro, L.F.S. 2011. Effect of Soil Temperature and Plant Age at Time of Inoculation on Progress of Root Rot and Foliar Symptoms of Sudden Death Syndrome. *Plant Disease, 95*(4): 436-440.

Gowen, S.R. 1997. Chemical control of nematodes: efficiency and side effects. *Plant Nematode Problems and their Control in the Near East Region.* Food and Agriculture Organization of the United Nations, Rome.

Grabau, Z. 2016. Fumigant and Nonfumigant nematicides labeled for agronomic crops in Florida. University of Florida Extension.

Hartman, G.L., Chang, H.X., Leandro, L.F. 2015. Research advances and management of soybean sudden death syndrome. *Crop Protection, 73:*60-66.

Hawk, T., Faske, T.R. 2020. Effect of seed applied fluopyram on *Meloidogyne incognita* infection and maturity in cotton and soybean. *Journal of Nematology, 52.*

Hobbs, T.W., Schmitthenner, A.F., Kuter, G.A., 1985. A new *Phomopsis* species from soybean. *Mycologia*, *77*(4): 535-544.

Ingham, E.R. Soil Nematodes. *USDA Natural Resources and Conservation Service-Soils*.www.nrcs.usda.gov/wps/portal/nrcs/detailfull/soils/health/biology/?cid=nrcs142p2_053866

Jackson, C.S., Faske, T.R., Kirkpatrick, T.L. 2014. Assessment of Fluopyram for Suppression of Root-Knot Nematode (*Meloidogyne incognita*) in Soybean. "Arkansas Soybean Research Studies 2014", pp 65-68. *Research Series.*

Jackson, C.S., Faske, T.R., Kirkpatrick, T.L. 2015. Assessment of ILEVO for Management of *Meloidogyne incognita* on Soybean, 2015. "Arkansas Soybean Research Studies 2015", pp 75-78. *Research Series.*

Jackson, E., Fenn, P., Chen, P. 2005. Inheritance of Resistance to Phomopsis Seed Decay in Soybean PI 80837 and MO/PSD-0259 (PI 562694). *Crop Science, 45*(6).

Jones, J., Kleczewski, N., Desaeger, J., Meyer, S.L., Johnson, G.C. 2017. Evaluation of nematicides for southern root-knot nematode management in lima bean. *Crop Protection, 96:* 151-157.

Kandel, Y.R., Bradley, C.A., Chilvers, M.I., Mathew, F.M., Tenuta, A.U., Smith, D.L., Wise, K.A., Mueller, D.S. 2019. Effect of Seed Treatment and Foliar Crop Protection Products on Sudden Death Syndrome and Yield of Soybean. *Plant Disease, 103*(7): 1712-1720.

Keeling, B L. 1988. Influence of temperature on growth and pathogenicity of geographic isolates of *Diaporthe phaseolorum* var. *caulivora*. *Plant Disease, 72*(3): 220-222.

Keeling, B.L. 1982. A Seedling Test for Resistance to Soybean Stem Canker Caused by *Diaporthe phaseolorum* var. *caulivora*. *Phytopathology, 72*(7), 807-809.

Kilen, T.C., Hartwig, E.E. 1987. Indication of Single Genes Controlling Resistance to Stem Canker in Soybean. *Crop Science, 27*(5): 863-864.

Kmetz, K.T., Schmittenhenner, A.F., Ellett, C.W. 1978. Soybean Seed Decay: Prevalence of Infection and Symptom Expression Caused by Phomopsis sp., Diaporthe phaseolorum var. sojae, and D. phaseolorum var. caulivora. *Phytopathology, 68*: 836-840.

Kruger, G.R., Xing, L., LeRoy, A.R., Westphal, A. 2008. *Meloidogyne incognita* Resistance in Soybean under Mid-West Conditions. *Crop Science, 48*: 716-726.

Lahm, G.P., Desaeger, J., Smith, B.K., Pahutski, T.F., Rivera, M.A., Meloro, T., Kucharczyk, R., Lett, R.M., Daly, A., Smith, B.T., Cordova, D., Thoden, T., Wiles, J.A. 2017. The discovery of fluazaindolizine: A new product for the control of plant parasitic nematodes. *Bioorganic & Medicinal Chemistry Letters, 27*(7): 1572-1575.

Lehman, S.G., 1923. Pod and stem blight of soybean. *Annals of the Missouri Botanical Garden*, *10*(2): 111-178.

Li, S. 2018. Development of a seedling inoculation technique for rapid evaluation of soybean for resistance to *Phomopsis longicolla* under controlled conditions. *Plant Methods, 14*(8): 1-12.

Li, S., Kurtzweil, N.C., Grau, C. R., Hartman, G.L. 2004. Occurrence of soybean stem canker (*Diaporthe phaseolorum* var. *meridionalis*) in Wisconsin. *Plant Disease, 88*(5): 576.

Li, S., Sciumbato, G., Rupe, J., Shannon, G., Chen, P., Boykin, D. 2017. Evaluation of Commercial Soybean Cultivars for Reaction to Phomopsis Seed Decay. *Plant Disease, 101*(12): 1990-1997.

Mai, W.F., Abawi, G.S. 1987. Interactions Among Root-Knot Nematodes and Fusarium Wilt Fungi on Host Plants. *Annual Review of Phytopathology, 25*: 317-338.

Mancebo, M.F., Bazzalo, M.E., Reid, R.J., Kontz, B., Mathew, F.M. 2019. First Report *Diaporthe gulyae* Causing Phomopsis Stem Canker of Sunflower (*Helianthus annus*) in Argentina. *Plant Disease*, *103*(7).

Mathew, F. M., Castlebury, L. A., Profile, S., Alananbeh, K. 2015. Identification of *Diaporthe longicolla* on Dry Edible Pea, Dry Edible Bean, and Soybean in North Dakota. *Plant Health Progress, 16*(2): 71-72.

McCarville, M.T., Marett, C.C., Mullaney, M.P., Gebhart, G.D., Tylka, G.L. 2017. Increase in Soybean Cyst Nematode Virulence and Reproduction on the Resistant Soybean Varieties in Iowa from 2001 to 2015 and the Effects on Soybean Yield. *Plant Health Progress, 18*(3):146-155.

Mena, E., Stewart, S., Montesano, M., Ponce de Leon, I. 2020. Soybean Stem Canker Caused by *Diaporthe caulivora*; Pathogen Diversity, Colonization, Process, and Plant Defense. *Frontiers in Plant Science, 10:* 1733.

Mitowski, N.A., Abawi, G.S. 2011. Root-knot nematodes. *The Plant Health Instructor*.

Mueller, D.S., Wise, K.A., Sisson, A.J., Smith, D.L., Sikora, E.J., Robertson, A.E., Bradley, C.A. 2016. A Farmer's Guide to Soybean Diseases. American Phytopathological Society, St. Paul, MN.

Mulrooney, R.P. 1985. Soybean disease loss estimates for Southern United States in 1983. *Plant Disease*, *69*(1): 92.

Mulrooney, R.P. 2011. Soybean Cyst Nematode. University of Delaware Cooperative Extension.

Niblack, T. L. 2005. Soybean cyst nematode management reconsidered. *Plant Disease, 89*(10): 1020-1026.

Olson, T. R., Gebreil, A., Micijevic, A., Bradley, C. A., Wise, K. A., Mueller, D. S., Chilvers, M.I., Mathew, F. M. 2015. Association of *Diaporthe longicolla* with black zone lines on mature soybean plants. *Plant Health Progress, 16*(3): 118-122.

Perry, R.N., Moens, M., Starr, J.L. 2009. *Root-Knot Nematodes*. CABI.

Peruzzo, A.M., Hernández, F.E., Pratta, G.R. Ploper, L.D. Pioli, R.N. 2019. Identification and inheritance of an *Rdc* gene resistance to soybean stem canker (*Diaporthe phaseolorum* var. *caulivora*). *European Journal of Plant Pathology* *154***,** 1179–1184.

Petrović, K., Skaltsas, D., Castlebury, L.A., Kontz, B., Allen, T.W., Chilvers, M.I., Gregory, N., Kelly, H.M., Koehler, A.M., Kleczewski, N.M. and Mueller, D.S. 2021. Diaporthe Seed Decay of Soybean [*Glycine max* (L.) Merr.] Is Endemic in the United States, But New Fungi Are Involved. *Plant Disease*, *105*(6): 1621-1629.

Petty, MA. 1943. Soybean disease incidence in Maryland in 1942 and 1943. *Plant Disease Reporter*, *27*:347-349.

Pioli, R. N., Morandi, E.N., Martínez, M.C., Lucca, F., Tozzini, A., Bisaro, V., Hopp, H.E. 2003. Morphologic, molecular, and pathogenic characterization of *Diaporthe phaseolorum* variability in the core soybean-producing area of Argentina. *Phytopathology, 93*(2): 136–146.

Pudake, R.N., Swaminathan, S., Sahu, B.B., Leandro, L.F., Bhattacharyya, M.K. 2013. Investigation of the *Fusarium virguliforme fvtox1* mutants revealed that the FvTox1 toxin is involved in foliar sudden death syndrome development in soybean. *Current Genetics, 59*:107-117.

Rocha, L.F., Gage, K.L., Pimentel, M.F., Bond, J.P., Fakhoury, A.M. 2021. Weeds Hosting the Soybean Cyst Nematode (*Heterodera glycines* Ichinohe): Management Implications in Agroecological Systems. *Agronomy, 11*(1): 146.

Roy, K.W., Rupe, J.C., Hershman, D.E., Abney, T.S. 1997. Sudden Death Syndrome of Soybean. *Plant Disease, 81*(10): 1100-1111.

Santos, J.M., Vrandečić, K., Ćosić, J., Duvnjak, T., Phillips, A.J.L. 2011. Resolving the *Diaporthe* species occurring on soybean in Croatia. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, *27*(1): 9-19.

Smith, S., Fenn, P., Chen, P., Jackson, E. 2008. Inheritance of Resistance to Phomopsis Seed Decay in PI 360841 Soybean. *Journal of Heredity, 99*(6): 588-592.

Triantophyllou, A.C. 1973. Environmental Sex Differentiation of Nematodes in Relation to Pest Management. *Annual Review of Phytopathology, 11*: 441-462.

Tylka, G.L., Marett, C.C. 2021. Known Distribution of the Soybean Cyst Nematode, *Heterodera glycines*, in the United States and Canada in 2020. *Plant Health Progress, 22*(1): 72 – 74.

Udayanga, D., Castlebury, L.A., Rossman, A.Y., Chukeatirote, E., Hyde, K.D., 2015. The *Diaporthe sojae* species complex: Phylogenetic re-assessment of pathogens associated with soybean, cucurbits and other field crops. *Fungal Biology*, *119*(5): 383-407.

USDA ERS. 2022. Soybean U.S. stocks Soybean U.S. stocks: On-farm, off-farm, and total by quarter, U.S. soybean acreage planted, harvested, yield, Soybean and soybean meal production, value, price and supply and disappearance, prices 1999/00-2021/22-Year 2020. https://www.ers.usda.gov/data-products/oil-crops-yearbook/oil-crops-yearbook/#Soy%20and%20Soybean%20Products

USDA NASS. 2017. Census of Agriculture – Maryland: Historical Highlights. https://www.nass.usda.gov/Publications/AgCensus/2017/Full_Report/Volume_1,_Chapter_1_State_Level/Maryland/st24_1_0001_0001.pdf

USDA NASS., Delaware Department of Agriculture. 2019-2020 Delaware Agricultural Statistics Bulletin. pp: 7. https://www.nass.usda.gov/Statistics_by_State/Delaware/Publications/Annual_Statistical_Bulletin/2019/2019-2020-Delaware-Agricultural-Statistics-Bulletin.pdf

USDA NASS., Maryland Department of Agriculture. 2019-2020 Agricultural Statistics Bulletin – Maryland. pp: 6. https://www.nass.usda.gov/Statistics_by_State/Maryland/Publications/Annual_Statistical_Bulletin/2019/2019_2020_MD_Annual_Bulletin_Final_rev.pdf

van Rensburg, J.C.J., Lamprecht, S.C., Groenewald, J.Z., Castlebury, L.A., Crous, P.W. 2006, Characterisation of *Phomopsis* spp. associated with die-back of rooibos (*Aspalathus linearis*) in South Africa. *Studies in Mycology,* 55: 64-74.

Wang, J., Niblack, T.L., Tremain, J.A., Wiebold, W.J., Tylka, G.L., Marett, C.C., Noel, G.R., Meyers, O., Schmidt, M.E. 2003. Soybean Cyst Nematode Reduces Soybean Yield Without Causing Obvious Aboveground Symptoms. *Plant Disease, 87*: 623-628.

Winstead, N.N., Skotland, C.B., Sasser, J.N. 1955. Soybean Cyst Nematode in North Carolina. *Plant Disease Reporter, 39*(1): 9-11

Wolf, F.A., and Lehman, S.G. 1920. Notes on new or little-known plant diseases in North Carolina in 1920. North Carolina Agr. Expt. Sta. Rept. 43: 55-58.

Wrather, J. A., Shannon, J. G., Stevens, W. E., Sleper, D. A., Arelli, A. P. 2004. Soybean cultivar and foliar fungicide effects on *Phomopsis* spp. seed infection. *Plant Disease, 88*(7), 721-723.

Wrather, J.A., Anand, S.C., Dropkin, V.H. 1984. Soybean Cyst Nematode Control. *Plant Disease, 68*(9): 829 – 833.

Zhang, A.W., Riccioni, L., Pedersen, W.L., Kollipara, K.P., Hartman, G.L. 1998. Molecular identification and phylogenetic grouping of *Diaporthe phaseolorum* and *Phomopsis longicolla* isolates from soybean. *Phytopathology, 88*: 1306-1314.

Zimmerman, M.S., Minor, H.C. 1993. Inheritance of Phomopsis Seed Decay Resistance in Soybean 417479. *Crop Science, 33*(1).

Zorrilla, G., Knapp, A. D., McGee, D. C. 1994. Severity of Phomopsis seed decay, seed quality evaluation, and field performance of soybean. *Crop Science, 34*(1), 172-177.

Chapter 2

DISTRIBUTION AND PATHOGENICITY OF DIAPORTHE SPECIES ISOLATED FROM SOYBEAN STEM AND ROOT TISSUE IN DELAWARE AND MARYLAND

Abstract

Diaporthe species are widespread, but poorly understood soybean (*Glycine max*) pathogens within Delaware (DE) and Maryland (MD), responsible for diseases such as northern and southern stem canker, pod and stem blight, and Diaporthe/Phomopsis seed decay. Following elevated levels of *Diaporthe*-like diseases in 2018, a two-year fungal survey was conducted from 2019-2020 to characterize *Diaporthe* species within soybean stem and root tissue. One hundred and fifty-four *Diaporthe* isolates were collected and identified by sequencing the nuclear ribosomal internal transcribed spacer (ITS) gene region. Four species were identified, *D. longicolla* (97%), *D. aspalathi* (1%), D. *sojae* (1%) and *D. ueckerae* (1%). Koch's postulates confirmed the pathogenicity of *D. ueckerae* and the association of zone lines in DE and MD with *D. longicolla.* Aggressiveness of *D. longicolla, D. aspalathi,* and *D. ueckerae* was assessed on cultivars Hawkeye, GH3934X, and S39G2X by conducting a greenhouse pathogenicity assay using a toothpick inoculation method. *D. longicolla* had the highest disease severity score within each hybrid, and *D. aspalathi* had the lowest. The observations of multiple *Diaporthe* species associated with stem and root tissue support the need for continued understanding of the role of this pathogen within the region.

Introduction

Soybeans are a major agronomic commodity of the United States (U.S.) and for the states of Delaware (DE) and Maryland (MD). In 2020, these states produced over 789,250 MT (29 million bu) of soybeans with a value over 247 million dollars for the region (USDA-NASS, 2020; https://www.nass.usda.gov/). Several diseases within the Diaporthe complex can impact soybean yield and quality each season. From 2010 to 2014, approximately 1 million MT were lost to *Diaporthe*-associated diseases (Allen et al. 2017). In 2018, above average rainfall led to widespread *Diaporthe* infection across the Mid-Atlantic region (Leathers 2018). This was also observed across the U.S., where yield loss from Diaporthe/Phomopsis seed decay was estimated at 2,367,700 MT (87 million bu) (Bradley et al. 2021). The genus *Diaporthe* Nitschke [syn. *Phomopsis* (Sacc.) Bubák] includes over 1,000 species described as endophytes, saprophytes, or pathogens on a broad host range worldwide (Gomes et al. 2013). A number of these species are pathogens of soybean and to date, the U.S. has five *Diaporthe*-associated diseases including pod and stem blight, northern stem canker, southern stem canker, stem disease, and Diaporthe/Phomopsis seed decay. Northern stem canker (*D. caulivora* (Athow & Caldwell) Santos, Vrandecic & Phillips), southern stem canker (*D. aspalathi* Jansen, Castlebury & Crous) and stem disease (*D. gulyae* Shivas, Thompson and Young) are typically associated with only single species (Ghimire et al. 2019). Pod and stem blight can be caused by two species, *Diaporthe sojae* Lehman (Lehman 1923; Udayanga et al. 2015) or *D. longicolla* (Hobbs) Santos, Vrandecic & Phillips (Cui et al. 2009; Hobbs et al. 1985; Mathew et al. 2015; Santos et al. 2011; Udayanga et al. 2015). Diaporthe/Phomopsis seed decay was long associated with *D. longicolla* (Udayanga et al. 2015; Ghimire et al. 2019), but Petovic et al. 2021 recently found over ten species which may be causal agents of seed decay. Of the species identified, a newly described species, *D. flavescens* Petrović, Skaltsas & Mathew, was isolated from Kent County, Delaware.

Life cycles for members of the *Diaporthe* complex begin with the overwintering of fungal structures on and in soybean debris left in the field. When proper temperature and rainfall conditions are met early in the growing season, at least 20ºC at high humidity for 4-5 days or 25ºC with 50% humidity for 3 days based on greenhouse experiments, the pathogen produces perithecia and pycnidia. These fruiting bodies then release ascospores and conidia (Balducchi and McGee 1987). These can be rain-splashed onto surrounding plants, infecting the rest of the field through leaves and wounded stems (Backman et al. 1985). The infection of the soybean occurs in the early vegetative stage, before pod formation and fill, and remains latent until late reproductive stages. Spores are spread via rain splash during summer precipitation (Lehman 1923; Backman et al. 1985).

