IMPACT OF *RAPHANUS* AND *BRASSICA* CULTIVARS ON *HETERODERA GLYCINES* (Nematoda) POPULATION DEVELOPMENT

By

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ABSTRACT

IMPACT OF *RAPHANUS* AND *BRASSICA* CULTIVARS ON *HETERODERA GLYCINES* (Nematoda) POPULATION DEVELOPMENT

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Heterodera glycines, Soybean Cyst Nematode (SCN), is a devastating pathogen to Soybeans (*Glycines max).* The objectives of this M.S. Thesis were to: 1) Identify cover crops that are non-hosts for SCN other than the Gramminaceae*,* with special references to radishes and mustards, under greenhouse conditions. 2) Select promising cultivars and evaluate them under field conditions. 3) Make initial observations on potential trap crops for SCN management. All soybean cultivars tested were found to have higher SCN population densities at the end of the experiments, compared to the *Brassica* and *Raphanus* cultivars. The 2017 research sites had greater mean numbers of SCN eggs compared to the susceptible soybean cultivars. Additionally, PI 88788 soybean at East Lansing in 2016 and Edwardsburg in 2017 had a greater reproductive factor than the susceptible soybean. There was little variability in the impact of *Brassica* and *Raphanus* cultivars on SCN under greenhouse conditions. While there was some variability in the field experiments, *Brassica* and *Raphanus* cultivars were often not significantly different from each other. They also never resulted in more than 500 eggs per 100 cm^3, which indicates all cultivars can safely be used in SCN infested fields.

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KEY TO ABBREVIATIONS

Pi: Initial SCN population density at the beginning of the experiment.

Pf: Final SCN population at the end of the experiment.

Reproductive Factor (RF): Pf/Pi.

Good Host: A plant cultivar nematodes are able to increase their populations by 2-fold or greater.

Moderate Host: A plant cultivar where the nematodes are able to increase their populations by more than 0.10-fold but less that 2-fold.

Poor Host: A plant cultivar where the nematodes are able to increase their populations 0.10-fold or less.

Non Host: A plant cultivar where the nematodes are not able to increase their populations .

CHAPTER 1:

Literature Review

Soybean

History - Soybeans are an important global agricultural commodity with a long history of use. The earliest records place their cultivation in China at around the 11^{th} century BC (Hymowitz, 1970). Soybeans were a staple food source in China and Japan for centuries. They eventually made their way to Europe through trade contacts in the 18^{th} century. Soybeans quickly became popular in Europe, assuring a steady demand for this crop. Soybean uses expanded to animal feed, vegetable oil and many others (Hartman *et al.,* 2011). Soybeans were introduced to North America in 1765 by Samuel Bowen (Shurtleff and Akiko, 2014). Bowen, was an English entrepreneur and farmer. He settled in Savannah, Georgia.

Henry Ford is an important figure in North American soybeans. He was born on July 30, 1863, on a farm in Michigan. After his success in the automotive industry, he made it a personal goal to improve the quality of life for the American farmer. To this end he sought agriculture crops that could be processed and converted into industrial products. Ford believed that agriculture and industry were natural partners (Shurtleff and Akiko, 2014). After evaluating various crops at his laboratory at Greenfield Village in Dearborn, Michigan, he decided that soybeans had the most potential. Ford convinced farmers near his automotive plant in Ypsilanti, Michigan to grow soybeans. This was achieved by promising them a market for their crops. Concurrently, Ford's laboratory focused on soybean industrial research. The researchers quickly became pioneers for procedures and equipment for oil extraction. The first use of soybean products in the automotive industry was synthetic paints made with 35% soy oil. Later, the laboratory discovered how to convert soy meal into plastic and fiber (Shurtleff and Akiko, 2014).

Henry Ford's fascination with soybeans also applied to food. At the 1934 Chicago World's Fair, he had a demonstration soybean farm. Part of this soybean demonstration included a soy-based food dinner for journalists. Ford was dressed in a suit made from soybean products (Shurtleff and Akiko, 2014). He was also excited about soy milk and flour and set-up a soy-based bakery near his factory. Henry Ford was fond of testing and promoting new soy foods. (Shurtleff and Akiko, 2014).

<u>Soybean Uses</u> - Eight countries produce about 97% of the world's soybeans (Wrather *et al.,* 2010). In descending order these are: The United States, Brazil, Argentina, China, India, Paraguay, Canada, and Bolivia. Overall, about 6% of the world's arable land is used for soybean production. This ranks soybeans as one of the most important crops globally. About 230 million metric tons (MMT) of soybeans were produced in 2008 (Hartman *et al.,* 2011), with 83.4 MMT produced in the U.S. (Wrather *et al.,* 2010). The value of the U.S. 2015 crop was $35 billion (Ash, 2017). Michigan grows two million acres of soybean annually. It ranks 12th out of 31 U.S. soybean producing states. In 2010, Michigan soybean export value was $589 million, more than that of corn (Wills, 2013).

The high protein, low cholesterol, and low saturated fat content of soybean is the reason for its popularity. It is also a source of complete protein. Soybeans are used in many products. Of all grain crops, soybean has the highest protein content (40%) and second highest fat content (20%). This makes soybeans an important plant-based food source. Its protein is of high quality, making soy a key part of vegetarian diets (Raghuvanshi and Bisht, 2010). Soybeans are processed into meal or oil. Soybean meal contains complete protein, unlike other meals. Approximately 98% of soybean meal is used as animal feed. About 1% is used for human consumption and is used in products such as soy milk and tofu (Wills, 2013).

Soybean oil has many uses, ranging from human consumption to industrial products. The majority of soybean oil, (*ca* 88%) is consumed by humans, mostly as a vegetable oil. The remaining 12% has many commercial and industrial uses (United Soybean Board 2018). Soybean products are becoming important alternatives to petroleum and used for biodiesel production (Wills, 2013). Soybean oil is also used in the making of rubber and plastic.

For over 60 years, soybean oil has been used as a vital ingredient in oil-based paints. Soy-based adhesives were developed to replace products containing formaldehyde. The United Soybean Board funded research on soy-based fibers, resulting in the development of a soy-polyethylene fiber. Soy fiber research designed to realizing the full range of applications is still ongoing. (United Soybean Board, 2018).

Cropping – Crop rotation is a common practice in soybean production. Without crop rotation, bean yields frequently decrease and continuous soybean cropping is usually considered unsustainable. Research has shown that soybeans following crops, such as corn or wheat, at least once in a four-year cycle, provide as much as an 8% increase in bean yield (Ashworth, 2017).

Rotation crops can have profound effects on nematode populations. Non-host crops can be used for management of several types of plant parasitic nematodes. Non-hosts result in lowering population densities through starvation (Grabau and Chen, 2016). Each crop used in a rotation will change the soil-borne nematode community structure and dynamics. This can be beneficial for maintaining the bacterial and fungal feeding nematodes involved in nutrient mineralization (Grabau and Chen, 2016: Ingham *et al.,* 1985).

While nematicides can be valuable in crop production, most are broad spectrum and do not discriminate between phytopathogenic and beneficial species. Recent soybean-related research has shown that various nematicides, such as Nimitz® (Fluensulfone), greatly decreased populations of both bacterial and fungal feeding nematodes, which participate in nutrient cycling in the soil (Grabau and Chen, 2016).

<u>Infectious Diseases</u> - On a global basis, there are numerous soybean diseases that significantly reduce bean yield. More than 300 pathogens are known to attack soybeans. The majority of these however, are negligible and would likely never be noticed by soybean growers (Hartman and Hill, 2010). In the U.S., the number one yield-robbing disease of soybeans is caused by SCN. Other key diseases that cause problems for U.S. soybean growers include root and stem rots including white mold, seedling diseases, sudden death syndrome and charcoal rot (Wrather *et al.,* 2010). These will be discussed in this section, while SCN and sudden death syndrome are the focus of the next part of this Literature Review. Soybean rust is an important infectious disease in many parts of the world. In the U.S., however, it rarely has a significant impact on bean yields. Research on this disease focuses on breeding for resistance (Hartman *et al.* 2005).

Stem and root rots of soybeans are caused by the oomycete *Phytophthora sojae* (Kaufman & Gerdemann 1958) and the fungus *Sclerotinia sclerotiorum* (de Bary,1884). In 2006, *P. sojae* was responsible for an estimated 1,424 thousand metric tons (TMT) of soybean yield loss and *S. sclerotiorum* for 362 TMT of loss (Wrather *et al.,* 2010). Once present, *P. sojae* quickly spreads to susceptible plants. Fields that are most at risk have poor drainage or are known to flood. The water-soaked environment is optimal for the proliferation of this pathogen. If infection happens soon after planting, seedlings will rot or damp-off. In some cases, this can result in the need to replant the crop. If soybean plants are established prior to infection, the oomycete infects the root tissue before moving into the stem, causing both stem and root rot. The severity of infection and possible plant mortality relates to the genetics (resistance) of the cultivar. Fields planted with cultivars without resistance can have upwards of 50% stand loss. Cultivar selection is the most important management practice. Multiple sources of resistance have been discovered. Some cultivars have partial resistance. Seed treatment with a fungicide is another option. Cultural practices are also used to prevent establishment of the disease. This is primarily through management designed to assure proper air and water drainage within the field (Dorrance *et al.,* 2007; Hershman, 2012).

White mold is less prevalent than other root and stem rots, but can still be significant. It is caused by the pathogen *Sclerotinia sclerotiorum*. White mold thrives in wet fields that have poor drainage, similar preferred conditions as Phytophthora. White mold can be readily identified in the field as white, cottony mycelial growth on the host. Symptoms begin with water-soaking that eventually turn into lesions. In later stages, it causes rot and blight of the foliage (Heffer Link and Johnson, 2012). Management options for white mold are similar to those for *Phytophthora.* These include cultural controls designed to reduce field moisture and eliminate sources of infection. Fungicides can be applied as seed treatments and crop rotation helps by reducing fresh sources of inoculum. Finally, there are resistance genes in certain host species and cultivars. Use of resistant soybean varieties is always recommended (Diers *et al.,* 2006).

Other soybean seedling diseases are caused by a wide variety of pathogens. In 2006, these caused an estimated yield loss of 1085 TMT (Wrather *et al.,* 2010). For example, Charcoal rot, is caused by a soil-borne fungus *Macrophomina phaseolina* (Tassi) Goid,1947). It caused nearly 700 TMT of yield losses in 2006 (Wrather *et al.,* 2010). Charcoal rot can be difficult to detect. Many of its symptoms overlap with those of other prominent soybean diseases. The pathogen thrives in hot, dry field conditions. It is able to survive in soil in the absence of a host. Infected seeds act a source of inoculum and can be difficult to detect. As infection spreads, the fungus grows through the stem. Its hyphae cause a physical impediment to water and nutrient uptake. There are a few management options available. No-till systems have been shown to have less infection than tilled systems. Irrigation can help prevent the conditions conducive to infection. Rotation with non-hosts is also another important part of Charcoal rot management (Smith *et al.,* 2014).

