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DIAPORTHE, SOYBEAN CYST NEMATODE, AND SOYBEAN APHID: AN EVALUATION OF POTENTIAL INTERACTIONS OCCURING AMONG PESTS ON SOYBEAN IN SOUTH DAKOTA

BY

JOHN PHILLIP POSCH

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Plant Science

South Dakota State University

2017

DIAPORTHE, SOYBEAN CYST NEMATODE, AND SOYBEAN APHID: AN EVALUATION OF POTENTIAL INTERACTIONS OCCURING AMONG PESTS ON SOYBEAN IN SOUTH DAKOTA

JOHN PHILLIP POSCH

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

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This work is dedicated to my parents Jeffery Posch and Deborah Posch, and to my siblings Kaley Wilkinson, Mathew Posch, Megan Balfany and Anna Posch. Without their love and support I would not be the man I am today. My father has always encouraged me and has always given me valuable advice throughout the years. He has never once pushed me to be something I am not, and he has always supported what I wanted to be. He has taught me integrity and that I should never give up and always be the best that I can possibly be. My mother provided me with the emotional support I needed to get through the hard times during my stay in Brookings, SD. Without her constant need to build her children up emotionally, I don't think I would have made it far in my education. Thank you, mom, for helping me get through the hard times. I would also like to thank my siblings, they were always there for me when I came home for the holidays. Without them, I don't know if I could have ever been distracted enough over my breaks to relax. I would also like to say thank you to my brother Mathew for face timing me during graduate school, and for convincing me not to join the military in order to continue my education. Thank you all for your love and support. I would also like to dedicate this work to my cousin Jeremy Jacobson. I've thought about you quite a bit since we lost you in April 2016. I think of you whenever life throws me a curve ball and I remember that saying you always used to use "It is, what it is." Life is not always going to go your way and you understood that better than most people. You were my cousin, but you were also a good friend whom I shared good times with. I will never forget you Jeremy, or your nomadic lifestyle.

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ABSTRACT

DIAPORTHE, SOYBEAN CYST NEMATODE, AND SOYBEAN APHID: AN EVALUATION OF POTENTIAL INTERACTIONS OCCURING AMONG PESTS ON SOYBEAN IN SOUTH DAKOTA

JOHN PHILLIP POSCH

2017

Soybean *Glycine max* (L.) Merr., is an important crop grown in South Dakota, with an estimated production of $2.33 billion in 2015. However, soybean production in South Dakota is compromised by four pests, the fungal pathogens *Diaporthe longicolla* (Hobbs) Santos, Vrandecic, and Phillips and *Diaporthe caulivora* (Athow and Caldwell) Santos, Vrandecic, and Phillips, the soybean cyst nematode *Heterodera glycines* Ichinohe (SCN), and the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae). Additionally, these pest can co-occur in fields within South Dakota and the implications associated with interactions among these pests are unknown. We hypothesized that both *Diaporthe* species would interact with *H. glycines*, and that *D. longicolla* would not interact with *A. glycines*. To test our hypotheses, both studies were set up as a completely randomized design in the greenhouse. For the *Diaporthe*-SCN interaction, five treatments were designed (*Diaporthe* alone, SCN alone, co-inoculation of SCN and *Diaporthe*, and *Diaporthe* inoculated either 15 days before or after SCN). For the *D. longicolla- A. glycines* interaction, eight treatments were designed that consisted of two infestation times (Inducer: V1; response: seven days later). Plants were infested with either five *A. glycines*, 2000 eggs of SCN, and a four mm plug infested with *Diaporthe* depending on the treatment and experiment. We assessed stem length, lesion length, and SCN reproduction for the *Diaporthe*-SCN interaction and lesion length and aphid counts for

the *D. longicolla- A. glycines* interaction. Our results showed that SCN reproduction was reduced by 90% when either fungus preceded SCN. Additionally, when SCN preceded *D. longicolla*, we see an increase in lesion length of 76% or greater on soybean stem. When SCN preceded *D. caulivora* for experiment one, we see a decrease in lesion length of 35%. Additionally, *D. longicolla* and *A. glycines* did not interact on soybean. However, we observed a potential compensatory effect from soybean plants in the concomitant infestation of both *D. longicolla* and *A. glycines*, where aphid counts were reduced by 47% when both pests were introduced together. Interactions among pests is relatively understudied, such studies may lead to new management strategies for soybean pest and disease complexes.

Chapter 1: Literature Review

History and Production of Soybean

Soybean, *Glycine max* (L.) Merr., is one of the most important crops worldwide; it is considered an excellent source of high quality protein and oil (Xiaomin et al. 2016). A study by Guo et al. (2010) suggests that the modern landrace soybean may have originated from wild progenitors in South China; and this finding was based on the phylogenetic and genotype assignment analysis of wild and landrace soybean using microsatellites and nucleotide sequences (Guo et al. 2010).

From the early 1900s to 1935, soybean production was mostly confined to China, Indonesia, Korea, and Japan (Burtis 1950; Hymowitz 1970). It was not until the 1950's that the United States became a major soybean producing country, and over took China in production. By 1968, farmers in the United States were growing 76 percent of the soybean produced worldwide (Hymowitz 1970). Over the past 10 years, soybean production has notably increased. The area of soybean planted has increased from 31.1 million hectares (77 million acres) in 2006 to an area of 33.5 million hectares (83 million acres) in 2016 (United States Department of Agriculture-National Agricultural Statistics Service 2016). As of 2016, United States ranks as the number one producer of soybean in the world in terms of area planted and area harvested (United States Department of Agriculture-Foreign Agricultural Service, 2016). More than 80 percent of the soybean acreage in the United States is concentrated in the Eastern Corn Belt which is located in the Midwest; this includes Iowa, Illinois, Indiana, Minnesota, Missouri, and Ohio (Hymowitz 1970).

In South Dakota, soybean production accounted for an estimated two million hectares (5.12 million planted acres) planted and approximately $2.33 billion of revenue

in 2015 (United States Department of Agriculture-National Agricultural Statistics Service 2016).

Soybean Uses

Soybean can be used in the production of food, industrial products, and oils. For instance, since soybean is a high-quality protein source, it is used in a variety of foods such as tofu and soymilk (Stanojevic et al. 2011). There are also industrial uses for soybean. For example, DuPont Pioneer (Johnston, IA) has developed transgenic soybean that produces seeds with an oleic acid content of roughly 80% (Kinney 1997), which are used for the production of biodegradable lubricant formulas (Cahoon 2003).

South Dakota Soybean Production

Planting Dates

South Dakota has a relatively short growing season when compared to other Midwestern states. As a result, planting is usually done between May 8th and June 21st with May 15th through June 11th being the most active time for planting in South Dakota (United States Department of Agriculture- National Agricultural Statistics Service 2010).

Planting Depth and Row Spacing

Planting depth of soybean is usually at a range between 2.54 cm to 4.45 cm and no deeper than 6.35 cm. The preferred row spacing for soybean is less than 50.80 cm due to the advantages for narrow row spacing that provides increased yields, weed control, and easier harvesting. However, in some cases conventional spacing (> 76.20 cm) is preferred to help reduce disease development in soybean fields (Yelverton et al. 1991).

Seeding Rate

Seeding rates for soybean varies depending on the row spacing used, percentage that seed germinates, and the percentage of plants that emerge. The recommended plant populations per acre vary depending on these factors (Robinson and Conley 2007). For example, a study conducted by Weber et al. (1965) showed that planting the soybean cultivar 'Hawkeye' in 25.40 cm rows with 104,544 plants per 0.40 hectare (1 acre) increased yield when compared to planting in 12.70 cm rows with 208,088 plants per 0.40 hectare (1 acre). Planting too many plants with less row spacing can reduce yield due to competition between plants. The seeding rate and row spacing used depends on the environment of the field. If the field is known to be high yielding then a row spacing of 20.30 cm will produce greater yields then a 76.20 cm row spacing. If moisture is typically known to be low then a row spacing of 76.20 cm would be more beneficial than a closer spacing. In high yielding conditions yield is maximized with a lower seeding rate such as 115,000 seeds per 0.40 hectare (1 acre) with 76.20 cm rows whereas seeding rates of 200,000 or more per 0.40 hectare (1 acre) was required to maximize yields at a row spacing of 20.32 cm (Devlin et al. 1995).

Types of Varieties

Soybean varieties belonging to maturity groups 00, 0, I, II, and III are grown in the Northern Great Plains (United States Department of Agriculture-National Agricultural Statistic Service 2016). Due to the relatively short growing season of the Great Plains area, maturity groups 0, I, and II are most commonly planted in South Dakota with maturity group II being the most utilized maturity group (United States Department of Agriculture-National Agricultural Statistic Service 2016). The type of variety grown depends on the

producer's targeted market. For example, a producer may grow soybeans for human or animal consumption, and so the producer may select varieties with high isoflavone content due to the health benefits associated with isoflavones. If the producer's target market is for the production of industrial products or oils then they would consider growing soybean with qualities that would fit those needs. Additionally, soybean pests may also influence the type of seed variety a producer will select. For example, if a producer struggles with yield loss associated with the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) every year, then they may want to select a soybean variety with resistance to soybean aphid. Soybean varieties that contain *Rag* genes would provide a degree of crop resistance to *A. glycines*, however, virulent biotypes of *A. glycines* have been identified that can overcome *Rag* gene mediated resistance (Hesler et al. 2013), so this is not necessarily a completely viable method for the management of *A. glycines* in every situation. Soybean producers use multiple practices for the management of soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, such as rotation to non-host crops and use of SCN-resistant varieties that can provide some defense against SCN (Niblack 2005).

Soil Factors

The optimal range of soil pH for growing soybean is between 6.3 and 6.5. Nutrient availability and nitrogen fixation of the plant is maximized at this pH range (Staton 2012). In addition, a soil temperature range of 25°C to 30°C is optimum for soybean emergence and for symbiotic activity (Zhang et al. 1996).

Fertilizers

Fertilizer rates are determined by the composition of the soil and are based off soil tests. Recommendations for fertilizer applications for soybean potassium and phosphorus

rates are determined by the crop planted the previous year and the current nutrient levels of the soil (Franzen 2016).

Water management

Soybean plants require good soil moisture to grow adequately. Having low soil moisture can result in reduced soybean yield. Additionally, low soil moisture at R6 growth stage (full seed in pod) can significantly reduce yield by speeding up plant maturity (Lozovaya et al. 2005). Water table management (WTM) has been known to increase soybean yield and also has been shown to encourage the conservation of resources (e.g., control water and agro pollution). Water table management allows producers to lower and raise the water table using drainage (Mejia et al. 2000).

Tillage

Tillage practices have been implemented in South Dakota for soybean production since the beginning of the 20th century. Recently, producers have been implementing no-till practices in South Dakota. No-till systems reduce soil erosion, equipment deterioration, and fuel and labor costs (Carpenter-Boggs et al. 2003). In addition to this, no-till systems also can contribute to an increase in organic matter in the soil and benefit soil biota that is beneficial to field crops. These soil organisms can benefit crops by releasing forms of nutrients from organic and inorganic sources in the soil, fixing nitrogen in plant roots, increasing phosphorous uptake, and participating in pathogen antagonism (Angers et al. 1993; Carpenter-Boggs et al. 2003). In 2013, tillage practices used by South Dakota producers mostly consisted of no-till practices (45%), which was followed by reduced tillage (19%) and mulch tillage (19%). Conventional tillage was used the least within the

State among producers (17%) (United States Department of Agronomy- Natural Resources Conservation Service 2014).

Pest management

Soybean production is threatened by a number of pests that include insects, plant pathogens, and weeds. It is important to monitor fields for pests considering the economic loss they can cause. Integrated pest management practices can be utilized to control multiple diseases and other pests in soybean fields. Integrated pest management practices include the use of natural predators, chemical controls, resistant cultivars, as well as cultural practices.

Crop Rotation

Rotation from soybean to other crops (e.g., wheat and corn) is important to protect crops from pests that would otherwise be present in continuous crop monoculture (Liebman and Dyck 1993). Rotating crops helps to reduce primary inoculum of certain pathogens by alternating from host to non-host crops. Alternating crops can also reduce insect populations and help control weeds (Brust and Stinner 1991; Liebman and Dyck 1993).

Harvest

Soybean is typically harvested between September 22nd and November 3rd with September 28th through October 24th being the most active time in which soybean is harvested in South Dakota (United States Department of Agriculture- National Agricultural Statistics Service 2010).

Pests as a Limiting Factor in Soybean Production

Pests of soybean can be yield limiting and should be a concern to soybean producers. Currently, there are more than 200 pathogens that are known to negatively affect

soybean and of those 200, 35 pathogens are thought to be economically important. Loss estimates are constantly changing as well due to changing pathogen virulence, host susceptibility, and the environment (Hymowitz et al. 2015). In addition, there are a wide variety of herbivorous insect pests that are also yield limiting. These insects include phloem feeders with piercing-sucking mouth parts and insects that feed on plants with chewing mouthparts. The potential yield loss of crops due to all herbivorous insects is estimated to be approximately 18% of all crops globally. Additionally, the potential yield losses of soybean due to insect feeding was estimated to be approximately 11% for soybean globally (Oerke 2006).

Soybean aphid (*Aphis glycines*)

Taxonomy of *A. glycines*

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Hexapoda

Class: Insecta

Order: Hemiptera

Suborder: Sternorrhyncha

Family: Aphididae

Genus: *Aphis*

Introduction

Of the insect pests that feed on soybean in South Dakota, the most economically important is the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae). *Aphis glycines* is an introduced pest that originated from Asia (Blackman and Eastop 2000). It

was first observed in Wisconsin in the United States in 2000. The majority of researchers studying *A. glycines* and soybean agree that the insect most likely was present in the United States for many years prior to its discovery in 2000 (Ragsdale et al. 2004). Since 2000, *A. glycines* has spread to 30 soybean-producing states in the Midwest, including South Dakota and also three Canadian providences (Alleman et al. 2002; Ragsdale et al. 2011). *Aphis glycines* is a concern for soybean producers because in some cases they have been known to reduce soybean yields by as much 40% when left unmanaged (Ragsdale et al. 2011).

Prior to its arrival, there was a great concern regarding the introduction of *A. glycines* into the United States. How *A. glycines* arrived into the United States is unknown. However, based off the facts from the United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ), interceptions of *Aphis* spp. from permit cargo was 43% and from baggage was 36% from 1985 to 2002. Additionally, *Aphis* spp. intercepted from ship stores only accounted for 10%. This suggests that *A. glycines* most likely entered the United States through permit cargo or on baggage (Venette and Ragsdale 2004).

Biology

Aphis glycines has both wingless (apterous) and winged (alate) morphs that may be observed simultaneously on soybean. The wingless morphs are pear-shaped and are roughly 1.5 mm long. Both the nymph and adult stages of *A. glycines* vary in color from yellow to bright green. Although present on all life stages, the black cornicles present on the posterior end of the abdomen are more easily observed on the adults (Tilmon et al. 2011).

Aphis glycines feed using piercing-sucking mouthparts. These mouthparts are used to pierce the vascular tissues of a plant and to feed on the phloem from the stem, leaves, and pods on soybean. Early in the season, *A. glycines* are typically found on the underside of developing trifoliates of the soybean plant. The success of an aphid colony is greatly influenced by environmental factors such as temperature, humidity, and also the presence of natural enemies (Tilmon et al. 2011). The optimal developmental conditions for *A. glycines* are a temperature of 27.8 °C and a relative humidity below 78%. Additionally, the growth stage of the host also affects the reproduction of *A. glycines*. *Aphis glycines* have greater reproduction rates during the vegetative and late reproductive soybean developmental growth stages (Tilmon et al. 2011).

Aphis glycines is a host-alternating organism that goes through sexual reproduction for only part of its life cycle (Ragsdale et al. 2004). Incidentally, a number of buckthorn (*Rhamnus*) species act as the primary host for *A. glycines* in North America (Voegtlin et al. 2005), and soybean acts as a secondary host (Ragsdale et al. 2004). *Aphis glycines* overwinters on *Rhamnus* spp. (Voegtlin et al. 2005).

Aphis glycines has a heteroecious, holocyclic life cycle. In the spring, nymphs hatch on buckthorn and later become wingless fundatrices, which produce the next generation that is made up of primarily wingless females. The third generation that is produced on buckthorn is made up of alates that search for soybean plants. During the summer on soybean, *A. glycines* may asexually reproduce as many as 15 generations of apterous and alate morphs. At this stage, the reduced temperatures and photoperiod induce the production of winged females known as gynoparae that migrate back to their primary host. The gynoparae will feed and produce nymphs that develop into oviparae. Male aphids, and

roparae, are produced on soybean and must find oviparae and then mate with them. The oviparae will deposit eggs on buckthorn that will overwinter until the next season (Ragsdale et al. 2004). *Aphis glycines* can both give live birth and lay eggs (Ragsdale et al. 2004; Tilmon et al. 2011).