Pod and stem blight (*D. sojae*) was first recorded on soybean in the U.S. in North Carolina in 1920 (Lehman 1923). By the 1970s, pod and stem blight was considered the main disease associated with diminished seed quality in the Midwestern U.S. and in DE and MD (Athow and Laviolette 1973). Stem canker was first described in MD in 1943 (Petty 1943). During the 1950s, cultivars with susceptibility to stem canker were widely planted and yield losses of up to 50% were reported until a shift away from susceptible cultivars Hawkeye and Blackhawk (Backman 1985). The first report of stem canker in the southern United States came from Mississippi in 1975, and by the mid-1980s, stem canker was reported across much of the southern U.S. (Keeling 1982; Mulrooney 1985). Originally believed to be a single pathogen, differences in symptomology, cultural characteristics, and increased aggressiveness of southern isolates led to the proposition to divide stem canker into two diseases, northern and southern stem canker. It was first suggested to recognize the causal organisms as separate forma speciales of *D. phaseolorum (*f.sp. *caulivora* for northern and f. sp. *meridionalis* for southern), and random amplified polymorphic DNA (RAPD) analysis later supported sorting each organism as a variety of *D. phaseolorum* (Fernandez and Hanlin 1996). However, further molecular and phylogenetic analysis raised *D. aspalathi* (van Rensburg et al. 2006) and *D. caulivora* (Santos et al. 2011) to species status. The similarities in both species' signs and symptoms make field diagnosis difficult in transition zones across the U.S. When plants with stem canker are found in the field, species are often assumed based on geographic location. Previous studies have shown *D. caulivora* to be less tolerant to high temperatures than *D. aspalathi* despite it being reported as far north as Wisconsin (Keeling 1988; Li et al. 2004). In transition zones like DE and MD in the Mid-Atlantic, it is assumed that both species are present, but there has been no molecular confirmation of species distribution or abundance in this region. Within infected plants, zone lines are often visible, but no molecular confirmation on causal agent has been conducted for DE or MD. Proper identification of *Diaporthe* species within the region is needed to clarify the *Diaporthe* complex and to avoid misdiagnosis with other fungal diseases that can cause similar symptoms.

To improve understanding of *Diaporthe* distribution and pathogenicity, a two-year fungal survey was conducted across DE and MD followed by greenhouse pathogenicity trials. The objectives of this study were to (i) determine the species composition and pathogenicity of *Diaporthe* associated with soybean stem and root diseases in the Mid-Atlantic and (ii) determine which *Diaporthe* species are associated with zone lines in the region.

Materials and Methods

Isolate Collection and Storage

Isolates of *Diaporthe* were collected from soybean fields in DE and MD during the summers of 2019 and 2020. Sixty field sites were selected in 2019 and 45 in 2020. Sites included randomly selected fields recommended by extension agents and crop consultants, fields from grower calls of concern, and fields with a history of *Diaporthe* in previous soybean crops. Whole plant samples were taken from areas of the field with visible signs or symptoms. Plants were dug, gently shook to remove excess soil and debris, bagged, and taken back to the lab where 1 cm sections of root, stem, or canker (if present) tissue samples were excised. Three root tips were cut per plant (typically necrotic), and three stem tissue pieces with discoloration or *Diaporthe* signs were sliced with a scalpel. Excised stem and root pieces were surface-disinfested in

0.825% sodium hypochlorite for 30 s, rinsed in deionized sterile water for a minute, and plated onto Petri dishes containing potato dextrose agar (PDA; Difco) amended with 0.05 mg Penicillin G (100 ug ml^{-1}) and Streptomycin sulfate (100 ug ml^{-1}). Petri dishes were incubated at room temperature (22°C) for 4 to 5 days, and actively growing hyphae with characteristics of *Diaporthe* were transferred to new PDA plates. Stems were split to check for the presence of zone lines in plants with interveinal chlorosis and/or wilting. To isolate the causal pathogen(s), stems were rinsed, and stem tissue was cut into 1 cm sections, surface disinfested as descried above, and placed on PDA. Petri dishes were incubated at 22°C, and cultures were examined for the presence of *Diaporthe* based on morphology and production of fruiting bodies (Udayanga et al. 2015). For long-term storage, a colonized hyphal plug of each isolate was transferred to a new 9 cm diam Petri dish containing PDA, and four sterile 2.5 cm diam filter paper discs were places around the plug (Thermo Fisher Scientific, Waltham, Massachusetts). Isolates were grown at 22°C until the discs were fully colonized. Discs were removed with sterile forceps, placed into coin envelopes, and stored at -80 C.

DNA Extraction and Sequencing

Three 6.75 mm plugs from each pure culture were transferred into 100 x 15 mm Petri dishes containing 20 ml of potato dextrose broth (PDB) (Difco). Plates were left at 23°C until fully colonized. Isolates showing no contamination were vacuum-aspirated through autoclave-sterilized filter paper in a Buchner funnel. Dried tissue was separated into 1.5 ml microcentrifuge tubes and stored at -2°C for DNA extraction.

Genomic DNA from dried, frozen tissue was extracted by macerating tissue with 400 µl of cell lysis solution (Qiagen, Hilden, Germany) using autoclave-sterilized plastic pestles. Following the addition of 3 µl Puregene proteinase K (Qiagen) to each sample, tubes were vortexed for 20 s, and incubated at 55°C for 1 h. The supernatant of micro centrifuged samples was treated with RNase (3 µl; Qiagen) and incubated at 37°C for 30 min followed by the addition of 200 µl of protein precipitation solution (Qiagen) and 1 h incubation on ice. Tubes were then centrifuged for 5 min and supernatant was pipetted into microcentrifuge tubes containing 400 µl isopropanol to precipitate DNA. Tubes were centrifuged for 3 min, the supernatant was poured off, and the DNA pellet was washed with 200 µl ethanol. After centrifuging, the supernatant was poured off and samples were allowed to dry on a heating block before being rehydrated with 50 µl of sterile, nuclease-free water. DNA concentration and purity were measured by spectrophotometry and standardized to a concentration of 20 ng ml^{-1} before storage at -2°C.

For each isolate, the nuclear rDNA internal transcribed spacer (ITS1-5.8S-ITS2 =ITS) gene region was amplified with polymerase chain reaction (PCR) (Santos et al. 2011; Zhang et al. 1998). PCR reactions were 50 µl and consisted of 10 X DreamTaq Master Mix, 10 µM of forward and reverse primer: ITS5 (5’-GGAAGTAAAAGTCGTAACAAGG-3’) and ITS4 (5’-

TCCTCCGCTTATTGATATGC-3’) (White et al. 1990) and 20 ng of genomic DNA. Thermal cycling parameters were as follows: 94°C for 5 min followed by 35 cycles of DNA denaturation for 45 s at 94°C; primer annealing for 30 s at 53°C; and primer extension for 90 s at 72°C. A final extension step of 72°C for 10 min completed the cycle.

Amplification was verified using gel electrophoresis, at 90 V for 40 min. Aliquots of amplified products were cleaned with ExoSAP-IT (USB products Affymetrix, Inc. ThermoFisher Scientific, Walthan Massachusetts). Samples were sequenced at Eurofins Genomics (Louisville, KY). Analysis of sequence data was completed with CLC Main Workbench 20.0.4 (CLC Bio: a Qiagen Company, Germany) and copied into the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) for comparison against published, identified specimens. Generated sequences were deposited in GenBank (Table 2.1).

Koch’s Postulates and Zone Lines

Koch’s postulates were conducted for any newly identified species and a representative set of isolates recovered from zone line tissue. For each 15 cm pot filled with soilless media (Lambert, Québec, Canada), a single soybean seed of Hawkeye was planted and maintained in a greenhouse (32 ± 5 ◦C; relative humidity 85%; in natural light conditions with 14 h of light and 10 h dark and watered twice daily. Inoculum was prepared following the protocol of Ghimire et al. 2019. Flat toothpicks were autoclaved and placed onto PDA plates amended as described above, with approximately 10 to 15 toothpicks per plate. Four fungal plugs of each selected isolate were placed around the toothpicks and sealed with parafilm. Inoculated plates were stored at 26ºC for 5-10 days or until toothpicks were fully colonized. Infested toothpicks were inserted approximately 50 mm below the first trifoliate node and the area around the wound was sealed with petroleum jelly. Sterile non-infested toothpicks were inserted at the same location on control plants and sealed with petroleum jelly. Five plants were inoculated per isolate and inoculations were replicated twice. At 37 days after inoculation, stem wound and vasculature tissue samples were taken from each of the plants, surface disinfested with 0.825% sodium hypochlorite, rinsed with sterilized water, and plated onto PDA. Once in pure culture, isolates underwent DNA extraction, sequencing, and analysis as outlined above.

Pathogenicity on soybeans

Greenhouse trials were conducted to determine the aggressiveness of isolated *Diaporthe* species. Previous studies have shown that inoculation method can significantly impact symptom development of various *Diaporthe* species. Following the results of Ghimire et al. 2019, a toothpick inoculation method was selected. Toothpick inoculum was prepared as described above using three species identified in the survey. Additional treatments included a sterilize non-infested toothpick control and a non-wounded control. For this experiment, three soybean varieties were selected; Hawkeye (susceptible), GH3932X (rated best for pod and stem blight rating), and NK S39-G2X (rated best for southern stem canker rating). For each variety, a single seed was planted into each of five replicate 15 cm pots filled with soilless media (Lambert, Québec, Canada). Plants were maintained in a greenhouse (32 ± 5 ◦C; relative humidity 85%; in natural light conditions with 14 h of light and 10 h dark and watered twice daily. After soybeans were inoculated with infested toothpicks, wounds were sealed using petroleum jelly (Ghimire et al. 2019). Plants were inoculated at the V2-V3 growth stage (Campbell et al. 2017) and were evaluated for disease at 37 days after inoculation. Plants were evaluated for disease severity (0 = little to no visible lesion, 0.5 = lesion present with plant still alive, 1.0 = whole plant death). The entire trial was repeated twice. After rating, 1 mm samples were taken from stem pieces at the lesion site and evaluated for zone lines, two samples from each of two plants per replication. These samples were processed as indicated above.

Statistical analyses were performed separately for each soybean hybrid in the greenhouse trial. Mixed-model analysis of variance (ANOVA) was conducted using PROC GLIMMIX in SAS (version 9.4). Data were analyzed for disease severity with isolate as a fixed effect and run and replication as random effects. Fixed effects were tested for significance at $\alpha = 0.05$ and (least squares means (LSmeans)) were separated using paired *t* tests.

Results

Environmental Conditions

Weather conditions in 2019 were not conducive for widespread *Diaporthe* infection. The average temperature from July to September (three-month period of the survey) was 24.1ºC with temperatures reaching as high as 36.6ºC. The average rainfall per month during that time was 5.3 cm (2.09 in) (DEOS 2019). Total rainfall throughout the season amounted to 15.9 cm (6.27 in). The warm, dry weather was less conducive to *Diaporthe* infections, but did favor *Macrophomina phaseolina* (Charcoal Rot).

Environmental conditions in 2020 were more conducive to *Diaporthe* infection, with frequent rain events. The average temperature between July and September was 23.9ºC with temperatures reaching as high as 35.2ºC. July – September had an average of 13.6 cm (5.37 in) of rainfall per month (DEOS 2020). The amount of rainfall during July through September was the major environmental factor promoting *Diaporthe* infection. The total rainfall for those months in 2020 was 40.9 cm (16.12 in), more than twice the total rainfall from 2019. In 2020, conditions were not favorable for *M. phaseolina*.

Isolate Collection and Identification

In total, 105 field sites were visited across both years. Plants displaying symptoms of interveinal chlorosis, lack of vigor, and/or zone lines were targeted. In total, 630 stem and root pieces were plated out. From these, 556 isolates were obtained. Within these, 28% were *Diaporthe,* 8% *Fusarium* spp., 4% *Macrophomina phaseolina,* 60% other (Figure 2.2). Isolates categorized as other were not morphologically identified as soybean pathogens in the region and were discarded.

Out of the 167 isolates collected in 2019, 94 were characterized as potential plant pathogens and advanced for sequencing. Isolates were grouped as *Diaporthe* spp. (26%) *M. phaseolina* (22%) *Fusarium* spp. (50%) and other (2%). The *Diaporthe* species collected in 2019 included *D. longicolla, D. aspalathi*, and a novel isolate, *D. ueckerae*. In 2020, 28% (130) of 462 isolates collected were identified as a *Diaporthe.* Ninety eight percent of the *Diaporthe* isolates from 2020 were *D. longicolla*. The remaining 2% of *Diaporthe* isolates were identified as *D. sojae* and *D. ueckerae.* Of the total 154 *Diaporthe* isolates from both 2019 and 2020, 149 (97%) were identified as *D. longicolla,* 2 (1%) *D. aspalathi,* 1 (1%) *D. sojae,* and 2 (1%) *D. ueckerae* (Table 2.1).

Koch's Postulates and Confirmation of Zone Lines

In the survey, one species was identified that had been previously unknown to the region. Koch's postulates were completed in the greenhouse and confirmed the pathogenicity of *D. ueckerae* on soybean. *D. ueckerae* was successfully isolated from stem lesions of infected plants, grown into pure culture, and sequenced with ITS4/5 primers.

In total, 41 field samples with zone lines were collected. Zone lines were observed more often when stems were split (Figure 2.1). They tended to be present on dried down soybeans, however, in cases where plants with low vigor and interveinal chlorosis were collected in 2020, zone lines could be induced by splitting stems and placing them in a moisture chamber box for 2-3 days at room temperature. From these samples, 13 isolates were recovered, of which all were identified as *D. longicolla.* A representative isolate was selected for Koch's Postulates. In all cases, zone lines were observed, and *D. longicolla* was reisolated.

Pathogenicity trials

From the survey, three species were selected for greenhouse trials, *D. aspalathi*, *D. ueckerae,* and *D. longicolla* (Figure 2.3). For this experiment, disease severity was analyzed by seed variety across two trials. Symptoms observed included necrotic lesions at, extending above, or extending below the inoculation site, as well as minimal pod development, wilting, whole plant death, and pycnidia covering the stem of the plant. For each variety, *D. longicolla* had the highest disease severity rating (Table 2.2). GH3934X (pod and stem blight tolerant) had significant differences among treatments, *D. longicolla* and *D. ueckerae* had higher disease severity than *D. aspalathi*. In S39G2X, disease severity scores of all three species were significantly different, with highest severity in *D. longicolla* and lowest in *D. aspalathi.* Hawkeye (the susceptible) had significant differences among treatment as well; *D. ueckerae* and *D. longicolla* had higher disease severity scores than *D. aspalathi*.

Discussion

Four *Diaporthe* species were identified across DE and MD in this survey. One of the initial questions of this work was sorting out the distribution of southern and northern stem canker, but interestingly, very few stem canker (*D. aspalathi* or *D. caulivora*) isolates were recovered. Only two *D. aspalathi* isolates were recovered in 2019, and no *D. caulivora* isolates were recovered. Several samples positively identified as *D. longicolla* also had linear lines of pycnidia on the stem of poorly growing or dead plants (Abdelmagid et al. 2019), a symptom typically associated with *D. sojae* (Chen et al. 2009). These similarities in symptoms and signs could lead to misdiagnosis of *Diaporthe* species or likely indicate that *D. sojae* and *D. longicolla* are more closely associated than the other *Diaporthe* complex species. No molecular confirmation of *Diaporthe* species had ever been conducted in the region. *D. sojae* was first reported in Snow Hill, MD, in 1942 as *Diaporthe* stem canker. No symptoms were described, but it is assumed that lesions were present in this field. Unfortunately, the field was plowed under before further observations were noted (Petty 1943).

Considering that *D. longicolla* has been shown to produce stem cankers, there is a chance this report misdiagnosed *D. longicolla* as *D. sojae* before *D. longicolla* was recognized as a separate species, and if that was the case, then there could be a long-standing history of *D. longicolla* infection in Lower Eastern Shore Maryland. It is likely that *D. longicolla*, because of symptom similarity to *D. sojae*, went under-reported from stem and root tissue isolation for many years until improved molecular tools were introduced for diagnosis.

Incidentally, *D. longicolla* was the dominant species recovered in our survey, accounting for 97% of samples. In Petrović et al. 2021, 28 of the 48 recovered *Diaporthe* isolates from soybean were *D. longicolla* (58%) across eight states. Ghimire et al. 2019 reported similar findings: 67.1% of 152 isolates collected were *D. longicolla.* In addition to *D. longicolla,* Petrović et al. 2021 recovered more than 10 other *Diaporthe* species from seed isolations. Samples in this survey came only from stem and root tissue. While *D. longicolla* has often been associated with Diaporthe/Phomopsis seed decay, recent surveys have identified that *D. longicolla* is frequently recovered from stem tissue. There have been recent reports of *D. longicolla* also causing or being associated with stem lesions in soybeans in Uruguay and Manitoba, Canada (Mena et al. 2020; Abdelmagid et al. 2022). These reports parallel our findings in the Mid-Atlantic. Isolation sources play a critical role in the recovery of *Diaporthe* species and diagnosis; therefore, more work is needed regarding the isolation of *Diaporthe* from stem, root, and seed. For example, *D. caulivora* and *D. aspalathi* tend to be isolated more often in literature from stem tissue bordering lesions. *D. longicolla*, has been isolated from both seed and stem, as mentioned previously, and Petrovic et al. 2021 was able to isolate *D. ueckerae*, among other *Diaporthe* species from seed alone (Mena et al. 2020; Li et al. 2016; Olson et al. 2015).

Zone lines had been associated with multiple pathogens, including *D. longicolla, M. phaseolina,* and *Fusarium* spp., but *D. longicolla* was confirmed as the causal organism in Illinois, Indiana, and Iowa. Michigan, and South Dakota (Olson et al. 2015). While it was presumed *that D. longicolla* was also the primary species involved in the Mid-Atlantic, our work is the first to investigate zone lines in the Mid-Atlantic. Only *D. longicolla* was isolated from soybean stems with zone lines present in the survey. Plants used in the greenhouse aggressiveness trials were left in the greenhouse for an extended period after the trial to dry down, and no zone lines were observed in plants inoculated with *D. aspalathi* or *D. ueckerae.*

Diaporthe ueckerae was identified as a new species in the region. This species was first reported as a soybean pathogen in Brazil in 2013 (Gomes et al. 2013; Udayanga et al. 2015), having also been listed a pathogen of melon (Udayanga et al. 2015; Dissanayake et al. 2017). Since 2019, *Diaporthe ueckerae* has been reported as a soybean pathogen in Louisiana, Missouri, and Columbia (Petrović et al. 2021; Lopez-Cardona et al. 2021). This is the first report of *D. ueckerae* in Maryland. Along with understanding the virulence of these pathogens, this was also an opportunity to observe symptom development in *D. ueckerae* infections. Currently, not much is understood about *D. ueckerae* infection within soybean plants. The discovery of the novel pathogen *D. ueckerae* in Maryland suggests that more *Diaporthe* species may be involved in the complex than initially thought. While not found in this survey, a novel seedborne *Diaporthe* species was identified in Petrović et al. 2021, from Delaware: *D.*

flaviscens. It produces similar conidia to *D. sojae* in culture and is placed into the same clade as *D. longicolla* and *D. sojae.*

In all three soybean varieties (stem blight tolerant, southern stem canker tolerant, susceptible), *D. longicolla* had the highest average disease severity score compared to the other treatments, meaning it was the most aggressive isolate. Although *D. longicolla* and *D. sojae* are closely related, resistance responses may not be transferable from one species to another. There is a need for regionally adapted varieties with resistance to corresponding regionally impactful fungal species. Further screening with multiple *D. longicolla* isolates is required to confirm these observations.