Soybean Cyst Nematode

Classification, Morphology and Pathology - SCN is a sedentary endoparasite described as *Heterodera glycines* by Ichinohe in 1952. It is classified in the family Heteroderidae.

Phylum: Nematoda

Class: Chromadorea

Subclass: Chromadoria

Order: Tylenchida

Infraorder: Tylenchomorpha

Suborder: Tylenchina

Superfamily: Tylenchoidea

Family: Heteroderidae

Genus: *Heterodera*

Species: *Heterodera glycines* (Ichinohe, 1952)

SCN is responsible for the most devastating soybean disease worldwide (Wrather *et al.,* 2010). The nematode is characterized by sexual dimorphism (Hunt, 2008). Males are vermiform. Females are lemon-shaped and become protective cysts at death. White females can be seen on soybean roots due to their size and distinct color. After death, the female becomes a dark brown cyst (Davis and Tylka, 2000). The metacorpus of the female esophagus is enlarged and fills the neck. SCN has a terminal anus and subterminal vulva. Males have strong spicules and do not have a bursa. Second-stage juveniles have a strong stylet, with basal knobs. The tail uniformly tapers to a rounded end point (Hunt, 2008; Davis and Tylka, 2000).

History – SCN is thought to be native to the home of the soybean; China. The earliest evidence for its origin is from *The Annals of Liü Buwei.* One of these books mentions "three robbers" of crops and provides management strategies for them. One of the robbers, "the land stealing the crops" is thought to be referencing soil-borne pathogens. Soybeans are among the crops featured and it is suspected that SCN was one of the robbers (Riggs, 2004).

The first modern report on SCN is from 1899 in northeastern China. The publication covers a soybean disease known locally as "fire-burned seedling". It had been known for years. The symptoms of the disease and time frame of the publication led to the conclusion that SCN is native to China. (Riggs, 2004). During this time frame, Japan was conducting investigations on a similar disease of soybeans. A 1916 report on "Moon-night disease", which had been observed since 1881, is attributed to SCN (Riggs, 2004). In subsequent years, Moon-night disease was reported in several locations in Japan. In 1951, SCN was classified, as *Heterodera gottingiana* (Liebscher 1892*).* One year later, Ichinohe described SCN as a new species, *Heterodera glycines* (Ichinohe, 1952). The reason for the initial classification was the morphological similarities of these two species.

In 1954, SCN was detected in the United States, in North Carolina. It was most likely introduced in the late 1930's when a wave of nutritional deficiencies afflicted soybean crops. While symptoms of SCN damage were first observed in North Carolina, within a few years, it was reported from surrounding states (Riggs, 2004). As of 2017, SCN has been confirmed in all soybean producing regions of the U.S. (Tylka and Marett, 2017).

The speed at which SCN detection increased inhibited investigating SCN's origin in the U.S. The introduction was most likely from infested soil. The 1954 confirmation was from a field containing bulb-grown flowers, some of which came from Japan. Another possible source was the practice of dusting soybean fields with soil taken from Japanese soybean cultivar sites. This process was used to introduce *Bradyrhizobium,* a nitrogen-fixing bacterium. Introductions and movement of soil and soybeans across North America, however, make it impossible to know the actual source of SCN (Riggs, 2004).

SCN was first detected in Michigan in 1987, in Gratiot County (Warner and Bird, 2000). Since 1987, it has spread to every soybean producing county in Michigan's lower peninsula, except Presque Isle. It causes an estimated $40 million in losses a year in Michigan (Tylka and Marett, 2017).

<u>2018 Distribution</u> – SCN has a worldwide distribution. It can survive a wide range of environmental conditions. It is known to be present in many countries where soybeans are prominent, including Japan, China, Brazil, Canada, and the United States (Wrather *et al.,* 2010). It has been confirmed in most soybean producing states and spreads to new counties every year. In 2017, a total of 37 new counties were added to the United States SCN distribution. It is currently the leading cause of soybean yield losses in the U.S. (Tylka and Marett, 2017: thescncoalition.com).

<u>Host Range</u> – SCN has a relatively wide host range. Many leguminous plants are hosts, including hairy vetch, mung beans, snap bean and pigeon pea. Some members of the *Caryophyllaceae,* such as common chickweed, figworts, common mullein, purple deadnettle

and other flowering annual plants have also been confirmed as hosts (Hunt, 2008). Many of these, such as common chickweed and purple deadnettle, can be found in Michigan fields. Some populations have the ability to reproduce on sugar beets and certain tomato cultivars (Riggs, 2004). Fortunately, Graminaceae spp. are not hosts of SCN.

Life Cycle – The life cycle of SCN can be complete in 30 days or less under optimal environmental conditions. They thrive at temperatures between 28-31C. The first molt, first-stage juvenile (J1) to second-stage juvenile (J2), takes place in the egg, in the cyst. When the correct chemical cues are received, the J2s emerge from the egg and then the cyst. The J2s follow chemical cues to the root zone of an appropriate host (Hunt, 2008; Davis and Tylka, 2000). Once a plant host root system is located, the juvenile penetrates the root tissue through use of its stylet. It migrates intracellularly, seeking the vascular cylinder. When an appropriate feeding site is reached, the J2 nematode adopts a sedentary lifestyle. Secretions from the nematode results in the formation of specialized feeding cells that sustain juveniles through the sausage-shape J2, the swollen J3 and J4 stages and into early adulthood (Hunt, 2008: Davis and Tylka, 2000).

SCN females remain at the feeding site for their entire adult lives. As SCN goes through its juvenile stages, it becomes progressively larger, assuming a sausage shape (Hunt, 2008). At the final molt into adulthood, the female is white and lemon shaped. Females produce 200-250 eggs. Most of the eggs are stored in the female’s body cavity (Davis and Tylka, 2000). Other eggs are deposited outside of the female in a gelatinous matrix. After death, the female body hardens, forming a cyst and becomes a protective barrier that assists in prolonging egg viability. SCN eggs can be viable for up to 20 years (Hunt, 2008: Davis and Tylka, 2000). It is interesting to note that in 2018, it was reported that a currently unidentified nematode species remained viable for more than 40,000 years in Siberian permafrost (Siberian Times, 2018).

SCN males feed on the nurse cells through the J4 stage. At this time, they undergo metamorphosis and become vermiform males. Males exit the root tissue. They move throughout the rhizosphere, fertilizing any adult female SCN they come across, until their death (Hunt, 2008: Davis and Tylka, 2000).

Symptomology – Symptomology is dependent on the severity of infection and environmental conditions. Light to moderate SCN population densities may result in few or no symptoms. Fields with fine textured (clay or silt) soils and adequate moisture are often unlikely to have above ground symptoms from SCN. Stunting and yellowing are the most common symptoms. Fields with high populations and sandy, dry soil tend to see more severe above ground symptoms and sometimes even plant death. In these fields, plants will be yellow and stunted. Root systems are usually stunted and often lack nitrogen-fixing nodules (Davis and Tylka, 2000).

Disease Complex – Under field conditions, Sudden Death Syndrome (SDS) is usually a soybean disease complex caused by the interaction of SCN and the fungal pathogen *Fusarium virguliforme (Aoki et al., 2003)*. SDS was first discovered in Arkansas in 1971 and has since spread across the United States. *F. virguliforme* is the primary causal agent of SDS. When SCN is present, SDS symptoms are more severe and occur earlier in the season. Due to this complex, managing SCN is an important aspect of SDS management (Westphal *et al.,* 2008). It has been suggested that the SCN female body may serve as on over-wintering protective body for the fungus (Personal Communication, George Bird, 2018).

While SDS management options exist, there is a need to continue investigating new control practices. The genomes of both soybean and *F. virguliforme* have been sequenced. Due to this development, biotechnology is likely to play a role in the future of SDS management. There is current promising research on SDS management, including plant stress response and F. *virguliforme* resistance in soybeans (Hartman *et al.,* 2015).

Management – The best SCN management strategy is avoidance. Since the eggs of this nematode can remain viable in protective cysts in soil for as long as 20 years, population reduction is a challenge. Good management practices that reduce risk of infestation include, cleaning equipment to avoid transfer of SCN between fields and use of SCN-free seed (Warner and Bird, 2016). Unfortunately, more than 50% of the soybean fields in Michigan have already been infested with SCN (Schumacher-Lott, 2011).

SCN Scouting - SCN scouting is essential for formulating an appropriate management plan. Typical SCN scouting (nematode sampling) involves taking as many soil cores as practical at random from the field or a specific portion of the field (Perez-Hernandez and Giesler, 2017). The fall is the optimal time to sample. These cores are then mixed before SCN extraction in order to homogenize the soil samples. This process is important to account for the nematode variability within locations (Perez-Hernandez and Giesler, 2017). The SCN coalition also outlines this information, and will be discussed in a later paragraph.

SCN Management - Crop rotation is an important aspect of a SCN management plan. One to two years of a non-host crop is recommended. While SCN eggs in cysts can remain viable as much as 20 years, egg viability decreases in the absence of a host by about 20% per year (Davis and Tylka, 2000). It should be noted, however, that these decreases are unpredictable and are heavily dependent upon environmental conditions (Davis and Tylka, 2000). Corn and wheat are commonly used as rotation crops in Michigan soybean systems. Sugar beet is also an option in parts of the state.

Use of SCN resistant cultivars is the most common SCN control procedure used, usually in conjunction with crop rotation. While over 100 genetic sources of SCN resistance have been identified, only seven have been developed for sale in commercial cultivars (Mitchum, 2016). Only three, however, are currently commercialized for use in soybean cultivars grown in the North Central region of North America. These sources are PI 548402 (Peking), PI 88788, and PI 437654. Most states, including Michigan, have used cultivars from a single source of resistance, PI 88788, for the past 20 years. In some years, as much as 95% of the market share in the North Central United States came from this line (Mitchum, 2016). Overuse of a single source of resistance has led to highly aggressive SCN populations. Research has shown that PI 88788 in particular is becoming less effective and resulting in increases in SCN reproduction (McCarville *et al.,* 2017). In 2018, a new SCN Coalition was formed to combat this problem. The SCN coalition is a partnership of universities from 27 states and Ontario, checkoff organizations, and the agricultural industry (Begeman, 2018). Additional information about the Coalition can be accessed at www.scncoalition.com.