Aphid management

Biological control. There are many natural enemies that are known to feed on *A. glycines*. The two most important natural enemies of soybean aphids are *Harmonia axyridis* and *Orious insidiosus* (Varenhorst and O'Neal 2012). Other natural enemies include several other species of ladybeetles, predatory flies, lacewing larvae, damsel bugs, and several species of parasitoid wasps (Tilmon et al. 2011). When natural enemies are not present, *A. glycines* populations can grow 2-7 times faster than when predation of the aphids is occurring (Costamagna and Landis 2006).

Genetic resistance. Soybean varieties that contain *Rag* genes confer a degree of resistance to soybean aphids. However, aphid biotypes have been identified to overcome *Rag1* and *Rag2* genes, which indicates that host plant resistance towards the soybean aphid has been overcome (Tilmon et al. 2011). However, the frequency of the virulent alleles in the North American *A. glycines* population is thought to be low.

Chemical control. The use of broad spectrum insecticides are commonly used for the control of *A. glycines* (Tilmon et al. 2011). Insecticides should only be used once *A. glycines* populations have reached their economic threshold which is when *A. glycines* are considered to be economically damaging. The economic threshold for *A. glycines* is 250 aphids per plant on over 80% of the plants in a field (Ragsdale et al. 2007).

Diaporthe

Taxonomy of *Diaporthe*

Kingdom: Fungi

Phylum: Ascomycota

Class: Sordariomycetes

Subclass: Sordariomycetidae

Order: Diaporthaceae

Family: Diaporthaceae

Genus: *Diaporthe*

Introduction

Diseases caused by the *Diaporthe/Phomopsis* complex on soybean includes northern stem canker, southern stem canker, Phomopsis seed decay, as well as pod and stem blight of soybean. In the United States, soybean losses associated with northern and southern stem canker and stem blight are estimated to be approximately 345,672 metric tons (12.7 million bushels) and 353,837 metric tons (13.0 million bushels) or approximately a combined monetary loss of $271 million (Bradley et al. 2014). Stem and pod blight of soybean was first documented during 1920 in North Carolina in the United States; and the causal agent of the disease was *Diaporthe sojae* (Lehman) Wehmeyer. In 1985, *Diaporthe longicolla* (Hobbs) Santos, Vrandecic and Phillips was described as the causal agent of Phomopsis seed decay on soybean (Hobbs et al. 1985). However, more recently it has been associated with stem and pod blight on soybean (Cui et al. 2009). Northern stem canker of soybean caused by *Diaporthe caulivora* (Athow and Caldwell) Santos, Vrandecic and Phillips was first described in Iowa in 1948 by J. M. Crall and has

been known to cause yield losses of up to 50% (Crall 1950; Keeling 1988). Southern stem canker of soybean caused by *Diaporthe aspalathi* Jansen, Castlebury, and Crous gained importance after the first outbreak that occurred in 1992, and yield losses up to 50% have been observed (Wrather et al. 2010). Throughout this thesis, given the nomenclatural transition from "*Phomopsis*" to "*Diaporthe*" (Wingfield et al. 2012), "*Diaporthe*" will be used when referring to *Diaporthe* species or isolates.

Diaporthe longicolla the Causal Agent of Stem Blight

The fungus *D. longicolla* is the causal agent of Phomopsis seed decay (Zhang et al. 1998) and stem blight (Cui et al. 2009). In South Dakota, *D. longicolla* has been associated with only stem blight (Gebreil et al. 2015). The fungus is characterized by alpha (α) and beta (β) conidiospores. The β conidia are unicellular and filiform in structure and range in length from 17.48 µm to 29.83 µm with a width of 1.0 µm (Vidic et al. 2013), whereas the α conidia are unicellular and oval shaped in structure and range in length from 5.9 µm to 8.1 µm with a width of 1.8 µm to 2.4 µm (Santos et al. 2011). When grown on potato dextrose agar (PDA), *D. longicolla* will produce pycnidia and α conidia, however, β conidia is not seen. The life cycle of *D. longicolla* is considered imperfect since the sexual structures have not been observed on the fungus under any environmental conditions (Santos et al. 2011). However, Santos et al. (2011) demonstrated the presence of both mating-types, which suggests that a sexual cycle exists and has yet to be identified.

Symptoms

In soybean, stem blight is observed most commonly on the stems, but can be seen on other tissues of the plant. The stem blight pathogen infects the host early in the growing season and does not initially produce noticeable symptoms on the plant. A moist or humid

environment (relative humidity between 85-90%) and a temperature of 20°C or greater will favor the development of stem blight and allow it to develop fruiting structures on the stem. Under field conditions there are no definite lesions formed on the stem, but pycnidia from the pathogen can be seen. Wet conditions can promote the development of pycnidia on the surface of the plant. When conditions are dry, the pycnidia formed by the fungus are usually confined near the nodes and are occasionally confused with northern stem canker (Sinclair et al. 2015).

Diaporthe caulivora the Causal Agent of Northern Stem Canker

Diaporthe caulivora (Athow and Caldwell) Santos, Vrandecic and Phillips is the causal agent of northern stem canker of soybean (Crall 1950). The fungus is characterized by the black beak shaped perithecia (165-340 x 282-412 µm in size) that germinate from the over-seasoned stems of soybean. The mycelium produced by *D. caulivora* is white and has random tuffs of fluffy growth on PDA. The perithecia of *D. caulivora* produces asci that are between 5.6-5.8 x 25.7-28.7 µm in size and are clavate in shape. The two celled ascospores produced are roughly 2.3-2.5 x 8.1-8.4 µm in size, are hyaline and two celled in structure (Li and Hartman 2015).

Symptoms

In soybean, northern stem canker is observed during flowering and the other reproductive growth stages (R growth stages) (Grau 2006), however, infection can also occur during the early vegetative growth stages (Li and Hartman 2015). The characteristic symptoms include elongated, reddish-brown cankers that have black margins and are slightly sunken into the stem. The surface of the cankers have randomly distributed black spots with groupings of black, long-necked pycnidia (Vidic et al. 2013). As the infection

continues eventually the nodes will become infected, and the lesions will appear darker than those observed on the stem (Vidic et al. 2013). The severity of northern stem canker depends on the time at which infection occur with early infections during first to second trifoliate (V1-V2) growth stages leading to more severe disease (Zhang 2004). The disease is favored by moist, humid (relative humidity of 85-90%), and cool conditions. Sporulation usually occurs after harvest at a temperature of 20°C or more with adequate moisture (Li and Hartman 2015).

Identification of species of *Diaporthe*

The fungal pathogens belonging to the *Diaporthe/Phomopsis* complex may be characterized based on morphological characteristics such as color of the colony and its appearance, shape and size of the stromata, and the size and shape of the conidia that the fungus produces. However, due to the variability and overlap in the cultural characteristics of the species of *Diaporthe*, it is difficult to characterize these pathogens by morphology (Zhang et al. 1998).

Currently, molecular techniques are used to confirm the identity of species of *Diaporthe*. In general, species of *Diaporthe* are identified through phylogenetic analyses of the nuclear ribosomal internal transcribed spacers (ITS), elongation factor 1-α (EF1-α), beta-tubulin (TUB) and histone-3 (HIS) gene regions (Santos et al. 2011; Udayanga et al. 2015). Among the gene regions used for species identification, Santos et al. (2010) suggested that the EF1-α gene region is the best because it represents the biological species boundaries better while allowing the study of phylogenetic relationships of different *Diaporthe* isolates and species to confirm the identity of the fungus.

In addition to phylogenetic analyses, real time PCR assays, or quantitative PCR (qPCR) assays, have been developed for the molecular identification of *D. longicolla* and *D. caulivora* on soybean as well. For example, in a study by Kontz et al. (2016), qPCR assays were used to detect *D. longicolla* and *D. caulivora* from soybean plant tissues with similar effectiveness as using DNA from pure fungal cultures.

Disease cycle

Species of *Diaporthe* mainly disseminates via the wind or rain. The primary source of inoculum of *D. longicolla* are spores (conidia) that are produced in fruiting bodies (pycnidia) that developed on infected plants or overwintered on plant debris (Garzonio and McGee 1983). In addition, *D. longicolla* can disseminate long distances when it infects soybean seeds (Xue et al. 2007). For *D. caulivora*, it has been proposed that seed infection may act as a possible means for spreading northern stem canker (Li and Hartman 2015). The primary inoculum of *D. caulivora* are ascospores which are produced in sac-like asci at the base of a perithecium (Pioli et al. 2002). The fungus overwinters as stromata on plant residue (Li and Hartman 2015).

Management of *Diaporthe*

Tillage. Conventional tillage systems can help reduce the primary inoculum of *Diaporthe* in fields by disrupting spore dissemination. Additionally, the use of conservation tillage practices may increase the chance of disease re-emerging following years (Li and Hartman 2015).

Rotation. Rotation to non-host crops is an effective means for reducing the primary inoculum in the field. For the control of *Diaporthe* rotating soybean with non-legume non-host crops is recommended (Li et al. 2010)

Chemical control. Fungicide applications during pod-fill developmental soybean growth stage can help manage *D. longicolla* from infecting the seeds to limit the development of Phomopsis seed decay (Sinclair and Hartman 2015), though, no information is available on the efficacy of fungicides to manage *D. longicolla* as a stem pathogen at this time. The application of foliar fungicides to manage northern stem canker is not effective once symptoms have begun to develop on soybean (Li and Hartman 2015).

Genetic resistance. There are cultivars available with resistance to northern stem canker of soybean. For example, soybean cultivars have been screened for resistance to *D. caulivora* and resistance has been identified (Keeling 1988). Additionally, cultivars have also been screened for resistance to *D. longicolla* as the causal agent of Phomopsis seed decay (Jackson et al. 2005). Furthermore, there are cultivars that provide resistance to stem and pod blight caused by *D. sojae* (Hepperly et al. 1979), but information on resistance to stem blight associated with *D. longicolla* is currently not known.

Soybean Cyst Nematode (SCN)

Taxonomy of SCN

Kingdom: Animalia

Phylum: Nematoda

Class: Chromadorea

Order: Tylenchida

Family: Heteroderidae

Genus: *Heterodera*

Description

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is currently regarded as the number one pest of soybean in the United States based on soybean yield losses. In 2014, yield losses from SCN were approximately 3.4 million metric tons (125 million bushels) in the United States or approximately $1.6 billion (Bradley et al. 2014). The first population of SCN that were discovered in the United States, were found in North Carolina in 1954, but how SCN first arrived to the United States is unknown. However, it may have possibly arrived on imported plant or soil material (Davis and Tylka 2000). Since then, SCN has spread to multiple soybean producing states in the United States, including South Dakota (Davis and Tylka, 2000; Doupnik 1993). As of 2016, SCN has been identified in 29 out of the 66 counties in South Dakota (Acharya et al. 2016).

Soybean cyst nematode is typically identified by enumerating cysts from soil samples taken from fields. Cyst densities from the soil are fairly easy to determine and for this reason cysts have become the primary life stage of SCN used in laboratory assessment. Soybean cyst nematode does not necessarily produce visual recognizable damage to soybean fields, therefore fields should not be scouted for symptoms like stunting and chlorosis of the plants that can be caused by SCN. This is because SCN can cause yield losses up to 30% in soybean fields without causing any noticeable symptoms. Soybean cyst nematode can also be identified by pulling plants from fields in question and observing the roots for the lemon shaped cysts that SCN forms, however, in order to determine the level of SCN infestation in a field, soil samples need to be sent in for laboratory assessment (Niblack 2005).

Life cycle

Soybean cyst nematode is a plant-parasitic nematode that survives by parasitizing soybean root systems (Davis and Tylka 2000). The life cycle of SCN is relatively complex. The nematode requires optimal conditions in order to complete its lifecycle; this includes soil temperature of approximately 25°C, a suitable host, and proper soil conditions such as soil moisture, soil fertility, and soil type (Niblack 2005). In addition, soil type has a large impact on the severity of damage caused by SCN. Light sandier soil allows for less restricted movement of SCN, and as a result damage caused by the nematode is greater. However, SCN can be found in all soil types (Davis and Tylka 2000; Niblack 2005).

SCN reproduction is sexual. However, there is still much that is unknown regarding the hatching mechanisms of SCN or why some of them are mediated differently (Niblack 2005). Sex ratios are usually observed as a 1:1 ratio for SCN, so approximately 50% of the nematodes are male and 50% are female; although certain factors can effect this ratio and set it off balance (Colgrove and Niblack 2005).

As part of the SCN life cycle, the eggs hatch within the cyst in response to the organic molecules (eclepins) produced by the host plant, and this is known as root diffusate based hatching (Davis and Tylka 2000; Rasmann et al. 2012). The first stage juveniles (J1) will transition into second stage juveniles (J2) while still in the egg. The J2 juveniles are able to find the host plant using a form of chemo taxis by sensing organic molecules (eclepins) produced by the plant (Davis and Tylka 2000; Rasmann et al. 2012). The J2 juveniles will then migrate through the soil and penetrate soybean roots to form a feeding site. The penetration site on the roots is typically in or near the vascular tissues of the plant, this area depends on the water status of the soybean plant (Johnson et al. 1993; Niblack

2005). The SCN that have penetrated the roots then become immobile as they feed due to swelling as they molt through J3 and J4 stages. The females will continue to swell as they develop and males will become vermiform in shape. The females eventually become lemon shaped, roughly at this time the males will leave the roots and fertilize the females. Eggs will develop and the female will die and form a protective cyst which later breaks away from the roots of the plant. Each female can produce anywhere from 40 to 600 eggs, but the average is approximately 200 eggs per female. The plants will experience greater stress depending on how many J2 females have successfully attached themselves to the roots. Incidentally, the males do not feed on the soybean roots and are only needed for reproduction (Davis and Tylka 2000; Niblack et al. 2008).

Symptoms

The infestation symptoms of SCN include yellowing of the leaves that occurs approximately 30-45 days after juvenile females have attached themselves to the roots of the plant, and also dwarfing of the soybean plants. However, symptoms may not necessarily be visible, so it is difficult to identify the presence of SCN in a field without taking soil samples. The severity of damage caused by SCN is determined by the population density and moisture levels. Typically, larger populations of SCN in the soil will correlate to greater field damage (Niblack 2005). Additionally, high moisture levels can help spread SCN eggs, juveniles, and cysts through surface water and increase disease incidence in the field (Davis and Tylka 2000).

Management of SCN

Rotation. Crop rotation is one of the main strategies for managing SCN. The cultural practice of rotating from soybean to non-host crops such as corn or wheat can reduce SCN population densities (Niblack 2005).

Biological control. There are currently no widely accepted biological controls for the management of SCN. However, studies suggest that biological controls may be a viable option for the control of SCN in the near future. For example, in a study performed by Kloepper et al. (1992) it was shown that rhizosphere bacteria had an antagonistic effect on SCN.

Chemical control. There are currently nematicide seed treatments that are available for commercial use that can help manage SCN, however, they do not give season-long management (Davis and Tylka 2001).

Genetic resistance. Soybean cyst nematode is primarily managed with resistant varieties in the United States. Currently, three sources of genetic resistance to SCN are incorporated into commercial soybean varieties and marketed. Cultivars that are derived from the plant introduction lines PI 548402 (also referred to as Peking), PI 88788, and PI 437654 provide some degree of resistance to SCN (Mitchum et al. 2016; Niblack et al. 2008). In more recent years, more than 95% of marketed lines for SCN resistant varieties are derived from PI 88788 and the remaining varieties are derived from Peking and PI 437654 (Mitchum et al. 2016). This practice of overusing the same source of SCN resistance has led to the development of SCN populations that are capable of infecting varieties derived from PI 88788. For example, populations of SCN have been shown to adapt to the SCN resistance allele *rhg1* from PI 88788. A field survey conducted in Illinois

by Niblack et al. (2008) demonstrated that most of the SCN populations in Illinois have adapted to PI 88788 to some degree.

Interaction among Pests

Interactions among organisms is common in nature and this is also true for pests of soybean. El-Borai (2001) has described interaction as "Both quantitative and qualitative responses resulting from two or more factors involved in plant diseases." Associations among different pests such as nematodes, insects, and fungus can be categorized into three types of different interactions. The interaction can be synergistic where the association between the two pests causes greater plant damage than the damage caused by the pests individually (1+1>2); the interaction can be antagonistic where the association of both pests cause less damage than the sum of the individual pests (1+1<2); and the interaction can be neutral where the association between pests cause damage that is equal to the damage caused by the pests individually (1+1=2) (Back et al. 2002).