In 2020, growers in the Mid-Atlantic region experienced widespread *Diaporthe* infection in soybean. The ability of *Diaporthe* species to overwinter on debris in the field may be a contributing factor to the rise in *Diaporthe* observations over recent years (TeKrony et al. 1983; Rothrock et al. 1988; Damicone et al. 1990; Reis et al. 2011). Interveinal leaf chlorosis symptoms caused by *Diaporthe* infection can mimic symptoms of other diseases, such as soybean sudden death syndrome (SDS), caused by *Fusarium virguliforme*. This similarity can confuse field diagnosis and is further complicated by the possibility of coinfections, which have been observed in the region. SDS tends to occur sporadically within the Mid-Atlantic, but the appearance of SDS can be associated with periods of above-average rainfall (Leandro et al. 2018). Soybean cyst nematodes are also widely present in the region and can further exacerbate SDS symptoms and other fungal symptoms.

In greenhouse trials, coinfection of soybeans with SCN and *D. longicolla* lead to increased lesion lengths on the stem of the infected plant (Posch 2017). In many of the 2019-2020 survey fields, soil samples to enumerate nematode populations were also collected. Twenty-five of the field sites that tested positive for *Diaporthe* spp. also had nematode survey data. Within these fields, 72% of the field sites also had soybean cyst nematodes present. Periods of heavy rainfall, the rise of conservation tillage, and the reduction of deep plowing can favor accumulation of nematode populations and *Diaporthe* species. Continued investigation on the role of coinfection could aid in elucidating the impact of early season infection rate on end of season disease severity and yield losses.

This survey sought to determine the distribution of stem canker pathogens *D. caulivora,* and *D. aspalathi* within the region. *D. caulivora* was never detected in the two-year survey, while only two *D. aspalathi* isolates were recovered in 2019. Instead, *D. longicolla* was the dominant pathogen observed. Isolations were made from stem lesions and root tissue, indicating *D. longicolla* may play a broader role than just a seed decay pathogen. Additionally, the lack of *D. aspalathi* and *D. caulivora* isolates found throughout both years implies that stem canker is not as prevalent as previously thought, most likely confused with the other, lesser-known symptoms of *D. longicolla* infection. Further work is needed to improve understanding of the yield impacts of *D. longicolla* in the region and other novel species, such as *D. ueckerae*.

REFERENCES

Abdelmagid, A., Hafez, M., Lawley, Y., Rehal, P.K., Daayf, R. 2022. First Report of Pod and Stem Blight and Seed Decay Caused by *Diaporthe longicolla* on Soybean in Western Canada. *Plant Disease, 106*(3).

Abdelmagid, A., Hafez, Y., Lawley, Y., Adam, L.R., Daayf, F. 2019. First Report of Northern Stem Canker Caused by *Diaporthe caulivora* on Soybean in Western Canada. *Plant Disease, 103*(2).

Allen, T.W., Bradley, C.A., Sisson, A.J., Byamukama, E., Chilvers, M.I., Coker, C.M., Collins, A.A., Damicone, J.P., Dorrance, A.E., Dufault, N.S., Esker, P.D., Faske, T.R., Gielser, L.J., Grybauskas, A.P., Hershman, D.E., Hollier, C.A., Isakeit, T., Jardine, D.J., Kelly, H.M., Kemerait, R.C., Kleczewski, N.M., Koenning, S.R., Kurle, J.E., Malvick, D.K., Markell, S.G., Mehl, H.L., Mueller, D.S., Mueller, J.D., Mulrooney, R.P., Nelson, B.D., Newman, M.A., Osborne, L., Overstreet, C., Padgett, G.B.,Phipps, P.M., Price, P.P., Sikora, E.J., Smith, D.L., Spurlock, T.N., Tande, C.A., Tenuta, A.U., Wise, K.A., Wrather, J.A. 2017. Soybean Yield Loss Estimates Due to Diseases in the United States and Ontario, Canada, from 2010 to 2014. *Plant Health Progress, 18*(1): 19-27.

Athow, K.L. and Laviolette, F.A., 1973. Pod protection effects on soybean seed germination and infection with *Diaporthe phaseolorum* var. *sojae* and other microorganisms. *Phytopathology*, *63*(8), pp.1021-3.

Backman, P.A., Weaver, D.B., and Morgan-Jones, G. 1985. Soybean Stem Canker: An Emerging Disease Problem. *Plant Disease, 69*(8): 641 – 647.

Balducchi, A.J., McGee, D.C. 1987. Environmental Factors Influencing Infection of Soybean Seeds by *Phomopsis* and *Diaporthe* Species During Seed Maturation. Plant Disease, *79*(3): 209–212.

Bradley, C.A., Allen, T.W., Sisson, A.J., Bergstrom, G.C., Bissonnette, K.M., Bond, J., Byamukama, E., Chilvers, M.I., Collins, A.A., Damicone, J.P. and Dorrance, A.E., Default N.S., Esker P.D., Faske T.R., Fiorellino N.M., Giesler L.J., Hartman G.L., Hollier C.A., Isakeit T.I., Jackson-Ziems T.A., Jardine D.J., Kelly H.M., Kemerait R.C., Kleczaeski N.M., Koehler A.M., Kratochvil R.J., Kurle J.E., Malvic D.K., Markell S.G., Mathew F.M., Mehl H.L., Mehl K.M., Mueller D.S., Mueller J.D., Nelson B.D., Overstreet C., Padgett G.B., Price P.P., Sikora E.J., Smith D.L., Spurlock T.N., Tande C.A., Telenko D.E.P., Tenuta A.U., Thiessen L.D., Warner F., Wiebold W.J., Wise K.A. 2021. Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2015 to 2019. *Plant Health Progress, 22*(4).

Chen, C., Wang, T., Seo, M. 2009. First report of soybean pod and stem blight caused by *Diaporthe phaseolorum* var. *sojae* in Taiwan. *Plant Disease, 93*: 202.

Cui, Y.L., Duan, C.X., Wang, X.M., Li, H.J., Zhu, Z.D., 2009. First report of *Phomopsis longicolla* causing soybean stem blight in China. *Plant Pathology*, *58*(4): 799-799.

Damicone, J.P., Snow, J.P., and Berggren, G.T. 1990. Spatial and temporal spread of soybean stem canker from an inoculum point source. *Phytopathology, 80*: 571-578.

DEOS (Delaware Environmental Observing System). 2020. Monthly Data for Georgetown – REC. http://www.deos.udel.edu

DEOS (Delaware Environmental Observing System). 2021. Monthly Data for Georgetown – REC. http://www.deos.udel.edu

Dissanayake, A.J., Phillips, A.J.L., Hyde, K.D., Yan, J.Y., and Li, X.H. 2017. The current status of species in *Diaporthe*. *Mycosphere, 8*(5): 1106-1156.

Fernández, F.A., Hanlin, R.T. 1996. Morphological and RAPD Analyses of *Diaporthe phaseolorum* from Soybean. *Mycologia, 88*(3): 425-440.

Ghimire, K., Petrović, K., Kontz, B., Bradley, C.A., Chilvers, M.I., Mueller, D.S., Smith, D.L., Wise, K.A., Mathew, F.M. 2019. Inoculation Method Impacts Symptom Development Associated with *Diaporthe aspalathi, D. caulivora,* and *D. longicolla* on Soybean (*Glycine max*). *Plant Disease, 103*(4): 677 – 684.

Gomes, R.R., Glienke, C., Videria, S.I.R., Lombard, L., Groenewald, J.Z., Crous, P.W. 2013. *Diaporthe*: A genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia, 31*: 1-41.

Hobbs, T.W., Schmitthenner, A.F., Kuter, G.A., 1985. A new *Phomopsis* species from soybean. *Mycologia*, *77*(4): 535-544.

Keeling, B L. 1988. Influence of temperature on growth and pathogenicity of geographic isolates of *Diaporthe phaseolorum* var. *caulivora*. *Plant Disease, 72*(3): 220-222.

Keeling, B.L. 1982. A Seedling Test for Resistance to Soybean Stem Canker Caused by *Diaporthe phaseolorum* var. *caulivora*. *Phytopathology, 72*(7), 807-809.

Leandro, L.F., S., Eggenberger, S., Chen, C., Williams, J., Beattie, G.A., Liebman, M. 2018. Cropping System Diversification Reduces Severity and Incidence of Soybean Sudden Death Syndrome Caused by *Fusarium virguliforme*. 2018. *Plant Disease, 102*(9): 1748 – 1758

Leathers, Dan. 2018. 2018 Summer Season Warm with Near Normal Precipitation Across Delaware. Delaware Climate Office.

Lehman, S. 1923. Pod and Stem Blight of Soybean. *Annals of the Missouri Botanical Garden, 10*(2): 111-178.

Li, S., Kurtzweil, N.C., Grau, C. R., Hartman, G.L. 2004. Occurrence of soybean stem canker (*Diaporthe phaseolorum* var. *meridionalis*) in Wisconsin. *Plant Disease, 88*(5): 576.

Li, X., Song, Q., Martins, A.M., Cregan, P. 2016. Draft Genome of *Diaporthe aspalathi* isolate MS-SSC91, a fungus causing stem canker in soybean. *Genomics Data, 7*: 262-263.

Lopez-Cardona, N., Guevera-Castro, A., Ganan-Becancur, L., Amaya-Gomez, C.V. 2021. First Report of *Diaporthe ueckerae* Causing Stem Canker on Soybean (*Glycine max)* in Colombia. 2021. *Disease Note*.

Mena, E., Stewart, S., Montesano, M., Ponce de Leon, I. 2020. Soybean Stem Canker Caused by *Diaporthe caulivora*; Pathogen Diversity, Colonization Process, and Plant Defense Activation. *Frontiers in Plant Science, 10*.

Mulrooney, R.P. 1985. Soybean disease loss estimates for Southern United States in 1983. *Plant Disease*, *69*(1): 92.

Olson T.R., Gebreil, A., and Micijevic, A. 2015. Association of *Diaporthe longicolla* with Black Zone Lines on Mature Soybean Plants. *Plant Health Progress, 16*(3): 118-122.

Petrović, K., Skaltsas, D., Castlebury, L. A., Kontz, B., Allen, T. W., Chilvers, I. M., Gregory, N., Kelly, H. M., Koehler, M. A., Kleczewski, N., Mueller, S. D., Price, P. P. (Trey), Smith, L. D., Mathew, F. M. 2021. Diaporthe seed decay of soybean [Glycine max (L.) Merr.] is Endemic in the United States, But New Fungi Are Involved. *Plant Disease, 105*(6): 1621-1629.

Petty, M.A. 1943. Soybean Disease incidence in Maryland in 1942 and 1943. *Plant Disease Reporter, 27*:347-349.

Posch, J.P. 2017. *Diaporthe,* Soybean Cyst Nematode, and Soybean Aphid: An Evaluation of Potential Interactions Occurring among Pests on Soybean in South Dakota (Accesion No. 1190) [Master's Thesis, South Dakota State University]. *Electronic Theses and Dissertations.*

Reis, E.M., Baruffi, D., Remor, L., Zanatta, M. 2011. Decomposition of corn and soybean residues under field conditions and their role as inoculum source. *Summa phytopathology, 37*(1).

Rothrock, C.S., Phillips, D.V., Hobbs, T.W. 1988. Effects of Cultivar, Tillage, and Cropping System on Infection of. Soybean by *Diaporthe phaseolorum* var. *caulivora* and Southern Stem Canker Symptom Development. *Phytopathology, 78:* 266-270

Santos, J.M., Vrandečić, K., Ćosić, J., Duvnjak, T., Phillips, A. J. L. 2011. Resolving the *Diaporthe* species occurring on soybean in Croatia. *Persoonia*, *27*, 9-19.

TeKrony, D.M., Egli, D.B., Stuckey, R.E., Balles, J. 1983. Relationship between weather and soybean infection by *Phomopsis* sp. *Phytopathology*, *73*(6): 914-918

Udayanga, D., Castlebury, L.A., Rossman, A.Y., Chukeatirote, E., Hyde, K.D. 2015. The *Diaporthe sojae* species complex: Phylogenetic re-assessment of pathogens associated with soybean, cucurbits and other field crops. *Fungal Biology*, *119*(5): 383-407

USDA-NASS, 2020; https://www.nass.usda.gov/

van Rensburg, J.C.J., Lamprecht, S.C., Groenewald, J.Z., Castlebury, L.A., Crous, P.W. 2006, Characterisation of *Phomopsis* spp. associated with die-back of rooibos (*Aspalathus linearis*) in South Africa. *Studies in Mycology,* 55: 64-74.

White, T.J. et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego, CA. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhang, A.W., Riccioni, L., Pedersen, W.L., Kollipara, K.P., and Hartman, G.L. 1998. Molecular Identification and Phylogenetic Grouping of *Diaporthe phaseolorum* and *Phomopsis longicolla* Isolates from Soybean. *Phytopathology, 88*(12): 1306-1314.

Table 2.1: Summary of *Diaporthe* isolates collected from 2019-2020

Isolate ID	Year Collected	Location	Species	ITS GenBank Accession
>SeqUD1	2019	Queen Anne's, MD	*D. longicolla*	ON179826
>SeqUD2	2019	Sussex, DE	*D. longicolla*	ON179827
>SeqUD3	2019	Wicomico, MD	*D. ueckerae*	ON179828
>SeqUD4	2019	New Castle, DE	*D. longicolla*	ON179829
>SeqUD5	2019	New Castle, DE	*D. longicolla*	ON179830
>SeqUD6	2019	New Castle, DE	*D. longicolla*	ON179831
>SeqUD7	2019	New Castle, DE	*D. longicolla*	ON179832
>SeqUD8	2019	New Castle, DE	*D. longicolla*	ON179833
>SeqUD9	2019	New Castle, DE	*D. longicolla*	ON179834
>SeqUD10	2019	New Castle, DE	*D. longicolla*	ON179835
>SeqUD11	2019	New Castle, DE	*D. longicolla*	ON179836
>SeqUD12	2019	New Castle, DE	*D. longicolla*	ON179837
>SeqUD13	2019	New Castle, DE	*D. longicolla*	ON179838
>SeqUD14	2019	Kent, DE	*D. aspalathi*	ON179839
>SeqUD15	2019	Kent, DE	*D. aspalathi*	ON179840
>SeqUD16	2019	Sussex, DE	*D. longicolla*	ON179841
>SeqUD17	2019	Sussex, DE	*D. longicolla*	ON179842
>SeqUD18	2019	Sussex, DE	*D. longicolla*	ON179843
>SeqUD19	2019	Sussex, DE	*D. longicolla*	ON179844
>SeqUD20	2019	Sussex, DE	*D. longicolla*	ON179845
>SeqUD21	2019	Sussex, DE	*D. longicolla*	ON179846
>SeqUD22	2019	Sussex, DE	*D. longicolla*	ON179847
>SeqUD23	2019	Sussex, DE	*D. longicolla*	ON179848
>SeqUD24	2019	New Castle, DE	*D. longicolla*	ON179849
>SeqUD25	2020	Kent, DE	*D. longicolla*	ON179850
>SeqUD26	2020	Kent, DE	*D. longicolla*	ON179851
>SeqUD27	2020	Kent, MD	*D. longicolla*	ON179852
>SeqUD28	2020	Kent, MD	*D. longicolla*	ON179853
>SeqUD29	2020	Kent, MD	*D. longicolla*	ON179854
>SeqUD30	2020	Kent, MD	*D. longicolla*	ON179855

Isolate ID	Year Collected	Location	Species	ITS GenBank Accession
>SeqUD31	2020	Kent, MD	*D. longicolla*	ON179855
>SeqUD32	2020	Kent, MD	*D. longicolla*	ON179856
>SeqUD33	2020	Kent, MD	*D. longicolla*	ON179857
>SeqUD34	2020	Kent, MD	*D. longicolla*	ON179858
>SeqUD35	2020	Kent, MD	*D. longicolla*	ON179859
>SeqUD36	2020	Kent, MD	*D. longicolla*	ON179860
>SeqUD37	2020	Kent, MD	*D. longicolla*	ON179861
>SeqUD38	2020	Kent, MD	*D. longicolla*	ON179862
>SeqUD39	2020	Kent, MD	*D. longicolla*	ON179863
>SeqUD40	2020	Sussex, DE	*D. longicolla*	ON179864
>SeqUD41	2020	Sussex, DE	*D. longicolla*	ON179865
>SeqUD42	2020	Wicomico, MD	*D. longicolla*	ON179866
>SeqUD43	2020	Wicomico, MD	*D. longicolla*	ON179867
>SeqUD44	2020	Wicomico, MD	*D. longicolla*	ON179868
>SeqUD45	2020	Wicomico, MD	*D. longicolla*	ON179869
>SeqUD46	2020	Wicomico, MD	*D. longicolla*	ON179870
>SeqUD47	2020	Wicomico, MD	*D. longicolla*	ON179871
>SeqUD48	2020	Wicomico, MD	*D. longicolla*	ON179872
>SeqUD49	2020	Wicomico, MD	*D. longicolla*	ON179873
>SeqUD50	2020	Wicomico, MD	*D. longicolla*	ON179874
>SeqUD51	2020	Wicomico, MD	*D. longicolla*	ON179875
>SeqUD52	2020	Wicomico, MD	*D. longicolla*	ON179876
>SeqUD53	2020	Wicomico, MD	*D. longicolla*	ON179877
>SeqUD54	2020	Wicomico, MD	*D. longicolla*	ON179878
>SeqUD55	2020	Wicomico, MD	*D. longicolla*	ON179879
>SeqUD56	2020	Wicomico, MD	*D. longicolla*	ON179880
>SeqUD57	2020	Sussex, DE	*D. sojae*	ON179881
>SeqUD58	2020	Sussex, DE	*D. longicolla*	ON179882
>SeqUD59	2020	Sussex, DE	*D. longicolla*	ON179883
>SeqUD60	2020	Sussex, DE	*D. longicolla*	ON179884
>SeqUD61	2020	Sussex, DE	*D. longicolla*	ON179885
>SeqUD62	2020	Sussex, DE	*D. longicolla*	ON179886
>SeqUD63	2020	Sussex, DE	*D. longicolla*	ON179887