According to the SCN Coalition the majority of soybeans planted in the Midwest derive their resistance from PI 88788, since that is what readily available. Soybean cultivars with PI88788 resistance use multiple copies of the rhg1 locus in order to convey resistance. There is evidence that when multiple copies of this gene are present, there is greater nematode suppression (Cook et al., 2012). Cultivars with PI 548402 (Peking) resistance employ two genes for resistance. This requires Rhg1 and Rhg4 to convey resistance (Lee et al., 2015). PI 437654 resistance is also found primarily on the Rhg1 and Rhg4, although there are five minor locus that have been mapped and are thought to be involved (Concibidio *et al.,* 2004). Studies suggests that there are different functional alleles for Rhg1 on PI 88788 and PI 437654 (Brucker *et al.,* 2005).

In order to halt development of aggressive SCN populations, alternate sources of resistance need to be incorporated into commercial cultivars. In many cases it has been difficult to breed for both SCN resistance and superior agronomic traits. Frequently, alternate sources of resistance have resulted in "yield drag". This issue needs to be resolved. Researchers are currently investigating increasing effectiveness by stacking Rhg1 and Rhg4 as, well increasing the number of Rhg1 copies (Mitchum, 2016).

An additional management strategy is the promotion of suppressive soils. It has been observed that a long-term soybean monoculture (> 6 years) can result in a decrease in in soil-borne pathogens (Hamid *et al.,* 2017). Continuous soybean cropping can result in soils suppressive to SCN. A suppressive soil is defined as when a pathogen is present but causes little crop damage due to the presence of antagonistic organisms. This has been observed in some long-term soybean production systems in China and the United States since the 1980's

(Chen, 2006). One question researchers have tried to answer is what specific organisms and processes cause this phenomenon. As a result of continuous cropping, there is a shift in the microbiota composition of the soil microbiome. This shift causes a rise in the abundance and diversity of pathogen suppressive microbial species (Hamid *et al.,* 2016). A group of researchers have compared fungal populations in a continuous soybean field with a soy-corn-wheat rotation (Li *et al.,* 2016). The researchers looked for parasitic fungi within SCN cysts. It was found that in the continuous soybean field there was an increase in the diversity of SCN-parasitic fungi, as well as higher populations of those fungi, compared to the rotation. Bacterial population density and diversity can play a similar role in regards to development of suppressive soil (Hu *et al.,* 2017). Microbial communities and their diversity are an important component in the formation of suppressive soils (Li *et al.,* 2016; Hu *et al.,* 2017).

Cover Crops

A cover crop is a plant that is grown to provide one or several agronomic, soil health or pest protection benefits. Cover crop use is increasing in the U.S. but is still slow to be incorporated into the Midwest. According to a 2015 survey of corn and soybean acres cover crops were used on 2.3% of the acreage in Illinois, 2.6% in Iowa, and 7.1% in Indiana (Rundquist and Carlson, 2017). Cover cropping is a complex management strategy and its effectiveness depends on many factors. Soil type, microbiota, field history, and crops present are all factors that influence the effect that cover crops have on soil health (Bowman *et al.,* 2007). It is also necessary to identify specific objectives and select specific cover crop cultivars to achieve them. Cover crops can be planted individually or as blends of cultivars or plant species. Additionally, it is also important to consider planting date, life cycle, and the environmental tolerances of a potential cover crop to make sure it will survive and fulfill the objective (Jimenez-Alfaro *et al*., 2018). In some cases, it is possible to inter-seed cover crops with the cash crop. While cover crops are used by a significant number of Michigan farmers, cover crops are starting to be used in soybean systems (Davis, 2010). An important element in cover crop usage is that it needs to fit into the crop rotation. In the Midwest this can be difficult in systems that only use corn and soybean due to the lateness of harvest. Introducing a crop that comes off earlier, opening a window for cover crop planting in August, such as wheat, cucumbers, dry beans, or seed corn creates a cover crop seeding opportunity. Iowa in particular has many sources provided by the Practical Farmers of Iowa (Rundquist and Carlson, 2017).

There are many reasons for using a cover crop and some cultivars provide multiple benefits. If a rotation leaves a field fallow, a cover crop will prevent wind and rain erosion. It has been shown that using certain cover crops, such as barley and vetch, can increase the water holding capacity of soil while also being able reduce nitrate leaching when compared to a winter fallow field. (Garcia-Gonzalez *et al*., 2018). Building organic matter is another important function and is the reason cover crops are also known as "green manures". Organic matter building is especially vital when the majority of plant material is removed during harvest. Legumes comprise a significant number of available cover crops and are valued for nitrogen fixation (Curran *et al.,* 2006: Clark, 2015). Nutrient scavenging (fixation) is another reason for using a cover crop. Nutrient loss in groundwater under fall, winter, and spring conditions can be reduced through successful cover crop use. Habitat establishment for promotion of beneficial organisms is a benefit of cover crops that has been a focus in recent years. This includes the establishment of natural pollinators, predators, earthworms, and microorganisms that can provide long-term benefits to a cropping system. Weed suppression can also be obtained through cover cropping since they provide additional resource competition (Curran *et al.,* 2006: Clark, 2015). The effects of cover crops on soil pore size has also been examined. Soil pore parameters can be important factors in soil health and can affect soil temperatures and water infiltration. The cover crops tested included, hairy vetch (*Vicia villosa*), cereal rye (*Secale cereale*), and Austrian winter pea (*Pisum sativum* subsp. *Arvense*). All of the cover crops tested resulted in an increase in the number of macropores from 25 to 75% when compared to the no-cover treatments. This recent study shows that cover crops can have positive soil health effects by affecting soil pore parameters (Cercioglu *et al*., 2018).

Due to the variety of uses and types of cover crops, farmers must have an objective or reason for wanting to plant a cover crop so the correct cover crop(s) can be selected. Therefore, the first and most important step is to identify the purpose or objective for seeding the cover crop. The next step is to select the proper cultivar to achieve the desired objective. Lastly, the cover crop must be managed in a way designed to achieve the objective (Personal Communication George Bird, 2017).

Farmers have difficulty in fitting a cover crop into their crop rotation, in particular, if the farmer in growing only corn, or corn and soybeans. Finding a time to seed the cover crop (frequently called a window) is difficult (Clark, 2015). In addition, it is important to know how a selected cover crop should be terminated. It is important to insure some species don't go to seed because the cover crop could potentially become a weed in subsequent crops. Herbicides, mowing, or tillage can be used to prevent this. Alternatively, fall seeding of a winter-killed cover crop can achieve this without additional cost and time, but this results in no cover during spring which may lead to soil erosion from spring winds and rains. Finally, it is important to know which pathogens are present and how they will interact with the cover crop. Planting cover crops that are a host for certain pathogens may offset the benefits of the cover crop (Curran *et al.,* 2006).

There are multiple ways to seed cover crops. Tillage prior to seeding is recommended for some cover crops as it prepares a seedbed and disrupts established weeds. Cereal rye and small grains, however, can benefit from a no-till approach. No-till plantings are recommended for late plantings or where erosion is a major concern. No-till drills are used to ensure uniformity, depth, and proper soil contact (Curran *et al.,* 2006). Planting depth is determined by the cover crop seed size (Curran *et al.,* 2006).

Broadcast seeding has the lowest rate of establishment of the cover crop planting methods. This method works best with small sized seeds. Multiple seedings may be needed to get an adequate stand. Aerial seeding can be used to cover large acreages. Aerial seeding or high clearance broadcast seeding are necessary methods of inter-seeding a field where another crop is already established (Curran *et al.,* 2006).

There are multiple ways cover crops influence various types of organisms. Rivers *et al.* (2018) examined the use of cover crops in a wheat-corn-soybean rotation to promote arthropod predators. The planting time of these cover crops occurred after wheat harvest for one crop and after corn harvest for the second. Cover crops were planted in the fall and roller-crimper terminated in the spring. The presence of cover crops increased the abundance of arthropod predators in the immediate area (Rivers *et al*., 2018).

A South African study examined the effect of various *Brassica* and *Raphanus* cover crops managed for biofumigant properties on the development of various *Meloidogyne* spp. populations. One cultivar examined was Terranova radish, which is included in my SCN thesis research (Daneel, *et al*., 2018). The effect of the cover crops on nematode species was variable both between the cover crops tested as well as the *Meloidogyne* sp. used. Terranova radish did not result in significant changes in the *Meloidogyne* populations (Daneel, *et al*., 2018).

Many options are available for cover crop selection. The most common include legume, Graminaceae and brassica species. Common winter annuals include crimson clover, hairy vetch, fields peas, etc. Perennials include red or white clover. Sweet clover is used as a biennial. Cowpeas are grown as a winter annual (Clark, 2012). Grasses make up another major group of available cover crops. They are often used as an annual cover crop. Cereal rye, spring oats, sorghum-Sudan grass, and annual ryegrasses are found under this category (Curran *et al.,* 2006). A wide variety of brassicas are used for nutrient scavenging, reduction of soil compaction and biofumigation. Cultivars of rape, kale, turnip, and radish are available. Some of these cover crops also have the possibility of being harvested to help recoup seed costs. Buckwheat is another prominent cover crop that doesn't fall into the categories discussed above (Curran *et al.,* 2006).

Cover Crops and Nematodes – Nematodes fill a wide range of niches in the soil and are involved in the transport and transformation of matter and energy among plants, fungi and bacteria. The majority of soil-borne nematodes are beneficial. Nematode community composition is dependent on the soil and environment, including primary producers. Adding cover crops to a rotation increases the soil biodiversity and thus impacts nematode community structure and population dynamics. The addition of soil organic matter results in increases in fungal and bacterial decomposers and both fungal and bacterial feeding nematodes. (Warner and Bird 2018).

There has been a significant amount of research on the effect of cover cropping on nematode populations. This includes both plant parasitic and beneficial species (Ito *et al.,* 2015). Increased microbial diversity allows for a positive response to soil-borne pathogens. As diversity increases, the likelihood of organisms antagonistic to pathogens increases. There are many roles that need to be filled in the soil food web for proper nutrient cycling and decomposition. Promoting soil biological diversity helps ensure these are filled (Ito *et al.,* 2015).

Specific cover crops reduce certain parasitic nematode populations. The most common method to achieve this is through use of a non-host cover crop. Non-hosts act as pest-starvers. These, however, are less effective for cyst nematodes because of the long viability in cysts in soil (Warner and Bird 2018, Bessey 1911).