Interactions of insect pests with other organisms have been documented in previous studies. For example, in a study conducted by McCarville et al. (2014), it was demonstrated that *A. glycines* and *H. glycines* may have an indirect effect on one another when they co-infest soybean. McCarville et al. (2014) compared *H. glycines* populations on a *H. glycines* resistant (DeKalb 27-52, PI 88788 derived) and *H. glycines* susceptible (Kenwood 94) cultivar in the presence of *A. glycines*. They observed that *H. glycines* reproduction increased on the resistant cultivar and decreased on the susceptible when *A. glycines* was feeding on the plants (McCarville et al. 2014). The decreased populations that were observed on the susceptible soybean cultivar were believed to be the result of competition between the thriving SCN and *A. glycines* populations. This is a prime example of multiple

pests interacting on soybean, where an interaction of one pest indirectly, or directly, affects a second pest by stimulating a response from the host on the physiological level (McCarville et al. 2014; Raven and Johnson 2002).

Insects have also been known to interact with fungal organisms on plants. Past research has showed that certain types of endophytic fungi have underpinning mechanisms that can influence insect behavior and ultimately effect insect feeding preferences, reproduction, and growth. In addition to underpinning mechanisms, insects and fungi can have mechanical interactions as well. For example, herbaceous insects have been known to act as transports for endophytic fungi during insect feeding on plant tissues (Hartley and Gange 2009). Additionally, insects can even help promote fungi in some cases. For example, aphids produce and secrete a substance known as honeydew that can promote the development of black sooty fungi that can negatively affect a plants ability to perform photosynthesis (Hill et al. 2006).

In the case of plant-parasitic nematodes, interactions with other organisms and influences in disease development as a result of the interaction have been observed. For example, in a study by Russin et al. (1989), they observed that the presence of *H. glycines* can reduce stem canker severity caused by *D. caulivora* on soybean plants. This is an example of a negative effect on the fungal pathogen *D. caulivora* as a result of SCN altering the disease pathway of the soybean plants (Raven and Johnson 2002; Russin et al. 1989). However, nematodes do not always have a negative effect on fungal pathogens. For instance, Hasan (1993) demonstrated that the disease severity caused by Fusarium wilt of cotton (*Gossypium hirsutum*), which is caused by the fungal pathogen *Fusarium*

oxysporum f. sp. *vasinfectum* (Atk.) Snyder and Hans is greater in the presence of the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chit wood.

Interactions are not always responsible for a negative effect resulting in reduced plant vigor, some may benefit the plant and give rise to commercialized biological controls for use in integrated pest management practices. For instance, it has been demonstrated that *Lecanicillium* spp. can have an antagonistic effect on insects, mites, and plant parasitic nematodes and bio-controls have been established and commercialized due to these antagonistic relationships (Goettel et al. 2008).

Management of Pest Interactions

Multiple pests on soybean can result in the occurrence of interactions that may be more yield limiting than the pests by themselves. For example, McCarville et al. (2014) found that concomitant infestations of *H. glycines* and *A. glycines* improved the quality of the host for both pests on *H. glycines* resistant variety (DeKalb 27-52, PI 88788 derived). These findings suggest that the management of *A. glycines* may also aid in reducing the impact that *H. glycines* may have on a soybean field when planting that particular variety. Improving current *A. glycines* resistant cultivars with PI 88788 may provide a degree of resistance to both these pests, but little is known about how *A. glycines* would respond to PI 88788 or how they would respond to potential cultivars with both *Rag* gene resistance and minor genes from PI 88788 (McCarville et al. 2012).

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Chapter 2: Interaction between *Heterodera glycines* and species of *Diaporthe* on soybean

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Abstract

Soybean, *Glycine max* (L.) Merr., is an important crop grown in South Dakota, with an estimated production of $2.33 billion in 2015. However, one of the biggest yield limiting factors in soybean production is the soybean cyst nematode *Heterodera glycines* Ichinohe (SCN). While SCN can affect soybean, it can also interact with fungal pathogens thus compromising more yield. In this study, we examine the interaction between SCN and two fungal pathogens, *Diaporthe longicolla* (Hobbs) Santos, Vrandecic and Phillips, and *Diaporthe caulivora* (Athow and Caldwell) Santos, Vrandecic, and Phillips on soybean. To examine the interaction, a greenhouse experiment was performed in a completely randomized design with five treatments and five replications per experiment. Treatments included *Diaporthe* alone, SCN alone, co-inoculation of SCN and *Diaporthe*, and *Diaporthe* inoculated either 15 days before or after SCN. Plants were either inoculated with 2000 SCN eggs/ml, a four mm fungal plug, or both. At R4 growth stage, SCN reproduction, stem length, and lesion length produced by fungus on soybean stems was assessed. Our results showed that SCN reproduction was reduced by 90% or greater when either fungus preceded SCN. Additionally, when SCN preceded *D. longicolla* for two experimental repeats, we see an increase in lesion length of 76% or greater on soybean stem. When SCN preceded *D. caulivora* for

experiment one, we see a decrease in lesion length of 35%. Information from this study will provide new insights regarding how these pests can interact on soybean.

Introduction

Soybean, *Glycine max* (L.) Merr., is one of the most important crops grown in South Dakota. The primary uses of soybean in South Dakota include livestock and fish feed, industrial products, and oils. For instance, soybean is incorporated into livestock feed for dairy cows to help increase milk yield for human consumption. In 2015, monetary gains associated with soybean production in South Dakota were approximately $2.33 billion (USDA National Agricultural Statistics Services 2016; https://www.nass.usda.gov/).

Among the pests that affect soybean production in the United States, soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is the single most damaging. In 2014, monetary losses associated with SCN were estimated to be $1.6 billion in the United States alone (Bradley et al. 2014). In South Dakota, SCN has been identified in 29 soybean producing counties and continues to be a major problem for soybean producers (Acharya et al. 2016). The nematode was first documented in North Carolina in 1954, and has since become an established pest in most soybean producing states (Davis and Tylka 2000). Under field conditions, SCN can infest plants at a relatively young growth stage and can complete their first generation before soybean plants are able to form nodules (Melakeberhan 2007). Symptoms of SCN infestation include the yellowing and stunting of soybean, however, SCN does not always cause noticeable symptoms. In the absence of visible symptoms, yield losses of 10-20% may still occur from SCN infestation (Davis and Tylka 2000). At this time, SCN is primarily managed by using

resistant soybean varieties. Approximately 95% of the soybean varieties that are marketed to manage SCN are derived from PI 88788. However, the continued use of a single resistance source (PI 88788) has led to SCN populations that are capable of surviving on soybean varieties with PI 88788 (Mitchum et al. 2016).

Soybean cyst nematode is known to interact with fungal pathogens on soybean. For example, in a study by Russin et al. (1989) it was determined that the lesion length caused by *Diaporthe caulivora* (Athow and Caldwell) Santos, Vrandecic and Phillips (the causal fungus of northern stem canker of soybean) was reduced up to 40% when soybean roots were colonized with SCN as compared to the soybean roots that were not colonized by SCN. Additionally, SCN showed a reduction in both cyst rating (0-4 rating scale were 0 is no cysts and 4 is >75 cysts) and in the number of juveniles when *D. caulivora* was also present on the plants. However the study by Russin et al. (1989) was conducted using only a SCN-susceptible cultivar (Bragg) and it was hypothesized that SCN may cause subtle physiological changes in soybean plants because the lesion length caused by *D. caulivora* is reduced on soybean. The agronomic effects on how *D. caulivora* and SCN can affect soybean were investigated by Pacumbaba (1991) in a field trial that was conducted using the soybean cultivar Bragg. Pacumbaba (1991) reported co-infestations of *D. caulivora* and SCN did not significantly reduce the soybean yield when compared to the non-inoculated control. This indicated that there was likely an antagonistic interaction occurring between the two pests, as each one individually is capable of causing significant yield loss.

Historically, species of *Diaporthe* have caused more yield losses than any other fungal pathogens on soybean (Sinclair 1993). Four pathogens have been reported on soybean in the United States. *D. caulivora*, *Diaporthe longicolla* (Hobbs) Santos,

Vrandecic and Phillips, (causal agent of Phomopsis seed decay and pod and stem blight), *Diaporthe sojae* (Lehman) Wehm, (causal agent of pod and stem blight), and *Diaporthe aspalathi* van Rensburg, Castlebury and Crous (causal agent of southern stem canker) (Xue et al. 2007). The monetary losses associated with the disease that are caused by these pathogens is estimated to be a total of $363.9 million in losses for the United States (Bradley et al. 2014). Pathogens from the *Diaporthe-Phomopsis* complex can attack soybean at all stages of plant development, however symptoms are not observed until later in the season during the reproductive stages (Xue et al. 2007).

In South Dakota, *D. caulivora* causes scattered outbreaks on soybean in commercial fields (Zhang and Chase 2004). The estimated reduction in soybean yield has increased from 0.1 to 0.2 million bushels during 1996-1998 to approximately 0.1 to 0.8 million bushels during 1999-2002 in South Dakota (Wrather et al. 2001; Wrather et al.2003). Recently, Gebreil et al. (2015) and Kontz et al. (2016) identified *D. longicolla* as a causal agent of pod and stem blight in South Dakota. Additionally, Kontz et al. (2016) determined that the presence of *D. caulivora* and *D. longicolla* as a complex on soybean in South Dakota.

In South Dakota, the distribution of SCN overlaps with that of fungal diseases that affect soybean through the reproductive stages (F. Mathew, *unpublished*). However, there is limited information available on how the outcome of the interaction between SCN and causal agents of fungal diseases (such as *D. caulivora* and *D. longicolla*) that can affect soybean production and growth. The objectives of this study are to evaluate (i) the effect of interaction between SCN and species of *Diaporthe* on stem length of soybean; (ii) the lesion length caused by *D. caulivora* and *D. longicolla* on soybean in the presence of SCN;

and (iii) SCN reproduction on soybean plants in the presence of *D. caulivora* and *D. longicolla*. For the purpose of this chapter, interaction will be defined as "the effects organisms in a shared community have on one another" (Raven and Johnson 2002).

Materials and Methods

Diaporthe longicolla and *Diaporthe caulivora* inoculum

In this study, a *D. caulivora* isolate (SD-23; Hamilin County, SD; GenBank accession number KT895384 and KT895398) and a *D. longicolla* isolate (SD-16; Union County, SD; GenBank accession number KT895379 and KT895393) were used. These isolates were identified to be *D. caulivora* and *D. longicolla* respectively by Gebreil et al. (2015) by sequencing the internal transcribed spacer (ITS) gene region (White et al. 1990) and the elongation factor 1-alpha (EF1-α) gene region (Carbone and Kohn 1999).

For the preparation of fungal inoculum, the two *Diaporthe* isolates SD-16 and SD-23 were cultured on potato dextrose agar (PDA) amended with 0.60 g streptomycin sulfate for 10 days with a photoperiod of 12:12 light: dark at approximately 21°C. Potato dextrose agar was made from fresh potatoes (*Solanum tuberosum* L.) according to methods described by Leslie and Summerell (2006). Briefly, the potato infusion was prepared by boiling 400 g of potatoes in deionized water, after which the infusion was strained through cheese cloth and the volume was brought up to 2000 ml using deionized water, 40 g of dextrose anhydrous (VWR Analytical, Radnor, PA) and 40 g of agar (Alfa Aesar, Ward Hill, MA) was added into the infusion. The mixture was autoclaved (121°C at 15 psi for 15 minutes) and allowed to cool to 50°C before adding 0.60 g of streptomycin sulfate (AMRESCO, Solon, OH).

SCN population

Populations of *H. glycines* HG type 0 were extracted from a soil sample collected from Clay County, SD, which was determined to be HG type 0 in a study conducted by Acharya et al. (2016). The HG type 0 population of *H. glycines* was selected for this study because it is the most common HG type recovered in South Dakota (Acharya et al. 2016). Populations of *H. glycines* were increased on the susceptible soybean cv. Williams 82 in a greenhouse water bath set at a temperature of approximately 26°C and a greenhouse temperature of approximately 23.8-30°C with a 16 hour photo period (16 light: 8 dark).

Interaction between SCN and *Diaporthe* on soybean

For this study, our hypothesis was that the lesion length formed as a result of infection by *D. longicolla* and *D. caulivora* on soybean would be reduced in the presence of SCN, as demonstrated by Russin et al. (1989). To test our hypothesis, experiments were conducted in a greenhouse water bath. Seeds of cv. Williams 82 were surface sterilized with 10% bleach and planted into cone-tainers containing a steam pasteurized 3:1 (sand: clay) mixture. Cone-tainers were inserted into 12 buckets (7.75L) containing sand. Buckets were placed into a greenhouse water bath that was maintained at a temperature range of 23.8-27.2°C. The ambient air temperature of the greenhouse was maintained at 20-22°C. Seedlings were thinned down to one plant per cone-tainer after plant emergence.

For *D. caulivora* and *D. longicolla*, the experiment was set up independently as a completely randomized design (CRD) and consisted of five treatments (fungal check, SCN check, concomitant infestation of SCN and fungus, fungus 15 days before SCN, and SCN 15 days before fungus (Table 1)). The treatments were replicated a total of 5 times

per experimental repeat, and each soybean plant was considered as a replication. The experiment was performed twice.

For *Diaporthe* inoculations, the cut seedling assay was used for *D. caulivora* (Thicket et al. 2007) and *D. longicolla* (Li et al. 2010). The cut seedling assay was performed by making a wound on the soybean stem with a sterile micro-pipette tip (200 µL) between the unifoliate node and the first trifoliate node. A four mm mycelial plug from a 10 day old culture of either *D. caulivora* or *D. longicolla* was cut from the advancing edge of the plate and was secured to the wound with Parafilm.

For SCN inoculations, cysts were ground using a drill press with a rubber stopper and a number 60 screen to release eggs (Alfa Aesar, Haverhill, MA). Grinding of cysts was performed over two sieves to capture the cysts that were released. A NO. 200 sieve with a 75 µm screen was placed on top of a NO. 500 sieve with a 25 µm screen to capture eggs (Faghihi et al. 1986). Eggs were enumerated using a hemocytometer and 2000 eggs were added to each cone-tainer at the base of each soybean plant receiving SCN when plants reached the cotyledon (VC) growth stage for the SCN check and for the treatment where SCN precedes the fungus. Infestation with SCN also took place 15 and 30 days after the first SCN infestation for the concomitant infestation where SCN and fungus were introduced at the same time (15 days after) and when fungus preceded SCN (30 days after).

The experiment was terminated 72 days after planting (pod development stage; approximately R3-R4 growth stage). The lesion length (lesion on the stem) caused by *D. longicolla* and *D. caulivora* as well as the stem length (from the seed attachment to the top of the plant) were measured. The reproduction of SCN on soybean was assessed by

counting the eggs and juveniles extracted from soybean roots for all experiments. Enumeration of SCN was performed using a nematode counting slide (Chalex Cooperation, Portland, OR) under a dissecting microscope at 40X magnification (Nikon SMZ745T, Nikon Instruments, Canada). Fresh root weight of the plants was measured to calculate SCN eggs per root gram.

Statistical Analysis

To determine the effect of SCN on either *D. longicolla* or *D. caulivora* and to determine the effect both *Diaporthe* species had on SCN, data (lesion length, stem length and SCN eggs counts) collected at 72 days after planting was analyzed using the linear mixed model analysis in R version 3.2.4 (http://cran.rproject.org/bin/windows/base/) (R Core Team, 2015) using the *lme4* package (Bates et al. 2015). While treatment was regarded as the fixed effect in the mixed model, replication was regarded as the random effect in the model. In order to obtain *P*-values, a likelihood ratio test of the full model with the effect in question (treatment) was tested against the reduced model without the effect in question (Winter 2013). The fixed effect was considered significant if the difference between the full model and the reduced model was significant ($P<0.05$).

The homogeneity of variance was tested between the two experimental repeats in R, and it was determined that the data obtained from the two experiments could not be pooled for least significant difference (LSD) analysis. Because homogeneity of variances was not satisfied, the two experimental repeats were analyzed separately. Stem length, lesion length and SCN egg count data was subjected to analysis of variance (ANOVA) and treatment means were separated using Fisher's LSD test at $P<0.05$ in R using the *Agricolae* package (de Mendiburu 2014).

Results

Interaction between SCN and *Diaporthe* on Soybean

Experiment One

Diaporthe longicolla

Lesion length: Test statistics indicated that the treatments did not significantly affect the lesion length produced by *D. longicolla* on plants of cv. Williams 82 (χ^2=4.24, df=3; *P*=0.23). Based on the ANOVA using LSD analysis, significant differences in lesion length were not observed among treatments (LSD=74.50; *P*=0.32) (Fig. 1). However, a 38% increase in lesion length was observed on soybean stems when SCN and *D. longicolla* were introduced at the same time as compared to the *D. longicolla* check. When SCN preceded *D. longicolla* on soybean, lesion length was increased by 76% on soybean stems, and when *D. longicolla* preceded SCN, lesion length increased by 15% on soybean stems when compared to the *D. longicolla* check (Fig. 1).