Isolate ID	Year Collected	Location	Species	ITS GenBank Accession
>SeqUD64	2020	Sussex, DE	*D. longicolla*	ON179888
>SeqUD65	2020	Sussex, DE	*D. longicolla*	ON179889
>SeqUD66	2020	Sussex, DE	*D. longicolla*	ON179890
>SeqUD67	2020	Sussex, DE	*D. longicolla*	ON179891
>SeqUD68	2020	Sussex, DE	*D. longicolla*	ON179892
>SeqUD69	2020	Sussex, DE	*D. longicolla*	ON179893
>SeqUD70	2020	Sussex, DE	*D. longicolla*	ON179894
>SeqUD71	2020	Sussex, DE	*D. longicolla*	ON179895
>SeqUD72	2020	Sussex, DE	*D. longicolla*	ON179896
>SeqUD73	2020	Sussex, DE	*D. longicolla*	ON179897
>SeqUD74	2020	Sussex, DE	*D. longicolla*	ON179898
>SeqUD75	2020	Sussex, DE	*D. longicolla*	ON179899
>SeqUD76	2020	Sussex, DE	*D. longicolla*	ON179900
>SeqUD77	2020	Sussex, DE	*D. longicolla*	ON179901
>SeqUD78	2020	Sussex, DE	*D. longicolla*	ON179902
>SeqUD79	2020	Sussex, DE	*D. longicolla*	ON179903
>SeqUD80	2020	Sussex, DE	*D. longicolla*	ON179904
>SeqUD81	2020	Sussex, DE	*D. longicolla*	ON179905
>SeqUD82	2020	Sussex, DE	*D. longicolla*	ON179906
>SeqUD83	2020	Sussex, DE	*D. longicolla*	ON179907
>SeqUD84	2020	Sussex, DE	*D. longicolla*	ON179908
>SeqUD85	2020	Sussex, DE	*D. longicolla*	ON179909
>SeqUD86	2020	Sussex, DE	*D. longicolla*	ON179910
>SeqUD87	2020	Sussex, DE	*D. longicolla*	ON179911
>SeqUD88	2020	Sussex, DE	*D. longicolla*	ON179912
>SeqUD89	2020	Sussex, DE	*D. longicolla*	ON179913
>SeqUD90	2020	Sussex, DE	*D. longicolla*	ON179914
>SeqUD91	2020	Queen Anne’s, MD	*D. longicolla*	ON179915
>SeqUD92	2020	Queen Anne’s, MD	*D. longicolla*	ON179916
>SeqUD93	2020	Queen Anne’s, MD	*D. longicolla*	ON179917
>SeqUD94	2020	Kent, DE	*D. longicolla*	ON179918
>SeqUD95	2020	Kent, DE	*D. longicolla*	ON179919
>SeqUD96	2020	Sussex, DE	*D. longicolla*	ON179920

Isolate ID	Year Collected	Location	Species	ITS GenBank Accession
>SeqUD97	2020	Sussex, DE	*D. longicolla*	ON179921
>SeqUD98	2020	Sussex, DE	*D. longicolla*	ON179922
>SeqUD99	2020	Sussex, DE	*D. longicolla*	ON179923
>SeqUD100	2020	Wicomico, MD	*D. ueckerae*	ON179924
>SeqUD101	2020	Wicomico, MD	*D. longicolla*	ON179925
>SeqUD102	2020	Wicomico, MD	*D. longicolla*	ON179926
>SeqUD103	2020	Wicomico, MD	*D. longicolla*	ON179927
>SeqUD104	2020	Wicomico, MD	*D. longicolla*	ON179928
>SeqUD105	2020	Wicomico, MD	*D. longicolla*	ON179929
>SeqUD106	2020	Wicomico, MD	*D. longicolla*	ON179930
>SeqUD107	2020	Wicomico, MD	*D. longicolla*	ON179931
>SeqUD108	2020	Wicomico, MD	*D. longicolla*	ON179932
>SeqUD109	2020	Wicomico, MD	*D. longicolla*	ON179933
>SeqUD110	2020	Wicomico, MD	*D. longicolla*	ON179934
>SeqUD111	2020	Kent, DE	*D. longicolla*	ON179935
>SeqUD112	2020	Kent, DE	*D. longicolla*	ON179936
>SeqUD113	2020	Kent, DE	*D. longicolla*	ON179937
>SeqUD114	2020	Kent, DE	*D. longicolla*	ON179938
>SeqUD115	2020	Sussex, DE	*D. longicolla*	ON179939
>SeqUD116	2020	Sussex, DE	*D. longicolla*	ON179940
>SeqUD117	2020	Sussex, DE	*D. longicolla*	ON179941
>SeqUD118	2020	Sussex, DE	*D. longicolla*	ON179942
>SeqUD119	2020	Sussex, DE	*D. longicolla*	ON179943
>SeqUD120	2020	Sussex, DE	*D. longicolla*	ON179944
>SeqUD121	2020	Sussex, DE	*D. longicolla*	ON179945
>SeqUD122	2020	Sussex, DE	*D. longicolla*	ON179946
>SeqUD123	2020	Sussex, DE	*D. longicolla*	ON179947
>SeqUD124	2020	Sussex, DE	*D. longicolla*	ON179948
>SeqUD125	2020	Sussex, DE	*D. longicolla*	ON179949
>SeqUD126	2020	Sussex, DE	*D. longicolla*	ON179950
>SeqUD127	2020	Sussex, DE	*D. longicolla*	ON179951
>SeqUD128	2020	Sussex, DE	*D. longicolla*	ON179952
>SeqUD129	2020	Sussex, DE	*D. longicolla*	ON179953

Isolate ID	Year Collected	Location	Species	ITS GenBank Accession
>SeqUD130	2020	Sussex, DE	*D. longicolla*	ON179954
>SeqUD131	2020	Sussex, DE	*D. longicolla*	ON179955
>SeqUD132	2020	Kent, DE	*D. longicolla*	ON179956
>SeqUD133	2020	Kent, DE	*D. longicolla*	ON179957
>SeqUD134	2020	Kent, DE	*D. longicolla*	ON179958
>SeqUD135	2020	Kent, DE	*D. longicolla*	ON179959
>SeqUD136	2020	Kent, DE	*D. longicolla*	ON179960
>SeqUD137	2020	Kent, DE	*D. longicolla*	ON179961
>SeqUD138	2020	Sussex, DE	*D. longicolla*	ON179962
>SeqUD139	2020	Sussex, DE	*D. longicolla*	ON179963
>SeqUD140	2020	Sussex, DE	*D. longicolla*	ON179964
>SeqUD141	2020	unknown	*D. longicolla*	ON179965
>SeqUD142	2020	Kent, DE	*D. longicolla*	ON179966
>SeqUD143	2020	Kent, DE	*D. longicolla*	ON179967
>SeqUD144	2020	Kent, DE	*D. longicolla*	ON179968
>SeqUD145	2020	Kent, DE	*D. longicolla*	ON179969
>SeqUD146	2020	Kent, DE	*D. longicolla*	ON179970
>SeqUD147	2020	Sussex, DE	*D. longicolla*	ON179971
>SeqUD148	2020	Sussex, DE	*D. longicolla*	ON179972
>SeqUD149	2020	Sussex, DE	*D. longicolla*	ON179973
>SeqUD150	2020	Sussex, DE	*D. longicolla*	ON179974
>SeqUD151	2020	Sussex, DE	*D. longicolla*	ON179975
>SeqUD152	2020	Sussex, DE	*D. longicolla*	ON179976
>SeqUD153	2020	Sussex, DE	*D. longicolla*	ON179977
>SeqUD154	2020	Sussex, DE	*D. longicolla*	ON179978

Table 2.2: Disease severity in soybean varieties following inoculation with multiple *Diaporthe* species

	Disease Severity Score[z]		
Inoculation Source	Hawkeye[y]	GH3934X	S39G2X
D. longicolla	0.7 A[x]	0.75 A	0.9 A
D. ueckerae	0.45 AB	0.65 A	0.65 B
D. aspalathi	0.3 B	0.2 B	0.25 C
p-value	0.04	0.0026	<0.0001

[z] Disease severity was measured as 0 = plant showed no lesion expansion beyond toothpick wound; 0.5 = plant showed lesion elongation above or below wound site compared with non-inoculated plants, but no plant death; and 1 = dead plant.
[y] Varieties included Hawkeye (susceptible), GH3934X (rated for pod and stem blight), and S39-G2X (rated for southern stem canker).
[x] Treatment effects were analyzed using PROC GLIMMIX in SAS (9.4); LS Means were separated using paired t tests (α=0.05). Means followed by the same letter within each column are not significantly different.

Figure 2.1: Zone lines associated with *D. longicolla* collected during 2020.

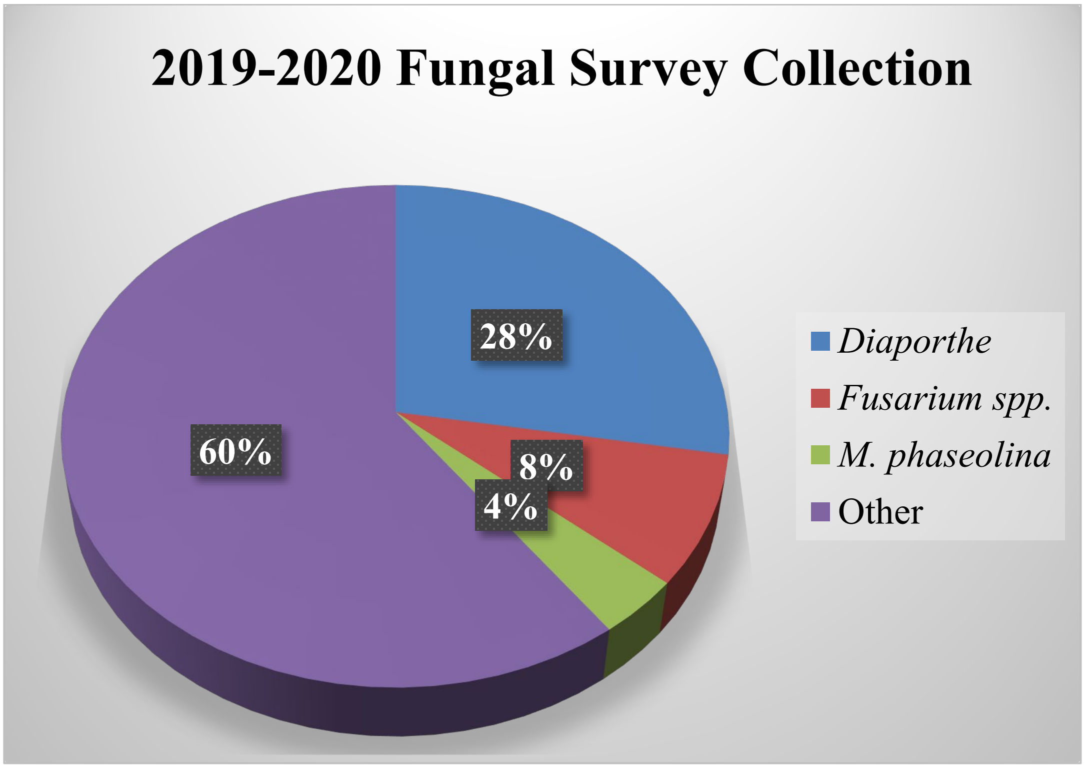

Figure 2.2: Summary of 2019-2020 *Diaporthe* collection.

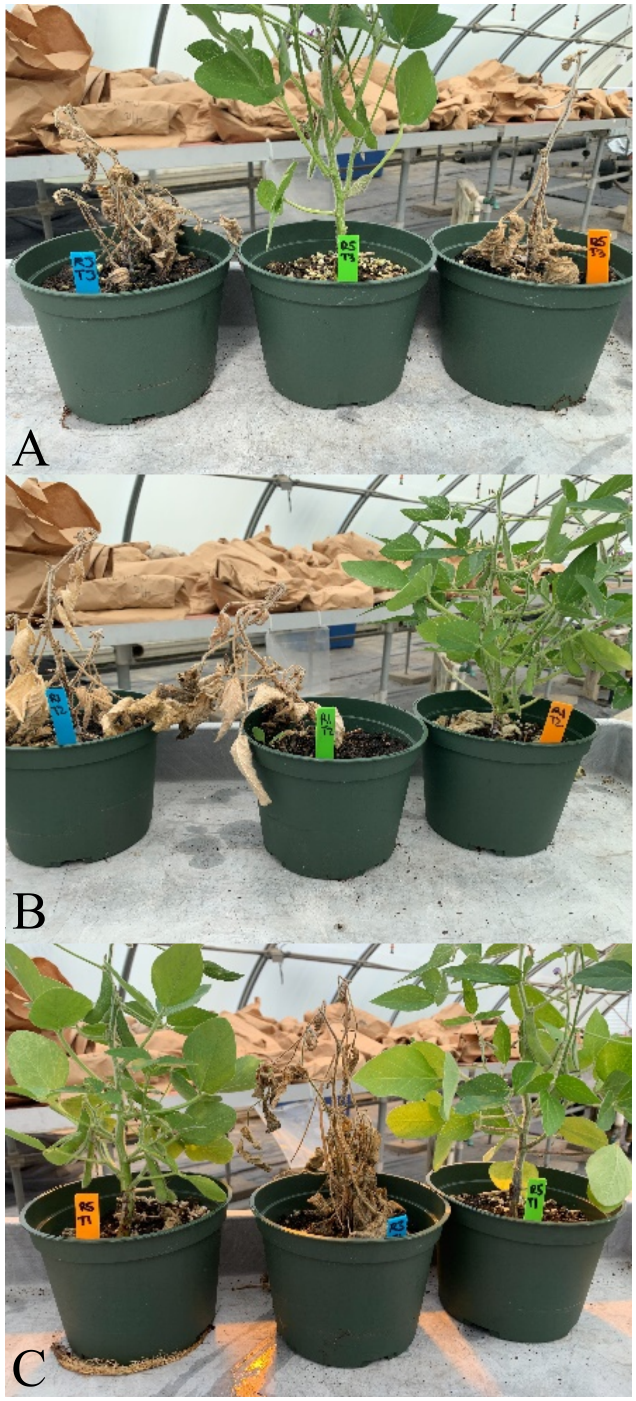

Figure 2.3: Three soybean varieties inoculated with *Diaporthe* species. a) *D. longicolla*, b) *D. ueckerae*, c) *D. aspalathi*. Blue tag (Hawkeye), green tag (GH3934X), orange tag (S39-G2X).

Chapter 3

A SURVEY OF PLANT PATHOGENIC NEMATODES OF SOYBEANS IN DELAWARE AND MARYLAND 2019 – 2021

Abstract

Soybean cyst nematode is the most yield-limiting pathogen of soybean across Delaware (DE) and eastern shore Maryland (MD). To update nematode distribution and population data, a three-year soil survey was conducted from 2019 to 2021. Soybean fields across DE and nine MD counties were sampled from August through October. Three hundred and eleven soil samples were collected, including two hundred and 22 predictive samples and 89 diagnostic samples. Across all samples, nine nematode taxa were identified. Soybean cyst nematode (*Heterodera glycines,* 53.38%), root-knot nematode (*Meloidogyne incognita,* 18.33%), lesion nematode (*Pratylenchus* spp., 57.23%), and spiral nematode (*Helicotylenchus* spp., 54.34%) were most abundant in the region. Among these taxa, 66% of SCN samples had populations above economic threshold; 39% of RKN; and 14% of lesion. Results from this survey highlight the continued challenge of SCN in the region and that other nematode taxa like RKN and lesion should be monitored. Many fields have multiple nematode genera present, and interactions are poorly understood.

Introduction

Soybean (*Glycine max* L. Merrill*)* is a major agronomic commodity for the Mid-Atlantic region, with over 190,500 and 571,500 harvested in Delaware (DE) and Maryland (MD), respectively in 2020 (USDA NASS 2020). Favorable environments (temperature and moisture), soils with high sand content, and frequent rotation of agronomic and vegetable crops have favored the widespread occurrence of numerous nematode genera. For decades, soybean cyst nematode (*Heterodera glycines* Ichinohe*,* SCN) has been considered the most yield limiting disease of soybean production in DE (Mulrooney et al. 1997; Allen et al. 2021; Bradley et al. 2021). Estimations of crop loss due to plant parasitic nematodes are difficult to attain since underground symptoms can easily go undiagnosed. From 1996 to 2016, when comparing all soybean pathogens, SCN caused the greatest economic loss per hectare across the entire US (Bandera et al. 2020). Crop rotation to non-host crops can help lower field populations, however, SCN can persist in the soil for years, even in the absence of their host crop (Riggs 2004).

Surveys for SCN in DE were conducted from 1993-94 and in 2009-10 (Mulrooney et al. 1997; Mulrooney and Gregory 2010). They were primarily focused on SCN, which has historically been the nematode of greatest economic concern in soybeans. Symptoms of SCN infection include stunting, yellowing of the plant, and plant death. Severe damage is often observed in sandy soils, which are widely prevalent in DE and Eastern Shore MD (Davis and Tylka 2021). PI 88788 has been used as the predominant SCN source of resistance since the 1990s; historically, it kept SCN reproduction on plants below 10%, however, there has been a noticeable trend of increased reproduction on this line, starting in the Mid-West in the early 2000s (McCarville et al. 2017). When reproduction increases above the 10% threshold, yield damage increases on resistant soybean cultivars. As anticipated, the regional surveys confirmed the widespread presence of SCN, but the 2009-10 survey also identified an increase in SCN population sizes, and reduced effectiveness of the resistance gene PI88788, with reproduction levels averaging 67% (Mulrooney and Gregory 2010). While SCN has been the focus of previous studies, other nematodes such as the southern root-knot nematode, *Meloidogyne incognita* ((Kofoid and White, 1919) Chitwood, 1949) (RKN) and lesion nematode *Pratylenchus* spp. (Cobb 1917) may also be of concern to the region, particularly in soybean fields rotated with vegetable production. Populations of RKN or lesion nematode were not evaluated in the 1993-94 or 2009-10 Delaware surveys. Presence of lesion nematode was noted in Maryland in 1993: 78% of 463 samples taken across eight MD counties were positive for lesion nematode, but no data was collected on percent of samples above economic threshold (Sindermann et al. 1993). No surveys have been conducted since 2009 for SCN or other potentially damaging nematodes in the region. To update the understanding of nematode distribution and prevalence in the Mid-Atlantic region, soil samples were collected across DE and MD in a three-year survey project from 2019-2021. The objectives of this project were to i) update SCN distribution and population data for the region and ii) determine if additional nematode genera are present above soybean economic thresholds and if they contribute to yield loss.

