IMPACTS OF SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES)* ON SUGARBEET (*BETA VULGARIS)* AND INTERACTIONS WITH *RHIZOCTONIA SOLANI*

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Soybean cyst nematode (SCN), *Heterodera glycines* is in the same genus as the sugarbeet cyst nematode (SBCN) *Heterodera schachtii*, there has been concern that SCN could also penetrate sugarbeet roots. The objectives were: 1) determine if SCN eggs hatch in the presence of sugarbeet seedlings and the larvae penetrate the roots, 2) determine at what age sugarbeet seedling are most susceptible to SCN penetration, 3) determine if SCN can penetrate sugarbeet roots under field conditions and 4) and determine if SCN could increase incidence of Rhizoctonia root rot in sugarbeet seedlings. This study demonstrated that SCN can penetrate the roots of a variety of sugarbeet. The results from the field demonstrated that SCN could penetrate sugarbeet seedlings under normal field growing conditions, and that penetration occurred in both SBCN susceptible and resistant sugarbeet cultivars. Penetration by SCN increased sugarbeet seedling root damage by *R. solani* under controlled conditions.

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Introduction

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is currently the most economically important pathogen of soybean worldwide (Wrather et. al., 1997). Reports show that in the United States from 2003 to 2009, SCN caused more yield loss than any other soybean disease (Wrather and Koenning, 2006 and Koenning and Wrather, 2010). Soybean cyst nematode was first reported in the U.S. in North Carolina (Winstead et al., 1955) and was discovered North Dakota in 2003 (Bradley, 2004).

Soybean cyst nematode is in the same genus, *Heterodera*, as the sugarbeet cyst nematode (SBCN), *Heterodera schachtii* Schmidt. Since SCN infects soybean, dry bean, legumes and some non-leguminous plants (Agrios, 2005), and it is in the same genus as *H. schactii* (Radice et. al., 1988), it is possible that SCN could also infect or attempt to infect sugarbeet. Soybean cyst nematode can remain viable in the soil for up to 12 years in cyst form (Niblack, 2005). During this time-frame there is a high probability in this region that soybeans and sugarbeet will be grown in the same fields. Since there are known *Heterodera*-fungal interactions in plants (Agrios, 2005), there is a potential that SCN could interact with fungal pathogens of sugarbeet, increasing disease incidence in seedlings.

Soybean cyst nematode egg densities in North Dakota (Bradley, 2004) are as high as or higher than those found in other states (Donald and Niblack, 2004 and Noel, 1985). These high

egg densities and the widespread occurrence of SCN in the Red River Valley warrants a look into the impact that SCN may have on sugarbeet grown in this sugarbeet production area .

The objectives of this research were the following: 1) determine if SCN eggs hatch in the presence of sugarbeet seedlings and if larvae penetrate sugarbeet seedling roots, 2) determine at what age sugarbeet seedlings are most susceptible to SCN penetration, 3) determine if SCN can infect or attempt to infect sugarbeet under field conditions and 4) explore the possibility that SCN could increase incidence of Rhizoctonia disease in sugarbeet seedlings

Literature Review

Beta vulgaris

Sugarbeet is classified in the class Dicotyledoneae, subclass Caryophyllidae, order Caryophyllales, family Chenopodiaceae, and species *Beta vulgaris* L. From the family Chenopodiaceae, only a few species such as sugarbeet, spinach (*Spinacia oleracea*), and quinoa (*Chenopodium quinoa*) are agricultural crops. A few noxious weeds such as fireweed (*Kochia scoparia*), Russian thistle (*Salsola kali*), and lambsquarters (*Chenopodium album*) are in the family Chenopodiaceae. Sugarbeets, like many of the other members of the family Chenopodiaceae, are halophytes. The ancestral form of sugarbeets was previously known as *Beta maritime,* but has since been classified as *B. vulgaris* subsp. *maritima*, the wild sea beet. The ancestral sugarbeet was used in Greek and Roman culture as a food source for both humans and animals (Cooke and Scott, 1993).

Andreas Marggraf was first credited with the extraction of sugar from white beetroot in Europe (Prussia) in 1744, and by the 19th century sugarbeet production had increased throughout Europe (Harveson et al., 2009). It was during this period that German immigrants began planting sugarbeet in the United States. This introduction of a new crop led to the spawn of the first successful sugar factory built in California. It was not until 1926 that large scale sugarbeet production came to North Dakota and Minnesota (American Crystal Sugar Company, 1998).

The gross estimated economic value of the sugarbeet industry in the Red River Valley is close to $3 billion, and it involves two growers' cooperatives: American Crystal Sugar Company and Minn-Dak Farmers Cooperative. Together, they account for nearly 60% the US sugarbeet production in the U.S. (Bangsund and Leistritz, 2004). The United States ranks as the second largest producer of sugarbeet worldwide with an annual production of 29.5 million tons and an economic value of $1.28 billion (United States Department of Agriculture, 2010). Reports from the 2009 and 2010 cropping seasons show that, of the top ten sugarbeet producing states, Minnesota ranks number one and North Dakota ranks third. Sugarbeet acreage is smallin comparison to the other agricultural crops of North Dakota and Minnesota, while the economic contribution is significant (United States Department of Agriculture, 2010).

Life cycle of *Heterodera glycines*

The soybean cyst nematode, *Heterodera glycines*, is currently the most economically important pathogen of soybean worldwide (Wrather et. al., 1997). The life cycle of SCN begins with the hatching of eggs and ends with the mature female producing fertile eggs (Agrios, 2005). Egg hatch is mediated by several factors such as temperature, host, time, and some eggs from the same cyst may hatch at different rates depending on the amount of time that has elapsed (Yen et al. 1995). Hatching factors for *H. glycines* may also be influenced by electrical potential or

pH (Pike et al., 2002). While zinc was determined to be a stimulus for the emergence of second stage juvenile (J2) larvae from eggs, egg shell depolarization and pH are not emergence signals. Therefore, applied zinc fertilizers could not induce hatching (Pike et al., 2002). Another hatching factor is glycoprotein A, which has been isolated from kidney bean roots. (Masamune et al., 1982).

After hatching, the J2 larva locates a root or suitable host by chemolocation and uses a piercing stylet and esophageal enzymes to puncture the root tissue (Davis et al., 2004). Once the J2 enters the vascular tissue it identifies a suitable place to establish a feeding site also known as a syncytium. The amount of water present in the root tissue influences where the syncytium is established within the root (Johnson et al., 1993). A SCN larva takes a direct path from the surface of the root to the vascular cells, causing cellular necrosis along that path (Gheysen and Fenoll, 2002). Once a vascular cell has been chosen for a feeding site, the nematode uses enzymes to dissolve cell walls to form the syncytium that may incorporate up to hundreds of root cells (Davis and Mitchum, 2005).

Feeding of *H. glycines* and *H. schachtii* can be divided in five phases: exploration, stylet insertion into host cell, esophageal gland secretions through stylet, ingestion of cytoplasmic nutrients via stylet, and retraction of the stylet. When an adequate feeding site is located by a J2 larva, the cell wall is quickly penetrated by rapid thrusting of the stylet through the cellular wall. The formation of a syncytium happens when partial dissolution of cellular walls takes place and hundreds of cells are incorporated into one large feeding site. A syncytium caused by a penetrating J2 is characterized by the enlarged nuclei and nucleoli, a denser cytoplasm, and an increased number of subcellular organelles (Hussey and Williamson, 1998). When the formation of a syncytium begins, cells that are adjacent to the initial feeding cell become metabolically

hyperactive, causing the cell walls of outlying cells to dissolve (Klink et al., 2009). Nuclei of feeding cells and adjacent cells are more active at 1-2 days postinoculation than at nine days post inoculation. The cytoplasm is denser and nuclei are enlarged at two days post inoculation (de Almeida Engler et al., 1999).