Stem length: Test statistics indicated that the treatments did not significantly affect stem length on plants of cv. Williams 82 (χ^2=3.71; df=3;*P*=0.29). Based on the ANOVA using LSD analysis, no significant differences in stem length was observed among treatments (LSD=27.30; *P*=0.47) (Fig. 2). However, a 3% decrease in stem length was observed when *D. longicolla* and SCN were introduced as compared to the *D. longicolla* check. When SCN precedes *D. longicolla*, there is an 11% decrease in stem length when compared to the *D. longicolla* check. Additionally, when *D. longicolla* precedes SCN, stem length was reduced by 7% when compared to the *D. longicolla* check (Fig. 2).

SCN egg counts: Test statistics indicate that treatments had a significant effect on SCN egg counts on plants of cv. Williams 82 (χ^2=11.45; df=3; *P*=0.009). Based on the ANOVA using LSD analysis, significant differences in SCN egg counts (per root gram) were observed among treatments (LSD=13848.00; *P*=0.02) (Fig. 3). When both pests were introduced at the same time on cv. Williams 82, SCN populations were significantly reduced by 65% when compared to the SCN check. When SCN preceded *D. longicolla*, SCN reproduction was significantly increased by 95% when compared to the SCN check. Additionally, when *D. longicolla* preceded SCN, SCN reproduction was significantly reduced by 98% when compared to the SCN check (Fig. 3).

Diaporthe caulivora

Lesion length: Test statistics did not indicate a significant effect of the treatments on lesion length caused by *D. caulivora* on plants of cv. Williams 82 (χ^2=4.45, df=3; *P*=0.22). Based on ANOVA using LSD analysis, significant differences in lesion length were not observed among treatments (LSD=71.60; *P*=0.32) (Fig. 4). When both pests were introduced at the same time, there was a 16% decrease in lesion length on the stems of cv. Williams 82 when compared to the *D. caulivora* check. When SCN preceded *D. caulivora*, a 35% decrease in lesion length was observed. Additionally, when *D. caulivora* preceded SCN, a 29% decrease in lesion development was observed on soybean stems when compared to the *D. caulivora* check.

Stem length: Test statistics indicated a significant effect of treatments on stem length on plants of cv. 'Williams 82' (χ^2=23.90, df=3; *P*<0.001). Based on the ANOVA using LSD analysis, significant differences in stem length were observed among

treatments (LSD=18.4; *P*=0.0002) (Fig. 5). Stem length was significantly reduced by 2% when both pests were introduced at the same time when compared to the *D. caulivora* check. When SCN preceded *D. caulivora*, stem length was significantly reduced by 24% when compared to the *D. caulivora* check. When *D. caulivora* preceded SCN, stem length was significantly reduced by 15% when compared to the *D. caulivora* check (Fig. 5).

SCN egg counts: Test statistics indicate a significant effect of treatments on egg counts on cv. Williams 82 (χ^2=20.36; df=3; *P*<0.001). Based on ANOVA using LSD analysis, significant differences in SCN reproduction were observed among treatments (LSD=9063.00; *P*=0.0005) (Fig. 6). Soybean cyst nematode egg counts per root gram were significantly reduced by 95% when both pests were introduced together when compared to the SCN check. When SCN preceded *D. caulivora*, SCN egg counts per root gram were significantly reduced by 45% when compared to the SCN check. Additionally, when *D. caulivora* preceded SCN egg counts per root gram were significantly reduced by 97% when compared to the SCN check (Fig. 6).

Experiment Two

Diaporthe longicolla

Lesion length: Test statistics indicate a significant effect of treatment for lesion length caused by *D. longicolla* on plants of cv. Williams 82 (χ^2=12.17,df=3, *P*=0.006). Based on the ANOVA using LSD analysis, significant differences in lesion length were observed among treatments (LSD=73.24; *P*=0.02) (Fig. 7). When compared to the *D. longicolla* check, lesion length was significantly increased by 231% in the concomitant infestation. When SCN preceded *D. longicolla*, lesion length was significantly increased

by 546% when compared to the *D. longicolla* check. When *D. longicolla* preceded SCN, lesion length was significantly increased by 272% when compared to the *D. longicolla* check (Fig. 7).

Stem length: Test statistics indicate no significant effect of treatment for stem length on plants of cv. Williams 82 (χ^2=3.67, df=3; *P*=0.29). Based on the ANOVA using LSD analysis, significant differences were not observed among treatments (LSD=29.2; *P*=0.5) (Fig. 8). However, stem length was increased by 7% in the concomitant infestation when compared to the stem length of the *D. caulivora* check. When SCN preceded *D. longicolla*, stem length was decreased by 3% and when *D. longicolla* preceded SCN, stem length was decreased by 2% when compared to the stem length of the *D. caulivora* check (Fig. 8).

SCN egg counts: Test statistics indicate a significant effect of treatment for egg counts on plants of cv. Williams 82 (χ^2=28.5, df=3; *P*<0.001). Based on the ANOVA using LSD analysis, significant differences were observed among treatments (LSD=5870; *P*<0.001) (Fig. 9). When both pests were introduced concomitantly, egg counts per root gram were significantly reduced by 21% when compared to the SCN check. When SCN preceded *D. longicolla*, egg counts per root gram were significantly reduced by 23% when compared to the SCN check. Additionally, when *D. longicolla* preceded SCN, egg counts per root gram was significantly reduced by 97% when compared to the SCN check (Fig. 9).

Diaporthe caulivora

Lesion length: Test statistics indicated no significant effect of treatment for lesion length caused by *D. caulivora* on plants of cv. Williams 82 (χ^2=3.77, df=3; *P*=0.28). Based on our ANOVA using LSD analysis, there was no significant interaction among treatments (LSD=112.00; *P*=0.41) (Fig. 10). However, there was a numerical difference among treatments when compared to the *D. caulivora* check. For example, when both pests were introduced at the same time, lesion length was increased by 39% when compared to the *D. caulivora* check. When SCN preceded *D. caulivora*, lesion length increased 198%, and when *D. caulivora* preceded SCN, lesion length was increased by 15% when compared to the check (Fig. 10).

Stem length: Test statistics indicated a significant effect of treatment for stem length on plants of cv. Williams 82 (χ^2=6.74, df=3; *P*=0.08). Based on the ANOVA using LSD analysis, no significant differences in stem length were observed among treatments (LSD=35.40, *P*=0.13) (Fig. 11). However, when both pests were introduced at the same time, stem length increased by 2% when compared to the stem length of the *D. caulivora* check. When SCN preceded *D. caulivora*, stem length of soybean cv. Williams 82 was increased by 3% when compared to the stem length of the *D. caulivora* check. Additionally, when *D. caulivora* precedes SCN, stem length was reduced by 17% when compared to the stem length of the *D. caulivora* check (Fig. 11).

SCN egg count: Test statistics indicated a significant effect of treatment on SCN egg counts on plants of cv. Williams 82 (χ^2=28.7; df=3; *P*<0.001). Based on ANOVA using LSD analysis, significant differences in SCN egg counts per root gram were observed among treatments (LSD=5869.00; *P*<0.001) (Fig. 12). When both pests were

introduced at the same time we saw a decrease of 43% in SCN egg counts per root gram when compared to SCN the check. When SCN preceded *D. caulivora,* SCN egg counts per root gram were only reduced by 4% when compared to the SCN check. Additionally, when *D. caulivora* preceded SCN, egg counts were reduced by 90% when compared to the SCN check (Fig. 12).

Discussion

The objective of this study were to investigate interactions between SCN and *D. caulivora*, as well as between SCN and *D. longicolla* on soybean in the greenhouse. For *D. longicolla*, having SCN precede *D. longicolla* resulted in the greatest lesion length in experiment one and two. For SCN reproduction, having *D. longicolla* preceded SCN resulted in a significant reduction in egg counts per root gram when compared to the SCN check for both experiments. In addition, we did not observe significant reductions on stem length for any of the treatments in both *D. longicolla*- SCN interaction experiments. For *D. caulivora*, having SCN precede *D. caulivora* resulted in the greatest lesion length reduction in the treatment were SCN preceded *D. caulivora* in experiment one. For SCN reproduction, having *D. caulivora* precede SCN resulted in the greatest reduction in SCN egg counts per root gram than any other treatment for both experiments. In addition, we observed significant reductions in stem length in experiment one of *D. caulivora*- SCN interaction study, but not in experiment two in the treatments where either of the pests preceded the other we see the greatest reduction in stem length.

The interaction between SCN and *D. longicolla* did not have a significant effect on stem length for both experiments. However, when SCN preceded *D. longicolla* by 15 days, we observed the greatest reduction in stem length for the *D. longicolla*- SCN

interaction in both experiments. For the SCN- *D. caulivora* interaction, we observed the greatest reduction in stem length when SCN precedes *D. caulivora* by 15 days for experiment one, but not in experiment two. It is quite possible that in both our interaction experiments, SCN was reducing the stem length of the soybean plants as opposed to *D. caulivora* or *D. longicolla*. Similar observations were made by Pacumbaba et al. (1992) when they measured the plant height of soybean plants while investigating SCN- *D. caulivora* interaction in the field. Pacumbaba et al. (1992) determined that infestations of SCN on its own reduced soybean stem length (plant height) more than infestations of *D. caulivora* for all three years in their study.

While studying the effect SCN had on the development of lesion length caused by the two *Diaporthe* species on soybean, we observed different outcomes for *D. longicolla* and *D. caulivora*. When we compared the results for both the experiments in the SCN- *D. longicolla* interaction, we observed greater lesion length caused by *D. longicolla* when preceded by SCN by 15 days than when compared to the *D. longicolla* check (Figs. 1, 3, 7, and 9). In contrast to the SCN- *D. longicolla* interaction, we observed an overall reduction in lesion length caused by *D. caulivora* among all treatments in the presence of SCN in experiment one of the SCN- *D. caulivora* interaction. For example, when we consider the treatment where SCN precedes *D. caulivora* (Fig. 4), we observed a 35% decrease in lesion length when compared to the *D. caulivora* check in experiment one. The decrease in lesion length caused by *D. caulivora* on cv. Williams 82 may be due to the fact that Williams 82 is moderately resistant to *D. caulivora* (McGee and Biddle 1987). However, SCN is known to decrease in lesion length caused by *D. caulivora* when SCN was colonizing the roots of the soybean plants (Russin et al. 1989). Russin et al.

(1989) hypothesized that SCN may be causing subtle physiological changes in the soybean plants which is influencing the lesion length produced by *D. caulivora* on soybean. Soybean cyst nematode can induce gene expression in susceptible soybean plants during infection, and many of the induced genes are not well understood (Alkharouf et al. 2006). Hence, it is possible that soybean plants infested with SCN are up-regulating or down-regulating genes that is affecting the ability of *D. caulivora* or *D. longicolla* to establish on soybean stems. For example, in a study by Ithal et al. (2006), it was demonstrated that SCN can down regulate jasmonic acid biosynthesis and signaling of the plant during syncytia formation. Jasmonic acid is a small signaling molecule that plays a role in basal resistance against necrotrophic pathogens (Pozo et al. 2005). As for *D. caulivora* or *D. longicolla*, if the up-regulation or down regulation of jasmonic acid biosynthesis by SCN allows for the infection of soybean by the two pathogens need further research.

When investigating the effect *D. caulivora* or *D. longicolla* had on SCN on soybean, we observed that both the pathogens reduced the ability of SCN to reproduce on plants of cv. Williams 82 in this study. When *D. caulivora* or *D. longicolla* is introduced before SCN, the reproduction of SCN is reduced by 90% or greater in both the experiments. Necrotrophic fungi, like *Diaporthe* species, are known for the production of secondary metabolites (Tan and Zou 2001). For example, *D. longicolla* produces metabolites that produce disease symptoms on soybean, which has been demonstrated in a study by Ivanovic and Sinclair (1989). Additionally, research by Lalitha et al. (1989) has shown that *D. caulivora* produces metabolites (toxins) that play a key role in the disease development of northern stem canker on soybean. Such metabolites may affect

the ability of other soybean pathogens like SCN from establishing on soybean roots. Another possible reason for the reduced SCN egg counts on the root of the soybean plants colonized by *D. caulivora* or *D. longicolla* is the possibility of the fungus manipulating the release of chemical exudates (eclepins) from the host plant, which enables SCN to hatch and sense soybean plants (Davis and Tylka 2000; Rasmann et al. 2012). Further investigations of *Diaporthe*- SCN interactions are needed to determine the precise cause of the reduction in SCN reproduction on soybean when *D. caulivora* or *D. longicolla* is present.

In summary, our results demonstrate that there is a possible interaction occurring between SCN and *Diaporthe* on soybean. Despite our best efforts, our results were not reproducible between the two experiments. For example, the lesion length caused by *D. longicolla* and *D. caulivora* was greater in experiment one than experiment two for most treatments, probably because greenhouse conditions influencing lesion development differed between repetitions. This was likely due to low relative humidity in the greenhouse when the experiments were performed. Typically, pathogenicity tests involving *Diaporthe* species are at a high relative humidity of 85-90% in the greenhouse (McGee and Biddle 1987). Our greenhouse study was conducted at a relative humidity of 30-40%. However, these greenhouse studies demonstrate that an interaction is occurring between *Diaporthe* and SCN, and the interaction should be tested under field conditions. Past studies have demonstrated that concomitant infestations of *D. caulivora* and SCN have an antagonistic relationship in the field and actually resulted in greater yield of soybean than when both pests were introduced separately (Pacumbaba 1992). However, no field trials have been conducted to look at potential interactions between *D. longicolla*

as a stem pathogen and SCN in natural conditions. At this time, SCN is managed by planting soybean varieties with resistance to the nematode (e.g. PI 88788). As for managing species of *Diaporthe*, most varieties grown in the North Central United States including in South Dakota have resistance to *D. caulivora*, however it is unclear if these varieties have resistance to *D. longicolla* also. However, if the soybean farmers use cultivars with resistance to SCN in combination with rotation to non-host crops (e.g. corn or wheat), it is possible to manage the disease complex caused by the two pathogens and protect yield in their fields.

Acknowledgements

We would like to thank Alec Weber, Brian Kisely, and Paul Okello for help with setting up the experiment and collecting data. We also thank the South Dakota Soybean Research and Promotional Council and the North Central Soybean Research Program for funding this project.

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Table 1.1. Timing for infestations of fungus and SCN on soybean place at 15, 30, and 45 days after planting in the greenhouse.

	Days after plant germination		
Treatment	15 days	30 days	45 days
SCN check	SCN	None	None
Diaporthe check	None	Fungus	None
SCN 15 days before Fungus	SCN	Fungus	None
Fungus 15 days before SCN	None	Fungus	SCN
Concomitant infestation of both pathogens	None	Fungus and SCN	None

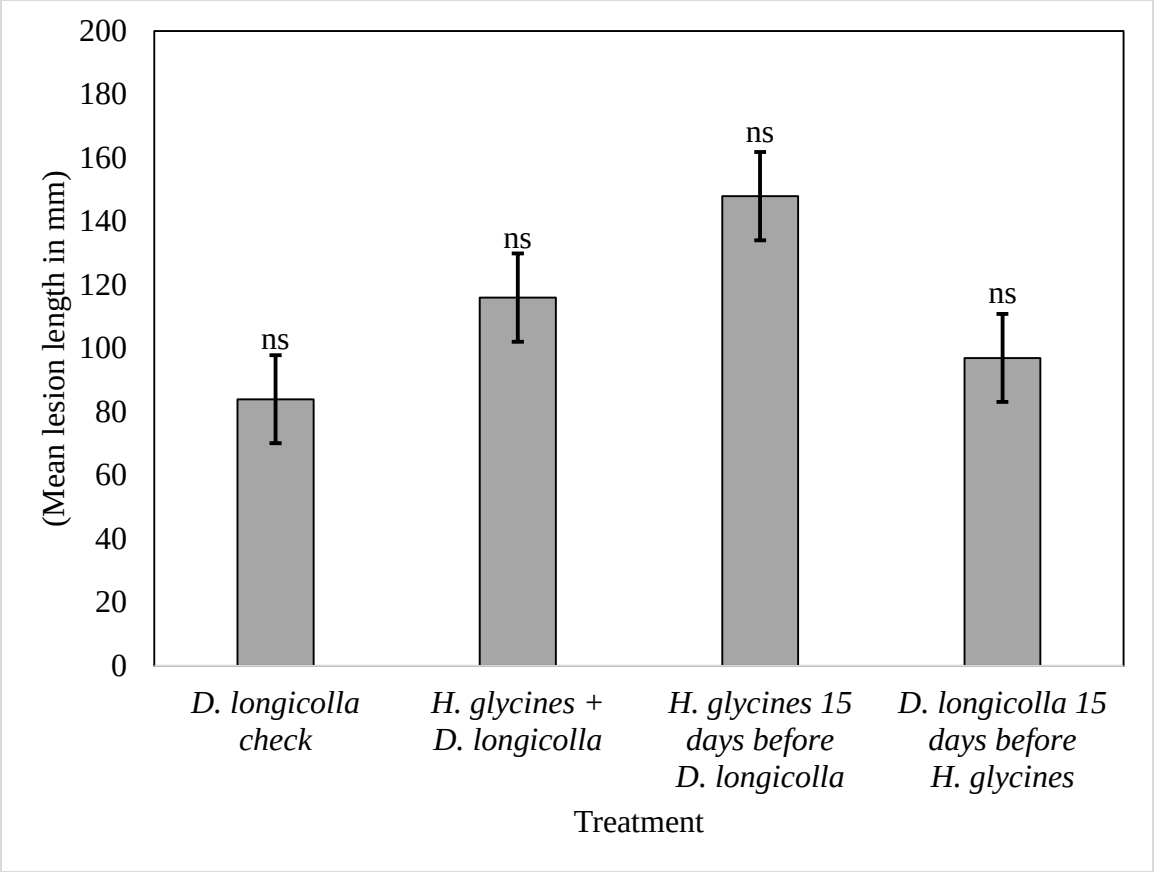

Fig. 1.1. The effect of SCN on lesion length caused by *D. longicolla* when the two pests infected soybean plants for experiment one. No significant differences in lesion length were observed among treatments (*P*>0.05).