Site Selection and Collection of Soil Samples

Nematode survey sites across 12 counties in DE and MD were selected based on correspondence with extension agents, crop consultants, and farmers. Each year, samples were collected from mid-August to October, using two sampling methods, predictive and diagnostic. Predictive sampling was used to determine the general distribution and species presence. In contrast, diagnostic sampling was used to confirm the presence of nematodes in fields with soybeans displaying symptoms of reduced vigor, stunting, or chlorosis. For predictive samples, fields were sampled from randomly following a zig-zag pattern to obtain 20-30 soil cores from the base of the plants at a depth of 10-15 cm. For diagnostic samples, 20-30 soil cores were collected from the base of plants in a problematic area, and a second sample of 20-30 soil cores was collected from a nearby area with no visible plant symptoms. Soil cores from each sample were mixed into a single plastic bag and stored at 18°C until they were shipped for processing. Samples were mailed to the North Carolina Department of Agriculture and Consumer Services' Nematode Assay Section. Five hundred cm^3 of soil from each sample was extracted using a combination of elutriation (Byrd et al. 1975) and sugar centrifugal flotation (Jenkins 1964). Each sample was screened for the presence of over 25 nematode genera. Counts from each sample were reviewed and compared to established economic damage thresholds (EDT) for soybean production in the Mid-Atlantic region (Mehl 2018; Langston and Koehler 2021). Historically, these thresholds were developed using a mathematical equation that factored the cost of materials for population reduction to a manageable level as well as regional population densities at that time (Ferris 1978). However, as cost of materials and population densities have changed, these thresholds have remained. Due to this, EDT values should be considered as more of a damage threshold or guideline; as a damage threshold or guideline, these guidelines serve to indicate levels at which visible or noticeable damage is likely in a field, but economic decisions will vary by field and situation.

Detection of Nematodes

During the 2019-21 survey, 311 samples were collected across 12 counties in DE and MD (Figure 1). Nematode genera identified included *Heterodera* (SCN), *Meloidogyne* (root-knot, RKN), *Pratylenchus* (lesion), *Hoplolaimus* (lance), *Xiphinema* (dagger), *Helicotylenchus* (spiral), *Trichodorus* (stubby root), *Tylenchorhynchus* (stunt), and *Criconemella* (ring). Soybean cyst nematode was recovered from 53% of samples, RKN from 18%, and lesion from 57%. Lance and spiral nematodes appeared in 57% and 82% of samples, respectively. The percentage of samples with populations above the economic threshold were highest for SCN and RKN (Table 3.1). Of samples positive for SCN, 66% had populations above the high EDT (EDT for severe damage), while 39% of RKN positive samples were above the high EDT. Of samples with lesion nematode, 14% had populations above the high EDT.

Predictive samples accounted for 222 out of the total 311 samples. Soybean cyst nematode was recovered in samples from nine counties, including all three counties in Delaware (Table 3.3). Recovery of SCN was highest in the two southern counties of DE: Kent and Sussex. Wicomico County and Talbot County had the highest recovery of SCN for Maryland. Caroline County was the only county without SCN recovery but had the smallest sample size. Root-knot nematode was recovered from seven of twelve counties, including three Delaware counties and four Maryland counties on both the Eastern Shore and in Western Maryland. Root-knot nematode populations were above the high EDT in four counties. Wicomico, MD had the lowest percentage of RKN samples with populations above the high EDT, and Queen Anne, MD had the largest percentage. Lesion nematode was recovered from 11 of 12 counties, including all three DE counties and eight MD counties. Five of the 11 counties had lesion nematode populations above the high EDT.

Eighty-nine diagnostic samples were collected with 13 sets in 2020 and 24 sets in 2021. Site comparisons of visually different parts of the same field were performed, to evaluate areas likely impacted by nematodes. Sets consisted of one-two symptomatic samples and one sample from a nearby more visually healthy area. In both 2020 and 2021, a higher percent of symptomatic samples had nematode levels above high EDT than samples from healthier looking field areas (Figure 3.2).

In 2021, seven additional diagnostic sets were collected at fields with patches of soybean sudden death syndrome (SDS), caused by the fungus *Fusarium virguliforme*. Infection by *F. virguliforme* typically occurs early in the season and major symptoms become visible at reproductive growth stages including interveinal chlorosis, necrosis, wilting, whole plant death, brown colored cortical tissue with a white pith, and necrotic roots. SDS is typically more severe when SCN is present (Xing and Westphal 2013). In these sample sites, no differences in percent of samples above high EDT were observed.

Discussion and Conclusions

Over the three-year survey, nine nematode genera were recovered (Table 3.2). While other surveys have been conducted in the region over the past decades, they primarily examined SCN (Sindermann et al. 1993; Mulrooney et al. 1997; Mulrooney and Gregory 2010). Soybean cyst nematode continues to be the most problematic nematode in the region. SCN tends to thrive in sandy loam soil textures consistent with the soils found in the Mid-Atlantic (Avendaño et al. 2004).

This survey also identified other nematode genera may be of economic concern, particularly RKN (present in 18% of samples) and lesion nematode (present in 57% of samples). A subset of seven samples with RKN were submitted for molecular confirmation of species, and all were identified as *M. incognita,* southern root-knot nematode. This is consistent with observations in vegetable systems in the region over the past decades (Everts et al. 2007; Jones et al. 2017). Lesion nematode populations were identified to genera, molecular identification to species could be further explored in future work. Based on this study, RKN and lesion populations should continue to be monitored in the region. Lance and spiral nematode populations were recovered from roughly 50% of all samples. However, lance and spiral populations rarely appeared above the recommended EDT and are likely not yield impacting.

Soybean cyst and RKN were recovered together or in conjunction with other potentially yield-impacting nematodes. There is little information of the interactions or effect on yield when multiple nematode taxa are present. Of 311 total soil samples, 41 (13%) had both SCN and RKN present. Further research is needed to understand the impact coinfection might have on soybean performance and yield. Similar questions regarding coinfection have been posed in corn systems. A survey of plant parasitic nematodes in Illinois corn fields during 2018 and 2020 found that over 90% of samples tested positive for having 2-4 nematode taxa in one sample (Han et al. 2021). As in soybeans, little is known about the impact of coinfection on corn growth or yield. In two studies, infection with sting nematode (*Belonolaimus longicaudatus*) and lesion nematode correlated with effects on stand count, yield and stalk weight, but other species did not have significant effect (Dickson and McSorley 1990; McSorely and Dickson 1989; Han et al. 2021). Coinfection by multiple nematode genera likely alters economic threshold rates. Considering that SCN and RKN cause the greatest damage in sandy soil types (Windham and Barker 1986; Davis and Tylka 2021), such as the soil in the Mid-Atlantic, a review of economic damage thresholds for the area may be warranted. Economic thresholds pose challenges due to the variability of nematode populations within a field at planting, along with variation in soil type and environmental conditions that may impact the level of damage observed. Due to these challenges, using action thresholds has been proposed as a more useful concept. In the case of SCN, a single cyst is viewed as enough for an action threshold in the Mid-Western United States (Niblack 2005). Given how widespread SCN is through the Mid-Atlantic, it could be explored if a low level of SCN infection would alter the EDT values of other genera like root-knot or lesion nematodes.

Lesion nematode was abundant across DE and MD, appearing in 11 of 12 counties, with recovery rates ranging from 50% – 100%. Despite being widely distributed across the surveyed region, populations rarely existed in the high economic damage threshold range. Lesion nematodes were extracted from soil samples; no root samples were examined for their presence. Root samples may prove to be a more accurate measurement of lesion population, because the lesion nematode life cycle can occur entirely within the root (Acosta and Malek 1981; Davis and MacGuidwin 2005). It is possible the percent of lesion samples above the high EDT may not reflect the actual population densities or the impact on yield in the fields where lesion nematode was recovered.

While SCN, RKN, and lesion nematode are all present within the region and impacting yield, nematode infection is likely also impacting soybean yield by coinfection with fungal pathogens like *F. virguliforme*. Appearance of SDS was limited throughout the region during 2019 and 2020, but widespread in 2021. Many fields were planted early in 2021 and cool spring conditions were conducive for development of SDS. SCN enhances the severity of SDS infections when *F. virguliforme* is present. In the 2021 SDS diagnostic sample sites, no differences in percent of samples above high EDT were observed. However, the areas with visual SDS symptoms had higher numerical populations than the nearby areas with no symptomatic plants. For example, in one site there were 1000 SCN per 500 cc soil in the SDS sample compared to 100 per 500 cc in the healthier sample. This suggests that total population may play a role in development of SDS symptoms rather than only considering the population above high EDT. Molecular screening of soybean tissue may also be useful to identify latent *F. virguliforme* infections that may not result in prominent foliar symptoms (Wang et al. 2015).

Over three years of surveys, nine nematode genera were identified from soybean fields in the Mid-Atlantic region, with SCN remaining a significant pest of concern. RKN and lesion nematodes were also frequently observed and should be monitored, particularly since corn (*Zea* mays) is a host to both taxa and the most common rotation partner with soybeans. Assessment of economic damage thresholds accounting for coinfection of multiple nematode genera may improve understanding of nematode impact in soybean. In this study 54 % of samples had SCN, 18% had RKN, and 13% of samples had both SCN and RKN. Management strategies targeting multiple nematode genera are recommended.

REFERENCES

Acosta, N., Malek, R.B. 1981. Symptomology and Histopathology of Soybean Roots Infected by *Pratylenchus scribneri* and *P. alleni*. *Journal of Nematology, 13*(1).

Allen, T.W., Bissonette, K., Bradley, C.A., Damicone, J.P., Dufault, N.S., Faske, T.R., Isakeit, T., Kemerait, R.C., Koehler, A., Langston, D., Mueller, J.D., Padgett, G.B., Price, P.P., Sikora, E.J., Small, I.M., Thiessen, L., Young, H., 2021. Soybean Disease Loss Estimates for 2020. *Southern Soybean Disease Workers*.

Avendaño, F., Ierce, F.J., Melakeberhan, H. 2004. The relationship between soybean cyst nematode seasonal population dynamics and soil texture. *Nematology, 6*(4): 511-525.

Bandera, A.Y., Weerasooriya, D.K., Bradley, C.A., Allen, T.W., Esker, P.D. 2020. Dissecting the economic impact of soybean diseases in the United States over two decades. *PLoS ONE, 14*(4): e0231141.

Bradley, C.A., Allen, T.W., Sisson, A.J., Bergstrom, G.C., Bissonnette, K.M., Bond, J., Byamukama, E., Chilvers, M.I., Collins, A.A., Damicone, J.P. and Dorrance, A.E., Default N.S., Esker P.D., Faske T.R., Fiorellino N.M., Giesler L.J., Hartman G.L., Hollier C.A., Isakeit T.I., Jackson-Ziems T.A., Jardine D.J., Kelly H.M., Kemerait R.C., Kleczaeski N.M., Koehler A.M., Kratochvil R.J., Kurle J.E., Malvic D.K., Markell S.G., Mathew F.M., Mehl H.L., Mehl K.M., Mueller D.S., Mueller J.D., Nelson B.D., Overstreet C., Padgett G.B., Price P.P., Sikora E.J., Smith D.L., Spurlock T.N., Tande C.A., Telenko D.E.P., Tenuta A.U., Thiessen L.D., Warner F., Wiebold W.J., Wise K.A. 2021. Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2015 to 2019. *Plant Health Progress*, *22*(4).

Byrd, D.W., Jr., Barker, K.R., Ferris, H., Nusbaum, C.J., Griffin, W.E., Small, R.H., Stone, C.A. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. *Journal of Nematology* 8: 206-212.

Davis, E.L., MacGuidwin, E. 2005. Lesion Nematode Disease. *Plant Health Instructor*.

Davis, E.L., Tylka, G.L. 2021. Soybean cyst nematode disease. *The Plant Health Instructor.*

Dickson, D.W., McSorely, R. 1990. Interaction of three plant parasitic nematodes on corn and soybean. *Journal of Nematology, 22*(4S): 783-791.

Everts, K.L., Sardanelli, S., Kratochvil, R.J., Armentrout, D.K. and Gallagher, L.E., 2006. Root-knot and lesion nematode suppression by cover crops, poultry litter, and poultry litter compost. *Plant disease*, *90*(4): 487-492.

Ferris, H. 1978. Nematode Economic Thresholds: Derivation, Requirements, and Theorhetical Considerations. *Journal of Nematology, 10*(4).

Han, J., Colgrove, A.L., Bowman, N.D., Schroeder, N.E., Kleczewski, N.M. 2021. A Sruvey of Plant-Parasitic Nematodes in Illinois Corn Fields, 2018 and 2020. *Plant Health Progress, 22*(4): 560-564

Jenkins, W. R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.

Jones, J.G., Kleczewski, N.M., Desaeger, J., Meyer, S.L., Johnson, G.C., 2017. Evaluation of nematicides for southern root-knot nematode management in lima bean. *Crop Protection*, *96*: 151-157.

Langston, D., Koehler, A. 2021. Disease and Nematode Management in Field Crops. *Virginia Cooperative Extension – Virginia Tech.*

McCarville, M.T., Marrett, C.C., Mullaney, M.P., Gebhart, G.D., Tylka, G.L. 2017. Increase in Soybean Cyst Nematode Virulence and Reproduction on Resistant Soybean Varieties in Iowa from 2001 to 2015 and the Effects on Soybean Yields. *Plant Health Progress, 18*(3): 146-155.

McSorley, R., Dickson, D.W. 1989. Effects and dynamics of nematode community on maise. *Journal of Nematology, 21*: 462-471

Mehl, H.L. 2018. Nematode Management in Field Crops. *Virginia Cooperative Extension – Virginia Tech.*

Mulrooney R., Gregory, N. 2010. 2009 Soybean Cyst Nematode Survey Results. UD Extension Bulletin #144: 15-17.

Mulrooney, R. Gregory, N.F., Caroll, R.B. 1997. Distribution and diversity of *Heterodera glycines* in Delaware Proceedings of the Southern Soybean Disease Workers. 24th Annual Meeting. March 15-16, 1997, Fort Walton Beach, FL.

Niblack, T.L., 2005. Soybean cyst nematode management reconsidered. *Plant disease*, *89*(10): 1020-1026.

Posch, J.P. 2017. *Diaporthe,* Soybean Cyst Nematode, and Soybean Aphid: An Evaluation of Potential Interactions Occurring among Pests on Soybean in South Dakota (Accesion No. 1190) [Master's Thesis, South Dakota State University]. *Electronic Theses and Dissertations.*

Riggs, R.D. 2004. *Biology and Management of the Soybean Cyst Nematode 2nd edition.*

Sindermann, A., Williams, G., Saranelli, S., Krusberg, L.R. 1993. Survey for *Heterodera glycines* in Maryland. *Journal of Nematology, 25*(4S): 887-889.

Wang, J., Jacobs, J.L., Byrne, J.M. and Chilvers, M.I., 2015. Improved diagnoses and quantification of Fusarium virguliforme, causal agent of soybean sudden death syndrome. *Phytopathology*, *105*(3), pp.378-387.

Windham, G.L., Barker, K.R. 1986. Effects of Soil Type on the Damage Potential of *Meloidogyne incognita* on Soybean. *The Journal of Nematology, 18*(3): 331-337.

Xing, L., Westphal, A. 2013. Synergism in the interaction of *Fusarium virguliforme* with *Heterodera glycines* in sudden death syndrome of soybean. *Journal of Plant Diseases and Protection, 120*(5): 209-217.

Table 3.1: Percentage of samples with nematode recovery and corresponding percent of recovered samples with populations above economic damage threshold from 2019 – 2021

Nematode	Total Samples	Percent of Samples with Recovery[z]	Soybean Economic Damage Threshold of High[y]	Number of Samples above the EDT	Percent of Samples with Populations above High EDT[x]
Soybean Cyst[w] (*Heterodera*)	166	53	> 60 per 500 cc soil	109	66
Root Knot (*Meloidogyne*)	57	18	>170 per 500 cc soil	22	39
Lesion (*Pratylenchus*)	178	57	>300 per 500 cc soil	25	14
Lance (*Hoplolaimus*)	174	56	>500 per 500 cc soil	4	2
Dagger (*Xiphinema*)	19	6	>300 per 500 cc soil	0	0
Spiral (*Heliocotylenchus*)	169	54	>3000 per 500 cc soil	5	3
Stubby Root (*Paratrichodorus*)	99	32	>250 per 500 cc soil	3	3
Stunt (*Tylenchorhynchus*)	139	45	>1000 per 500 cc soil	15	11
Ring (*Criconemella*)	3	1	>700 per 500 cc soil	0	0

[z] Percentage out of 311 soil samples
[y] Soybean economic damage thresholds as established by Virginia Tech Cooperative Extension https://hortintl.cals.ncsu.edu/sites/default/files/documents/spes-15.pdf
[x] Percentage out of samples testing positive for indicated genera
[w] Soybean cyst nematode counts represent cysts and second-stage juveniles

Table 3.2: Percent of soil samples testing positive for nematodes

Number of Nematode Taxa	Number of samples (% of total samples)
0	25 (8.0)
1	6 (1.9)
2	82 (26.4)
3	66 (21.2)
4	73 (23.5)
5	17 (5.5)
6	36 (11.6)
7	6 (1.9)
8	0 (0)
9	0 (0)
Total	**311 (100)**

Table 3.3: Percentage of predictive samples with nematode recovery and populations above economic damage threshold in each county sampled from 2019 – 2021

County	State	% of Samples with SCN recovery[y]	% of Samples above EDT for SCN[z]	% of Samples with RKN recovery[y]	% of Samples above EDT for RKN[z]	% of Samples with Lesion recovery[y]	% of Samples above EDT for Lesion[z]
New Castle n = 60	**DE**	23	43	2	0	92	7
Kent n = 36	**DE**	69	19	17	33	81	43
Sussex n = 52	**DE**	71	68	23	33	58	10
Cecil n = 2	**MD**	0	0	0	0	100	0
Kent n = 10	**MD**	10	0	0	0	0	0
Queen Anne n = 15	**MD**	20	67	13	50	67	10
Caroline n = 1	**MD**	0.	0.00	0	0	100	0
Talbot n = 11	**MD**	73	75	0	0	73	0
Dorchester n = 12	**MD**	58	100	0	0	58	43
Somerset n = 3	**MD**	33	100	33	0	67	0
Wicomico n = 8	**MD**	100	100	62.5	20	100	0
Garrett n = 6	**MD**	0	0	17	0	50	0

[z] Percentage out of positive samples with populations above the high economic damage threshold established by Virginia Tech Cooperative Extension https://hortintl.cals.ncsu.edu/sites/default/files/documents/spes-15.pdf
[y] Percent recovery from 222 predictive samples