Formation of the syncytium allows the J2 larva to feed and continue transforming to subsequent juvenile stages (Noel, 2004). Following syncytium establishment, the feeding cycle of SCN happens in 3 steps. Esophageal secretions from the stylet into the cytoplasm of host cells form a straw-like structure that aids in nutrient uptake. Nutrient uptake happens via a metacarpal pump and the final step is the removal of the stylet from the dense cytoplasm. This three-step process can take 1-2 hours, and nutrient uptake is suspended for a few minutes between feeding sessions. The stylet tip is then re-inserted into the initial syncytial cell, and another nutrient uptake cycle is started (Hussey and Williamson, 1998).

After establishing a feeding site, the J2 larva continues to grow and swell to a "sausage-stage" and will complete the final two molts at 4to 6 day intervals. Following the final molt, males exit the root into the soil where they may fertilize SCN females, after which they soon die (Agrios, 2005). Females continue to grow until they can no longer be contained within the root, although the head and neck still remain attached to the feeding site (Niblack, 2005). Under extremely optimal conditions juveniles can reach sexual maturity in 14 days or less (Lauritis et al., 1983).

Reproduction for SCN is obligately sexual, and reports of observed sex ratios of 1:1 may be otherwise influenced by plant host resistance or environmental conditions, whereas it was previously thought that sex determination was genetic (Niblack, 2005). Mating between the

sedentary females and the motile males takes place in the soil outside of the plant root while the females are still attached to their feeding sites (Anand et al., 1995). Typically females will produce between 300-600 eggs (Agrios, 2005). About one third of the eggs produced by the female are deposited outside of her body in a gelatinous matrix (egg mass), and two-thirds are encysted within the hardened female body (Sipes et al., 1992). Egg-mass eggs are shown to hatch faster than encysted eggs by up to 16 days. Egg-mass eggs are less sensitive to hatch inhibitors, and egg-mass eggs contain more vermiform juveniles than encysted eggs (Thompson and Tylka, 1997).

Molecular aspects of SCN pathogenicity

Genes expressed specifically during the parasitic J2 stage were shown to be down-regulated once the sedentary feeding stage was reached following the establishment of a syncytium (Elling et.al., 2009). Expression of parasitism genes fluctuated throughout the SCN life cycle. Genes expressed primarily for parasitism were also detected in juveniles during the sedentary feeding stage (Gao et al., 2003). Expression of cDNA clones SYV46, SY20 and SYS65 from the dorsal esophageal gland cell of juveniles of parasitic SCN could only be detected after *H. glycines* had parasitized its host (Wang et al., 2001).

The expressed gene *Hg4F01* from the esophageal gland of *H. glycines*, which codes for an annexin-like effector, has a homologue which has been isolated from *H. schachtii* (Patel et. al., 2010). Another secretory protein found in *H. glycines* with homologues that have been cloned from *H. schachtii* is 10A06, which is secreted as an effector during the early stages of plant parasitism. As a result of 10A06 protein secretion, there is increased spermidine content and increased polyamine oxidase activity (Hewezi et al., 2010).

SCN management practices

Arelli et al. (2000) found that 118 PIs from exotic soybean germplasm were resistant to combinations of the SCN races 1, 2, 3, 5, and 14. The PI that exhibited nearly complete resitance to all SCN races was PI 437654 (Anand et al., 1988), which was confirmed by Diers et al. (1997). From a survey done by Anand (1991) on 130 cultivars with resistance to SCN, 69 were traced from 'Peking', 24 from PI 88788, and 31 were traced to both 'Peking' and PI 88788. Diers and Arelli (1999) reported that the majority of soybean grown in the Midwest had SCN resistance from the source PI 88788. Sheir (2008) showed that more than 95% of SCN resistant cultivars planted in Illinois in 2009 received SCN resistance from PI 88788. Caldwell et al. (1960) showed that the resistance that is inherited from 'Peking' follows a three gene recessive modes, with the symbols *rhg1*, *rhg2*, and *rhg3* being designated.

The mechanism of resistance to SCN in soybean is through preventing the formation of a syncytium by stopping the spread of the necrotic layer of cells around the forming syncytium, or by nuclear degradation prior to cytoplasmic degradation (Kim et al., 1987). Breeding for resistance in soybean to sexually reproducing plant parasitic nematodes such as *Heterodas*pp. can be difficult due to genetic variation among races and responses to resistance genes (Williamson and Hussey, 1996).

There is considerable emphasis placed on the threshold levels of SCN eggs within a field that will result in yield losses for soybean. Reported threshold levels vary by the region in which soybean is produced, which is most likely based on the variance of optimum conditions (Niblack, 2005). The threshold egg numbers for soybean production areas in Illinois are 600 eggs and J2 per 250 cm^3 soil (Noel, 1985). Missouri has economic thresholds set at 500 eggs/250 cm^3 of soil

(Donald and Niblack, 2004). Economic thresholds have not been determined yet in North Dakota.

Levels and densities of SCN can be affected by soil texture and tillage. Aggregated SCN populations subjected to no-tillage systems promoted aggregation of populations, while conventional tillage resulted in less-aggregated spatial patterns of SCN populations (Gavassoni et al., 2001). In a no-tillage versus conventional tillage study, no-tillage always had less SCN than conventional tillage (Tyler et al., 1983). Studies in southern states such as Arkansas and Missouri have shown that earlier planting dates can offset yield losses in soybeans. This is directly related to soil temperature. If soybean roots are more developed before the nematodes are active there is less yield loss throughout the growing season (Riggs et al. 2000).

Heterodera schachtii

The sugarbeet cyst nematode (SBCN), *Heterodera schachtii*, can be found almost everywhere sugarbeet is produced, and it is the most important nematode pest of sugarbeet. Sugarbeet cyst nematode is widespread in Europe and is found in Canada as well as the sugarbeet production areas in California, Utah, Colorado, Idaho and Michigan. Sugarbeet cyst nematode has been reported in 16 states and 4 Canadian provinces, and was recently reported in North Dakota (Harveson et al., 2009; Nelson et. al., 2012). Yield losses from SBCN can range from 25 to 50%, with higher loss occurring in late-planted crops or crops grown in warmer soils. Losses in sugarbeet are primarily due to reduced root weight or reduced sugar content in warmer climates (Müller, 1999). Common symptoms are patches of wilting or dead plants, and older sugarbeet that appear stunted. Older plants will produce many hair-like roots in response to being aggravated by SBCN (Agrios, 2005). Sugarbeet fields infested with SBCN display the same symptoms as soybean fields infested with SCN.

With the use of nematicides being highly restricted and the use of "trap" crops for plant parasitic nematodes often unreliable, there is an emphasis on using resistant cultivars to manage these pests (Agrios, 2005). Genetic resistance for SBCN has been identified with the gene $Hs1^{pro\text{-}1}$, which can only be found from the wild *Beta* species *B. procumbens* and the related species *B. webbiana* and *B. patellaris* (Cai et al., 1997).

Successful hybridization between *H. schachtii* and *H. glycines,* leading to fertile progeny, has proven successful, when *H. glycines* females were paired with males of *H. schachtii* (Potter and Fox, 1965). When comparing restriction fragment patterns of mitochondrial DNA from *H. schachtii* and *H. glycines*, 10% out of 90 scorable fragments were shared by both species (Radice et. al., 1988).