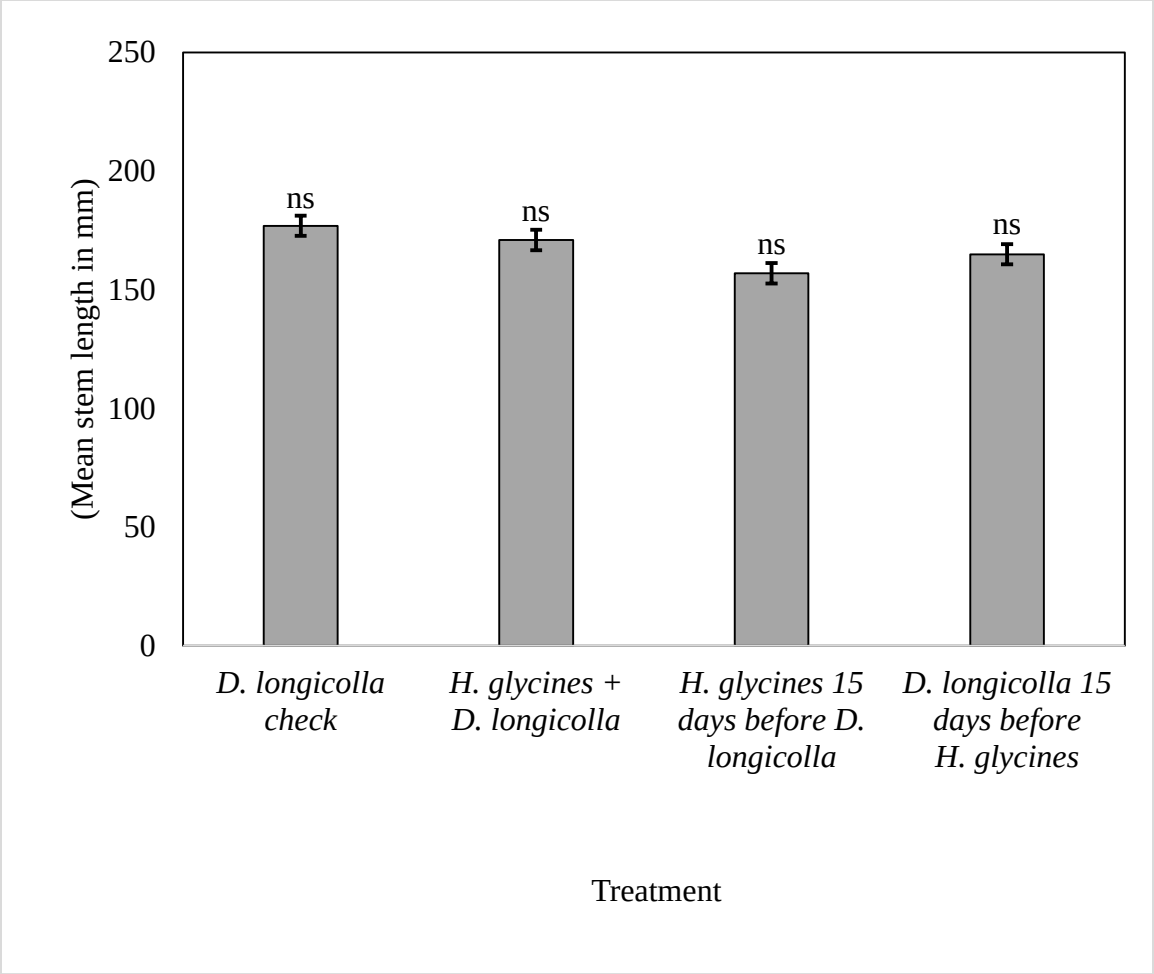

Fig. 1.2. The effect of *D. longicolla* and SCN on stem length when the two pests infected soybean plants for experiment one. No significant differences in stem length were observed among treatments (*P*>0.05).

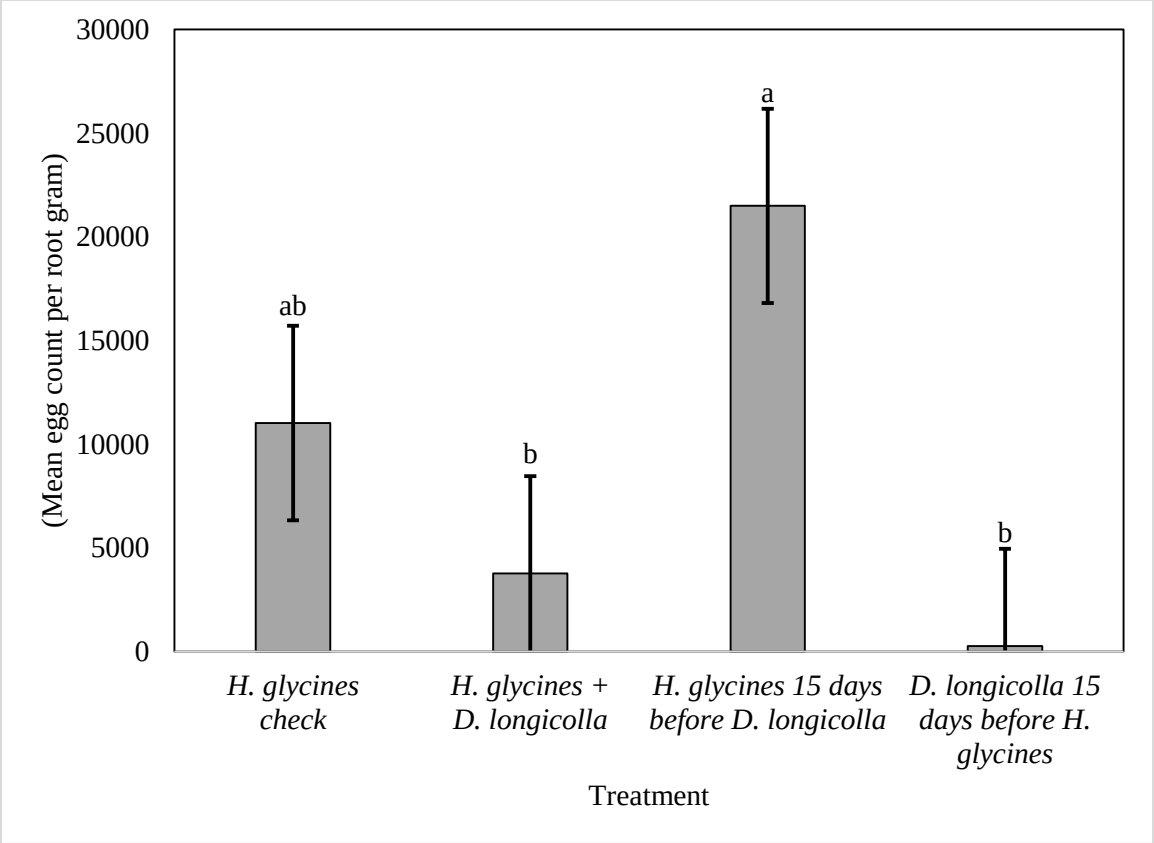

Fig. 1.3. The effect of *D. longicolla* on SCN reproduction when the two pests infected soybean plants for experiment one. Significant differences in egg counts per root gram were observed among treatments (*P*<0.05).

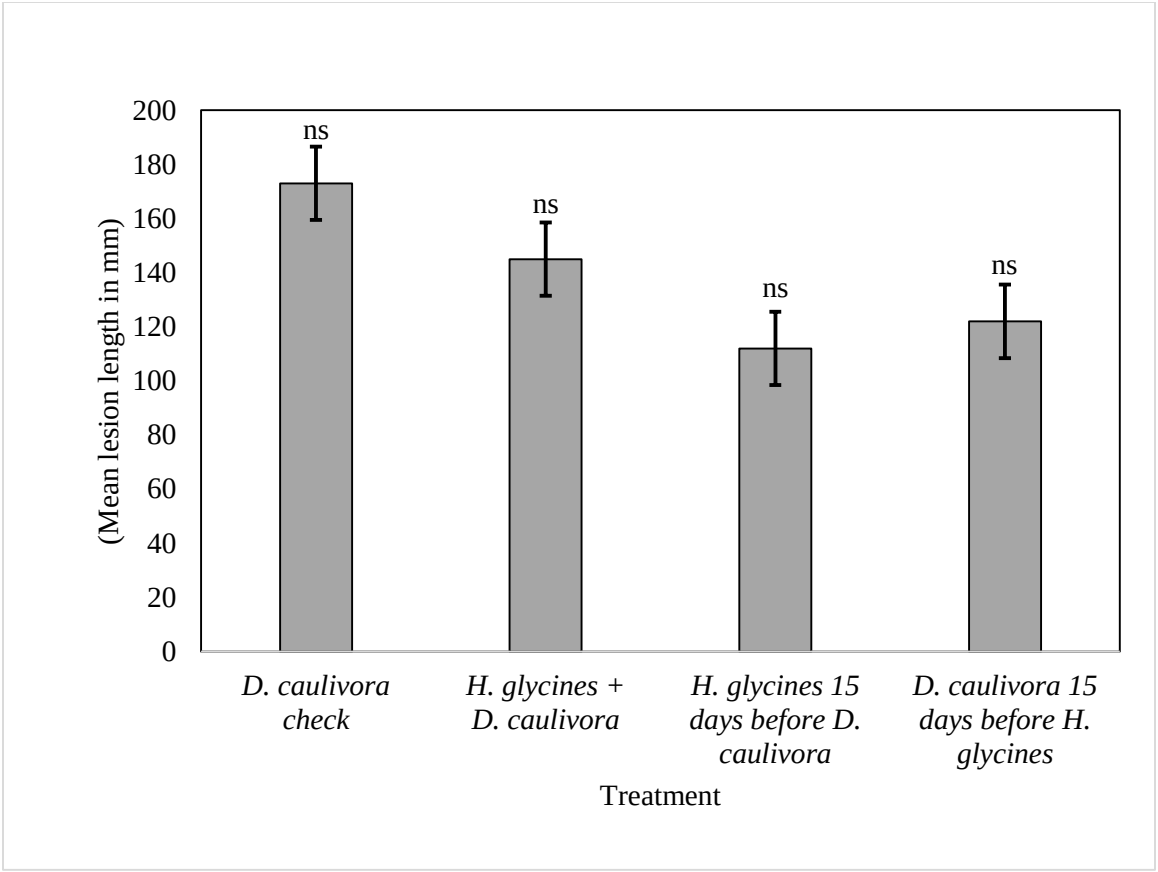

Fig. 1.4. The effect of SCN on lesion length caused by *D. caulivora* when the two pests infected soybean plants for experiment one. No significant differences in lesion length were observed among treatments (*P*>0.05).

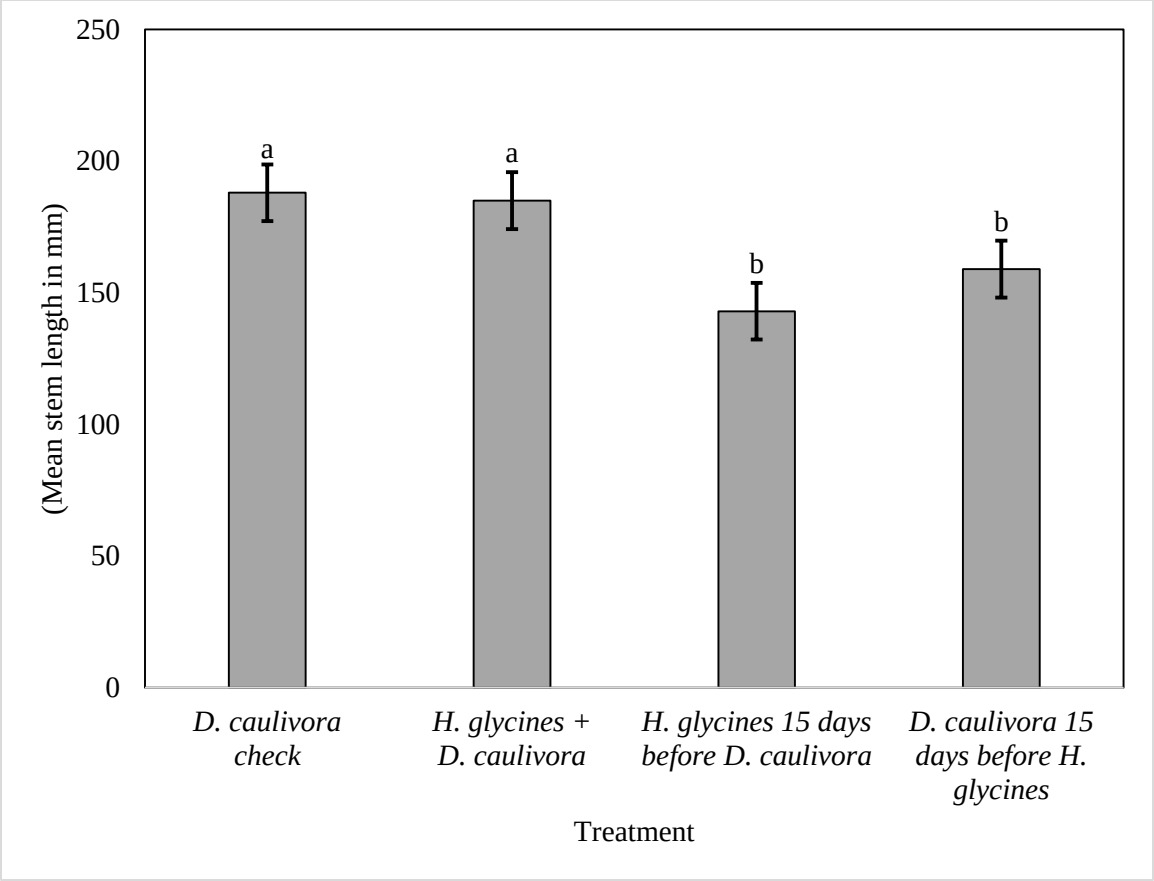

Fig. 1.5. The effect of *D. caulivora* and SCN on stem length when both pests infected soybean for experiment one. Significant differences in stem length were observed among treatments (*P*<0.05).