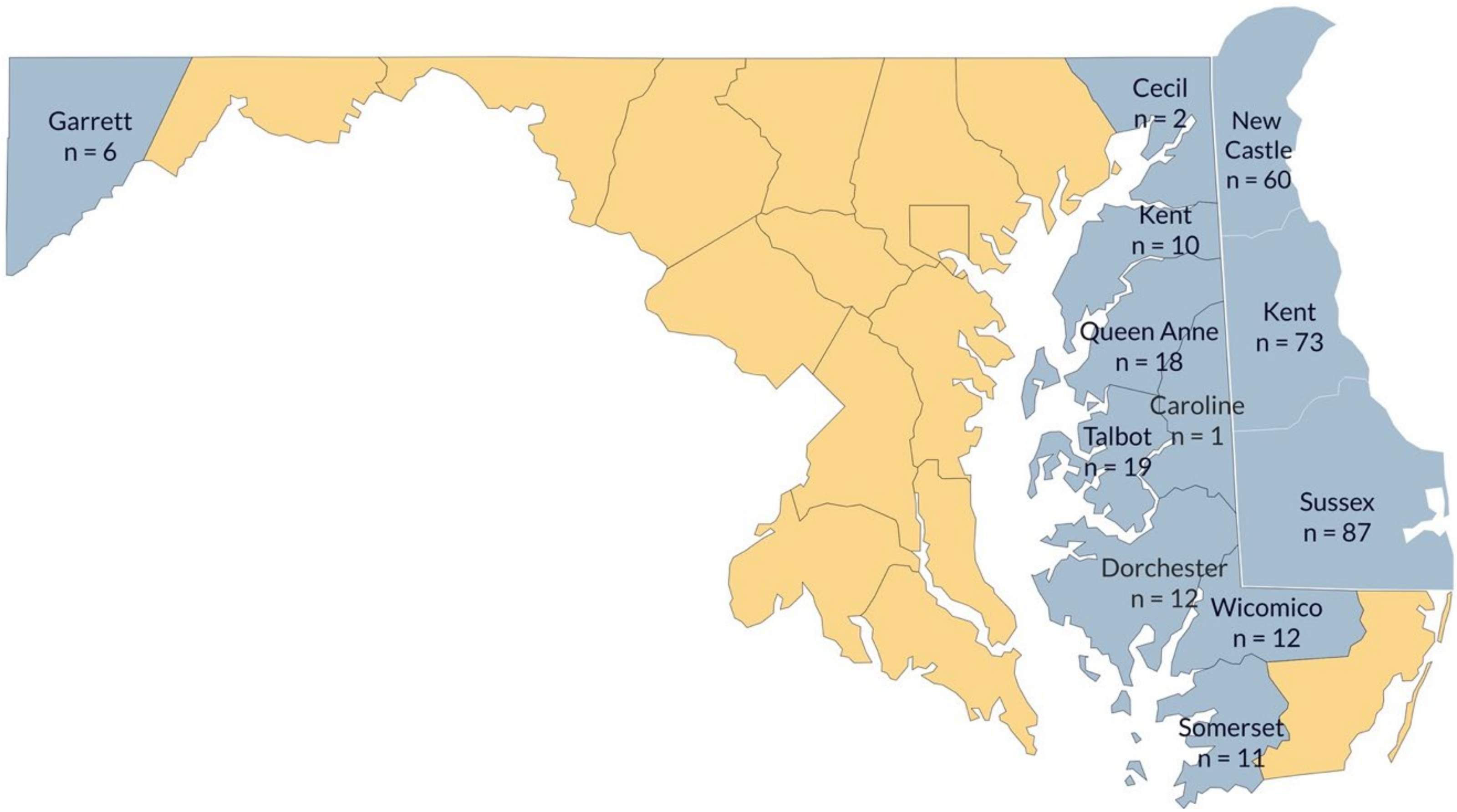

Figure 3.1: Map of Delaware and Maryland counties surveyed. Surveyed counties shaded blue.

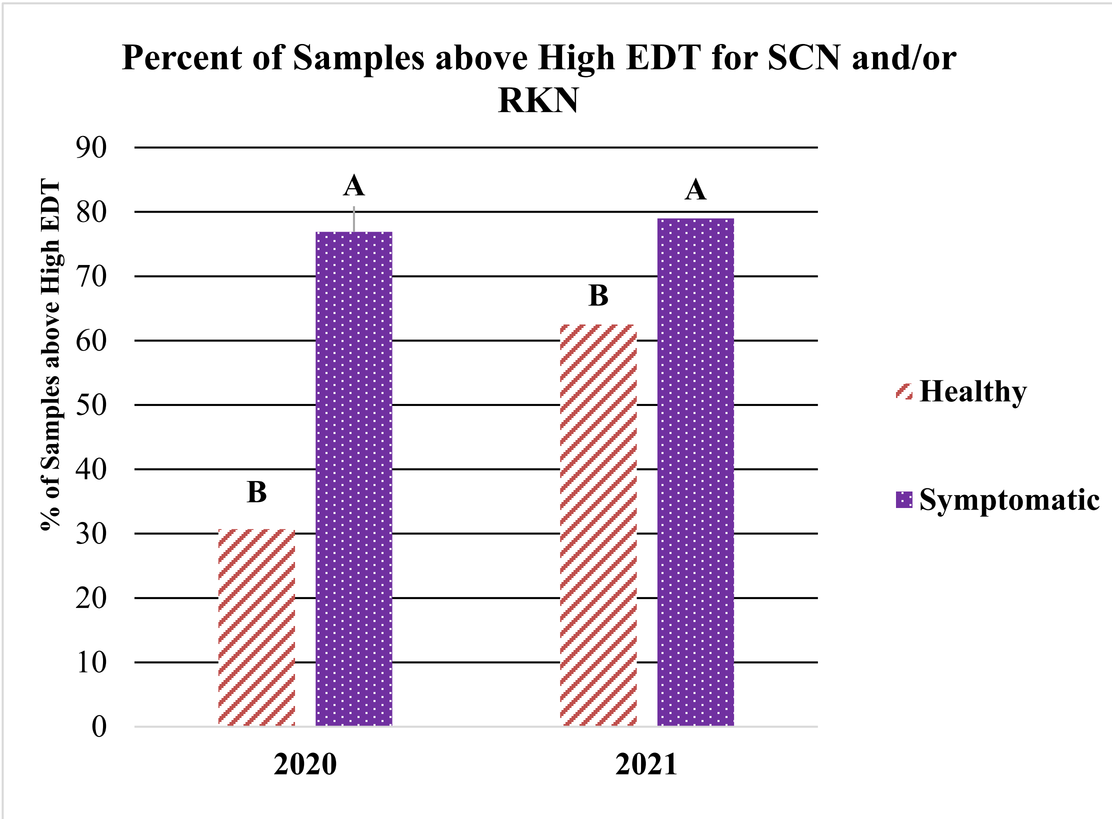

Figure 3.2: Percent of samples above high EDT for soybean cyst nematode (SCN) and/or root-knot nematode (RKN) in symptomatic and nearby healthier samples

Chapter 4

SEED TREATMENTS FOR MANAGEMENT OF SOYBEAN CYSTS NEMATODE, *HETERODERA GLYCINES* IN SOYBEAN PRODUCTION

Abstract

Soybean Cyst Nematode (SCN) is the most important pathogen of soybean in the Mid-Atlantic region. In recent decades, a decline in the effectiveness of genetic resistance has been observed and additional management approaches are needed. Seed treatments are of rising interest, but no local data on product response exists for the region. In 2020-2021, two experiments were conducted to observe the effects of chemical and biological seed treatment options. One experiment tested two fungicide seed treatments against non-treated for SCN suppression in three runs across two locations. The second experiment compared a variety of treatments: seed with a base treatment, chemical treatments, and biological treatments, to test the efficacy of different products against SCN. Seed treatments increased the percent emergence over control seed. Nematode reproductive factors and female counts from roots were collected but did not statistically differ between seed treatments. Yield differences were observed in Experiment 2 in 2021, but not in 2020 or in Experiment 1. Seed treatments may be beneficial as an element of integrated approaches to managing SCN.

Introduction

In 2019, United States (U.S.) soybean (*Glycine max* [L.] Merrill) production exceeded 9,664,239,000 MT covering 76.1 million acres (USDA ERS 2022). Soybeans are also an important crop within the Mid-Atlantic region and were the second crop grown by acre in Delaware (DE) and Maryland (MD) in 2019, accounting for over 60 million dollars of revenue (USDA ERS 2022). Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is the most yield-limiting pest of soybean in the U.S. and within the Mid-Atlantic (Allen et al. 2017; Bandara et al. 2020). Sandy soils in DE and Eastern Shore MD favor the proliferation of SCN, with yield loss estimates exceeding 7,076 MT (260,000 bu) across both states annually (Allen et al. 2021).

Hatched SCN juveniles (J2s) penetrate and embed themselves within the plant roots, where they establish a feeding site and consume cell contents to survive (Davis and Tylka 2021). Females break through the root surface, remaining attached, and swell full of eggs. A single female can fill with over 250 eggs (Lauritis et al. 1983). Symptoms of SCN infection includes stunting, yellowing of the plant, and plant death. Even in the absence of aboveground symptoms, SCN can reduce yields up to 30% (Noel 1992; Niblack 2005). As females die, their body walls harden, leaving a cyst around the eggs. This cyst provides a survival structure for the eggs after it is dislodged from the roots into the soil (Duan et al. 2009). Eventually, the cyst will burst, and the eggs will be released into the soil, continuing the cycle (Davis and Tylka 2021). Soybean cyst nematodes are often found in "hotspots" throughout a field and can be spread by mechanical equipment or rainwater runoff (Davis and Tylka 2021).

The ability of cysts to persist for long periods limits the effectiveness of short crop rotations with nonhost crops such as maize (*Zeae mays* L.) as a management method (Riggs 2004).

SCN management includes chemical, biological, and cultural methods. The first step in SCN management is to conduct soil samples or root observations to confirm the presence of SCN. Cultural methods include working in non-infested fields first and sanitizing equipment after entering SCN infested fields.

For many decades, host resistance was the primary tool for SCN management. Plant Introduction 88788 (PI 88788) is the most common source of genetic resistance (Niblack et al. 2008). Nationally, there are seven main breeding lines, but since 1990, most resistant cultivars have been developed using PI88788 (Davis and Tylka 2021). When first released, PI88788 kept SCN reproduction rates below 10%. Increased reproduction of SCN began to be observed in the Mid-West in the early 2000s, and nearly all surveys in that region over the past two decades have indicated increasing virulence of SCN on PI 88788 and decreasing yield in soybean varieties relying on PI88788 (McCarville et al. 2017). This same observation was made during a 2009-10 survey in DE where a shift in population was observed and reproduction levels on resistance gene PI88788 averaged 67% (Mulrooney and Gregory 2010).

Fumigants, such as Telone C-35 and chloropicrin, when used in combination with other controls, have been shown to increase yield in SCN infested fields (Bruin and Pedersen 2008). Not all soil-applied nematicides will kill SCN populations in fields, sometimes resulting in larger populations at the end of the season than at application due to the short window of efficacy and resurgence of population once root protection declines (Davis and Tylka 2021; Wrather et al. 1984). Fumigants are toxic to both humans and soil microfauna, due to a lack of specificity in the mode of action. Overuse can result in declining soil health and destruction of beneficial organism communities.

Seed treatments may include chemicals or biological control agents with varying modes of action. The most recently launched chemical seed treatments Saltro (pydiflumetofen, succinate dehydrogenase inhibitor (SDHI), Syngenta, Basel, Switzerland) and ILEVO (fluopyram, SDHI, developed by BASF, Ludwigshafen, Germany) are fungicides with nematstatic activity. Fluopyram was first registered for soybean seed treatment use in 2014 and pydiflumetofen in 2020. The SDHI class of fungicides inhibit mitochondrial respiration in fungi and both products are also labeled for control of *Fusarium virguliforme*, the causal agent of soybean sudden death syndrome (SDS) and *Septoria glycines,* causal agent of Septoria brown spot. Symptoms of SDS often appear earlier in the season and at greater severity in fields with SCN (McLean et al. 1993). In controlled environments, ILEVO has been shown to reduce SCN hatching, juvenile movement, and ability of juvenile SCN to penetrate soybean roots (Beeman and Tylka 2018; Xing and Westphal 2006). In greenhouse conditions, Saltro has also been shown to reduce egg hatching and number of SCN cysts per plant (Dhital 2020). To separate SDS and nematicide activity in the field, Kandel et al. 2017, recommend SDS disease index levels to be below 10 to determine if yield treatment effects are related to SCN management. Bissonette et al. 2020, assessed ILEVO seed treatment for impact on SCN population density in fields with low SDS index levels throughout Iowa. No seed treatment trials have been conducted for ILEVO or Saltro in DE. In recent years, several biological agents have also come on the market for application as soybean seed treatments, with no local data for the Mid-Atlantic. The objectives of this study were to i) compare performance of two chemical seed treatments recently labeled for management of SCN in soybean and ii) assess performance of biological seed treatments compared to chemical standards for management of SCN in Mid-Atlantic soybean production.

Materials and Methods

Experimental Design

From 2020-2021, two small-plot experiments were conducted to assess the impact of nematicide seed treatments on SCN population density and end of season yield. The first experiment was established in a field with pepperbox loamy sand and known SCN pressure in Georgetown, DE. Soybeans were planted in the two seasons prior (2018-19), and glyphosate herbicide was used for weed control. In 2021, an additional site with low SCN pressure was added in Wye Mills, MD at a field with Nassawango silt loam soil type that had been previously planted in corn. The soybean cultivar GH3934X (Syngenta) was planted at 272,000 sd/ha (110,000 sd/A) on April 19, 2020, and April 27, 2021, in Georgetown and June 4, 2021, in Wye Mills. The Georgetown trials were planted using a Kinze (Kinze, Williamsburg, IA) plot planter equipped with an Almaco cone-type seeder (Almaco, Nevada, Iowa) with four row plots 8.8 m in length on a 0.76 m spacing in a randomized complete block design with five replications. The Wye Mills trial was planted using a Great Plains EWNT-10 with six row plots 8.8 m in length on a 76.2 cm spacing in a randomized complete block design with five replications. Treatments included ILEVO (fluopyram at 0.15 mg/seed), Saltro (pydiflumetofen at 0.075 mg/seed), and a non-treated control. The second experiment was conducted from 2020-2021 in the same field in Georgetown, DE. The soybean cultivar 4444RXS (Progeny) was planted at 346,000 sd/ha (110,000 sd/A) on April 19, 2020, and April 27, 2021, in four row plots 8.8 m in length on a 0.76 m spacing with six replications. Treatments included completely non-treated control, a base treatment seed [Allegiance (Metalaxyl, 4 g/100kg, Bayer Crop Science), Stamina (pyraclostrobin, 7.5 g/100 kg, BASF), Systiva XS Xemium Brand (fluxapyroxad, 5 g/100 kg, BASF), Poncho 600 (clothianidin, 0.11 mg/seed, BASF), Flo Rite (66 ml/100 kg, BASF), and Color Coat Red (33 ml/100 kg, BASF)], and six nematicides that also received the base treatment. Nematicide treatments included ILEVO (fluopyram, 0.15 mg/seed, BASF), Saltro (pydiflumetofen, 0.075 mg/seed, Syngenta), BioST (heat-killed *Burkholderia rinojenses* and its spent fermentation media, 195 ml/100 kg, Albaugh), Aveo EZ (*Bacillus amyloliquefaciens* strain PTA-4838, 2ml/100,000 seed, Valent), Clariva (*Psateuria nishizawae,* 130 ml/100 kg seed, Syngenta), and Trunemco (*Bacillus amyloliquefaciens* strain MBI 600 and cis-Jasmone, 20.2 ml/100 kg seed, Nufarm). Both soybean cultivars contained the PI88788 source of resistance, and seed treatments were applied by the manufacturer.

In all experiments, the center two rows of each plot were harvested mechanically with a plot combine (Table 4.1). A Massey Ferguson 8XP plot combine (AGCO, Duluth, GA) was used at the Georgetown fields and an Almaco R1 was used at Wye Mills. Grain yield and moisture were collected, and grain yield was adjusted to 13% moisture. Weather data for these experiments was collected using the Delaware Environmental Observing System (DEOS), Georgetown-REC station in Georgetown, DE.

Stand Counts

Stand counts were recorded at 14 and 28 days after planting (DAP) or emergence (DAE) in all trials. All emerged seedlings were counted in rows two and three of each plot. Average seedlings from the middle two rows were divided by the initial seeding rate to determine a percent emergence value.

Quantifying SCN populations

Soil sampling was used to determine population levels in each treatment. Soil samples were collected immediately after planting to determine the initial population density [P_i] and at the end of the season prior to harvest for a final population density [P_f]. Soil cores were collected from the middle two rows of each plot by moving in a zig-zag pattern to obtain 20-30 soil cores from the base of soybean plants at a depth of 10-15 cm. Soil cores from each plot were mixed into a single plastic bag and stored at 18°C until they were shipped for processing. Samples from Experiment 1 were submitted to the North Carolina Department of Agriculture and Consumer Services' Nematode Assay Section, where 500 cm^3 of soil from each plot was extracted using a combination of elutriation (Byrd et al. 1975) and sugar centrifugal flotation (Jenkins 1964). Counts of cysts and second-stage juveniles were determined. Samples from Experiment 2 were submitted to SCN Diagnostics at the University of Missouri. From each soil sample, SCN cysts were extracted from 250 cm^3 of soil and collected on a 250 µm-pore sieve using modified wet-sieving and decanting methods (Gerdemann 1955). Eggs were extracted from cysts with a motorized rubber stopper (Faghihi and Ferris 2000), collected on a 25 µm-pore sieve, stained, and counted. The reproductive factor (RF) was calculated by dividing the P_f by P_i to generate a ratio to measure the change in number of SCN per volume of soil over the entire growing season for each plot. When RF=1, no change in SCN population density occurred in the season; if RF <1, there was a decrease in SCN population density over the season; if RF >1, there was an increase in SCN population density. An HG type test was conducted by SCN Diagnostics at the University of Missouri for the Georgetown field each season. HG type testing tests SCN reproduction growth on 7 indicator lines by using a calculated female index, which is determined by comparing the average number of females produced on the standard susceptible line to the number produced on the HG indicator type over a 30-day period (Niblack et al. 2002; Beeman et al. 2016). In addition to soil sampling, mid-season on-root female counts were quantified by destructively sampling five plants from the first row of each treatment at the Georgetown site. Plants were dug and roots were gently rinsed in field and brought back to the lab.

Stems were clipped to separate root and above ground plant parts. Roots were placed on an 850 µm mesh sieve over a 250 µm sieve and blasted with high pressure water to release females. The contents of the bottom sieve were transferred to a counting dish and SCN females were enumerated under a dissecting microscope (Stemi 508, Carl Zeiss Microscopy LLC, Thornwood, NY). Female counts and fresh root weights were recorded and used to calculate the number of females per gram of root weight.

Data Analyses

Within both experiments, there were significant trial by run interaction, so runs within each trial were analyzed individually for each year. Mixed-model analysis of variance (ANOVA) was conducted using PROC GLIMMIX in SAS statistical software (SAS version 9.4 (SAS Institute Inc. Cary, NC)). Data were analyzed for percent emergence, SCN RF, SCN female counts, females per gram of fresh root weight, and yield with seed treatment as a fixed effect, while replication and the overall error term were considered random effects. The RF data were transformed using the equation ($\log_{10}$ ('RF'+1)) prior to analysis to generate normal distribution values, but transformed and raw RF values were used for presentation purposes. Fixed effects were tested for significance at $\alpha = 0.05$ and LSmeans were separated using paired *t* tests.