Sugarbeet cyst nematode has been shown to increase yield losses caused by other sugarbeet pathogens, such as *Cercospora, Rhizoctonia,* and viruses (Agrios, 2005). In situations where fungal pathogens are causing disease in conjunction with nematodes, yield losses are usually higher than each pathogen alone. In nematode-fungal interactions involving *Rhizoctonia* and *Verticillium,* both of which are major sugarbeet pathogens, the SBCN does not vector the fungus, but promotes pathogen activity after root penetration (Agrios, 2005).

Interaction of *Heterodera* with fungal pathogens

Whether a nematode is an ecto- or endo- parasitic nematode, both types cause injury to the host during the process of feeding. The size of the wound caused by ectoparasitic nematodes is the same as the initial wound caused by endoparasitic nematodes, which is the size of the stylet (Bergeson, 1972). *Trichodorus* spp. and *Tylenchorhynchus* spp., both ectoparasitic nematodes, leave small wounds in the epidermis of the plant root. *Heterodera* spp. and other endoparasitic

nematodes have a more complex life cycle, which causes more disruption to the host root (Back et al. 2002). Through nuclear magnetic resonance imaging by Hillnhütter et al. (2012) confirmed that Rhizoctonia crown and root rot (RCRR) of sugarbeet caused by *Rhizoctonia solani* developed above and below the inoculation site when *H. schachtii* was present. The disease only developed above the inoculation site when *H. schachtii* was absent. Changes in sugarbeet tissue due to penetration from *H. schachtii* promote growth of *R. solani* in sugarbeet seedlings. Fungal growth within the seedlings is further influenced by nematode penetration even after fungal establishment within the root (Polychronopoulos et al., 1969).

Another economically important disease of soybean is sudden death syndrome (SDS), which is caused by *Fusarium virguliforme*. Sudden death syndrome occurs in most soybean-producing states and several South American countries. Yield losses from SDS can vary from 5 to 80%, depending on plant age at time of infection, environmental conditions or plant resistance (Agrios, 2005). In studies dealing with soil-borne disease complexes and a soybean-corn rotation, fields that contained *H. glycines* populations showed increased crop loss from SDS. Recommendations for the reduction of yield loss from SDS in fields with SCN include suppression of SCN through crop rotation or the use of cultivars resistant to SCN (Xing and Westphal, 2009).

In studies where SCN was present with *F. virguliforme*, infection was not dependant on SCN, yet SDS symptoms occurred earlier and more severely (Melgar et al., 1994, and Roy et. al., 1989). A micro plot study examining the relationship between SCN and *F. virguliforme* showed that foliar symptoms of SDS occurred on more plants developed 3 to 7 days earlier, and were more severe on plants compared to those inoculated with *F. virguliforme* only. It was also noted that population densities of SCN were lower in plots containing *F. virguliforme*, due to *F.*

virguliforme inhibiting growth of cysts and eggs or reduced root size from SDS, which hinders SCN infection (McLean and Lawrence, 1993).

Potential for SCN to interact with sugarbeet pathogens

All plant parasitic nematodes use stylets to obtain access inside the plant to obtain nutrients and complete their life cycle (Agrios, 2005). Puncturing of the plant's epidermis could lead to the leakage of root exudates that alert soil-borne plant pathogens to the presence of sugarbeet roots. Penetration by SCN could also leave wounds and openings which could provide entry for opportunistic soil-borne sugarbeet pathogens. Two diseases of sugarbeet, Fusarium root rot and Rhizoctonia root and crown rot, could potentially be impacted by an interaction with SCN and sugarbeet.

Fusarium Yellows, which is caused by *Fusarium oxysporum* f. sp. *betae*, is a fungal soil-borne pathogen of sugarbeet that causes yellowing and wilting. It can survive without a host in the soil as chlamydospores (Harveson et al., 2009). When temperatures become favorable (24-28°C) the chlamydospores germinate and infect the root (Harveson et al., 2009 and Kahn 2013). Once the germinating spores are able to penetrate the root and reach the vascular system, the fungus reproduces and moves in the vascular system. Wilting of the leaves and rotting of the root are symptoms characteristic of Fusarium Yellows (Agrios, 2005).

Rhizoctonia root and crown rot, caused by *Rhizoctonia solani* Kühn, is an endemic soil-borne disease of sugarbeet wherever the crop is grown (Harveson et al., 2009). *R. solani* is a basidiomycete fungus that produces thread-like hyphae with no asexual spores. Under favorable environmental conditions, sexual spores can be produced, but this is rarely seen in nature (Agrios, 1997). *R. solani* causes damping off of seedlings as well as crown and root rot. Poor

plant stands that occur due to damping off can lead to low yields. Major economic and yield losses can also occur if disease development by *R. solani* occurs later in the growing season (Harveson et al., 2009). Because previous research has shown that SBCN can affect the damage caused by *R. solani* on sugarbeet, one of the most likely sugarbeet diseases to be affected by SCN might also be RRCR.

***Rhizoctonia solani* Kühn**

The genus *Rhizoctonia* was first described in 1815 by DeCandolle. DeCandolle assigned *R. crocorum* (Pers.) DC. as the type species, while the most important species of *Rhizoctonia, R. solani,* was described by Kühn in 1858 (Sneh et al. 1996). The sexual stage of *R. solani,* also referred to as the teleomorph, is *Thanatephorus cucumeris* (Frank) Donk. The sexual stage was first reported and identified in 1956 (Sneh et al., 1996). It is classified in the Domain: *Eukaryota*; Kingdom: *Fungi*; Phylum: *Basidiomycota*; Order: *Ceratobasidiales;* and Family: *Ceratobisidiaceae* (Agrios, 1997; Sneh et al., 1996).

Rhizoctonia solani is further classified into anastomosis groups (AGs) based on the vegetative compatibility of reactions that occur when hyphae of similar isolates fuse together and there is an exchange of genetic material (Anderson, 1982). Two isolates where compatible fusion takes place are placed within the same AG. If no hyphal fusion occurs, the two isolates will be placed in separate AGs (Agrios, 2005). To date, 14 AGs have been identified (AG-1 to AG-13 and AG-BI), and 8 of the 14 contain subgroups (Matthew et al., 2012; Carling et al., 1994, Carling et al., 2002; Kuninaga et al., 1997; Sneh et al., 1996; Stodart et al., 2007). The term subgroup or intraspecific group (ISG) was first proposed by Ogoshi (1987) as a way to further classify subgroups of AGs for *R. solani*. Anastomosis groups 1, 2, 3, 4, 6, 7, 8, and 9 have been further subdivided into ISGs based on their fusion characteristics, differing

morphology, genetic makeup, nutrition requirements, virulence and host range (Carling et al., 2002; Vigalys and Cubeta, 1994; Sneh et al., 1996).

A distinguishing characteristic of *R. solani* is the 90-degree angle formed by hyphae branching off the main hyphae with slight constrictions observed at the point of branching (Harveson et al., 2009; Whitney and Duffus, 1986). Mycelium of *R. solani* is initially colorless; however as aging occurs, the color turns to a yellowish-brown (Agrios, 2005). Septa of Ascomycetes are homogenous and electron translucent, and the septal pores have rounded edges. Although the pore size will facilitate nuclear migration, the pores are flanked by one or more Wornin bodies that can block migration (Buller, 1933; Markham and Collinge, 1987). Basidiomycete septa differ from Ascoymycetes by being tripartite. Viewed under an electron microscope, the tripartite-septa appear to alternate colors between light-dark-light (Bracker and Butler, 1963). Kreger-van Rij and Veenhuis (1971) first made the distinction between ascomycete and basidiomycete septal morphology, and this is now an accepted fundamental indicator that distinguishes between these groups.