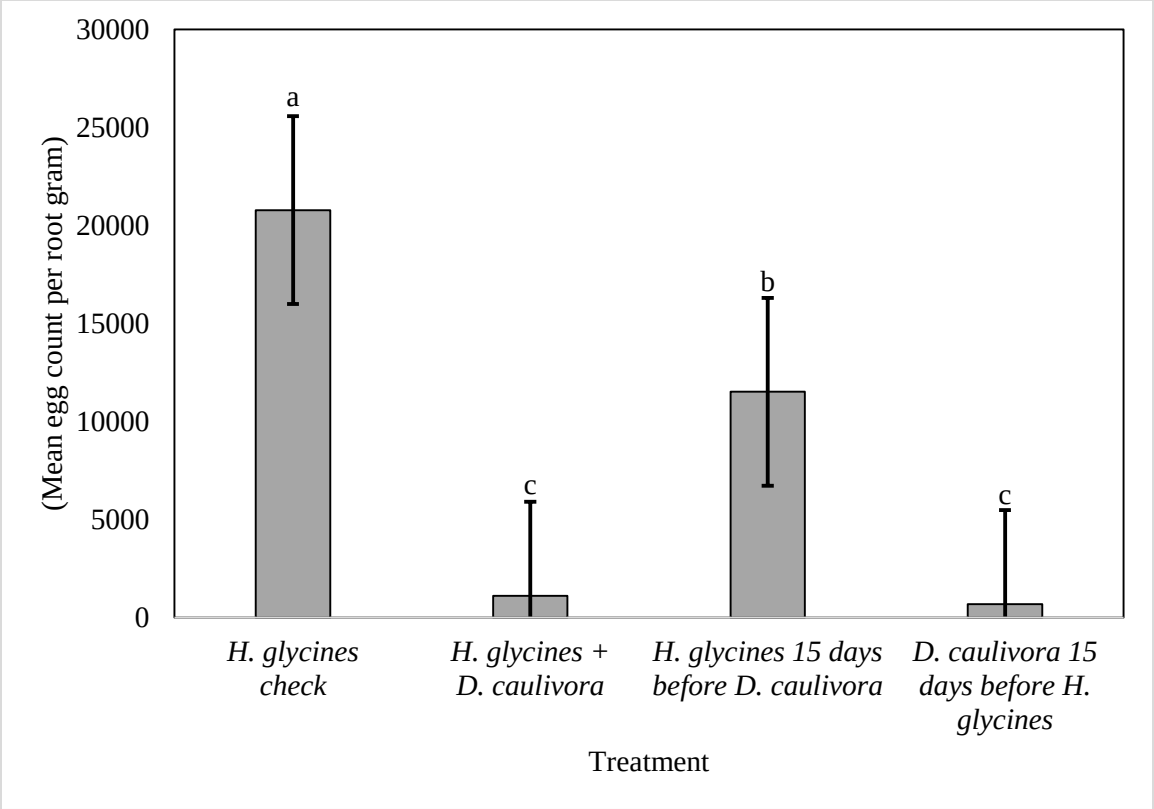

Fig. 1.6. The effect of *D. caulivora* on SCN reproduction when both pests infected soybean plants for experiment one. Significant differences in egg counts per root gram were observed among treatments (*P*<0.05).

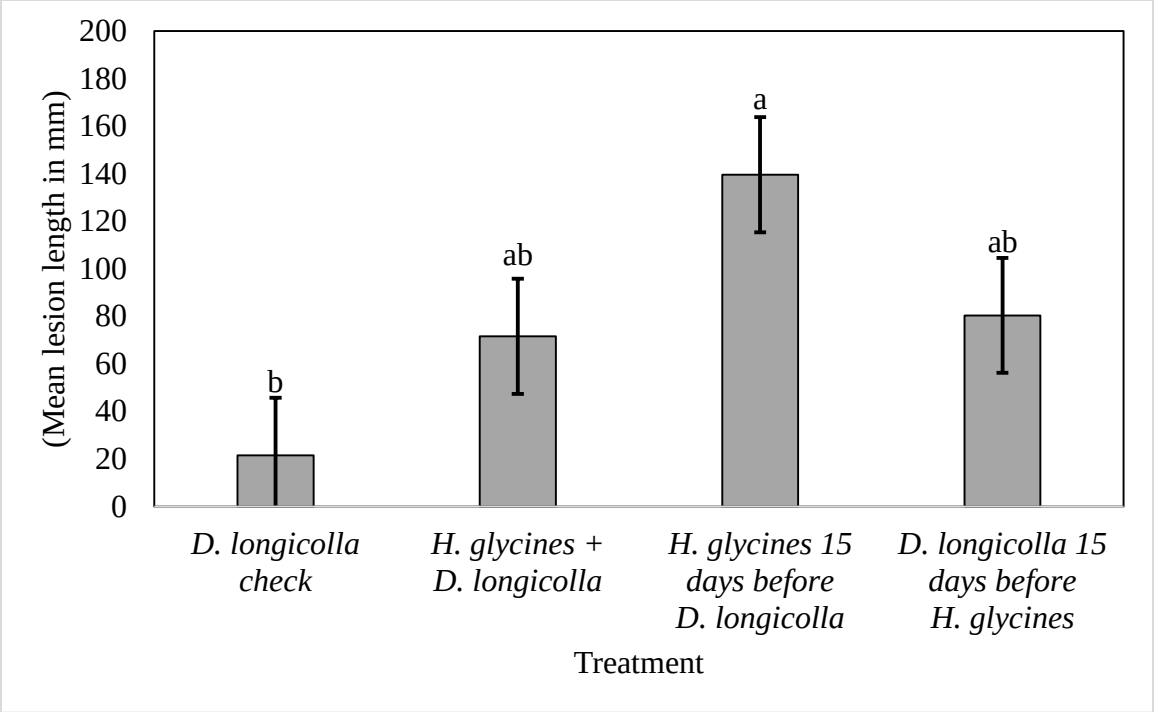

Fig. 1.7. The effect of SCN on lesion length caused by *D. longicolla* on soybean plants for experiment two. Significant differences in lesion length were observed among treatments (*P*<0.05).

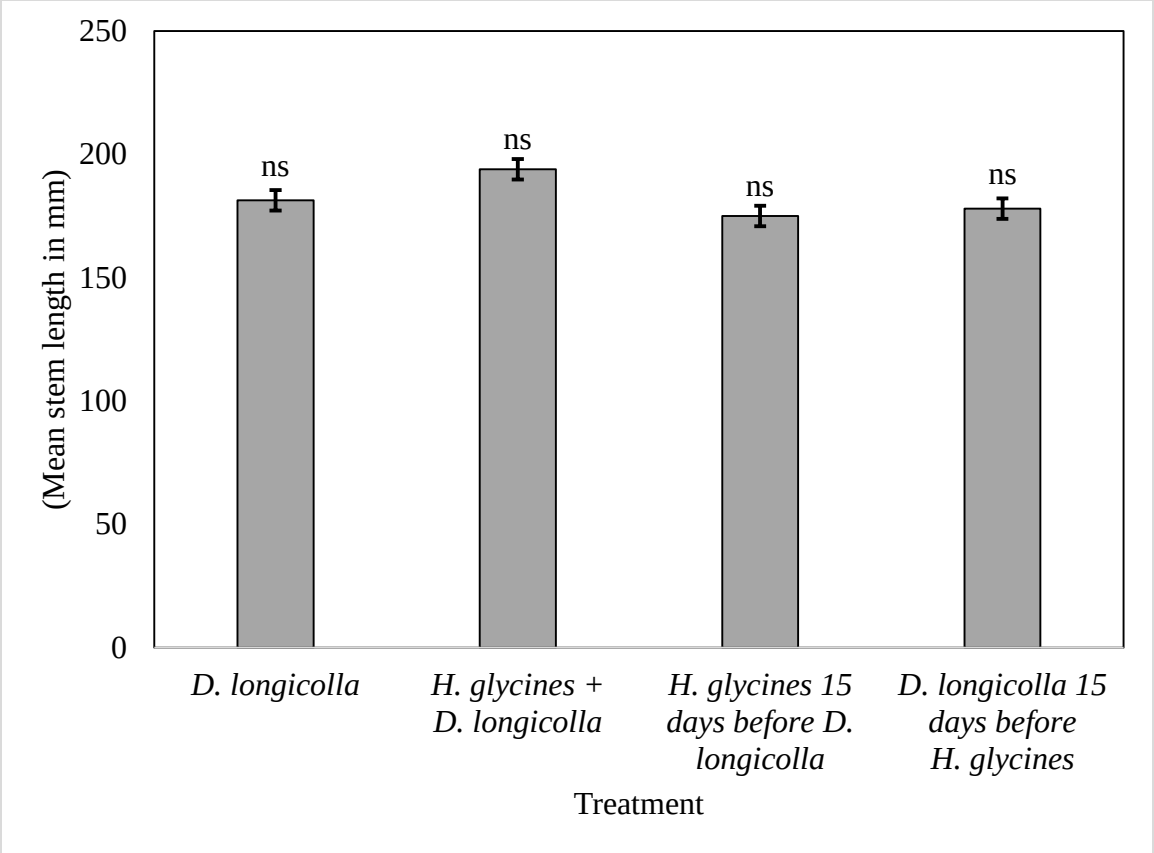

Fig. 1.8. The effect of *D. longicolla* and SCN on stem length when both pests infected soybean for experiment two. No significant differences in stem length were observed among treatments (*P*>0.05).

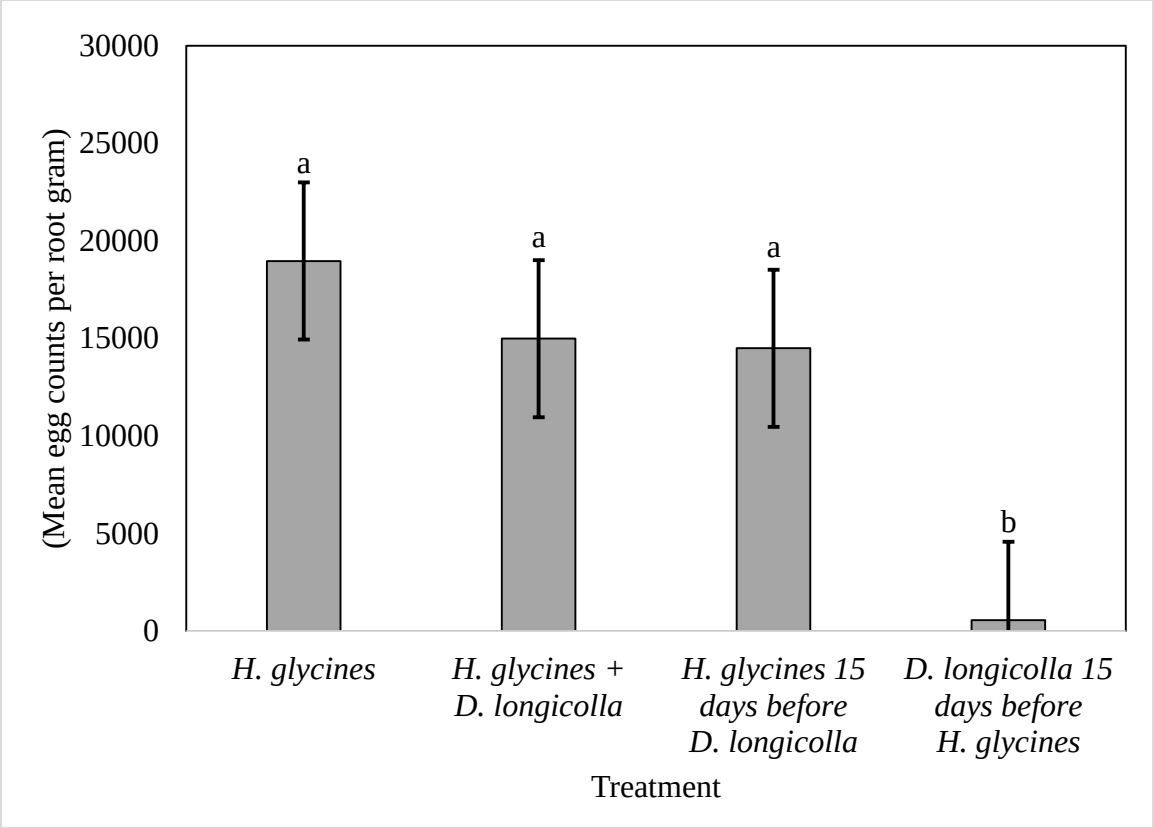

Fig. 1.9. The effect of *D. longicolla* on SCN egg counts per root gram when both pests infected soybean for experiment two. Significant differences in egg counts per root gram were observed among treatments (*P*<0.05).

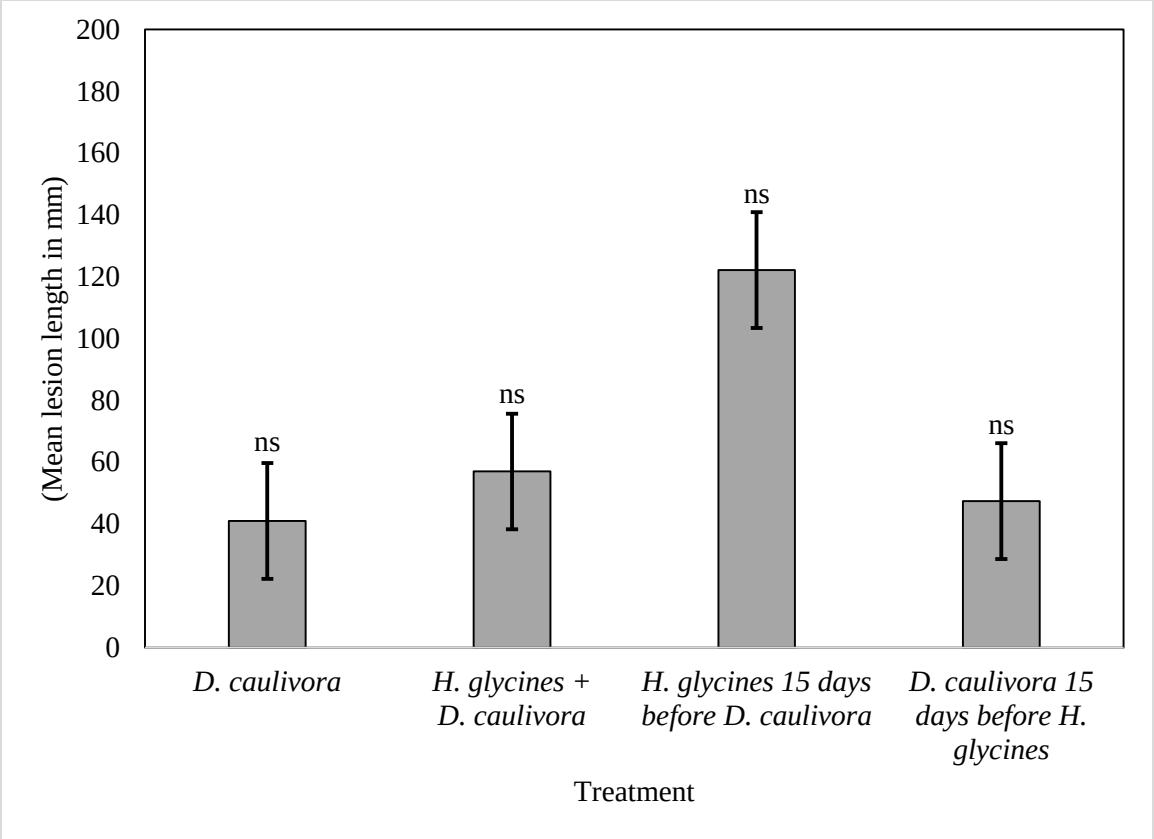

Fig. 1.10. The effect of SCN on lesion length produced by *D. caulivora* when both pests infected soybean for experiment two. No significant differences in lesion length were observed among treatments (*P*<0.05).