Brassica and *Raphanus* species can be managed as biofumigant crops by incorporating their foliage into soil. Mustards, radishes, rapeseed and others contain a high level of glucosinolates. These are volatile chemicals released when plant material is damaged or decomposed. At high concentrations these chemicals are toxic and provide localized pest suppression (Fourie *et al.,* 2015).

There are multiple species of the genus *Raphanus* (radish) and at least four subspecies of R. sativus. These include *R. sativus sativus* (garden radish), *R. sativus oleiferus* (oilseed radish), *R. sativus longipinnatus* (daikon radish) and *R. sativus niger* (horse radish). Daikon and oilseed radish are used as cover crops. A few cultivars of oilseed radish are used as trap crops in sugar beet systems. Mustards are highly variable and include Ethiopian mustard (*Brassica carinata*), wild mustard (*Sinapis arvensis*), White mustard (*Sinapis alba*), Yellow mustard (*Brassica hirta*), Brown/Oriental/ Indian mustard (*Brassica juncea*) and Black mustard (*Brassica nigra*). Various mustards have been used as cover crops and for biofumigation.

Trap Crops

Trap crops are plants that attract and prevent the reproduction of pathogens and pests. They may be cash crops or cover crops. The concept of trap crops began about 100 years ago. German scientists noticed sugar beet failures were being caused by nematodes. To combat this, they planted crops that were a host for the nematode and then terminated the crop before completion of the nematode's life cycle (Bessey, 1911). This is referred to as a Category 1 Trap Crop. The German researchers used summer rapeseed for the trap crop. When the process was repeated multiple times a year, there was a decrease of pest pressure on sugar beets in subsequent years (Bessey, 1911). In more recent times, garden radish has been used as a trap crop to manage *Meloidogyne* spp. The radishes are harvested prior to completion of the nematode life cycle.

Category 2 Trap Crops also attract and prevent reproduction of the target pest. For nematode management, Category 2 Trap Crops release appropriate cues to stimulate the emergence of the J2's from eggs and cysts. The nematodes are attracted to and penetrate root tissue. They locate a potential feeding site and attempt to incite nurse cell formation. The trap crop fails to respond to this stimulus and nurse cells are not formed. This results in nematode starvation of females before reproduction, thus preventing the next SCN generation.

Research on nematode trap crops has been undertaken in several laboratories. This includes, but is not limited to, SCN, *Heterodera schachtii* (Beet Cyst Nematode), *Globodera pallida* (potato cyst nematode), and *Meloidogyne hapla* (northern root-knot nematode). Several commercial oilseed radish cultivars have been developed as trap crops for beet cyst nematode. (Smith *et al.,* 2004: Peterka, 2004). Potato cyst nematode is a severe pest and a quarantine species in many parts of the world. Due to this, it is receiving a lot of research attention, particularly in Europe. Many members of the nightshade family have been explored and recommended as trap crops for BCN (Scholte and Vos, 2005: Timmermans *et al.,* 2007). Historically, garden radish has successfully been used as a trap crop for root-knot nematodes (*Meloidogyne* spp.) since the plant has a shorter life cycle than the nematode.

Control of SCN can be difficult. Using cover crops adds more variables into the production system. Many cash crops, cover crops, and weeds are hosts of SCN. These serve to increase SCN populations even when soybeans are not present. Care needs to be taken in cover crop selection. If a host is used then it needs to be terminated before SCN reproduction occurs. This allows for cover crops to be used as Category 1 Trap Crops. Additionally, some cover crop cultivars act as Category 2 Trap Crops for specific nematode species. For example, specific cultivars of oil seed radish have been developed for control of beet cyst nematode. These trap crops act as alternate hosts that will attract second stage juveniles, but not allow females to fully develop and reproduce. This research MS. thesis research evaluates the SCN host status of several *Brassica* and *Raphanus* cultivars as well exploring them as potential Category 2 Trap Crops for SCN management.

CHAPTER 2:

Reproduction of *Heterodera* glycines on *Raphanus* and *Brassica* Cover Crop Cultivars

Introduction

Soybeans (*Glycines max*) originated in China in 2800 B.C. (Hymowitz, 1970). Today, soybean is a dietary and economic staple in many parts of the world. The United States ranks as the top single soybean producer but is eclipsed by the combined production of Brazil and Argentina, with Brazil itself expected to take the lead in the coming years (George *et al.,* 2018). There are many challenges to successfully growing soybeans, including a diverse array of pests and diseases.

Heterodera glycines (Ichinohe, 1952), commonly known as soybean cyst nematode (SCN), has been a major problem for soybean growers in the United States since the 1950's. SCN can be extremely devastating. Soybean (*Glycines max*) yield losses can be as high as 75% in heavily infested fields, with an estimated $500 million loss annually in the United States. The most common and effective methods of control are resistant soybean varieties and use of non-hosts in crop rotation systems. While there are effective *H. glycines* (SCN) management practices, there is a pressing need to explore new technologies. *Heterodera schachtii* (sugar beet cyst nematode) is successfully managed with trap crop cultivars of *Brassica* species. Seeding *Brassica* and *Raphanus* cover crops for maintaining and renovating soil health is increasing in the United States (Roesch-McNally *et al.,* 2017), but there is limited research on how these cover crops influence SCN population development (Leslie *et al.,* 2017).

The objectives of this M.S. Thesis are to: 1) determine the impact of *Brassica* and *Raphanus* cultivars on SCN population dynamics, 2) determine the suitability of using *Brassica* and *Raphanus* cultivars as cover crops in SCN infested sites, and 3) attempt to identify appropriate *Brassica* and *Raphanus* cultivars as trap crops for SCN management.

Materials and Methods

The research was conducted in 2016 and 2017 in multiple experiments in the G. W. Bird Laboratory Nematology greenhouse and laboratory at Michigan State University. In addition, field research was done in 2015, 2016 and 2017 at three widely distributed commercial soybean sites in Michigan. One 2015 field experiment and four 2015 greenhouse runs were preliminary. These were considered failures and were used to develop research methodology. The results from the preliminary trials are included in Appendices A and B. In the main body of research, two mustards (Hood River Seeds, Evansville, IN) (white and Ethiopian), and two radish subspecies (Center Seeds, Sidney, OH), (daikon and oilseed), were selected for study. Several susceptible and resistant soybean cultivars were also selected (DF Seeds, Dansville, MI). For this paper the terms *Brassica* and *Raphanus* are used to refer to these radishes and mustards.

Greenhouse Research

Overview: Experiments were conducted at the MSU Plant Science greenhouses (42°43'17.2"N 84°28'34.5"W). Experiments where conducted in the greenhouse over the course of nine runs. Runs occurred during the fall and winter of 2016 and 2017. A max of two runs occupied the greenhouse at one time and were staggered so they were not terminated at the same time. A randomized complete block design was used with each treatment (plant cultivar) replicated six times in a single run. Conetainers, 20.95 cm x 4.12 cm, were used as vessels for growing the plants (Figure 1.). The conetainers were filled with media consisting of 50% playground sand and 50% mixed soil, provided by the MSU greenhouse, until one inch of empty space remained at the top. Each conetainer held 100 cm^3 of soil. Ten cultivars were evaluated in the final greenhouse experiments (Table 1). All seeds were provided either by the Michigan State University Plant Science and Microbial Sciences departments or Center Seeds.

Figure 1: Susceptible soybean in conetainers after 45 days. Photograph contains all six replicates of a single treatment from a single run of the host status experiment before being processed for SCN.

Plant Inoculation: SCN inoculant for greenhouse research was collected from greenhouse cultures. The cultures originated from an SCN population at the Michigan State University Department of Entomology Research Farm, East Lansing, Michigan (42°41'25.8"N 84°29'53.0"W). SCN cultures were established on susceptible soybean (DF 155) and allowed to increase for four months. One teaspoon of slow release fertilizer pellets (20-20-20) were added once every month to the soybeans of these cultures. SCN cysts were recovered using a modified centrifugal flotation method (Jenkins, 1964), using a heavy sugar solution, consisting of 1845 grams of sugar dissolved in 3.8L of water. The cysts recovered were examined using an inverted microscope (Nikon TMS, 20x). Based on the available inoculant and estimated number of SCN eggs/cyst, an inoculation standard of 20 cysts (40 eggs/cyst) per 1 ml of water was established. In the field this would be above the danger threshold of 500 eggs per 100 cm^3 of soil.

Table 1: Ten plant cultivars tested in 2016 and 2017 greenhouse and experiments. Industry names are followed by Latin names.

1) Susceptible Soybeans
DF5242R2Y®
DF155®
2) Resistant Soybeans
INA® - PI 437654 x PI 88788
DF242N® – PI88788
3) Biofum Summer® (Blend)
50% Brassica *carinata*
40% Raphanus *sativus* subsp. *oleifera*
10% *Sinapis alba*
4) Ground Hog® Daikon Radish –
Raphaus sativus subsp. *longipinnatus*
5) Cappuchino® Ethiopian Mustard –
Brassica carinata
6) Image® oil seed radish
Raphanus sativus subsp. *oleifera*
7) Braco® White Mustard-
Sinapis alba
8) Defender® oil seed radish –
Raphanus sativus subsp. *oleifera*
9) Action® White Mustard –
Sinapis alba
10) Maximus® Fodder Radish –
Raphanus sativus subsp. *oleifera*

Seeds of the eight *Brassica* and *Raphanus* cover crop cultivars selected for evaluation were germinated for two days at room temperature in a petri dish containing damp tissue paper. After germination, a single seedling was planted in each conetainer in a depression in the media made using a glass rod. Twenty SCN cysts in 1.0 ml of water were added immediately to the future rhizosphere of the seedlings with a disposable plastic syringe. The conetainers were placed in the greenhouse growing area. This process was used for all runs.

Plant Growth*:* Four *Brassica* and *Raphanus* and two soybean cultivars (SCN susceptible and SCN resistant) were used in eight of the runs. Five *Brassica* and *Raphanus* cultivars were included in the two runs. This was done to try to keep the number of treatments balanced. Despite this some treatments were removed due to having few replicates surviving. The *Brassica* and *Raphanus* cultivars were selected because of their potential as SCN trap crops. In every experiment, each cultivar was replicated six times. The containers were split into blocks, randomly arranged, and placed in half of a conetainer rack. The racks were maintained in the greenhouse for45 days. Plants were under 16 hours of light, maintained at 26-28 C, and watered once per day. PAR measurements were not taken. Plants were terminated after 45 days and SCN were extracted. Due to SCN's 30 days lifecycle this would ensure that SCN were in the second generation and would be present in the roots for staining.