Rhizoctonia solani on sugarbeet

Rhizoctonia solani has a wide host range, but AGs infect only certain groups of plants (Sneh et al., 1996). Of the AGs that attack sugarbeet, AG 2 is the most pathogenic. Of AG 2 there are five subsets: 2-1, 2-2, 2-3, 2-4, 2-BI, with the more virulent subsets being 2-1, 2-2 and 2-4 (Carling et al., 2002). While many AGs are able to colonize sugarbeet, the primary AG for Rhizoctonia root and crown rot in sugarbeet is AG-2-2 IV (Engelkes and Windels, 1996; Ogoshi, 1987; Sneh et al., 1996; Bolton et al., 2010). AG-2-2 IIIB is another AG group that causes disease on sugarbeets; however, AG-2-2 IIIB is also better known for causing disease on mat rush, rice, soybean, maize, and dry edible bean (Ithurrant et al., 2004; Nelson et al., 1996; Sneh

et al., 1991; Sneh et al., 1996). Isolates of *R. solani* AG-2-2 IIIB and AG-2-2 IV were found to be pathogenic on soybean, dry bean and sugarbeet, and pathogenicity was found to increase with crop rotation schemes (Engelkes and Windels, 1996). The prevalence of AG-2-2 IIIB and 2-2 IV in North Dakota and Minnesota is likely due to the distribution of the crops that are in a sugarbeet rotation.

Rhizoctonia solani can cause damping-off in sugarbeet seedlings, while in older and more mature plants a crown and root rot (Sneh et al., 1996). Seedling diseases of sugarbeet can be classified as any disease resulting in stand losses from seed decay or pre-emergence and post-emergence damping off. *Rhizoctonia solani* often results in injury to the hypocotyls, which results in stunted, deformed and under-developed plants (Windels and Jones, 1989). *Rhizoctonia solani* AG-2-2 IIIB is more aggressive than AG IV on sugarbeet (Harveson et al., 2009). Crown and root rot primarily occurs midway through the growing season, usually around the time of canopy closure. Symptoms on sugarbeet begin with black lesions on older petioles that are in contact with the soil. Lesions will then progress to the crown and roots of the beet resulting in wilting of the plant (Sneh et al., 1996).

Rhizoctonia solani is able to survive by overwintering in the soil as sclerotia, thickened hyphae or bulbils in remaining crop debris or soil (Harveson et al., 2009). *R. solani* is able to survive on crop residue such as barley and sorghum for at least 8 weeks at temperatures around 20ºC (Ruppel, 1985) and is found in the top 10 cm of field soil (Papavizas et al., 1975). Under favorable conditions of moisture and temperature, *R. solani* overwintering structures will germinate (Sneh et al., 1996). The temperature range for *R. solani* to infect sugarbeet is 15.6-33ºC, the optimum temperature range for infection by AG 2-2 IIIB is 21.1-26.7ºC (Whitney and Duffus, 1986; Harveson et al., 2009; Bolton et al., 2010; Khan et al., 2008).

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PENETRATION OF SUGARBEET SEEDLING ROOTS BY SOYBEAN CYST NEMATODE

Introduction

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is currently the most economically important pathogen of soybean worldwide (Wrather et al. 1997). In the United States, SCN caused more yield loss than any other soybean pathogen from 2003 to 2009 (Wrather and Koenning, 2006 and Koenning and Wrather, 2010). The SCN was first reported in the United States in North Carolina (Winstead et al., 1955), and it was recently discovered in North Dakota in 2003 (Bradley et al., 2004). Soybean is the principal host of SCN, but the nematode also infects dry bean and several other legumes (Davis and Mitchum, 2005).

The life cycle of SCN begins with the hatching of the eggs and ends with the mature female producing fertile eggs (Agrios, 2005). Hatching of the eggs is mediated by several factors such as temperature, host, and time, and eggs from within the same cyst may hatch at different rates (Yen et al., 1995). Certain root exudates are almost completely necessary for hatching of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*), while beet and cyst nematodes are only stimulated by root exudates (Perry and Wesemael, 2008). A unique characteristic of hatching stimuli of cyst nematodes is that found from the water-soluble glycinoclepins A, B, and C isolated from kidney bean root (Masamune et al., 1982).

Feeding by SCN can be divided in five phases: exploration, stylet insertion into host cell, esophageal gland secretions through stylet, ingestion of cytoplasmic nutrients via stylet, and retraction of the stylet (Hussey and Williamson, 1998). Upon hatching, the J2 larva locates the

root of a suitable host by chemotaxis and uses a piercing stylet with rapid thrusting to break through the cell wall (Hussey and Williamson, 1998 An attractive compound for *H. schachtii* is carbon dioxide, which is a byproduct of biological activity within the soil, while *Globodera pallida*, another plant parasitic nematode, is attracted to g-aminobutyric acid and L-glutamic acid (Rasmann et al., 2012). Esophageal enzymes assist the piercing stylet with puncturing the root tissue (Davis et al. 2004). Once the J2 enters the vascular tissue it identifies a suitable place to establish a feeding site called a syncytium (Gheysen and Fenoll, 2002). A syncytium is characterized by enlarged nuclei and nucleoli, and a denser cytoplasm with increased number of subcellular organelles compared to normal cells (Hussey and Williamson, 1998). A SCN larva takes a direct path from the surface of the root to the vascular cells, causing cellular necrosis along that path (Gheysen and Fenoll, 2002). Once a vascular cell has been chosen for a feeding site, the nematode uses enzymes to dissolve cell walls and form the syncytium that may incorporate up to hundreds of root cells (Davis and Mitchum, 2005). When the formation of a syncytium begins, cells that are adjacent to the initial feeding cell become metabolically hyperactive, causing the cell walls of outlying cells to dissolve (Klink et al, 2009).

After establishing a feeding site, the J2 larva continues to grow and swell to a “sausage-stage” and will complete the final two molts at 4to 6 day intervals in soybean. Following the final molt, males may fertilize the females, then they will exit the root into the soil and die (Agrios, 2005). Females continue to grow until they can no longer be contained within the root, although the head and neck still remain attached to the feeding site (Niblack, 2005). Under extremely optimal conditions, juveniles can reach sexual maturity within 14 days (Lauritis et al. 1983).

Soybean cyst nematode has been shown to penetrate the roots of non-host plants (Back et al., 2002). Non-host plants are plants in which the nematode cannot complete its life cycle. Although sugarbeet is considered a non-host for SCN, it is unknown if the nematode penetrates sugarbeet roots and attempts to establish a syncytium. The Red River Valley of North Dakota and Minnesota is a major sugarbeet production area, and SCN-infested fields are now common in this region (Harveson et al., 2009 and Bradley et al., 2004). Soybean cyst nematode has spread from the North Dakota/South Dakota border all the way into Manitoba. Field infestions of SCN in this area have been shown to approach densities of up to 10,000 eggs/100 cm^3 of soil (Bradley et al., 2004).

Sugarbeet is damaged by a number of important soil-borne fungal pathogens such as *Rhizoctonia solani* and *Fusarium* sp. (Agrios, 2005). *Rhizoctonia solani* Kühn is especially detrimental to sugarbeet in the Red River Valley. Soybean cyst nematode is known to interact with soil-borne fungal pathogens of soybean, resulting in greater damage than that caused by the fungal pathogens alone (Back et al., 2002). Sugarbeet and soybean are crops that are commonly grown in rotation. Thus sugarbeet can be grown in fields with high densities of SCN. A question that arises is whether SCN penetrates sugarbeet roots. If SCN penetration of sugarbeet root occurs, SCN might affect soil borne pathogen interactions with sugarbeets. The purpose of this study was to determine (1) if SCN penetrates the roots of sugarbeet, (2) which seedling root stages are most susceptible to penetration, and (3) will SCN penetrate sugarbeet seedlings growing in an SCN-infested field under normal growing conditions.