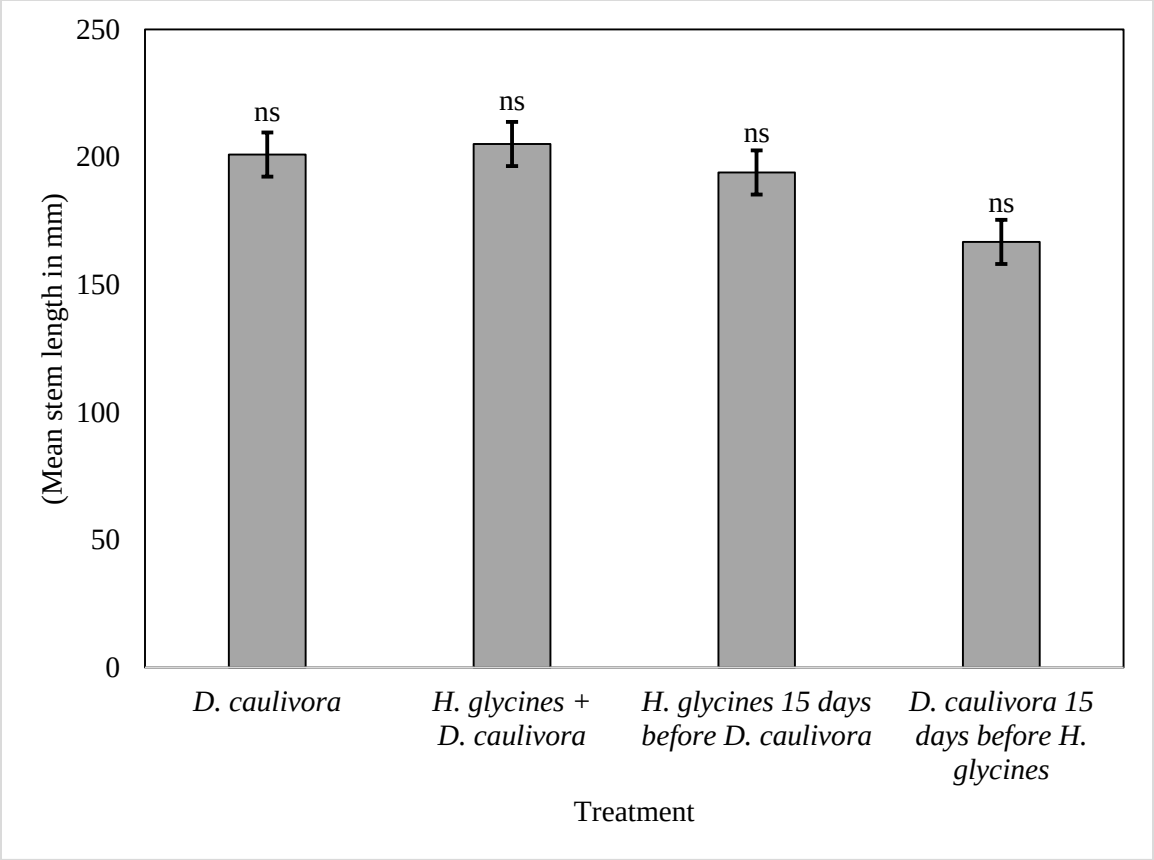

Fig. 1.11. The effect of *D. caulivora* and SCN on stem length when both pests infected soybean for experiment two. No significant differences in stem length were observed among treatments (*P*>0.05).

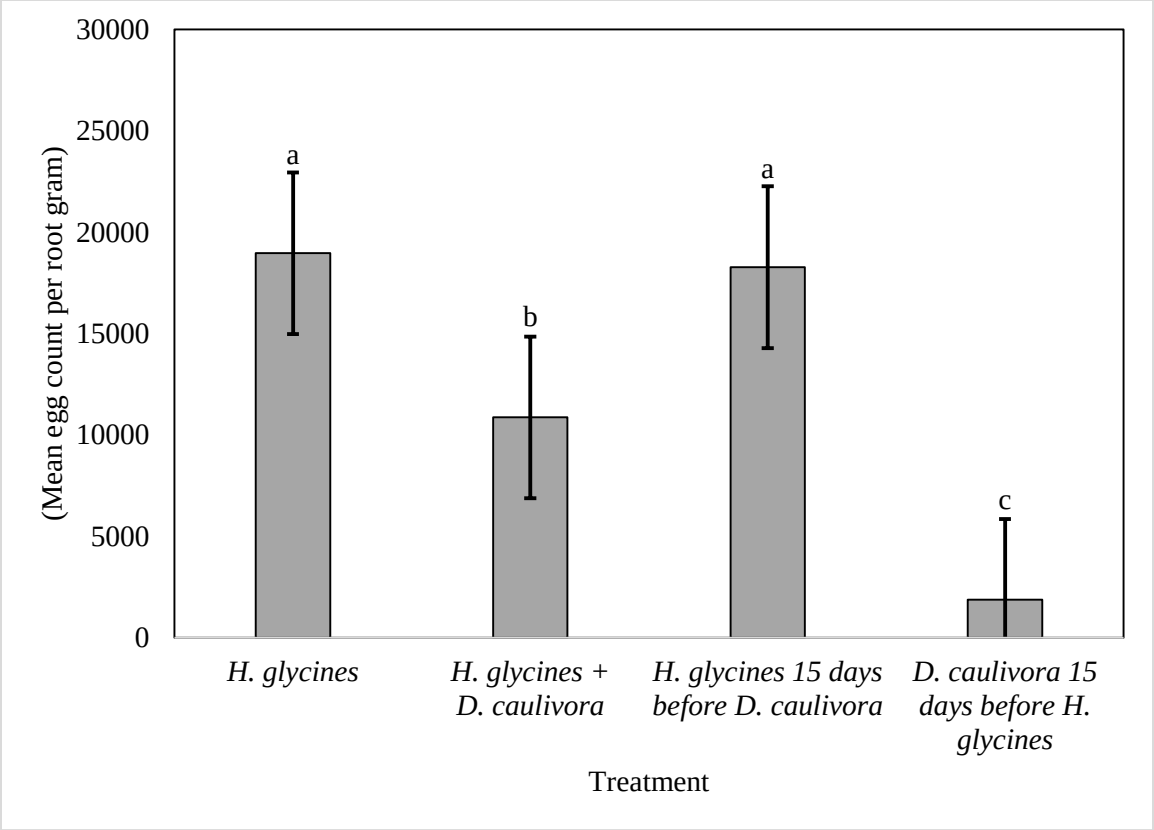

Fig. 1.12. The effect of *D. caulivora* on SCN when both pests infected soybean for experiment two. Significant differences in egg counts per root gram were observed among treatments (*P*<0.05)

Chapter 3: Effect of Soybean Aphid, *Aphis glycines* (Hemiptera: Aphididae), on Infection of Soybean by *Diaporthe longicolla*

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Abstract

Soybean, *Glycine max* (L.) Merr., is an economically important crop for South Dakota with an estimated $2.33 billion worth of soybeans produced in 2015. However, soybean production can be hindered by pests such as *Aphis glycines* Matsumura (Hemiptera: Aphididae) and *Diaporthe longicolla* (Hobbs) Santos, Vrandecic and Phillips. Additionally, these pests can co-occur in soybean fields in South Dakota and the implications associated with a potential interaction between these pests is unknown. We hypothesized that *A. glycines* and *D. longicolla* would not impact one another when co-inhabiting soybean plants. To test our hypothesis, we conducted an experiment using a completely randomized design with eight treatments. Each treatment consisted of two infestation times (Inducer: V1; response seven days later). Plants were either infested with five aphids or a four mm plug infested with *D. longicolla*. At 14 days after the introduction of the response populations, we determined that *A. glycines* and *D. longicolla* did not induce resistance or susceptibility of soybean cultivar 'Williams 82'. However, we observed a potential compensatory effect from soybean plants in the concomitant infestation of both *D. longicolla* and *A. glycines*, where *A. glycines* counts were reduced by 47% when both pests were introduced at the same time ($P<0.05$). Interactions among pests on soybean are a relatively understudied aspect of agricultural

science, such studies may lead to new targeted approaches for more inclusive management of soybean pests and disease complexes.

Introduction

Soybean, *Glycine max* (L.) Merr., is one of the most important crops in South Dakota. In 2015, soybean production accounted for approximately 235 million bushels that brought in approximately $2.33 billion in revenue for the state (USDA National Agricultural Statistics Services 2016; https://www.nass.usda.gov/). However, there are a number of abiotic and biotic factors that are capable of negatively affecting soybean production in South Dakota, among which diseases and insect pests are most important as they adversely affect plant vigor and yield.

Of the insect pests that can negatively affect soybean production, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is the most important in the Midwestern United States. *Aphis glycines* is capable of causing yield reductions of up to 40% through direct feeding, and causes additional losses through the indirect involvement in the growth of sooty mold and virus transmission (Ragsdale et al. 2011; McCarville et al.2014). Although first observed in Wisconsin in 2000, *A. glycines* has since been observed in 30 soybean producing states in the Midwest including South Dakota (Alleman et al. 2002; Ragsdale et al. 2011). In South Dakota, *A. glycines* infestation of soybean fields may occur at any time during the spring and summer. However, population outbreaks are typically observed during the end of July through August (Tilmon et al. 2011).

Varenhorst et al. (2015) found that inducer populations (i.e., the population that occurred on the soybean first) of avirulent *A. glycines* (i.e., biotype 1) improved the suitability of a susceptible soybean plant for response populations (i.e., subsequent

populations that were introduced to the plant). In the same study, they also determined that virulent *A. glycines* (i.e., biotype 2 that is known to overcome *Rag1* resistance) can induce susceptibility of *Rag1* resistant soybean for avirulent *A. glycines*. That is, induced susceptibility caused by a virulent biotype allowed the successful colonization of an avirulent biotype on a resistant soybean variety. This study indicated that *Aphis glycines* is capable of improving the soybean host. The induced susceptibility effect that was caused by *A. glycines* feeding suggests a potential for *A. glycines* to interact with other soybean pests during colonization of soybean. The induced susceptibility effect may be due in part by how aphids feed. *Aphis glycines* feed on the phloem of soybean plants using piercing-sucking mouth parts. While feeding *A. glycines* injects two types of saliva into the plant. In other aphid species, it is known that the watery saliva contains effector proteins (Hogenhout et al. 2011). Recently, Bansal et al. (2014) determined that *A. glycines* also have effector proteins. Although effector proteins are generally associated with pathogens, it is possible that *A. glycines* also utilizes these proteins to manipulate their host plant in order to make it a more suitable host.

During two independent studies, McCarville et al. (2012, 2014) observed that *A. glycines* is capable of interacting with the soybean cyst nematode, *Heterodera glycines* Ichinhoe (Nematoda: Heteroderidae) bean on soy (McCarville et al. 2012; McCarville et al. 2014). McCarville et al. (2012) observed a 5.24x increase in reproduction factor (RF) for *H. glycines* when soybean were also infested by *A. glycines* and the brown stem rot fungus (*Cadophora gregata* (Allington and Chamberlain) Harrington and McNew). These results indicated that *H. glycines* was benefiting from one or both of the pests. In a follow up study, McCarville et al. (2014), observed that an infestation of *A. glycines*

significantly increased *H. glycines* reproduction (1.34x) on soybean roots on a SCN resistant cultivar derived from PI 88788. These results suggest that *A. glycines* can possibly alter the host plant's defense pathways to make the soybean plant more suitable for *H. glycines* (McCarville et al. 2012, 2014).

It is also possible that *Aphis glycines* interacts with fungal pathogens. For example, McCarville et al. (2012) observed that the disease severity caused by *C. gregata* was reduced on SCN susceptible cultivars when *A. glycines* and *H. glycines* were both present on the plants. Previous studies involving interaction between *C. gregata* and *H. glycines* have shown that disease severity caused by *C. gregata* can increase on *C. gregata* resistant and susceptible cultivars. However, *H. glycines* resistant cultivars have some resistance to *C. gregata* when co-infested with *H. glycines* and *C. gregata* (Hughes et al. 2004), so it is likely that *A. glycines* may be affecting the disease caused by *C. gregata* in the multiple pest treatment of the study by McCarville et al. (2012). The reduced disease severity of *C. gregata* in the multiple pest treatment in the study may have been through an *A. glycines* colonization-induced modification of the pre-existing interaction that *C. gregata* and *H. glycines* have exhibited in past studies.

Among the fungal diseases affecting soybean in the United States, those caused by the *Diaporthe*/*Phomopsis* complex are steadily gaining importance. In 2014, diseases caused by the *Diaporthe*/*Phomopsis* complex, northern and southern stem canker, Phomopsis seed decay, pod and stem blight have contributed to a combined estimated monetary loss of $363.9 million in the United States alone (Bradley et al. 2014). In South Dakota, *Diaporthe longicolla* (Hobbs) Santos, Vrandecic and Phillips was recently identified as a causal agent of pod and stem blight (Gebreil et al. 2015). In general, pod

and stem blight can affect soybean early in the growing season, although they do not exhibit symptoms (Sinclair et al. 2015). The disease development is favored by temperatures around 20°C and high moisture that can promote the development of fruiting bodies (i.e., pycnidia) on the stem and leaves. Pod and stem blight can be managed by reducing weeds and rotating to non-host crops such as corn (Li et al. 2010).

In South Dakota, *A. glycines* and *D. longicolla* can potentially co-occur simultaneously in soybean fields within the state, given that their geographical distribution overlaps. At this time, there is limited information available on the interaction between *A. glycines* and *D. longicolla* on soybean and the implications an interaction between these pests may have for soybean production. In this study, we wanted to determine how *A. glycines* feeding of soybean plants will affect the development of the stem disease caused by *D. longicolla* on soybean. In addition, we also wanted to determine if the soybean plants inoculated with *D. longicolla* will have an effect on *A. glycines* population growth. In this study, interactions was defined as "the effects organisms have on one another in a shared community" (Raven and Johnson 2002).

Materials and Methods

Aphis glycines populations

For this study, avirulent *A. glycines* biotype-1 populations that originated from a colony maintained by Iowa State University (Ames, IA) were used. The colony originated from field populations that were collected in Ohio, and were initially maintained in colony at the Ohio State University. This aphid population was selected for use in this experiment based on its inability to effectively survive on *Rag* soybean

cultivars (Kim et al. 2008). The initial population was harvested and identified in Ohio using detached leaf assays (Michel et al. 2010). At South Dakota State University, populations of *A. glycines* were maintained on the susceptible soybean cultivar soybean SD01-76R in a colony (Brookings, SD). To maintain the colony, new SD01-76R soybean seedlings are placed into the colony cage and are naturally infested every week. Populations that were used for this experiment were based off of randomly selected leaves that were removed from the colony.

Diaporthe longicolla **Inoculum**

In this study, a representative isolate of *D. longicolla* (SD-16; Union County, SD; GenBank accession number KT895379 and KT895393) which was identified as *D. longicolla* by Gebreil et al. (2015) by sequencing the internal transcribed spacer gene region (White et al. 1990) and elongation factor 1-alpha (EF1-α) gene region (Carbone and Kohn 1999).

To prepare inoculum, the *D. longicolla* isolate was cultured on potato dextrose agar (PDA) made from fresh potatoes (*Solanum tuberosum* L.) according to Leslie and Summerell (2006). Briefly, PDA was prepared by boiling 200 g of diced potatoes in 1000 mL of water until they can be mashed. The potato infusion was strained with a cheese cloth, after which 20 g anhydrous dextrose (VWR Analytical, Radnor, PA) and 20 g of agar (Alfa Aesar, Ward Hill, MA) was added. The solution was then autoclaved at 121°C at 15 psi for 15 minutes. After cooling to 50°C, 0.30 g of streptomycin sulfate (AMRESCO, Solon, OH) was added to 1000 mL solution of PDA.

Interaction between *A. glycines* and *D. longicolla* on soybean

We hypothesized that the lesion length produced by *D. longicolla* will not be affected by inducer populations of *A. glycines* on soybean. Additionally, we hypothesized that *A. glycines* populations will not be affected by inducer infestations of *D. longicolla* on soybean. To test our hypothesis, we infested plants with either *D. longicolla* or *A. glycines*, or a combination of the two pests when soybean reached the V1 (Fehr et al. 1971) developmental growth stage (approximately 14 days after planting soybean); which was termed the inducer infestation. A subsequent population of either *D. longicolla* or *A. glycines*, or a combination of the two pests was introduced seven days after the inducer infestation (approximately 21 days after planting soybean); this population was termed the response infestation (Table 1).

The experiment was set up as a completely randomized design with a total of eight treatments and six replications (plants) per treatment. For each treatment, two seeds of cv. Williams 82 (*A. glycines* and *D. longicolla* susceptible) were planted approximately 12.7 mm deep into damp soil (Sunshine mix #1, Sun Grow Horticulture Products, Bellview, WA) in each of six classic 300S (3.78 L) plastic pots. Pots were placed onto 279.4 mm by 533.4 mm plastic flats. Cages, constructed out of 14 gauge wire and a fine mesh net, were placed over each pot and secured with a rubber band to prevent movement of *A. glycines* across treatments. The greenhouse had an ambient air temperature of approximately 23.8-25°C and a 16 hour photo period (16 light: 8 dark). Soybean plants were watered by filling the flats when the top soil of the pots began to dry. The plants were thinned down to one plant per pot upon reaching the VC

developmental growth stage (cotyledons have developed and unifoliates leaves fully unrolled; Fehr et al. 1971). The experiment was performed a total of three times.

Soybean were infested with biotype 1 *A. glycines* populations that were reared and maintained at South Dakota State University. Using a 000 fine tip paintbrush aphids were transferred to first fully developed trifoliate leaves. A mixed age population of five *A. glycines* were added to the first fully developed trifoliate leaves at the V1 growth stage for treatments receiving the *A. glycines* inducer treatment (first trifoliate; Fehr et al. 1973). A similar method was used to infest soybean that were receiving a response population seven days later. After 24 hours, soybean were checked to ensure establishment of *A. glycines* populations. The total number of *A. glycines* (nymphs and adults) were counted at 7, 14, and 21 days after the inducer infestations; counts at 7 and 14 days were performed to monitor *A. glycines* population growth.