SCN Extraction and Population Analysis: After 45 days, the soil and plants were removed from the conetainers. This was followed by gently removing the root systems from the soil. The roots were then rinsed and stored (4.4 C) for future root staining. SCN cysts were extracted from the entire volume (100 cm^3) of the media in each conetainer using the modified centrifugal flotation method (Jenkins, 1964), placing the 100 cm^3 of media in a bucket before adding three to four liters of water and mixing thoroughly. The solution was filtered using a 16-mesh over a 400-mesh sieve. The 16-mesh sieve was used to remove large debris. Media collected in the 400-mesh sieve was rinsed into a centrifuge tube. The sample was re-suspended using a spatula. The samples were then centrifuged at 4000 rpm for four minutes. The supernatant water was decanted and the sucrose solution added. The media was re-suspended using a spatula then centrifuged at 4000 rpm for two minutes. A 400-mesh sieve was used to collect the SCN cysts. The sieve was washed with tap water for 30 seconds to remove any remaining sucrose solution. The cysts were funneled into a test tube. The resulting samples consisted of SCN cysts and eggs in ~5.0 ml of water. Each sample was poured into a petri dish divided into ten segments to ensure accurate nematode population density determination. After all cysts were counted, the sample was funneled into a tissue macerator. Samples were ground for 20-30 seconds to break the cysts and leave the eggs undamaged. Each sample was poured into a 50.0 ml beaker. The tissue macerator was rinsed into this beaker and the sample diluted to 20.0 ml. A 1.0 ml aliquot was used to estimate the number of eggs in a sample. The SCN cysts and eggs were counted under a Nikon TMS, 20x inverted microscope.

Root systems were stained within one week after terminating the experiment using the procedure of Byrd *et al.* (1983). The entire root systems were placed in 600 ml flasks containing 50 ml of a 1:1 solution of Chlorox Regular Bleach and DI H_2O. They were soaked for four minutes with occasional agitation. Afterwards, they were rinsed with tap water to remove any remaining solution. Rinsed roots were soaked in tap water for 15 minutes to remove any remaining solution. Roots were then placed into 100 ml beakers in 30-50 ml of water containing a 1.0-2.0 ml stock acid-fuchsin-stain solution. The beakers were brought to a boil using a hotplate set to high. Boiling occurred for 30-60 seconds before beakers were removed from the hotplate and allowed to cool for ten minutes. Roots were rinsed with tap water and added to beakers containing 50 ml of acidified glycerin. The acidified glycerin was brought to a boil for 30-60 seconds before being removed from the hotplate. Roots were allowed to cool to room temperature before being examined. Three-to- four-cm-long root segments were inserted between two glass discs until all root segments were examined. Samples were inspected (Nikon SMZ18, 10x) for juvenile and adult nematodes. Nematodes observed were categorized by body shape (vermiform, sausage-shape or lemon-shape). This data was not presented due to all SCN observed on *Brassica* and *Raphanus* being vermiform.

Field Experiments:

Overview: Field research was conducted in 2016, and 2017. A preliminary 2015 experiment can be found in Appendix B. The 2016 and 2017 experiments were conducted in East Lansing, Dundee, and Edwardsburg MI. All experiments used a randomized complete block design. All seeds were planted at the following rates: 6.0 lb/A for *Brassica,* 8 lb/A for *Raphanus* and 140,000 seeds/A for soybeans. This was determined from recommendations from Michigan State University Extension.

2016 and 2017 Field Experiments:

***Locations**:* The 2016 field experiments were located at East Lansing, MI (42°41'25.8"N 84°29'53.0"W), Edwardsburg MI, (41°46'38.1"N 86°04'16.1"W), and Dundee, MI (41°56'42.8"N 83°39'08.4"W). The 2017 field experiments were located at East Lansing, Mi (42°41'25.8"N 84°29'53.0"W), Edwardsburg, Mi (41°46'38.1"N 86°04'16.1"W), and Dundee, Mi (41°59'24.9"N

83°43'50.9"W). All plantings occurred before August 15th, which is after winter wheat is harvested and a cover crop window in Michigan soybean production.

***Planting**:* Each cultivar was replicated six times in a randomized complete block design. Each block was 3.0 m^2, separated into nine 1.0 m^2 plots (Figure 2). Each segment contained one of three SCN controls and six *Brassica* and *Raphanus* cultivars chosen based on preliminary greenhouse data and the 2015 field experiment. One of the treatments was a SCN susceptible soybean cultivar. It was always located in the center plot. Also included was an SCN resistant soybean cultivar, a weedy fallow, and five *Brassica* and *Raphanus* cultivars. Since SCN only move a few cm in their lifetime this allows for a greater statistical analysis, as the population numbers in each plot are more likely to be similar. Soybean cultivars and resistance sources were chosen due to being commonly available and used in Michigan. This was done to better understand how treatments would work in current soybean systems. While the center segment in all six replicates was always the SCN susceptible control, the locations of the other eight plots were randomly assigned. All plantings were done in early August, to mimic the time farmers plant cover crops after harvesting winter wheat in Michigan. All seeds were planted by hand at the following rates: 6.0 lb/A for *Brassica,* 8 lb/A for *Raphanus* and 140,000 seeds/A for soybeans.

Figure 2: Example grid for the 2016 and 2017 field experiments. The grid is 3m^2 with each plot within it being 1m^2. The center plot is always a susceptible soybean. All other treatments are assigned a number 1-8 and randomly are distributed around the center.

3	6	5
8	Susceptible	2
1	4	7

Figure 3: Edwardsburg 2016 field, 90 days after planting. Figure shows three plots on one side of a grid. From Left to Right: Oilseed radish, weedy fallow, and white mustard.

Plant Growing Phase: Plots were not managed after planting. No fertilizer was applied and no weed management practices employed. Visual estimates put weed biomass as 30% of the plots in 2016 and 10% in 2017. At the end of the season the experimental plots were left to winter kill.

Post-Harvest Phase*:* Three soil samples were taken from each plot at each location at the time of planting, and 45 and 90 days after planting. These were then homogenized before nematode extraction. Of the soil collected, 100 cm^3 was processed and cysts extracted using the modified centrifugal flotation method (Jenkins, 1964). One hundred cm^3 of soil was placed in a bucket with three to four liters of water and mixed thoroughly. The solution was filtered using a 16-mesh over a 400-mesh sieve. The 16-mesh sieve was used to remove large debris. Nematode numbers were determined using the methods previously described. Plant biomass was considered as a part of this experiment.

Statistical Analysis:

Overview: Statistical analyses were performed using SPSS Grad Pack 25.0. Statistics was done with the aid of a consultant, Andrew Dennhardt, Michigan State University Center for Statistical Training and Consulting (CSTAT). The level of significance for all tests was set to $P = 0.05$.

Greenhouse Data Analysis: Data normality was checked using a histogram to evaluate for skewness. If normality was violated, a negative-binomial with loglink was used instead of a one-way ANOVA. Omnibus tests showed that this model worked significantly outperforms a one-way ANOVA. The negative-binomial with loglink was used to show differences between the cultivars, experiment, and Cultivar x Experiment interactions at $\alpha = 0.05$. Variables analyzed included population densities of SCN eggs, cysts, egg/cyst, and stained nematodes in root tissue. A histogram of the Cook's distances was visually inspected to check for outliers. No outliers were detected. The raw and Pearson residuals were plotted against the predicted values in order to check for violation of the homogeneity of variance assumption. If residuals were normally distributed then a general linear model was used for analysis. if any treatments were discovered to have zero counts they were removed from the analysis due to treatments with all zero counts having no variability. It was not determined if cysts recovered were from initial inoculation. There were no significant differences ($P<0.05$) between runs of different time periods and the data was analyzed together. Confidence Intervals of 95% are provided where appropriate.

Field Data Analysis: Statistics were conducted as outlined in the greenhouse section. Data from 2016 and 2017 was analyzed separately since there were significant ($P<0.05$) differences between years. Location and cultivar interactions were included when appropriate. If there was no significant site x cultivar interaction then data was combined over sites. The negative-binomial with loglink was used to show differences between the cultivars, locations, and Cultivar x Location interactions at $\alpha = 0.05$. The analysis for reproductive factor ($RF=P_f/P_i$) was conducted using a Univariate General Linear Model. RF Data was square root transformed to due to unequal variances. After transformation data was in accordance to equal variance assumptions. A square root transformation was chosen due to the low numbers and zeros present in count data. This was done because residuals plotted appeared to normally distrusted. Reproductive factor was determined from eggs recovered. One outlier was removed from Dundee RF in 2017.

Results

Cyst Development Under Greenhouse Conditions

Statistical analysis showed no significant ($P<0.05$) interactions among cultivars and dates the experiments were conducted; therefore, experiments are combined for presentation. The susceptible and PI 88788 resistant soybean cultivars had the highest mean population of cysts, with 94 and 44 per 100 cm^3 respectively, and were not significantly ($P<0.05$) different from each other (Table 2). All *Brassica* and *Raphanus* cultivars had significantly ($P<0.05$) fewer cysts than the two soybean cultivars, and did not differ significantly ($P<0.05$) from each other. The mean number of cysts for the *Brassica* and *Raphanus* cultivars was ~1.5 per 100 cm^3.

Table 2: SCN cysts recovered from soil of soybean, radish, and mustard cultivars under greenhouse conditions.

Treatment	Mean[1] (100 cm^3 of soil)	Standard Error	95% Confidence Interval	
			Lower	Upper
DF155® susceptible soybean	95 A[2]	17.70	65.61	136.71
DF242N® PI 88788 soybean	44 A	8.40	30.39	64.10
Groundhog® daikon radish	2 B	0.77	1.02	4.31
Braco® white mustard	2 B	0.72	0.92	3.98
Maximus® oilseed radish	2 B	0.75	0.85	4.10
Image® oilseed radish	2 B	0.71	0.65	3.82
Action® white mustard	1 B	0.46	0.54	2.52
Biofum® Summer blend[3]	1 B	0.41	0.45	2.23
Cappuchino® oilseed radish	1 B	0.41	0.45	2.23
Defender® oilseed radish	1 B	0.46	0.41	2.45

1: Means from greenhouse experiments after 45 days.
2: Means followed by same letter are not significantly different from each other.
3: 50% Brassica carinata, 40% Raphanus sativus var. oleiferac, 10% Sinapis alba

Egg Production Under Greenhouse Conditions

No significant ($P<0.05$) interactions were detected between cultivars and the dates the experiments were conducted; therefore results are combined for presentation. The susceptible and PI 88788 resistant soybeans cultivars had the highest egg density. There were no significant differences ($P<0.05$) in egg density between the susceptible and PI 88788 soybean cultivars (Table 3). SCN egg densities on *Brassica* and *Raphanus* cultivars were much lower ($P<0.05$), and did not differ among cultivars (Table 3).