Materials and Methods

Root-penetration study

Sugarbeet cultivars M832224 (SESVanderHave, Belgium, experimental line; susceptible to sugarbeet cyst nematode (SBCN)), 0957-22 (SESVanderHave, experimental line; SBCN resistant), ACH 117 (Crystal Beet Seed, Fargo, ND) and Beta 936 (Betaseed, Shakopee, MN) were planted into Sunshine mix #1 (Sungro Horticulture, Canada) in individual plastic "Cone-tainers" (Type SC10 Super Cell [3.8 cm in diameter, 21 cm in depth, volume of 164 ml], Stuewe and Sons, Inc., Corvallis, OR). There was one plant per Cone-tainer. Plants were maintained in the greenhouse on benches with the Cone-tainers being supported by RL 98 trays (Stuewe and Sons, Inc.). Plants were grown for 16 h under natural and supplemented light (19.9 W/m^2) and greenhouse temperatures ranged from 20-29ºC. At 10 days post emergence, the plants were inoculated with a 1 ml solution of SCN eggs in water at a concentration of 10,000 eggs ml^{-1}. Following inoculation plants were incubated in the greenhouse for 18-21 days then roots were harvested.

The roots were washed in tap water to remove potting mix and decrease the likelihood that SCN larvae was attached to the root exterior. Roots were then stained following the method described by Tylka (2012). Roots were soaked for 4 min in a 50 ml solution of a 5:1 water to bleach solution in glass beakers. Roots were then rinsed in tap water for 45 seconds, followed by 15 min incubation in tap water. Tap water was poured off and replaced with 40 ml of distilled water, and approximately 7-8 drops of acid fuchsin stain. The stain solution plus roots was placed on a hot plate and brought to a boil. After approximately 5 min, the beakers were removed from the heat and samples were allowed to cool. The roots were then rinsed in tap

water for 1 min, the excess water was carefully drained off, and 10-20 ml of acidified glycerin was added to the roots. Acidified glycerin was prepared by adding 5-10 drops of 1.0 M HCl to 10-20 ml of glycerin.

Roots were mounted in acidified glycerin on glass sides for viewing. Small roots were examined directly while larger roots were split longitudinally and sections of the roots were viewed. Stained root samples were examined with a Zeiss Stemi SV6 (Zeiss, New York, USA) microscope at up to 50x magnification for evidence of larvae inside the root tissue. Selected samples were also examined and photographed with a CARV confocal scanning unit attached to Nikon Eclipse E600W fluorescence light microscope. Five to ten plants were selected from each of the five sugarbeet cultivars and were processed for staining and microscopy work. This experiment was repeated once.

Seedling age for optimal SCN penetration of sugarbeet

Sugarbeet cultivars M832224, 0957-22 and SES VanderHave SBCN Resistant and Susceptible (4 cultivars, 2 resistant and 2 susceptible to SBCN) were planted into Sunshine mix #1 (Sungro Horticulture) in individual plastic Cone-tainers as described earlier. Plants were started four separate times at seve- day intervals and grown in the greenhouse under the conditions described previously. Plants were watered daily as needed to maintain a moist potting mix. After 5 weeks, when the plants ranged in age from 7 to 28 days post emergence, approximately 10,000 SCN eggs were added to the soil of each cone-tainer near the sugarbeet root. Following inoculation the plants were maintained in the greenhouse for 14 days at 20-29ºC. There were five replications of each treatment, and the experiment was repeated once.

After the incubation period, the roots were removed from the soil, cleaned of excess potting mix, freeze dried and ground to a homogenous powder in liquid nitrogen with a mortar and pestle. Genomic DNA was isolated using the CTAB method (Stewart and Via, 1993). Semi-quantitative polymerase chain reaction (PCR) was performed as described by Thomma et al (2006) with primers that were specific to SCN and sugarbeet actin (SCN forward: GTGCCCATCTACGAGGGTTA; reverse: ACAGGTCCTTACGGATGTCG; *Beta vulgars* actin forward: GATTTGGCACCACACCTTCT; reverse: TCTTTTCCCTGTTTGCCTTG). PCR was conducted with Go Taq Flexi DNA Polymerase kit (Promega, Madison, WI) following the manufacturer's instructions with a final $MgCl_2$ concentration of 2.5 mM. PCR conditions were as follows: an initial 95ºC denaturation step for 3 min followed by denaturation for 30 s at 95ºC, annealing for 30 s at 62ºC and extension for 30 s at 72ºC for 32 cycles. PCR products were run on 1% agarose gel made with 1x TBE with ethidum bromide added before the gel was set. The products were run @ ~120 mV for 60-90 minutes.

Detection of penetration in the field

Two field locations were selected to determine if SCN can penetrate sugarbeet roots under natural field conditions. Each location had a history of SCN and had soybean in the crop rotation. The first location was 11 miles east of Wheaton, MN (township/range: 127-45; SW quarter of section 13). The soil composition of this site was Bearden silt loam (~15%) and Hamerly Lindaas clay loams (~85%). The second location was 7 miles east of Herman, MN (township/range: 127-42; SE quarter of section 7) and the soil composition was Hamerly-Clay (32%), Vallers clay (27.5%), Aazdahl-Hamerly-Parnell complex (27%), Hamerly-clay loam (9.2%) and 4.2% other. Sugarbeet seedlings were collected during the 4-leaf stage and two

weeks later during the 6-leaf stage. Each location had two cultivars, Betaseed 0692N (Shakopee, MN, SBCN-resistant) and Hilleshog 4022 (Syngenta, Longmont, CO; SBCN-susceptible). At each location and growth stage, 4to 7 plants were collected from each cultivar from 10 different places in the field. All of the roots from each cultivar from each place in each field were pooled together so there were 10 bulked samples from each cultivar from each time period for each field for a total of 80 samples.

Plants at the 4-leaf stage were carefully washed to remove soil from the roots, then they were directly freeze dried. Roots collected at the 6 week stage were peeled with a standard kitchen peeler to remove the epidermis and a portion of the root tissue and the peelings were placed in plastic sample bags (10 cm x 10 cm). All samples were freeze dried in a Virtis FreezeMobile 35 xL (SP Scientific, Gardiner, NY) for 36-72 h. The freeze dried root samples were ground in liquid nitrogen with a mortar and pestle to a homogenous powder. Genomic DNA was extracted from homogenous root powder following a CTAB and phenol extraction method (Stewart and Via, 1993). PCR was performed with a primer set described above that were specific to SCN. PCR was conducted with Go Taq Flexi DNA Polymerase kit (Promega, Madison, WI) following the manufacturer's instructions with a final $MgCl_2$ concentration of 2.5 mM. PCR procedures were followed as described above.

Results

SCN penetration of roots

Stained SCN juveniles were observed in the upper portion of the main tap-root, with 25-50 juveniles observed per root. The juveniles in the root appeared bright pink in color with a vermiform shape. To ensure that the SCN juveniles observed in the upper portions of the taproot

were not on the root surface, a confocal microscope was used to verify juveniles were in the tissue. Upon finding a nematode in the root, the outline of a root cell above the juvenile was brought into focus and set as the upper point. Another outline of a root cell that was below the juvenile was brought into focus and set as the lower point. Once the upper and lower planes were set, the microscope automatically scanned ~20 points between the upper and lower points, thus scanning and focusing on the area within the root where the SCN juvenile was located (Fig. 1).