Results

Environmental Conditions

Experiment 1 and 2 of 2020 were planted on April 19, which ended up being twenty-one days before the final frost date, May 10, 2020. The average temperature from April 19 – April 31 ranged from 9.1ºC – 17.8ºC, with 9 accumulating growing degree days (GDD; days above 10ºC). The GDD for these 9 days ranged from 0.7 – 14.8. Average air temperature in the first 11 days ranged from 10.8ºC – 15.7ºC. Rainfall in April ranged from 0 – 2.4 cm (0 to 0.95 in) in a day, with the most occurring on April 24, 2020 (2.4 cm). Total rainfall amounted to 10.7 cm (4.2 in) in April. May 4, 2020, saw a total of 3.5 cm (1.4 in) of rainfall in one day. Total rainfall amounted to 9.7 cm (3.8 in) for May. Rainfall in late April and May contributed to soil crusting on the field pre-emergence. The average air temperature for May was 15.8ºC, a high of 30.2ºC and a low of 0.5ºC. The average soil temperature was 16.8ºC. June had an average air temperature of 73.8ºC, an average soil temperature of 75ºC, a monthly GDD of 715.7, and total rainfall of 6.4 cm (2.5 in). The average air temperature for the remainder of the season (July – September) ranged from 20.1ºC to 26.4ºC. The maximum temperature recorded was 35.22ºC. The total rainfall in those months was 40.9 cm (16.1 in). The most rainfall in one day occurred on July 10, with 7.8 cm (3.08 in). The average temperature of October was 15.5ºC and total rainfall for the month was 17.2 cm (6.76 in), contributing to the late harvest of soybeans across the region (DEOS April – October 2020).

Both Experiments 1 (Georgetown) and 2 were planted on April 27th, 2021, four days after the final frost date. The average air temperature on the day of planting was 17.6ºC, the average soil temperature 13.2ºC with a GDD of 11.6. The daily GDD for the remainder of April ranged from 11.6 to 26.1. During May, the average air temperature was 17.1ºC with a high of 32.5ºC. The average soil temperature was 17.4ºC, the total rainfall was 6.5 cm (2.57 in) and the month GDD was 400.1. The average air temperature for June was 22.9ºC with a high of 35.6ºC. The average soil temperature was 23.4ºC, the monthly GDD was 707.4 and rainfall accumulated to 12.3 cm (4.8 in) The most rainfall occurred on June 9th, when 6.1 cm (2.4 in) fell. For the remainder of the season, from July through September, the average air temperature ranged from 21.3ºC to 25.1ºC with the maximum temperature recorded 33.4ºC. The total rainfall during those months was 38.9 cm (15.3 in). The most rainfall in one day occurred on September 9th, when 4.5 cm (1.8 in) in fell. October had an average air temperature of 17.5ºC and total rainfall of 11.5 cm (4.5 in) (DEOS April – October 2021).

Experiment 1

In 2020, rainfall totaling 4.0 cm (1.6 in) in the week after planting led to crusting of the soil, making emergence for plants difficult (DEOS). Due to cold weather, no plants had emerged at 14 days after planting (DAP), so first counts were taken at 14 days after emergence (DAE) and again at 28 DAE. Significant treatment differences were observed among all three treatments at 14 DAE. ILEVO had the highest percentage of plants emerged (70%), Saltro had the second highest (54%), and the non-treated seed had lowest percent emergence (26%) (Table 4.3). At 28 days after emergence, treated seed emergence was higher than the non-treated seed, but not from one another. At planting, ILEVO plots had the highest SCN soil counts, averaging 1000 SCN per 500 cc of soil, Saltro plots had the second highest with 988 per 500 cc of soil, and plots of the non-treated seed had the lowest populations, averaging 820 per 500 cc of soil. At 30 days after emergence, ILEVO soil samples had larger nematode populations than soil samples from Saltro and non-treated seed plots ($p = 0.03$). ILEVO plots averaged 582 SCN per 500 cc of soil, Saltro plots averaged 106 per 500 cc of soil, and the non-treated plots at 102 per 500 cc of soil. Higher numbers of SCN females ($p = 0.03$) were present on roots from non-treated (189) and Saltro plots (179) compared to ILEVO plots (54). When converted to females per gram of root, Saltro plots (24) again grouped with the non-treated plots (19) having more SCN females than in ILEVO plots (5) ($p = 0.01$). RF factors were not significantly different from one another. In all treatments, a decline in nematode population density was observed from the initial counts. Differences in yield were not observed.

The trial was continued in 2021 in the same high-pressure SCN field in Georgetown, DE. Stand counts were taken at 14 and 28 days after planting. Treatment differences were observed in both 14 and 28 DAP emergence percentages. At 14 days after planting, Saltro plots had the highest average percent emergence, followed by plots with ILEVO, and non-treated seed. This emergence pattern was observed again at 28 days after planting. There were no differences in SCN soil or female counts throughout the trial. At planting, plots with ILEVO-treated plants had numerically higher SCN counts. At 30 days after planting, plots with ILEVO-treated plants still had the highest numerical counts. At harvest, ILEVO plants continued to have the highest numerical SCN counts, while the non-treated control had the lowest. RF values were not significant but indicated an increase in SCN population densities in all treatments over the season. No differences in female counts at 30 days after planting were observed and there were no differences in yield.

The trial was also conducted at a second location with low SCN pressure in Wye Mills, MD. Stand counts were taken at 14 and 28 days after planting. Treatment differences were observed in percent emergence. Saltro-treated seed had higher average percent emergence at 14 and 28 days after planting than both ILEVO-treated and non-treated seed. Plots with ILEVO-treated seed had numerically higher initial SCN soil count (54). At 30 days after planting, the plots with ILEVO-treated seed were numerically lower than the Saltro and non-treated plots. Saltro-treated seed had the highest SCN female root-blasting count at 30 days after replanting, while ILEVO seed the lowest. Since initial SCN populations were low with many Pi values of 0, the corresponding final RF values were large. Non-transformed RF values were 76 (non-treated seed), 46 (Saltro-treated seed), and 9 (ILEVO-treated seed). There was no significant difference in yield between treatments. With low SCN populations, the average number of females per gram of root was less than one SCN female for all treatments. Yields did not vary by treatment and ranged from 4815.2 kg/ha (71.6 bu/a) (Saltro) and 4909.3 kg/ha (73.0 bu/a) (ILEVO).

Experiment 2

Screening of HG types on SCN populations was conducted using a composite soil sample from Experiment 2 fields in 2020 and 2021. In 2020, the SCN population was designated as HG type 1.2, reproducing above 10% on indicator lines Peking (line 1; Female Index = 22%) and PI88788 (line 2; Female Index = 64.8%). The SCN populations for 2021 tested as HG type 2, capable of reproducing on PI88788 (line 2; 66.2%).

In 2020, there were differences in average emergence. Saltro had the highest average emergence (78%) and the BioST had the lowest (71%). No differences in egg counts were observed at any sample timing. Plots with Saltro-treated seed (41,500 eggs per 250 cm^3) and Clariva-treated seed (38,541.7 eggs per 250 cm^3) had numerically higher initial soil eggs counts while plots with base-treated seed (28,291.7 eggs per 250 cm^3) and Trunemco-treated seed (29,291.7 eggs per 250 cm^3) had lower initial egg counts. At 30 DAP, ILEVO and Saltro plot egg counts (25,958 and 20,042 eggs per 250 cm^3) were numerically higher than the BioST plots (12,750 eggs per 250 cm^3) and the non-treated control plots (13,416.7 eggs per 250 cm^3). SCN female counts on roots ranged from 380 (base treatment) to 201 (ILEVO-treated seed) with no differences among treatments (Table 4.4). No differences were observed in number of females per gram of roots. Initial nematode populations were high in 2020 and RF values indicated declining population densities across all treatments over the season. No differences in yield were observed. Yield ranged from 2750.6 kg/ha (40.9 bu/a) in Clariva plots to 2037.7 kg/ha (30.3 bu/a) in the base treatment.

In 2021, differences in percent emergence were observed at both 14 and 28 days after planting. Emergence was lowest in plots with non-treated seed at both evaluations. The addition of a base treatment improved emergence over the non-treated seed and emergence of base-treated seed did not differ from treatments with an additional nematicide component. There were no significant differences between treatments for initial soil egg counts, 30 days after planting soil egg counts, SCN female root counts or the number of females per gram of roots. Soil samples in 2021 began with low population densities and RF values indicated increasing SCN populations in the field over the season across all treatments. All RF values were greater than 1. Differences in yield were present ($p < 0.001$). Saltro-treated plots had the highest yield 3813.1 kg/ha (56.7 bu/a) and non-treated plots had the lowest 2885.1 kg/ha (42.9 bu/a).

Discussion

Over the five study sites, Experiment 1 in 2020 was the only trial with treatment differences for SCN soil egg counts, female root counts, and number of females per gram of roots. Yield differences were observed in Experiment 2 in 2021 where plots with Saltro-treated seed had the highest yield and plots with non-treated seed had the lowest yield. Numerical differences of yield in treatment plots compared to the non-treated plot of each trial ranged from 242.1 kg/ha (3.6 bu/a) reduction to a 712.9 kg/ha (10.6 bu/a) increase. This is consistent with previous work where ILEVO significantly reduced RF in a strip trial and small plots but yields only increased 52.2 kg/ha (0.8 bu/a) overall with use of seed treatment (Bissonnette et al. 2020).

In 2020, nematode populations at the Georgetown field were highest at planting and declined through the season. Initial populations at the Georgetown field in 2021 were lower than 2020 initial or final counts. This is likely due to the cold temperatures from December 2020 to February 2021. The average air temperatures ranged from 2.4ºC to 4.6ºC with the lowest temperature recorded at -7.9ºC. Soil temperatures ranged from 3.4ºC to 5.7ºC (DEOS). Colder winter temperatures can reduce SCN populations – the optimal temperature for SCN is 25ºC (Niblack et al. 2005). Differences in RF were not observed in either location or year. This is consistent with a field and greenhouse study in Michigan where fluopyram was used to suppress SCN population densities, though none of the recorded RF values were significant (Roth et al. 2020). This is also consistent with results from Bissonnette et al. 2020, in which ILEVO was able to increase yields but not significantly decrease SCN RF values across all 12 strip trials and 27 small plots. Bissonnette et al. 2020 contradicts previous results in which seed treatments, with the addition of a fungicide and insecticide base, were able to reduce some SCN reproduction, but not increase yield (Gaspar et al. 2014; Mourtzinis et al. 2017; Bissonnette et al. 2018). The RF values for the Wye Mills location were impacted by the lack of SCN pressure in that field, and as a result, RF values across all treatments showed drastic changes (from low initial counts) that were not reflective of the actual change. While there were no treatment differences regarding RF values in either Experiment 1 or 2, these values convey the degree of change observed over the season and could be used to further investigate the role of winter environmental conditions on initial populations.

Four of the treatments in Experiment 2 were biological materials including Clariva, Aveo EZ, BioSt, and Trunemco. These agents rely on biologic elements to suppress nematodes rather than chemical control to kill them. Constituents of these products include live or killed bacterium, spent media, metabolites, or plant volatiles (Table 4.2). Biological seed treatments have mixed efficacy for nematode management in field settings. There are several contributing factors, but one of the most important is the introduction of seed treatments into viable soil environments (Lamovšek et al. 2013). Soil environments that inhibit the establishment of seed treatment introductions reduce or nullify the effects of the seed treatment and provide little to no nematode control. Lund et al. 2018 found no consistent SCN reductions or yield benefits when using Clariva (*Pasteuria nishizawae*). This finding was consistent with other work showing the density of endospores may impact the efficacy of SCN parasitism (Bissonnette et al. 2018). In Iowa, Clariva has been shown to significantly decrease RF values of SCN in soybean and improve yield when compared CruisserMaxx Advanced plus Vibrance (Bissonnette et al. 2018). In Experiment 2, Clariva had the highest numerical yield during 2020. Aerial drone imagery identified that four replications of the plots with Clariva-treated seeds were randomly placed in a part of the field with visibly better stand growth, compared to other parts of the field. This likely contributed to the yield values for Clariva.

SCN population distribution tends to be variable in the soil and is strongly correlated with soil texture (Avendaño et al. 2004). Cyst populations tend to be higher and more consistent over time in loamy sand (Avendaño et al. 2004). A study from 1991 concluded that the number of SCN cysts increased in each experiment in infested wet silt loam (Heatherly and Young 1991). This is consistent with sandy loam and soils in the coastal Mid-Atlantic. It helps to explain why SCN pressure tends to be higher in certain areas of the region and is a consistent pest of concern to Mid-Atlantic soybean production. SCN resistant soybean varieties are the most efficient method of SCN management, however a decline in efficacy of varieties using resistance source PI88788 has been observed in recent decades. Reproduction levels on PI88788 lines in Hg tests from soil in these trials were 64.8% and 66.2% in 2020 and 2021. Elevated reproduction was also indicated on Peking lines in 2020. Peking is a second resistance source associated with low reproductive levels since 1957 (Ross and Brim, 1957). With movement away from nematicide applications and the decline in the effectiveness of SCN-resistant cultivars, seed treatments are an additional option for nematode management in a region with prevalent SCN populations.

Dhital 2020 established the effectiveness of Saltro regarding egg, cyst, and juvenile reduction in greenhouse soybeans. However, little work has been done to evaluate its efficacy in a field setting due to its recent commercial release (2020). This is the first study to examine the use of Saltro as a method of control against soybean cyst nematode in DE and MD. This is also the first study examining biological products for the region. The most notable effect of seed treatment was improved yield over non-treated seed, but differences in percent emergence of base-treated seed did not differ following the addition of a nematicide to the base. Nematode response varied across trials and by year. In 2020, nematode populations were reduced in plots with ILEVO-treated seed in one trial. In 2021, yields of Saltro-treated plots in one trial were higher than all other treatments. ILEVO and Saltro are both viable options for the region to use as part of an integrated management approach for SCN control. Both products are also labeled for root-knot and lesion nematode, which are other nematodes of concern found in the region. Future work could investigate the efficacy of these products against RKN and lesion, as well as examine the impact of using other resistance sources, such as PI89772 or Peking in combination with these products.

REFERENCES

Allen, T. W., Bissonnette, K., Bradley, C. A., Damicone, J. P., Dufault, N. S., Faske, T. R., Isakeit, T., Kemerait, R.C., Koehler, A., Langston, D., Mueller, J.D., Padgett, G.B., Price, P.P., Sikora, E.J., Small, I.M., Thiessen, L., Young, H. 2021. Southern United States Soybean Disease Loss Estimates for 2020. *Proceedings of the Southern Soybean Disease Workers*.

Allen, T.W., Bradley, C.A., Sisson, A.J., Byamukama, E., Chilvers, M.I., Coker, C.M., Collins, A.A., Damicone, J.P., Dorrance, A.E., Dufault, N.S., Esker, P.D., Faske, T.R., Gielser, L.J., Grybauskas, A.P., Hershman, D.E., Hollier, C.A., Isakeit, T., Jardine, D.J., Kelly, H.M., Kemerait, R.C., Kleczewski, N.M., Koenning, S.R., Kurle, J.E., Malvick, D.K., Markell, S.G., Mehl, H.L., Mueller, D.S., Mueller, J.D., Mulrooney, R.P., Nelson, B.D., Newman, M.A., Osborne, L., Overstreet, C., Padgett, G.B.,Phipps, P.M., Price, P.P., Sikora, E.J., Smith, D.L., Spurlock, T.N., Tande, C.A., Tenuta, A.U., Wise, K.A., Wrather, J.A. 2017. Soybean Yield Loss Estimates Due to Diseases in the United States and Ontario, Canada, from 2010 to 2014. *Plant Health Progress, 18*(1): 19-27.

Avendaño, F., Ierce, F.J., Melakeberhan, H. 2004. The relationship between soybean cyst nematode seasonal population dynamics and soil texture. *Nematology, 6*(4): 511-525.

Bandera, A.Y., Weerasooriya, D.K., Bradley, C.A., Allen, T.W., Esker, P.D. 2020. Dissecting the economic impact of soybean diseases in the United States over two decades. *PLoS ONE, 14*(4): e0231141.

Beeman, A.Q. and Tylka, G.L., 2018. Assessing the effects of ILEVO and VOTiVO seed treatments on reproduction, hatching, motility, and root penetration of the soybean cyst nematode, *Heterodera glycines*. *Plant Disease*, *102*(1): 107-113.

Beeman, A.Q., Harbach, C.J., Marett, C.C., Tylka, G. L. 2016. Soybean Cyst Nematode HG Type Test Results Differ Among Multiple Samples from the Same Field but the Management Implications are the Same. *Plant Health Brief, 17*(3): 160–162.

Bissonnette, K.M., Marett, C.C., Mullaney, M.P., Gebhart, G.D., Kyveryga, P., Mueller, T.A., Tylka, G.L. 2018. Effects of Clariva Complete Beans seed treatment on *Heterodera glycines* reproduction and soybean yield in Iowa. *Plant Health Progress*, *19:* 1-8.

Bissonnette, K.M., Marett, C.C., Mullaney, M.P., Gebhart, G.D., Kyveryga, P.M., Mueller, T.A.,Tylka, G.L., 2020. Effects of ILEVO seed treatment on *Heterodera glycines* reproduction and soybean yield in small-plot and strip-trial experiments in Iowa. *Plant Disease*, *104*(11): 2914-2920.

Byrd, D.W., Jr., Barker, K.R., Ferris, H., Nusbaum, C.J., Griffin, W.E., Small, R.H., Stone, C.A.1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. *Journal of Nematology* 8: 206-212.

Davis, E.L. and G.L. Tylka. 2021. Soybean cyst nematode disease. The Plant Health Instructor.

De Bruin, J.L., Pedersen, P. 2008. Soybean Cultivar and Planting Date Response to Soil Fumigation. *Agronomy Journal, 100*(4): 965-970

DEOS (Delaware Environmental Observing System). 2020. Monthly Data for Georgetown – REC. http://www.deos.udel.edu

DEOS (Delaware Environmental Observing System). 2021. Monthly Data for Georgetown – REC. http://www.deos.udel.edu

Dhital, B.P. 2020. Determining the Effects of Plant Extracts and Saltro Nematicide on Hatching, Mortality, and Reproduction of the Soybean Cyst Nematode (*Heterodera glycines*) (Accession No. 4082) [Master's Thesis, South Dakota State University]. *Electronic Theses and Dissertations*.

Duan Y., Chen, L., Zhou, X., Wang, Y., Sun, J. 2009. Effects of Abiotic Environmental Factors on Soybean Cyst Nematode. *Agricultural Sciences in China, 8*(3): 317-325

Faghihi, J., Ferris, J.M., 2000. An efficient new device to release eggs from *Heterodera glycines. Journal of Nematology*, *32*(4): 411.