Table 3: SCN eggs recovered from soil of soybean, radish, and mustard cultivars under greenhouse conditions.

Treatment	Mean[1]	Standard Error	95% Confidence Interval	
			Lower	Upper
DF155® susceptible soybean	4354 A[2]	810.8	3022.5	6271.6
DF242N® PI 88788 soybean	1794 A	334.1	1244.9	2584.0
Braco® white mustard	74 B	23.0	40.6	134.13
Groundhog® daikon radish	66 B	20.0	36.0	119.16
Image® oilseed radish	60 B	21.2	29.7	119.9
Maximus® oilseed radish	57 B	18.4	29.9	107.1
Defender® oilseed radish	35 B	11.3	18.2	65.8
Cappuchino® oilseed radish	31 B	9.2	17.6	55.6
Biofum® Summer blend[3]	29 B	8.6	16.5	52.0
Action® white mustard	27 B	7.8	15.0	47.5

1: Means from greenhouse experiments after 45 days.
2: Means followed by same letter are not significantly different from each other.
3: 50% Brassica carinata, 40% Raphanus sativus var. oleiferac, 10% Sinapis alba.

SCN Fecundity Under Greenhouse Conditions

The number of eggs per cyst was highly variable among treatments (Figure 4). The susceptible soybean and PI 88788 cultivars had the greatest mean number of eggs per cyst with 50 and 48 per 100 cm^3, respectively. They were significantly ($P<0.05$) greater than Cappuchino oilseed radish and Action white mustard which had means of 23 and 19, respectively. None of the *Brassica* and *Raphanus* cultivars were significantly ($P<0.05$) different from one another.

Table 4: SCN eggs per cyst recovered from soil of soybean, radish, and mustard cultivars under greenhouse conditions.

Treatment	Mean[1] (100 cm^3 soil)	Standard Error	95% Confidence Interval	
			Lower Bound	Upper Bound
DF155® susceptible soybean	50 A[2]	3.9	42.5	57.9
DF242N® PI 88788 soybean	49 AB	3.9	40.9	56.2
Braco® white mustard	34 ABC	6.3	21.9	46.8
Groundhog® daikon radish	30 ABC	6.3	17.7	42.5
Image® oilseed radish	29 ABC	7.0	15.5	42.9
Maximus® oilseed radish	26 ABC	6.6	12.6	38.6
Defender® oilseed radish	25 ABC	6.6	12.0	38.0
Biofum® Summer blend[3]	25 BC	6.0	13.1	36.9
Cappuchino® oilseed radish	23 C	6.0	11.4	35.2
Action® white mustard *hood*	19 C	6.0	7.3	31.1

1: Means from greenhouse experiments after 45 days.
2: Means followed by same letter are not significantly different from each other.
3: 50% Brassica carinata, 40% Raphanus sativus var. oleiferac, 10% Sinapis alba.

SCN Population Densities on Root Systems in the Greenhouse

Differences in SCN population densities were observed among the stained root systems. Susceptible and PI 88788 soybean cultivars did not differ significantly ($P<0.05$) from each other, and had the highest SCN densities (Figure 5). All of the *Brassica* and *Raphanus* cultivars had fewer nematodes, less than 1.0 per root system (Table 5).

Table 5: SCN recovered from whole root systems of soybean, radish, and mustard cultivars under greenhouse conditions.

Treatment	Mean[1]	Standard Error	95% Confidence Interval	
			Lower	Upper
DF155® susceptible soybean	49.7 A[2]	9.31	34.38	71.72
DF242N® PI 88788 soybean	36.4 A	6.86	25.20	52.71
Maximus® oilseed radish	0.7 B	0.35	0.27	1.84
Groundhog® daikon radish	0.5 B	0.25	0.16	1.31
Action® white mustard	0.3 B	0.19	0.11	1.03
Image® oilseed radish	0.2 B	0.17	0.05	1.03
Braco® white mustard	0.1 B	0.10	0.01	0.70
Defender® oilseed radish	0.0 B	0.00	0.00	0.00
Biofum® Summer blend[3]	0.0 B	0.00	0.00	0.00
Cappuchino® oilseed radish	0.00 B	0.000	0.00	0.00

1: Means from greenhouse experiments after 45 days.
2: Means followed by same letter are not significantly different from each other
3: 50% Brassica carinata, 40% Raphanus sativus var. oleiferac, 10% Sinapis alba.

Cyst Development Under Field Conditions

2016 – There was no significant ($P<0.05$) cultivar x site interaction for cyst development. There were, however, significant ($P<0.05$) differences between sites and cultivars. East Lansing and Edwardsburg had significantly ($P<0.05$) higher cyst densities than Dundee (Table 6). Biofum Summer blend, Braco white mustard, Maximus oilseed radish, and Action white mustard all had cyst densities that were significantly lower than the susceptible and PI 88788 soybeans (Table 7). No *Brassica* or *Raphanus* cultivars were significantly ($P<0.05$) different from the weedy fallow.

Table 6: SCN cysts recovered from three field sites across Michigan in 2016.

Field	Mean[1] (100 cm^3 soil)	Standard Error	95% Confidence Interval	
			Lower	Upper
Edwardsburg	7 A	1.00	5.10	9.09
East Lansing	6 A[2]	0.89	4.40	7.93
Dundee	2 B	0.41	1.74	3.39

1: Means of three field experiments after 90 days.
2: Means followed by same letter are not significantly different from each other

2017 - There was no significant ($P<0.05$) cultivar x site interaction for cyst development. Significant differences were observed between sites and cultivars. All three locations were significantly ($P<0.05$) different from each other (Table 8). Edwardsburg had the highest number of cysts recovered while East Lansing had the lowest. Braco white mustard was the only cultivar that was significantly ($P<0.05$) different from all three soybeans (Table 9). No *Brassica* or *Raphanus* cultivars were significantly ($P<0.05$) different from the weedy fallow.

Table 7: SCN cysts recovered from soil of soybean, radish, and mustard cultivars at three field sites across Michigan in 2016.

Treatment	Mean[1] (100 cm^3 soil)	Standard Error	95% Confidence Interval	
			Lower	Upper
DF155® susceptible soybean	24.5A	5.89	15.26	39.21
DF242N® PI 88788 soybean	16 AB[2]	3.89	9.90	25.74
Image® oilseed radish	4 BC	1.05	2.36	6.64
Defender® oilseed radish	4 BC	0.98	2.07	6.12
Biofum® Summer blend[3]	3 C	0.88	1.85	5.46
Braco® white mustard	3 C	0.85	1.75	5.27
Weedy fallow	3 C	0.83	1.73	5.15
Maximus® oilseed radish	3 C	0.81	1.72	5.06
Action® white mustard	2 C	0.60	1.11	3.58

1: Means from three field experiments after 90 days.
2: Means followed by same letter are not significantly different from each other.
3: 50% Brassica carinata, 40% Raphanus sativus var. oleiferac, 10% Sinapis alba.

Table 8: SCN cysts recovered from three field sites across Michigan in 2017.

Field	Mean[1] (100 cm^3 soil)	Standard Error	95% Confidence Interval	
			Lower	Upper
Edwardsburg	3 A[2]	0.49	2.19	4.12
Dundee	1 B	0.21	0.66	1.52
East Lansing	0.3 C	0.09	0.18	0.56

1: Means of three field experiments after 90 days.
2: Means followed by same letter are not significantly different from each other

Table 9: SCN cysts recovered from soil of soybean, radish, and mustard cultivars at three field sites across Michigan in 2017.

Treatment	Mean[1] (100 cm^3 soil)	Standard Error	95% Confidence Interval	
			Lower	Upper
DF242N® PI 88788 soybean	3 A[2]	0.75	1.42	4.53
INA PI 437654 x PI 88788 soybean	2 AB	0.55	0.79	3.12
Defender® oilseed radish	1 ABC	0.46	0.73	2.67
DF155® susceptible soybean	1 AB	0.49	0.67	2.77
Groundhog® daikon radish	1 ABC	0.35	0.46	1.93
Image® oilseed radish	0.7 BC	0.28	0.34	1.52
Weedy fallow	0.7 BC	0.30	0.28	1.61
TC JS001 blend[3]	0.7 BC	0.29	0.28	1.58
Braco® white mustard	0.4 C	0.20	0.13	1.06

1: Means from three field experiments after 90 days.
2: Means followed by same letter are not significantly different from each other
3: 35% INA soybean, 35% cereal rye, 30% Maximus oilseed radish

Egg Production Under Field Conditions

2016 – There was a significant ($P<0.05$) cultivar x site interaction (Table 10) for egg production. At East Lansing, the egg densities of the susceptible and PI 88788 soybeans were significantly ($P<0.05$) greater than all *Brassica* and Raphanus cultivars, with means of 4076 and 2057, respectively. No *Brassica* and *Raphanus* cultivars were significantly ($P<0.05$) different from each other. No *Brassica* or *Raphanus* cultivars were significantly (P<0.05) different from the weedy fallow. At Dundee, the susceptible and PI 88788 soybeans resulted in significantly (P<0.07) greater egg densities than all *Brassica* and *Raphanus* cultivars, with means of 4273 and 2073, respectively. Image oilseed radish egg densities, with a mean of 310, were significantly (P<0.05) greater than Braco white mustard and Defender oilseed radish with means of 37 and 40, respectively. No *Brassica* or *Raphanus* cultivars were significantly (P<0.05) different from the weedy fallow. At Edwardsburg the susceptible soybean was significantly (P<0.05) greater than all *Brassica* and *Raphanus* cultivars with a mean of 3333. The egg densities of Action white mustard, with a mean of 233, were the only *Brassica* and *Raphanus* cultivar that were significantly (P<0.05) lower than the susceptible and PI 88788 soybeans.

2017 – There was a significant cultivar x site interaction (Table 11) in egg production. At East Lansing the PI 88788 soybean, with a mean of 67, resulted in significantly (P<0.05) greater egg densities than the PI 437654 x PI 88788 soybean, Image oilseed radish, Groundhog daikon radish, and Braco white mustard; with means of 10, 10, 7, and 3, respectively. The weedy fallow also had significantly (P<0.05) greater egg densities than Groundhog daikon radish and Braco white mustard. The Dundee site had low egg densities on all cultivars. There are no significant (P<0.05) differences between cultivars. At Edwardsburg, the PI 88788 soybean was significantly (P<0.05) greater than all *Brassica* and *Raphanus* cultivars, with a mean of 1167. The TC JS001 blend and Braco white mustard had the lowest egg densities and were significantly (P<0.05) different from all three soybean cultivars. The weedy fallow was not significantly (P<0.05) different from any *Brassica* or *Raphanus* cultivar.