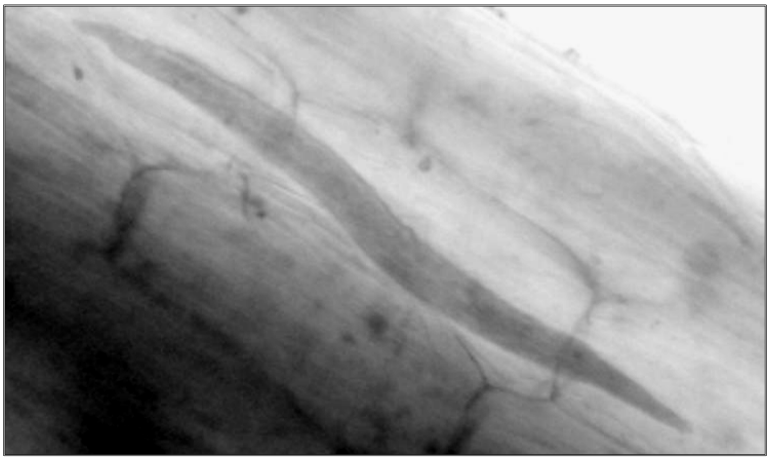

Figure 1. SCN stage 2 juvenile in sugarbeet root

Age study

Semi-quantitative PCR was utilized to quantify the amount of SCN in sugarbeet roots (Fig. 2). In cultivar M832224, SCN was found only in plants inoculated at 1 and 2 weeks postemergence. In SES Vanderhave SBCN susceptible, SCN was found in all four plant ages, with the 2 weeks postemergence showing the most SCN present. In cultivar 0957-22, SCN was found at 2 and 3 weeks postemergence, while in SES Vanderhave SBCN resistant, SCN was found at 2, 3, and 4 weeks post emergence. This experiment was repeated with similar results.

In general, plants inoculated at 2 to 3 weeks post emergence appeared to be most susceptible to SCN penetration.

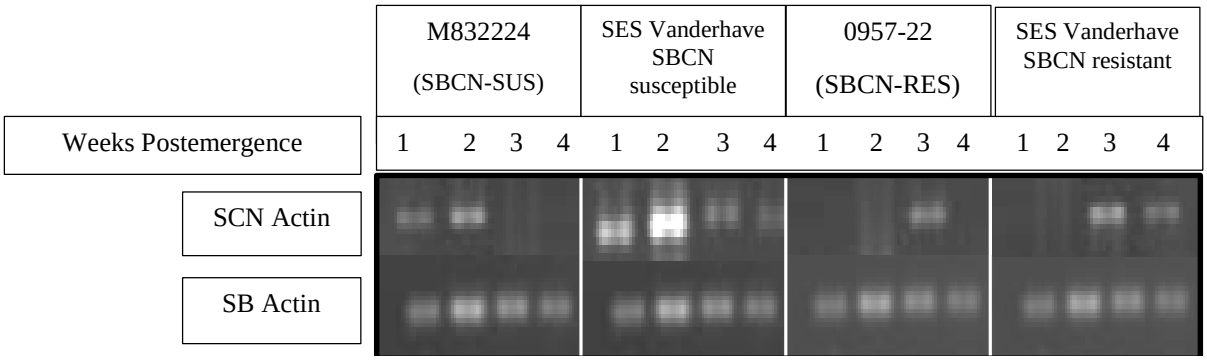

Figure 2. Effect of seedling age on penetration of sugarbeet by soybean cyst nematode

Samples were normalized in concentrations and primers that amplified sugarbeet actin through PCR were used to standardize all samples. Primers that amplified on SCN actin was run with PCR to show which varieties at what age (weeks post emergence) had the greatest SCN penetration. Bands that are brighter for SCN actin amplification show that there is a greater presence on SCN genomic DNA extracted from root tissue. The sugarbeet cyst nematode (SBCN) susceptible varieties of M832224 and SES VanderHave SBCN susceptible showed greater SCN penetration at the plants younger stages. The resistant sugarbeet varieties 0957-22 and SES VanderHave SBCN resistant showed greater SCN penetration in plants that were inoculated at a later plant age.

Field study

Polymerase chain reaction was conducted on all 80 samples taken from the two field locations. Analysis was performed to determine if there was a positive or negative amplification to genomic SCN actin DNA. A positive amplification indicated the presence of SCN within the sugarbeet root, while a negative amplification indicated no SCN within the root. As shown in table 2.1, 29 of the 80 samples had positive amplification of SCN actin. SCN was found within the sugarbeet roots at both locations, in both SBCN-resistant and SBCN-susceptible cultivars, as well as both time points.

Table 1. Identification of soybean cyst nematdoe (SCN) in sugarbeets growing under field condtions in soil naturally infested with SCN during 2011

	Location		Growth Stage		Cultivar	
	Wheaton, MN	Herman, MN	4 Leaf	6-Leaf	Hillshog 4022	BetaSeed 0692N
SCN Positive Plants	14	15	18	11	17	12
SCN Negative Plants	26	25	22	29	23	28

Plants were collected from the field, at two locations, Wheaton and Herman, Minnesota. Collections were made at two different times, the 4 and 6 leaf growth stage. At each location there were two varieties, a SBCN resistant (0692N) and a susceptible (4022). PCR was run on the genomic DNA extracted from the collected plants, primers specific to SCN amplification was used to determine if SCN was present or absent.

Discussion

J2 SCN larva were observed within the cells of the sugarbeet seedling root tissue. This study demonstrated that SCN can penetrate the roots of a variety of cultivars and move into the tissues, even though sugarbeet is known to be a non-host of SCN. The results suggest that SCN can penetrate sugarbeet seedling root tissue for at least four weeks after planting, but the optimal time for penetration may vary depending on the sugarbeet genotype. From the results of the PCR analysis, SCN penetration appeared to be most prevalent between 2 to 3 weeks postemergence from the semi-quantitative PCR results. In the cultivar M832224, penetration by SCN appeared strongest in the younger stages of the sugarbeet seedlings, 1 to 2 weeks post emergence, while in cultivar SES VanderHave SBCNsusceptible, the penetration appeared most intense at 2 weeks postemergence. In both the SBCN resistant cultivars 0957-22 and SES Vanderhave SBCN-resitant, penetration appeared most intense at 3 weeks postemergence. These studies point out an important aspect of the resistance in sugarbeet to SBCN. This resistance does not appear to confer resistance to penetration of the roots by SCN. Resistance to *H. schachtii* for *B. vulgaris* was developed through the hybridization of *B. vulgaris* to the wild type *B. procumbens*, which has resistance to *H. schachtii* (Savitsky, 1975). In soybean cultivars

resistant to SCN, the mechanism for resistance is to prevent a syncytium to be formed (Kim et al., 1987). Penetration by nematodes to roots of resistant host plants is known to cause damage to the roots through the mechanical penetration alone (Back et al., 2002).

The results from the first two experiments showed that SCN juveniles can penetrate sugarbeet seedlings growing in potting soil mix in a greenhouse under controlled environmental conditions. The results from the field demonstrated that SCN could penetrate sugarbeet seedlings under normal field growing conditions, and that penetration occurred in both SBCN susceptible and SBCN resistant sugarbeet cultivars. Polymerase chain reaction confirmed SCN at both field locations tested as well as both growth stages. No cysts were observed on the roots, which would also confirm observations from the initial study on penetration that found no development of SCN past the J2 stage.