To inoculate soybean plants with the fungus, the cut seedling assay was used for *D. longicolla* (Li et al. 2010). Briefly, a cut was made on each of the soybean plants between the unifoliate node and the first trifoliate node with a sterile micro-pipette tip (200 µL). A four mm plug was cut from the advancing edge of the fungus from a 10 day old PDA culture of *D. longicolla* and applied to the wound. The plug was secured to the wound using Parafilm. Lesion length and stem length were measured in mm 14 days after response inoculations.

Statistical Analysis

To address our hypothesis, data collected at 14 days after the response infestations were analyzed for *D. longicolla* and *A. glycines* using R statistical software version 3.2.4 (http://cran.rproject.org/bin/windows/base/) (R Core Team 2015) and the package *lme4*

(Bates et al. 2015) to perform a linear mixed effects model analysis of the relationship that inducer populations had on the response infestations. While treatment was regarded as the fixed effect, replication and experimental run were regarded as the random effects in the model. *P*-values were obtained by likelihood ratio tests of the full model with the effect in question (e.g. treatment) against the same model without the effect in question.

To address the hypothesis, the data analyses for the response infestations was combined from three experimental repeats after performing the homogeneity of variance tests in R. The *A. glycines* plant data at 14 days after response infestations were log transformed (LN (counts+1)) to reduce heteroscedasticity and figures were constructed from non-transformed data (*A. glycines* counts). A value of '1' was added to the log transformations because some counts were zero. The homogeneity of variance was tested between four experimental repeats in R, and it was determined that three of the data sets could be pooled for least significant difference (LSD) analysis. The data obtained for lesion length and stem length at 14 days after response infestations were not log-transformed. For response infestations, treatment means based on *A. glycines* numbers, lesion length, and stem length were separated using least significant difference (LSD) test at $P < 0.05$ using R (de Mendiburu 2014).

Results

Interaction between *A. glycines* and *D. longicolla* on soybean

We rejected our hypothesis that an inducer population of *D. longicolla* would have no impact on *A. glycines* response populations on soybean. The main effect interaction was significant and our results indicate that inducer infestations of *D. longicolla* significantly affected response populations of *A. glycines* on soybean (χ^2 =10.116, df=2, P=0.006).

Based on the ANOVA using LSD analysis, significant differences in *A. glycines* counts were observed among treatments (LSD=0.931; *P*=0.008) (Table 2; Fig. 1). We observed that there was significant reduction in *A. glycines* population (47% reduction) in the treatment where both pests were introduced as response populations when compared to the no inducer control and the *D. longicolla* inducer treatment (LSD=0.931; P=0.008) (Fig. 1). Although not significantly different, there was an 8% increase in *A. glycines* populations when *D. longicolla* acted as an inducer when compared to the no inducer control (Fig. 1).

We confirmed our hypothesis that inducer populations of *A. glycines* would have no impact on *D. longicolla* lesion length. Our results indicate that inducer populations of *A. glycines* did not significantly affect the lesion length of response infestations of *D. longicolla* (χ^2=0.797, df=2, *P*=0.67). Based on ANOVA using LSD analysis, significance differences in lesion development by *D. longicolla* was not observed among treatments (LSD=7.730; *P*=0.768) (Table 2; Fig. 2). However, we observed an 11% increase in lesion length when *A. glycines* acted as the inducer population for *D. longicolla* when compared to the no inducer control and a 4% increase in lesion length when both pests were present as the response (Fig. 2).

Discussion

Our results indicate that inducer infestations of *D. longicolla* do not increase the suitability of the soybean cultivar that was tested. Additionally, our results also indicate that inducer populations of *A. glycines* do not increase the suitability of soybean for *D. longicolla*. However, there was evidence that simultaneous infestation has negative impacts on *A. glycines* populations. Although *A. glycines* have been known to interact on soybean with themselves, nematodes, and potentially other stem pathogens, (McCarville et al. 2012; Varenhorst et al. 2015) the results from this study demonstrate that *A.*

glycines and *D. longicolla* do not promote population growth of one another when concomitantly present on soybean. When *A. glycines* and *D. longicolla* were introduced together, we observed a significant decrease of 47% in *A. glycines* populations on the plants (Fig. 1). This is most likely due to an over response from the plant rather than an interaction between the pests. Introducing multiple pests at the same time can result in a compensatory effect in plants and result in a greater plant host response than when both pests infest the plant individually (Gagic et al. 2016).

As previously mentioned, *D. longicolla* was unaffected by inducer populations of *A. glycines* or by the concomitant infestation in this study, even though lesion length did not reach expected lengths that were observed by Li et al. (2010). They observed an average of 40.2% lesion length on soybean stems in their study (Li et al. 2010). The results from this study are surprising because *A. glycines* potentially can interact with fungal stem pathogens. For example, McCarville et al. (2012) demonstrated that disease severity caused by *C. gregata*, the causal agent of brown stem rot, may have been decreased in the presence of *A. glycines* feeding on soybean, However, they were unable to determine if this was due to an interaction of the fungus with SCN or *A. glycines*. Previous studies have shown that an interaction between SCN and *C. gregata* can increase disease severity on *C. gregata* resistant and susceptible cultivars (Hughes et al. 2004), so it is likely that *A. glycines* was reducing the disease caused by *C. gregata* in the McCarville et al. (2012) study.

This lack of a clear interaction between *D. longicolla* and *A. glycines* could potentially be due to the two pests inducing different responses in the plant. For example, effector proteins can be found in the saliva of *A. glycines* which play a role in the

molecular interaction with their host plant (Basal et al. 2014). Effector proteins are molecules that can change the structure and function of host cells and have been known to suppress plant immunity (Hogenhout et al. 2011). Though effector molecules produced by insects have been known to modulate plant defenses making the host more beneficial for other herbivores, they may not be effective at altering plant defenses that affect *D. longicolla*. Additionally, *A. glycines* has been shown to increase peroxidase activity in plants due to the increase of reactive oxygen species (ROS) as a result of the stress caused by feeding. Plants that convey resistance to *A. glycines* are more successful in up-regulating peroxidases, most likely in an attempt to slow the deleterious effect and plant death associated with aphid feeding (Pierson et al. 2010). In addition, soybean cultivars have been developed (e.g., soybean cultivar Harovinton) that have high peroxidase activity in the seed coat that are still moderately susceptible to seed mold like *D. longicolla* (Buzzel et al. 1991), so increased peroxidase may have no real effect on *D. longicolla* as a stem pathogen as well. These two pests may be activating different disease pathways that do not affect the other pests.

This study is merely an investigation of potential interactions occurring between these pests in a controlled environment, and may not be a good representation of the interaction that could exist in the field. Furthermore, lesion length development by *D. longicolla* was less than expected based off of lesion length observed by Li et al. (2010), where they observed an average lesion development of 40.2% coverage on soybean stems. This may be due to the environmental conditions in the greenhouse. For example, when performing pathogenicity studies with *Diaporthe*, inoculated plants are usually placed in an environment with a relative humidity of 85-90%. The relative humidity in

our greenhouse space was 40%, which may be the cause of the reduced lesion length in our study. Additionally, we only looked at the potential for an induced effect on *A. glycines* and *D. longicolla* susceptible soybean (Williams 82) and not on resistant cultivars. Field trials should be performed in order to get the full picture on the interaction that could be occurring between these two pests. Once further research has been conducted, the information acquired from this study and potential future field trials could be beneficial for the development of new or improvement integrated pest management practices in South Dakota.

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Table 2.1. Showing treatment layout for the interaction study. Each treatment consists of two factors: An inducer infestation at V1 developmental growth stage and a response infestation seven days after the inducer infestations.

Treatment	Inducer (At V1 growth stage)	Response (Seven days after Inducer)
1	None	*A. glycines*
2	*A. glycines*	None
3	None	Fungus
4	Fungus	None
5	*A. glycines*	Fungus
6	Fungus	*A. glycines*
7	Fungus + *A. glycines*	None
8	None	Fungus + *A. glycines*

Table 2.2. ANOVA results for response infestations of *A. glycines* and *D. longicolla* on soybean cv. Williams 82.

***A. glycines* as a response infestation**					
	df	**Sum sq**	**Mean sq.**	***F*-value**	***P*-value**
treatment	2	20.34	10.17	5.254	0.008
residuals	51	98.74	1.93		
***D. longicolla* as a response infestation**					
	df	**Sum sq**	**Mean sq.**	***F*-value**	***P*-value**
treatment	2	71	35.39	0.265	0.768
residuals	51	6815	133.63		

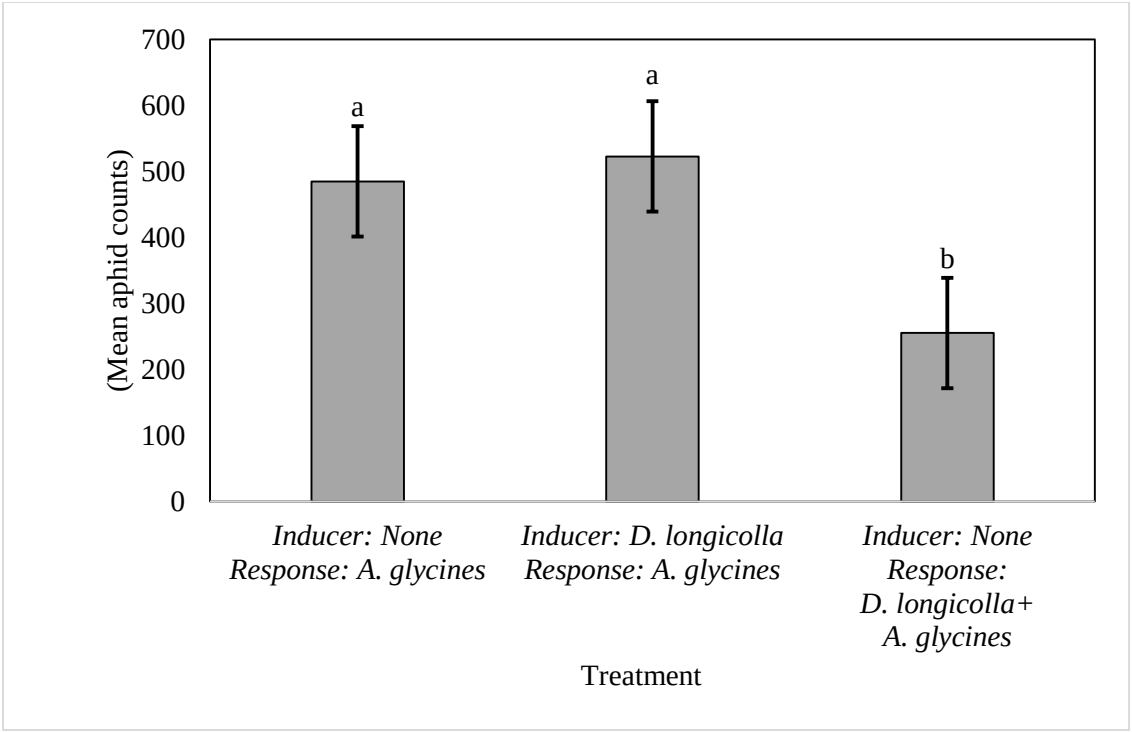

Fig. 2.1. The effect inducer infestations of *D. longicolla* had on response infestations of *A. glycines* at 21 days after inducer infestations. Significant differences in *A. glycines* counts were observed among treatments (*P*<0.05).

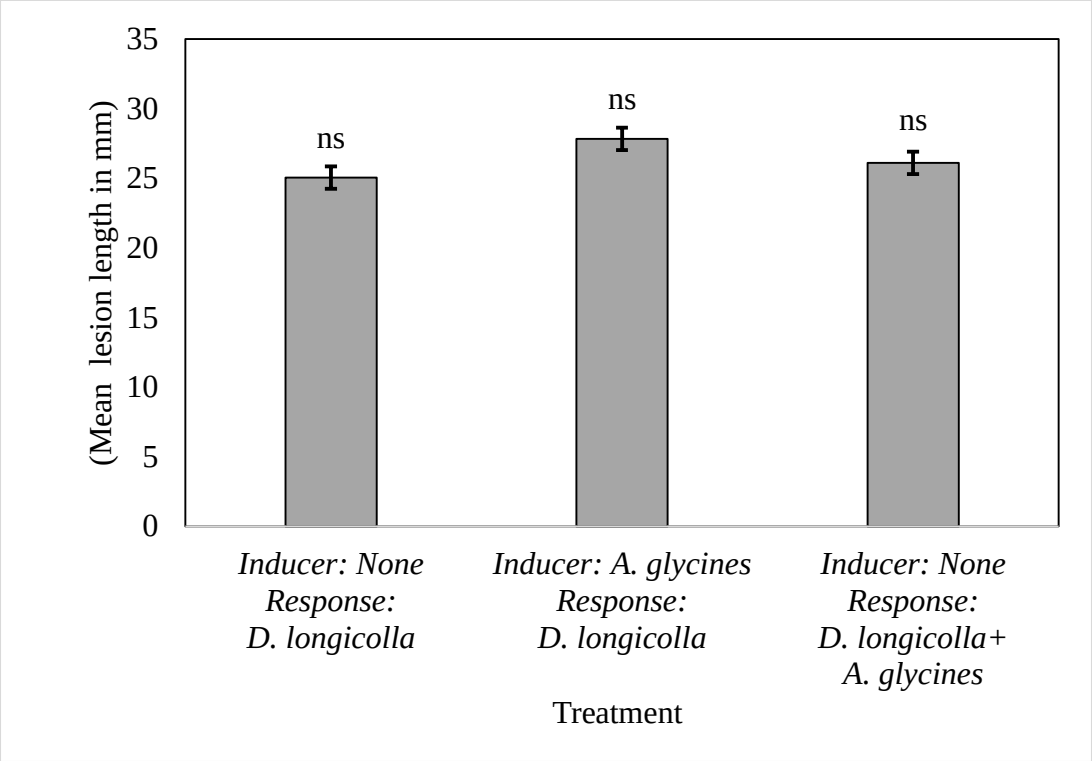

Fig. 2.2. The effect inducer infestations of *A. glycines* had on response infestations of *D. longicolla* lesion length at 21 days after inducer infestations. No significant differences in lesion length caused by *D. longicolla* were observed among treatments (*P*>0.05).

Chapter 4

General Conclusions and Recommendations

The goal of this research was to assess potential interactions among pests on soybean in South Dakota within a controlled greenhouse environment. Specifically, we investigated the potential interaction on soybean between SCN and *D. caulivora*, as well as the interaction between SCN and *D. longicolla*. Additionally, the aim of this research was to investigate the potential interaction that could be occurring between *A. glycines* and *D. longicolla* on soybean.

We investigated how SCN would interact with either *D. longicolla* or *D. caulivora* on soybean in a greenhouse water bath. When *Diaporthe* preceded SCN, we observed a 90% or greater decrease in SCN egg count per root gram in both the interaction between SCN and *D. longicolla* and the interaction between SCN and *D. caulivora*. Therefore, *D. longicolla* and *D. caulivora* negatively affect the ability of SCN to reproduce on soybean roots. Additionally, we determined that SCN can hinder the lesion length produced by *D. caulivora* on soybean plants. For example, when SCN preceded *D. caulivora*, we saw a decrease in lesion length of 35% in experiment one. We also determined that SCN can increase the lesion length caused by *D. longicolla* on soybean plants. For example, when SCN preceded *D. longicolla*, we observed an increase in lesion length of 76% or greater in both experimental repetitions. These results indicate that an interaction exists between SCN and *D. longicolla* or *D. caulivora* on soybean in a controlled environment.

We also examined the potential interaction between *A. glycines* and *D. longicolla* in the greenhouse. Our results indicated that no interaction is occurring between *A.*

glycines and *D. longicolla* on soybean. However, introducing both pests together did significantly reduce *A. glycines* population on soybean plants. We observed a decrease in *A. glycines* populations of 47% when compared to the *A. glycines* check. However, this was likely due to an over compensation effect from the soybean plants due to having two pests introduced at the same time given that *D. longicolla* did not induce resistance or susceptibility of soybean plants for *A. glycines* in the study.

This research has advanced current knowledge of pest interactions occurring on soybean in South Dakota. Currently, it is recommended that soybean producers utilize crop rotation to limit the three pests on soybean by rotating the crop to non-legume crops (e.g. corn and wheat). Soybean varieties are available to the producers in South Dakota that can provide some resistance to *D. caulivora*, *A. glycines*, and SCN. Additionally, seed treatments are labelled for managing *D. longicolla* and SCN, foliar insecticides for managing *A. glycines* as well as foliar fungicides for management of northern stem canker and pod and stem blight caused by species of *Diaporthe* on soybean.