Table 10: SCN eggs recovered from soil of soybean, radish, and mustard cultivars at three field sites across Michigan in 2016.

Treatment	East Lansing[1] (Standard Error)	Dundee[1] (Standard Error)	Edwardsburg[1] (Standard Error)
DF155® susceptible soybean	4076 A[2] (1664)	4273 A (1745)	3333 A (1361)
DF242N® PI 88788 soybean	2057 A (840)	2073 A (847)	1373 AB (561)
Braco® white mustard	280 B (115)	37 C (15.2)	300 BC (123)
Weedy fallow	163 B (67.9)	110 BC (45.1)	250 C (102)
Defender® oilseed radish	140 B (57.3)	40 C (17.8)	480 BC (196)
Biofum® Summer blend[3]	123 B (50.5)	120 BC (49.2)	343 BC (140)
Maximus® oilseed radish	120 B (49.2)	217 BC (88.7)	353 BC (144)
Image® oilseed radish	110 B (45.1)	310 B (127)	325 BC (133)
Action® white mustard	110 B (45.1)	100 BC (41)	233 C (95.5)

1: Means from field experiments after 90 days from 100 cm^3 soil.

2: Means followed by same letter within a column are not significantly different from each other

3: 50% Brassica carinata, 40% Raphanus sativus var. oleifera, 10% Sinapis alba

Table 11: SCN eggs recovered from soil of soybean, radish, and mustard cultivars at three field sites across Michigan in 2017.

Treatment	East Lansing[1] (Standard Error)	Dundee[1] (Standard Error)	Edwardsburg[1] (Standard Error)
DF242N® PI 88788 soybean	67 A[2] (4.3)	17 A (32.9)	1167 A (133.6)
DF155® susceptible soybean	17 ABC (7.3)	33 A (14.8)	553 AB (248.4)
INA PI 437654 x PI 88788 soybean	10 BC (27.4)	80 A (7.3)	327 ABC (476.5)
Defender® oilseed radish	13 ABC (53.2)	53 A (22)	130 BCD (53.3)
Groundhog® daikon radish	7 C (2.9)	30 A (12.5)	107 BCD (44)
Weedy fallow	43 AB (17.9)	23 A (9.7)	80 CD (32.9)
Image® oilseed radish	10 BC (4.3)	30 A (12.5)	73 CD (30.1)
TC JS001 blend[3]	13 ABC (5.8)	40 A (17.8)	38 D (16.9)
Braco® white mustard	3 C (1.6)	20 A (8.4)	33 D (13.8)

1: Means from field experiments after 90 days from 100 cm^3 soil.

2: Means followed by same letter within a column are not significantly different from each other

3: 35% INA soybean, 35% cereal rye, 30% Maximus oilseed radish

Fecundity Under Field Conditions

2016- There was no significant (P<0.05) cultivar x site interaction for eggs per cyst. There were significant (P<0.05) differences among cultivars, as well as the sites. Dundee had the greatest eggs per cyst with a mean of 72 (Table 12). This was significantly (P<0.05) greater than at East Lansing, which had a mean of 43 eggs per cyst. Susceptible soybean had significantly (P<0.05) higher eggs per cyst than all *Brassica* and *Raphanus* cultivars, with a mean of 133 (Table 13). With Biofum summer blend, Braco white mustard, and Defender white mustard, the number of eggs per cyst were all significantly (P<0.05) lower than the susceptible and PI 88788 soybeans. None of the *Brassica* and *Raphanus* cultivars were significantly (P<0.05) different than the weedy fallow. Groundhog daikon radish, with a mean eggs per cyst of 8, was the only *Brassica* and *Raphanus* cultivar that was significantly different from the three soybeans. No cultivars were significantly (P<0.05) different from the weedy fallow.

Table 12: SCN cysts recovered from soil of soybean, radish, and mustard cultivars at three field sites across Michigan in 2017.

Field	Mean[1] (100 cm^3 soil)	Standard Error	95% Confidence Interval	
			Lower	Upper
Dundee	72A[2]	9.8	54.8	93.9
Edwardsburg	58AB	7.9	44.2	75.8
East Lansing	43B	5.9	32.7	56.2

1: Means of three field experiments after 90 days.
2: Means followed by same letter are not significantly different from each other

Table 13: SCN eggs per cyst recovered from soil of soybean, radish, and mustard cultivars at three field sites across Michigan in 2016.

Treatment/Cultivars	Mean[1] (100 cm^3 soil)	Standard Error	95% Confidence Interval	
			Lower	Upper
DF155® susceptible soybean	134 A[2]	31.608	83.99	212.38
DF242N® PI 88788 soybean	100 AB	23.432	62.14	157.33
Maximus® oilseed radish	62 BC	14.643	38.66	98.16
Action® white mustard	52 BC	12.344	32.5	82.67
Image® oilseed radish	50 BC	11.79	31.02	78.94
Biofum® Summer blend[3]	43 C	10.347	27.19	69.24
Braco® white mustard	42 C	10.018	26.28	67
Weedy fallow	41 C	9.658	25.35	64.61
Defender® oilseed radish	37 C	8.794	23.04	58.78

1: Means from three field experiments after 90 days.
2: Means followed by same letter are not significantly different from each other.
3: 50% Brassica carinata, 40% Raphanus sativus var. oleiferac, 10% Sinapis alba.

2017 – There was a significant cultivar x site interaction (Table 14) for eggs per cyst. At Dundee and Edwardsburg there were no significant ($P<0.05$) differences between treatments. At East Lansing, PI 88788 soybean had the greatest eggs per cyst with a mean of 67. Groundhog daikon radish, defender oilseed radish, and Braco white mustard, with mean eggs per cyst of 7, 4, and 3 respectively, were significantly ($P<0.05$) different from both the PI 88788 soybean and the weedy fallow. No *Brassica* and *Raphanus* cultivars were significantly ($P<0.05$) different from each other or the susceptible and PI 437654 x PI 88788 soybeans.

Table 14: SCN eggs per cyst recovered from soil of soybean, radish, and mustard cultivars at three field sites across Michigan in 2017.

Treatment	East Lansing[1] (Standard Error)	Dundee[1] (Standard. Error)	Edwardsburg[1] (Standard. Error)
DF242N® PI 88788 soybean	67 A[2] (27.4)	5 A (2.4)	48 A (19.6)
Weedy fallow	43 AB (19.3)	20 A (8.8)	24 A (10)
DF155® susceptible soybean	17 ABC (7.0)	17 A (7.2)	54 A (22.1)
TC JS001 blend[3]	13 ABC (5.6)	21 A (8.7)	17 A (7.1)
INA PI 437654 x PI 88788 soybean	10 BC (4.3)	18 A (7.3)	53 A (21.7)
Image® oilseed radish	10 BC (4.3)	25 A (10.4)	40 A (16.5)
Groundhog® daikon radish	7 C (2.9)	8 A (3.4)	25 A (10.4)
Defender® oilseed radish	4 C (17.9)	8 A (8.4)	12 A (12.5)
Braco® white mustard	3 C (1.6)	20 A (8.4)	19 A (7.9)

1: Means from field experiments after 90 days from 100 cm^3 soil.
2: Means followed by same letter within a column are not significantly different from each other
3: 35% INA soybean, 35% cereal rye, 30% Maximus oilseed radish

Reproductive Factor in Field Conditions

2016 – There were significant (P<0.05) cultivar x site interactions (Table 15) for RF. At East Lansing, both the susceptible and PI 88788 soybeans had a significantly (P<0.05) greater RF than all the *Brassica* and *Raphanus* cultivars tested. None of the *Brassica* and *Raphanus* cultivars were significantly (P<0.05) different from the weedy fallow. At Dundee, only the susceptible soybean was significantly (P<0.05) greater than the *Brassica* and *Raphanus* cultivars. None of the *Brassica* and *Raphanus* cultivars were significantly (P<0.05) different from the weedy fallow. At Edwardsburg there were no significant (P<0.05) differences among treatments.

Table 15: Impact of soybean, radish, and mustard cultivars on Heterodera glycines reproductive factor under 2016 field conditions.

Treatment	East Lansing[1] (Standard Error)	Dundee[1] (Standard Error)	Edwardsburg[1] (Standard Error)
DF155® susceptible soybean	40.4 A[2] (37.3)	105.2 A (127.2)	3.5 A (4.4)
DF242N® PI 88788 soybean	48.8 A (56.1)	29.2 AB (37.9)	2.2 A (3.4)
Braco® white mustard	3.6 B (4.2)	0.4 B (0.3)	1.2 A (2.2)
Weedy fallow	5.7 B (10.0)	1.0 B (1.0)	7.2 A (15.7)
Defender® oilseed radish	1.4 B (1.6)	1.4 B (2.4)	5.4 A (8.6)
Biofum® Summer blend[3]	3.4 B (2.5)	1.3 B (1.1)	0.4 A (0.3)
Maximus® oilseed radish	5.3 B (7.3)	4.9 B (5.5)	1.37 A (1.7)
Image® oilseed radish	2.7 B (1.8)	2.3 B (1.7)	1.1 A (1.3)
Action® white mustard	2.5 B (3.0)	3.3 B (7.2)	6.5 A (14.5)

1: Means from field experiments after 90 days from 100 cm^3 soil.
2: Means followed by same letter within a column are not significantly different from each other. Pairwise analysis from square root transformation.
3: 50% Brassica carinata, 40% Raphanus sativus var. oleiferac, 10% Sinapis alba.

2017 – There was no significant (P<0.05) cultivar x site interaction. There were, however, significant (P<0.05) differences among locations of (Table 16). Edwardsburg had a significantly (P<0.05) greater RF than Dundee and Last Lansing. There were no significant (P<0.05) differences among experimental cultivars (Table 17).

Table 16: Impact of 2017 sites on *Heterodera glycines* on reproductive factor.

Field	Mean[1] (100 cm^3 soil)	Standard Error	95% Wald Confidence Interval	
			Lower	Upper
Edwardsburg	4.1 A[2]	7.9	-0.63	1.91
Dundee	1.2 B	2.0	-0.08	2.49
Collins	0.6 B	1.5	2.86	5.39

1: Means from field experiments after 90 days.
2: Means followed by same letter are not significantly different from each other

Table 17: Impact of soybean, radish, and mustard cultivars on *Heterodera glycines* reproductive factor under 2017 field conditions.