Since J2 larvae cause wounding in the outer root layer during the process of migrating into the root, the wounding they cause could be sites that fungal pathogens might use for access into the root. Furthermore, wounding by the nematode might result in increased amounts of root exudates that leak into the soil. Those compounds could stimulate the growth of dormant pathogen survival structures or enhance vegetative growth of pathogens. The amount of penetration by SCN larvae would be an important factor in determining the extent of damage to a seedling root. SCN-infested soybean fields in ND that have had susceptible cultivars can have egg densities exceeding 20,000 egg/100 cm^3 of soil (Berlin Nelson, unpublished data). Under high egg densities, sugarbeet seedling roots would likely be penetrated by large numbers of J2 SCN larvae.

Soil-borne pathogens cause serious diseases to sugarbeet in the production areas of ND and northern MN (Crane et al., 2012). The presence of SCN in sugarbeet fields where those pathogens are common could be a factor that enhances seedling disease caused by those pathogens. Two examples of pathogens where this might be a problem are *Rhizoctonia solani* and *Fusarium* spp. Soybean cyst nematode distribution is expanding in this area. Thus in the future more sugarbeet fields will be infested with SCN since soybean and sugarbeet are commonly grown in the same rotations. The results of this suggests that research should be conducted to determine if SCN could enhance seedling disease caused by these soil borne pathogens under field conditions.

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EFFECT OF SOYBEAN CYST NEMATODE ON THE INTERACTION OF SUGARBEET WITH *RHIZOCTONIA SOLANI*.

Introduction

Global agricultural losses from plant-parasitic nematodes account for an estimated $157 billion annually (Abad et al. 2008). Reports show that in the United States from 2003 to 2009, the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, caused more yield loss in soybean than any other soybean disease (Wrather and Koenning, 2006 and Koenning and Wrather, 2010). SCN was first reported in North Carolina in 1955 (Winstead et al., 1955) and was recently discovered in North Dakota in 2003 (Bradley, 2004). Soybean cyst nematode is now considered a major soybean disease in North Dakota and has spread from the southern to the northern border on the eastern side of the state where soybean production is prevalent. The predominant biotype of SCN in North Dakota is HG type 0. Soybean cyst nematode infects primarily legumes, but also some nonleguminous plants

Soybean cyst nematode is in the same genus, *Heterodera*, as the sugarbeet cyst nematode (SBCN), *H. schachtii* Schmidt. The two species are closely related and even hybridize (Miller, 1976; Potter and Fox, 1965; Radice et. al., 1988). Sugarbeet cyst nematode is a major pathogen of sugarbeet and completes its life cycle on the host (Harveson et al. 2009). SCN is not known to reproduce on sugarbeet, thus sugarbeet is considered a non-host. However, SCN is reported to penetrate the roots of non-hosts (Riggs, 1987; Schmitt and Riggs, 1991). It is possible that SCN could also penetrate or attempt to penetrate sugarbeet roots. Since there are known *Heterodera*-fungal interactions in plants (Adeniji et al, 1975; Tabor et al, 2003; Xing and Westphal, 2009),

there is a potential that SCN could interact with fungal pathogens of sugarbeet, increasing disease incidence or severity.

Rhizoctonia crown and root-rot (RCRR) caused by *Rhizoctonia solani* Kuhn, is a common and endemic soil-borne plant pathogen of sugarbeet that is widespread throughout the United States, Europe and Japan (Harveson et al., 2009). Dark circular lesions on the root surface are indications of RCRR damage, and younger plants exhibit lesions near the root tip (Agrios, 2005). Rhizoctonia root and crown rot is associated with anastomosis group (AG) 2-2. The group AG 2-2 can further be divided into IV or IIIB, with IIIB being more aggressive on sugarbeet (Harveson et al. 2009). The presence of SBCN has been shown to increase damage from *R. solani* (Polychronopoulos et al. 1969). Rhizoctonia crown and root-rot showed distinct development in root regions damaged from *H. schachtii* penetration (Hillnhütter et al. 2012). Through the use of nuclear magnetic resonance imaging it has been confirmed by Hillnhütter et al., (2012) that RCRR of sugarbeet developed above and below the inoculation site when *H. schactii* was present, whereas RCRR only developed above the inoculation site when *H. schachtii* was absent.

Upon hatching a nematode larva locates a root or suitable host by chemolocation and uses a piercing stylet and esophageal enzymes to puncture the root tissue (Davis et al., 2004). Once the larva enters the vascular tissue, it identifies a suitable place to establish a feeding site known as a syncytium. The amount of water present in the root tissue influences where the syncytium is established within the root (Johnson et al., 1993). Soybean cyst nematode larvae take a direct path from the surface of the soybean root to the vascular cells, causing cellular necrosis along that path (Gheysen and Fenoll, 2002). Penetration of susceptible sugarbeet by *H. schachtii* showed syncytial formation in the vascular cylinder of the sugarbeet. Mechanical damage from

SBCN tunneling causes necrosis of cells prior to the establishment of a syncytium (Bleve-Zacheo and Zacheo, 1987). The penetration, movement of larvae and establishment of the feeding site causes damage to roots which can be exploited by soil borne fungal pathogens. Not only do nematodes provide a point of entry for pathogens to enter sugarbeet root tissue, penetration by the nematode also provides a pathway for fungal colonization into the cortex of the root (Polychronopoulos et al. 1969). *Heterodera* spp. and other endoparasitic nematodes have a more complex life cycle compared to ectoparasitic nematodes, which causes more disruption to the host root (Back et al. 2002).

The objective of this study was to determine if SCN increases disease on sugarbeet seedlings caused by *R. solani* AG 2-2 IIIB. Experiments were conducted under greenhouse conditions where SCN and *R. solani* were simultaneously present.

Materials and Methods

SCN egg production

The methods of SCN cyst production on Barnes soybean and egg extraction described by Poromarto and Nelson (2009) were used in this research. Plants were grown in the greenhouse for 30 days under natural and supplemental light using high pressure sodium lamps (1,000 µE m-2 s-1) for 16h/day. Plants were in conetainers immersed in a water bath at 27 ± 3ºC as described by Poromarto and Nelson (2009). At 14 and 21 days after planting, plants were fertilized with 3 ml of a solution of Peters Hydro-Sol 5-11-26 (at the rate of 20 ml of Peters in 980 ml of water; W. R. Grace & Co.-Conn., Fogelsville, PA). Cysts were then washed from the roots and eggs extracted, suspended in distilled water, and used to inoculate sugarbeet.

Inoculum production of *R. solani*

Inoculum of *R. solani* was prepared as described by Pooran De-Suza (2010). About 500 cm^3 of barley grain plus 300 ml of water and 7 grams of potato dextrose broth (Sigma-Aldrich, St. Louis, MO, USA) were placed in a 1-L flask for 24 h and then the mixture was autoclaved for 2 h. The grain was then inoculated with an isolate of *R. solani* AG IIIB that was cultured on potato dextrose agar (Sigma-Aldrich, St. Louis, MO, USA) in a 100 x 15 mm petri dish. The inoculated grain was incubated for 3 weeks at room temperature. The grain inoculum was then air dried and then frozen at -20 C until needed for experiments. Before each experiment the grain inoculum was tested to determine that the inoculum was viable.

Co-inoculation study

Sugarbeet cultivars M832224 (experimental cultivar from SES Vanderhave; SBCN susceptible) and Hilleshog 4051 (*Rhizoctonia solani* tolerant) were planted into Sunshine mix #1 in individual plastic "Cone-tainers" (type D40; Stuewe and Sons, Inc., Corvallis, OR) and grown in the greenhouse under natural and supplemental light using 1000 watt mercury lamps for 16h/day at temperatures ranging from 20 to 29 ºC. Plants were watered daily as needed to maintain sufficient moisture for plant growth. At 14 days post emergence, 10,000 SCN eggs in a water solution were placed around the sugarbeet hypocotyl in each pot. Twenty-four hours after adding SCN, plants were inoculated with *R. solani* AG-2-2 IIIB by placing 2 infested barley kernels three cm from the plant hypocotyl at 1 cm below the surface of the potting mix. Inoculated plants were maintained in the greenhouse for 14 days.