Gaspar, A.P., Marburger, D.A., Mourtizinis, S., Conley, S.P. 2014. Soybean yield response to multiple seed treatment components across diverse environments. *Agronomy Journal*, *106*:1955-1962

Gerdemann, J.W., 1955. Relation of a large soil-borne spore to phycomycetous mycorrhizal infections. *Mycologia*, *47*(5): 619-632.

Heatherly, L.G., Young, L.D. 1991. Soybean and Soybean Cyst Nematode Response to Soil Water Content in Loam and Clay Soils. *Crop Science, 31*(1).

Jenkins, W. R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. *Plant Disease Reporter 48*: 692.

Kandel, Y., Wise, K.A., Bradley, C.A., Chilvers, M.I., Byrne, A.M., Tenuta, A.U., Faghihi, J., Wiggs, S.N., Mueller, D.S. 2017. Effect of Soybean Cyst Nematode Resistance Source and Seed Treatment on Population Densisities of *Heterodera glycines*, Sudden Death Syndrome, and Yield of Soybean. *Plant Disease, 101*(12): 2137-2143.

Lamovšek, J., Urek, G., Trdan, S. 2013. Biological Control of Root-Knot Nematodes (*Meloidogyne* spp.): Microbes against the Pests. *Acta Agriculturae Slovenica, 101*(2): 263-275.

Lauritis, J.A., Rebois, R.V., and Graney, S. 1983. Development of *Heterodera glycines* Ichinohe on soybean, *Glycine max* (L.) Merr., under gnotobiotic conditions. *Journal of Nematology, 15*(2): 272-281.

Lund, M.E., Mourtzinis, S., Conley, S.P., Ané, J.M. 2018. Soybean Cyst Nematode Control with *Pasteuria nishizawae* Under Different Management Practices. *Agronomy Journal, 110*(6): 2534-2540.

McCarville, M.T., Marett, C.C., Mullaney, M.P., Gebhart, G.D., Tylka, G.L. 2017. Increase in Soybean Cyst Nematode Virulence and Reproduction on the Resistant Soybean Varieties in Iowa from 2001 to 2015 and the Effects on Soybean Yield. *Plant Health Progress, 18*(3):146-155.

McLean, K.S. and Lawrence, G.W.1993. Interrelationship of *Heterodera glycines* and *Fusarium solani* in sudden death syndrome of soybean. *Journal of Nematology*, *25*(3): 434.

Mourtzinis, S., Marburger, D., Gaska, J., Diallo, T., Lauer, J., Conley, S.P. 2017. Corn and soybean yield response to tillage, rotation, and nematicide seed treatment. *Crop Science, 57*:1704-1712.

Mulrooney R., Gregory, N. 2010. 2009 Soybean Cyst Nematode Survey Results. UD Extension Bulletin #144: 15-17.

Niblack, T.L. 2008. Shift in Virulence of Soybean Cyst Nematode is Associated with Use of Resistance from PI 88788. *Plant Health Progress.* Plant Management Network.

Niblack, T.L., 2005. Soybean cyst nematode management reconsidered. *Plant Disease*, *89*(10): 1020-1026.

Niblack, T.L., Arelli, P.R., Noel, G.R., Opperman, C.H., Orf, J.H., Schmitt, D.P., Shannon, J.G., Tylka, G., 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. *Journal of nematology*, *34*(4): 279.

Noel, G. R. 1992. History, distribution, and economics. Pages 1-3 in: Biology and Management of the Soybean Cyst Nematode. R. D. Riggs and J. A. Wrather, eds. American Phytopathological Society, St. Paul, MN

Riggs, R.D. 2004. *Biology and Management of the Soybean Cyst Nematode 2nd edition.*

Ross, J.P., Brim, C.A. 1957. Resistance of soybeans to the soybean cyst nematode as determined by a double-row method. *Plant Disease Reporter, 41*: 923-924

Roth, M.G., Jacobs, J.L., Napieralski, S., Byrne, A.M., Stouffer-Hopkins, A., Warner, F., Chilver, M.I. 2020. Fluopyram Suppresses Population Densities of *Heterodera glycines* in Field and Greenhouse Studies in Michigan. *Plant Disease, 104*(5): 1305-1311

USDA ERS. 2022. Soybean U.S. stocks Soybean U.S. stocks: On-farm, off-farm, and total by quarter, U.S. soybean acreage planted, harvested, yield, Soybean and soybean meal production, value, price and supply and disappearance, prices 1999/00-2021/22-Year 2019. https://www.ers.usda.gov/data-products/oil-crops-yearbook/oil-crops-yearbook/#Soy%20and%20Soybean%20Products

Wrather, J.A., Anand, S.C., Dropkin, V.H. 1984. Soybean Cyst Nematode Control. *Plant Disease, 68*(9): 829-833

Xing, L. and Westphal, A., 2006. Interaction of *Fusarium solani* f. sp. Glycines and *Heterodera glycines* in sudden death syndrome of soybean. *Phytopathology*, *96*(7): 763-770.

Table 4.1: Experiment 1 and 2 Information

Year	Location	Experiment	Planting Date	Harvest Date	Previous Crop	Irrigation[z]	Sampling Date for SCN		Date of SCN Female Count	Initial SCN[y]
							At Planting	End of Season		
2020	Georgetown, DE	1	19 Apr	22 Oct	Soybean	Yes	19 Apr	14 Sept	16 Jun	936
2021	Georgetown, DE	1	27 Apr	5 Nov	Soybean	Yes	27 Apr	27 Aug	3 Jun	61
2021	Wye Mills, MD	1	4 Jun	12 Nov	Corn	No	18 May	1 Sept	3 Jun	31
2020	Georgetown, DE	2	19 Apr	22 Oct	Soybean	Yes	19 Apr	6 Oct	15 Jun	34263
2021	Georgetown, DE	2	27 Apr	5 Nov	Soybean	Yes	27 Apr	13 Sept	7 Jul	3917

[z] Water was applied through overhead irrigation during the summer months to supplement natural rainfall.
[y] Initial soybean cyst nematode (SCN) counts in spring prior to or at the time of planting. Experiment 1 is the average number of SCN J2s and cysts/500 cm^3 soil and Experiment 2 is the average number of SCN eggs/100 cm^3 soil.

119

Table 4.2: Seed Treatments used in Trial 1 and Trial 2 from 2020-2021

Name	Active Ingredient	Application Rate	Mode of Action	Group
Saltro[1,2]	Pydiflumetofan	0.075 mg/seed	SDHI	–
ILEVO[1,2]	Fluopyram	0.15 mg/seed	SDHI	N-3
Clariva[2]	*P. nishizawae*	130 ml/100 kg	Biologic	N-UNB
Trunemco[2]	*B. amyloliquefaciens* cis–jasmone	20.2 ml/100 kg	Biologic	N-UNB
Aveo EZ[2]	*B. amyloliquefaciens* strain PTA-4838	2ml/100,000 seed	Biologic	N-UNB
BioST[2]	*Burkholderia rinojenses*	195 ml/kg	Biologic	N-UNB
Base Treatment[2]	Metalaxyl Pyraclostrobin Fluxapyroxad Clothianidin Flo Rite Color Coat Red	4 g/100 kg 7.5 g/100 kg 5 g/100 kg 0.11 mg/seed 66 ml/100 kg 33 ml/100 kg	PA QoI SDHI nAChR	Group 4 Group 11 Group 7 Group 4(A)

[1] Indicates use in Experiment 1
[2] Indicates use in Experiment 2

Table 4.3: Experiment 1 2020 – Emergence, RF values, female counts, and yield, Georgetown, DE

Name	% Emergence 14 DAP	% Emergence 28 DAP	Nematode Reproductive Factor (Rf)	Transformed Nematode Reproductive Factor (RF)	Female Counts from Root Blasting	Females per (g) root weight	Yield Kg/ha	Yield Difference from Non-treated Seed
2020, Georgetown								
Non-treated	26.2c	54.0b	0.6	0.2	189.0a	19.1a	2125.1	--
Saltro	54.3b	90.5a	0.2	0.1	179.2a	23.9a	2313.4	+2.8
ILEVO	70.0a	86.4a	0.2	0.1	53.8b	5.4b	2447.9	+4.8
p-value	0.0001	0.0001	0.12	0.27	0.03	0.01	0.55	
2021, Georgetown								
Non-treated	53.3c	56.8c	7.6	0.8	531.6	125.8	2838	--
Saltro	82.4a	85.5a	3.6	0.6	473.4	94.8	3275.1	+6.6
ILEVO	68.9b	79.5b	6.6	0.7	482.2	115.3	3281.9	+6.5
p-value	<0.001	0.0001	0.38	0.56	0.92	0.63	0.18	
Wye Mills, 2021								
Non-treated	53.3c	56.8b	76.17	1.2	1.6	0.1	4848.8	--
Saltro	82.5	85.5a	46.2	1.1	2.6	0.1	4815.2	+0.95
ILEVO	68.9a	79.5a	8.51	0.7	0.8	0.05	4909.3	-0.46
p-value	<0.001	<0.0001	0.21	0.40	0.37	0.50	0.92	

Table 4.4: Experiment 2 – Emergence, RF values, Female counts, and yield, Georgetown, DE

Name	% Emergence 14 DAP	% Emergence 28 DAP	Nematode Reproductive Factor (Rf)	Transformed Nematode Reproductive Factor (RF)	Female Counts from Root Blasting	Females per (g) root weight	Yield bu/a	Yield Difference from Base Seed Treatment
Georgetown, 2020								
Non-treated	74.6ab	na	0.5	0.2	239	40.5	2353.8	+4.7
Fungicide + Insecticide Base	73.1ab	na	0.6	0.2	380	57.7	2037.7	--
BioST	70.6b	na	0.5	0.2	323	46.8	2454.6	+6.2
Aveo + Base	75.4a	na	0.7	0.2	270.8	39.2	2528.6	+7.3
Clariva + Base	76.2a	na	0.4	0.2	290.7	37.8	2750.5	+10.6
ILEVO + Base	77.0a	na	0.7	0.2	200.8	34.6	2219.3	+2.7
Trunemco + Base	76.0a	na	0.6	0.02	292.0	43.9	2313.4	+4.1
Saltro + Base	77.7a	na	0.6	0.2	333.0	50.0	2084.8	+0.7
p-value	0.1	na	0.9	0.9	0.3	0.3	0.5	
Georgetown, 2021								
Non-treated	47.3c	48.51c	6.7766	0.8	341.5	55.6	2885.1 d	-6.2
Fungicide + Insecticide Base	69.9ab	76.07ab	6.3766	0.9	450.8	71.5	3302 b	--
BioST	69.2ab	74.4ab	8.3981	0.9	392.7	52.1	3328.9 b	+0.4
Aveo + Base	70.3ab	78.8a	6.3748	0.8	394.9	55.4	3059.9 c	-3.6
Clariva + Base	76.1a	78.7a	6.9387	0.9	428.8	62.9	3080.1 c	-3.3
ILEVO + Base	68.0ab	74.0ab	7.9504	0.9	202.8	36.5	3402.9 b	+1.5
Trunemco + Base	73.5ab	76.6ab	8.7247	1.0	276.2	44.0	3295.3 b	-0.1
Saltro + Base	66.9b	72.5b	6.8743	0.9	305.5	46.3	3813.1 a	+7.6
p-value	<0.0001	<0.0001	0.94	0.97	0.17	0.24	0.0005	

Appendix A

FIRST REPORT OF *RHIZOCTONIA SOLANI* AG-4 CAUSING ROOT AND STEM ROT OF STEVIA ON THE DELMARVA PENINSULA

Published in *Plant Disease*

Kessler AC and Koehler AM

Department of Plant & Soil Sciences, University of Delaware, Georgetown, DE 19947

First report of *Rhizoctonia solani* AG-4 causing root and stem rot of stevia on the Delmarva Peninsula

Stevia (*Stevia rebaudiana* (Bertoni) Bertoni) is an emerging crop in the U.S. used as a nonnutritive sweetener. Symptomatic seedlings were observed in newly transplanted field sites in Georgetown, Delaware, and Salisbury, Maryland, throughout May and June 2019. Symptoms included wilting, stem lesions, shredded stem tissue, and root necrosis. Isolations from root and stem tissue were plated onto potato dextrose agar, amended with 50µg/ml penicillin G and streptomycin sulfate, incubated at 23ºC, and observed for colony morphology. Tan colonies had mycelia with right angle branching and septations near the branch consistent with the description of *Rhizoctonia solani* Kuhn (Sneh 1991). After extracting DNA, pathogen identification was confirmed by sequencing of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA using primers ITS4/ITS5 (White et al. 1990). Georgetown and Salisbury isolates were identified as *Rhizoctonia solani* AG-4 (99.5% sequence identity with GenBank Accession MN106332.1.) Koch's postulates were confirmed for each of the isolates by inoculating 15-week-old greenhouse grown stevia plants in 8-cm diameter pots. Colonized oat grains served as the inoculum. Three oat grains were buried at a depth of 5 mm, 2 cm from the base of each plant with four replicate plants for each isolate. Two non-inoculated plants served as the control. Plants were observed over a five-week period. Within two weeks, plants inoculated with the Delaware isolate exhibited symptoms of wilting, stem lesions, stem shredding, root necrosis, and plant death. Plants inoculated with the Maryland isolate developed symptoms at four weeks and non-inoculated plants did not develop symptoms. *R. solani* AG-4 was re-isolated from inoculated root and stem pieces and confirmed by culture morphology and sequencing (99.5% sequence identity with GenBank Accession No. MN106332.1). To our knowledge, this is the first report of *R. solani* AG-4 causing root and stem rot of stevia in North America. Hilal and Baiuomy (2000) reported root rot of stevia by *R.solani* in Egypt. Plant establishment is the most expensive component of US production, so it is important to monitor pathogens with potential to kill transplanted stevia and reduce overall plant stands.

Literature Cited:

Hilal A, Baiuomy M. 2000. J. Agric. Res. 78:1435-1448.

Sneh, B. et al. 1991. Identification of *Rhizoctonia* species. APS Press, St. Paul, MN.

White, T.J. et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego, CA. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Appendix B

EVALUATION OF FOLIAR FUNGICIDES FOR MANAGEMENT OF SOYBEAN DISEASE IN DELAWARE, 2020

Published in *Plant Disease Management Network*

Kessler AC and Koehler AM

Department of Plant & Soil Sciences, University of Delaware, Georgetown, DE 19947

Soybean (*Glycine max* L. Merr.; 'CZ 3930GTLL')
Purple seed stain; *Cercospora kikuchii*
Phomopsis seed decay; *Diaporthe longicolla*

A.C. Kessler and A.M. Koehler

University of Delaware, Georgetown, DE 19947

Evaluation of foliar fungicides for management of soybean diseases in Delaware, 2020.

A trial was established at the Carvel Research and Education Center in Georgetown, DE in a Pepperbox loamy sand that had previously been planted in soybeans. The experimental design was a randomized complete block with five replications. Soybeans treated with Liberty Link GT27 were planted on 11 May 2020 at a population of 150,000 seeds/A. Plots consisted of four, 30-in spaced rows, 25-ft in length. The trial was composed of twelve fungicide treatments and one non-treated control. Fungicides were applied using a CO_2 pressurized backpack sprayer equipped with extended range 8002VS flat fan nozzles calibrated to deliver 20 GPA at 40 psi and 2.5 mph walking speed. All treatments included 0.125% of non-ionic surfactant, Induce 90 SL. Fungicides were applied at the R3 growth stage on 30 Jul, and the R5 growth stage on 12 Aug. Plots relied on natural sources of inoculum for disease development. Prior to harvest, stems with green tissue present were counted in the center two rows of each plot to determine % green stems. Yield was then determined by harvesting the center two rows of each plots on 22 Oct and correcting to 13% moisture. A 10 g subsample from each plot was rated for disease incidence of purple seed stain (*Cercospora kikuchii*) and white/chalky seed characteristic of phomopsis seed decay (*Diaporthe longicolla*). Data were analyzed using ANOVA and means were separated using Fisher's least significant difference (LSD, α= 0.05)

Fungicides were applied at R3 and R5 and no phytotoxicity was observed in any treatment. Foliar disease pressure remained low throughout the entire season. Septoria brown spot and downy mildew were present at low levels, but no ratable foliar diseases established within the trial. None of the treatments influenced the percent of green stems remaining at harvest. No treatments resulted in yields significantly different from the non-treated control. Warm weather and excessive rainfall favored the development of purple seed stain and phomopsis seed decay. Miravis Neo and Quadris Top SBX applied at R3 resulted in lower avg. purple seed stain than the control. No treatment differences were observed for disease incidence for phomopsis seed decay.

Treatment, rate/A and timing *	% Green Stems at Harvest**	Test Weight	Moisture	Yield	Avg. Purple Seed Stain	Avg. Phomopsis Seed Decay
Non-treated Control	6.2	55.0	15.8	67.2 ab	2.4 ab	1.2
Revytek 3.33 SC, 8 oz at R3	8.3	55.4	15.6	69.7 ab	1.6 ab	0.4
Priaxor 4.17 SC, 4 oz at R3	4.9	55.5	15.6	65.4 b	0.8 ab	0.6
Veltyma 3.34 S, 7 oz at R3	10.0	55.2	15.6	75.7 ab	1.8 ab	1.0
Delaro 2.76 SC, 8 oz at R3	8.5	55.2	15.5	78.7 a	1.0 ab	0.8
Topguard EQ 4.29 SC, 5 oz at R3	6.2	55.1	15.8	68.2 ab	1.8 ab	0.6
Lucento 4.17 SC, 5 oz at R3	7.1	54.9	15.6	71.5 ab	1.8 ab	1.2
Miravis Neo 2.5 SE, 13.7 oz at R3	4.4	55.5	15.2	71.2 ab	0.6 b	1.0
Miravis Top 1.67 SC, 13.7 at R3	5.8	55.5	15.2	71.0 ab	1.0 ab	0.6
Quadris Top SBX 3.76 SC, 8 oz at R3	8.7	55.5	15.3	74.9 ab	0.6 b	0.6
Miravis Neo 2.5 SE, 13.7 at R5	6.9	55.1	15.4	71.9 ab	3.0 a	1.0
Miravis Top 1.67 SC, 13.7 oz at R5	7.0	55.0	15.4	70.7 ab	0.8 ab	0.4
Miravis Neo 2.5 SE, 13.7 oz at R3 Trivapro 2.21 SE, 13.7 oz at R5	7.4	55.3	15.6	66.3 ab	0.8 ab	0.4
p-value	0.18	0.14	0.30	0.04	0.02	0.84
LSD (α=0.05)	ns	ns	ns	7.52	1.35	ns

* All treatments included 0.125% non-ionic surfactant, Induce 90 SL.
** Means followed by the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; α=0.05). ns=not significant.