At 14 days post inoculation the roots were carefully removed from the potting mix and rinsed in tap water. Roots were rated for necrosis caused by *R. solani* on a modified 0-7 rating scale as described by Ruppel et al. (1979) and Pooran De-Suza (2010). The rating scale has the following categories: 0 = healthy roots with no lesions; 1 = <1% of root surface with visual lesions; 2 = 1-5% of root surface with visible lesions; 3 = 6-25% of root surface with dry root canker; 4 = 26-50% of root surface with dry root canker; 5 = 51-74% of the root surface with dry rot canker; 6 =75% of the root surface with dry root canker; 7 = 76-100% root rot or total root necrosis.

Experimental design and statistical analysis

The experimental design was a randomized complete block. There were a minimum of 20 plants per treatment (one plant = one replication) with both sugarbeet cultivars. There were two treatments, plants inoculated with SCN alone and plants inoculated with SCN and *R. solani*. The experiment was conducted three times with M832224 and six times with Hilleshog 4051. Each cultivar was considered a separate experiment. Because the scale used a percentage of damage on the root, for each of the seven categories the mid-point of the percentage was used as the estimate of damage to the root for each plant. For example, category 4 would be rated at 38%. That percentage was arcsin transformed for the statistical analysis. Data from individual experiments were analyzed with analysis of variance (ANOVA) with SAS (SAS Institute, Cary, NC) and the variances were compared between repeated experiments. Since variances did not differ by a factor of 10 or greater, the data was combined for each cultivar and analyzed with ANOVA. Differences between the two treatments were accepted if F tests were significant at $P \leq 0.05$.

Results

SCN interaction with an SBCN susceptible sugarbeet cultivar

When data from the cultivar M832224 were combined for analysis there were significant differences ($P \leq 0.05$) between treatments; when each experiment was analyzed separately there were also significant differences ($P \leq 0.05$) between treatments in each experiment. Plants exposed to both SCN- and *R. solani*-infested barley kernels, showed more disease based on necrosis ratings, than plants that were only exposed to *R. solani* infested barley kernels. Averaged over experiments, plants with added exposure to SCN had a mean root necrosis of 20.5 % whereas those not exposed to SCN and only *R. solani* had a mean root necrosis of 12 % (Table 2).

SCN interaction with a *Rhizoctonia solani* tolerant sugarbeet cultivar

In all six experiments with Hilleshog 4051, a *R. solani* tolerant cultivar, there were significant differences ($P \leq 0.05$) in root necrosis between plants that were co-inoculated with SCN and *R. solani* and those that were only inoculated with *R. solani*. The same results were found when the data was combined for analysis. Averaged over experiments, the plants that were exposed to *R. solani* and SCN had a mean root necrosis of 6.4 %, whereas plants that were only exposed to *R. solani* had a mean root necrosis of 2.1 % (Table 2).

Table 2. Effect of soybean cyst nematode (SCN) on root infection of two sugarbeet cultivars by *Rhizoctonia solani*

	Percentage of root surface with lesions	
	Cultivars[a]	
Treatment[b]	M832224	Hilleshog4051
R. solani	12.0[ac]	2.1[a]
R. solani + SCN	20.5[b]	6.4[b]

a. M832224 is susceptible to sugarbeet cyst nematode and Hilleshog4051 is tolerant to *R. solani*
b. Fourteen day old plants received 10,000 eggs per plant placed around the base of the hypocotyl. Twenty four hours later barley kernels infected with *R. solani* were placed 3cm from the hypocotyl. Roots were rated for disease after 14 days postinoculation using a 0-7 scale which estimated the percent or root with lesions. For each category, the midpoint of the percentages was used to estimate the lesion coverage. Plants inoculated with *R. solani* only did not receive any SCN eggs.
c. Data from three experiments with M832224 and six experiments with Hilleshog4051. Data from each cultivar was arcsin transformed for analysis with ANOVA. Means of transformed data were each transformed and presented in the table. Means followed by different letters were significantly different at α=0.05.

Discussion

The objective of this research was to determine if sugarbeet seedlings exposed to SCN could increase root damage from *R. solani*, an important soil-borne pathogen in the sugarbeet production area of North Dakota and north western Minnesota. Inagaki and Powell (1969) showed the importance of mechanical wounding with their work on the nematode *Pratylenchus brachyurus* on the development of black shank (*Phytopthora parasitica*) in tobacco (*Nicotiana tabacum*). They showed that plants with either artificial wounding, simultaneous nematode inoculation with the oomycete, or the nematode introduction 1 week prior to the oomycete all produced more disease on plants than when the oomycete was introduced alone (Inagaki and Powell, 1969). The work of Polychronopoulos et al. (1969) parallels work done by Inagaki and

Powell. Polychronopoulos et al. (1969) showed that *R. solani* hyphae took the path of least resistance created by the juveniles of *H. schachtii* in sugarbeet (*Beta vulgaris*) roots. Work done by Hillnhütter et al., (2012) also showed that penetration by *R. solani* followed the path of penetrating nematodes, first proposed by Polychronopoulos et al. (1969). The data from the current study showed that SCN can increase root damage from *R. solani* under the conditions used in these experiments. Not only was there a greater percentage of necrosis in the cultivar that was susceptible to *H. schachtii* (SBCN), the cultivar that was tolerant to *Rhizoctonia* also showed a higher percentage of necrotic tissue when exposed to SCN. The latter result may indicate that penetration by SCN can reduced the effectiveness of tolerance or resistance to *R. solani* in sugarbeet. Khan (1993) proposed that the physiological changes by invading nematodes can alter the functionality of genes that confer resistance.

Although this research has demonstrated that SCN can increase root damage from *R. solani*, whether this occurs in sugarbeet fields naturally infested with SCN and *R. solani* remains to be determined. In experiments conducted in this research, there was a large amount of eggs placed close to the seedlings prior to placing *R. solani* near the plant. The authors have observed egg levels of 10,000 eggs/100cm^3 or greater in fields where susceptible soybeans have been grown in SCN infested soil, therefore, egg levels similar to what the plants were exposed to in this research could also occur in the field. Lower egg levels may also cause an increase in root infection, but additional research will be needed to determine the effect of eggs levels on root infection by *R. solani*. In addition, plants in these experiments were only exposed to SCN and *R. solani* for 14 days, but in the field sugarbeet plants in soil infested with SCN and *R. solani* would be exposed to both pathogens over a much longer period. Thus, the damage to sugarbeet from

this potential interaction could be greater than indicated by these controlled, short term experiments.

Soybean is commonly used in rotations with sugarbeets and SCN is now well established in the eastern part of North Dakota and northern Minnesota in the Red River Valley where sugarbeet production is concentrated. Although soybean growers can manage SCN through the use of resistant cultivars and crop rotation, they often do not realize they have an SCN problem in soybean until egg levels reach high levels and there is highly visible damage to the crop. Therefore it is not unusual for egg levels to reach high levels in SCN infested fields planted to soybean. Rhizoctonia root rot is a major problem in sugarbeet production and is widespread in the production area. The presence of SCN and *R. solani* together in sugarbeet fields would not be unusual. The results of this study suggest that additional research on this potential problem is warranted and should focus on determining if root infection by *R. solani* is increased in the presence of SCN under field conditions common to commercial sugarbeet production.

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