Treatment	East Lansing[1] (Standard Error)	Dundee[1] (Standard Error)	Edwardsburg[1] (Standard. Error)
DF242N® PI 88788 soybean	0.27 A[2] (0.41)	1.54 A (3.19)	10.5 A (16.43)
DF155® susceptible soybean	0.71 A (.78)	1.67 A (2.07)	6.07 A (6.57)
INA PI 437654 x PI 88788 soybean	0.27 A (0.41	1.16 A (2.15)	8.57 A (10.01)
Defender® oilseed radish	0.67 A (0.82)	1.95 A (3.18)	0.94 A (1.34)
Groundhog® daikon radish	0.33 A (0.52)	0.567 A (1.20)	5.26 A (10.35)
Weedy fallow	2.27 A (4.35)	1.02 A (1.25)	1.91 A (2.10)
Image® oilseed radish	0.5 A (.84)	1.06 A (1.04)	1.16 A (1.44)
TC JS001 blend[3]	0.67 A (0.82)	1.57 A (2.61)	1.7 A (2.04)
Braco® white mustard	0.17 A (0.41)	0.28 A (0.38)	1 A (1.10)

1: Means from field experiments after 90 days from 100 cm^3 soil.
2: Means followed by same letter within a column are not significantly different from each other. Pairwise analysis from square root transformation.
3: 35% INA soybean, 35% cereal rye, 30% Maximus oilseed radish

Discussion and Conclusion

Discussion

The impacts of *Brassica* and *Raphanus* taxa tested on SCN were similar in all greenhouse experiments. Across sites, as well as years, results were more variable. A single SCN population and plant growth medium was used for all greenhouse experiments. This, however, was not true for the field experiments. The variability in the field appears to be associated with fundamental differences in the SCN populations, as well as the biological, chemical, physical and environmental attributes of the sites. Variability between years was likely caused by a variety of factors, site changes and weather conditions being two. The populations recovered in 2017 were much lower than those of 2016. In 2017 conditions were dry for the first 45 days of growth and waterlogged for the last 45 days. These conditions were not conducive to plant growth. These are also not the optimal conditions for SCN (Davis and Tylka, 2000). The results indicate that while greenhouse research can provide a basic understanding of host-parasite relationships, it does not represent field conditions. Site-specific research, therefore, is essential for developing accurate predictions and recommendations about the impacts of *Raphanus* and *Brassica* cultivars on SCN.

There is no evidence that any of the cultivars tested acted as a trap crop for SCN. One experiment evaluating mustard and radish trap crops for Beet Cyst Nematode resulted in very low reproductive factors on the cultivars evaluated (Smith *et al.,* 2004). In contrast, the reproductive factors of this experiment were much higher than these examples of trap crops. A similar experiment that also contained a fallow control returned similarly small RF's even while the fallow increased numbers (Wright *et al.,* 2018). The RF's of the cultivars evaluated in this experiment were also higher when compared to the Beet Cyst Nematode trap crops.

There are many known weeds host of SCN (Poromart *et al.,* 2015: Venkatesh *et al,* 2000). Several of these are winter annuals that are present in Michigan. While no weed identifications were made in the fallow plots, it is possible that one or more alternate hosts were present at the sites. By comparing the fallow plots of this experiment to the number of eggs recovered from past experiments on weed hosts, similarities can be observed (Poromart *et al.,* 2015: Venkatesh *et al,* 2000).

It has been observed that SCN's ability to reproduce on PI 88788 resistant soybean cultivars is increasing (McCarville *et al.,* 2017). These aggressive populations are present in Michigan due to the overuse of the PI 88788 resistance source. The main reason PI 88788 cultivars were used was to observe how many eggs were produced versus a susceptible soybean. Data from the MSU studies confirm this trend. The soybean cultivars in both the greenhouse and field experiments had the highest cyst and egg population densities, compared to the *Raphanus* and *Brassica* cultivars. In all experiments, the susceptible soybean and PI 88788 soybean cultivars were not significantly different from each other. In the greenhouse, as well as the 2016 field experiments, susceptible soybean cultivars had *circa* 50% more egg production, compared to the PI 88788 soybean cultivars. In the 2017 field experiments, the PI 88788 soybean had greater egg production than the susceptible soybean at Edwardsburg and East Lansing, Mi. At Edwardsburg, in particular, PI 88788 eggs were more than 2-fold greater than the susceptible soybean cultivar. The mean number of eggs per cyst was very similar between PI 88788 and susceptible soybeans in the greenhouse experiments. This was also true at the field sites. In 2017 at Edwardsburg, however, the PI 88788 soybean resulted in a greater number of eggs per cyst than the susceptible soybean. In 2017, INA (PI 437654 x PI 88788) was included in the research. The number of eggs recovered was variable among locations. In East Lansing and Edwardsburg, INA (PI 437654 x PI 88788) soybean had lower egg production than the susceptible and PI 88788 soybeans. At Dundee, however, INA (PI 437654 x PI 88788) resulted in more eggs than susceptible and PI 88788 soybeans. Despite this, it was never significantly ($P<0.05$) different from the susceptible soybean. It was significantly ($P<0.05$) different from the PI 88788 soybean at East Lansing for cysts, eggs, and eggs per cyst recovered, but not at the other locations. This indicates an increasing virulence of certain HG Types, as previously described by McCarville *et al.* (2017).

There were no statistically significant differences among *Raphanus* and *Brassica* cultivars in the greenhouse experiments. In the field, however, there was some variability. All of them resulted in lower SCN cysts, eggs, eggs per cyst, and RF than the soybean cultivars. Image oilseed radish at Dundee in 2016 resulted in greater egg densities than Braco white mustard and Defender oilseed radish. Image oilseed radish at East Lansing in 2016 had the lowest egg production of the *Brassica* and *Raphanus* cultivars while Braco had the greatest. Braco at East Lansing in 2016 had the greatest egg densities of the *Brassica* and *Raphanus* cultivars while at Dundee that same year it had the lowest egg densities. In both years, Defender oilseed radish had some of the highest SCN cysts and eggs recovered after 90 days, except at Dundee in 2016. In 2017 TC JS001 and Braco white mustard resulted in some of the fewest eggs and cysts recovered. When it comes to cover cropping with *Brassica* and *Raphanus*, the 2016 and 2017 field experiments indicate that there are many variables that determine the overall impact of the site on SCN populations. Variability in soil parameters, such as composition, permeability, and microbiome, are likely important factors. It may be that SCN populations interact differently with *Brassica* and *Raphanus* cover crop, similar to the differences between HG types.

Root staining was used to determine how many individuals infected root tissue. Once again, the two soybean cultivars were not statistically different from each other and high numbers of SCN were observed in root tissue. Furthermore, very few individuals were observed in the *Raphanus* and *Brassica* cultivars. No SCN were detected in roots of Defender oilseed radish, Biofum Summer blend, and Cappuchino oilseed radish. This is additional evidence that these *Brassica* and *Raphanus* cultivars are not hosts for SCN. Root staining analysis is a time-consuming process; however, it is the best method of examination. This is because there are fewer steps in the processing procedure from the field to the microscope, and therefore less opportunities for cysts and eggs to be lost.

The SCN reproductive factor (RF) was variable among experimental cultivars. At East Lansing and Edwardsburg in 2016, all *Brassica* and *Raphanus* cultivars had lower RFs than the weedy fallow. At Dundee and East Lansing, all *Brassica* and *Raphanus* cultivar RFs were much lower than the susceptible and PI 88788 soybeans. At Dundee in 2016 only Braco white mustard had a lower RF than the weedy fallow. It was the only *Brassica* and *Raphanus* cultivar at that location to decrease populations of SCN. Meanwhile, at East Lansing, Braco white mustard had the second greatest RF of the *Brassica* and *Raphanus* cultivars. The East Lansing and Edwardsburg weedy fallow plots had high RF values, with SCN experiencing on average a 5-fold or greater populations increase. This indicates that there were probably alternate hosts at these locations among the weeds, probably of the *Caryophyllacaea* family which is known to support SCN populations (Venkatesh, 2000). This could explain some of the high *Brassica* and *Raphanus* RF's, especially at Edwardsburg where all experimental cultivars resulted in reproduction. At Edwardsburg, both soybean cultivars had RF values that were lower than the weedy fallow. At this location Action white mustard was the only *Brassica* and *Raphanus* cultivar that had a greater RF than the soybean cultivars. Defender oilseed radish at Dundee and East Lansing had some of the lowest RFs of the *Brassica* and *Raphanus* cultivars, as well as being close to or below the weedy fallow. At Edwardsburg it had the second highest RF of the *Brassica* and *Raphanus* cultivars, but was still below the weedy fallow. Maximus oilseed radish at East Lansing and Dundee had RF's that were similar to the weedy fallow, indicating it is possibly a poor host for SCN. Overall host status appears to be site dependent.

RF values were lower in 2017, compared to 2016. At East Lansing, all cultivars tested had lower RFs than the weedy fallow and resulted in decreased populations. Braco white mustard and PI 88788 soybean had the lowest RFs, respectively. At Dundee, Groundhog daikon radish and Braco white mustard had RFs below that of the weedy fallow. At Edwardsburg, however, Groundhog Daikon radish was the only *Brassica* or *Raphanus* cultivar with a RF above the weed fallow. The TC JS001 blend, Braco white mustard, and Defender oilseed radish often. They may be poor hosts, allowing some reproduction. Image oilseed radish had a low RF at all three locations. They are most likely non-hosts; however, they may result in premature emergence as a way to explain to population decreases observed.

The results of the field experiments indicate a distinct need for a Cover Crop Evaluation Test to determine which cultivars are appropriate for specific SCN-infested sites. This should be similar to HG Type testing (Niblack *et al.,* 2002). Its primary purpose is to assure that specific cultivars are non-hosts for specific SCN field populations. This concept has not previously been proposed for cover crops in regards to nematode management or their general usage. It may explain a significant portion of the variability of cover crop use success. A greenhouse or field Cover Crop Type Test for SCN management should consist of the following seven components: 1) a susceptible soybean cultivar, 2) PI 88788 resistant soybean cultivar, 3) clean fallow, 4) daikon radish cultivar, 5) oilseed radish cultivar, 6) a white mustard cultivar and 7) a widely used non-host Graminacaeae cultivar such as Wheeler Rye. The greenhouse growing media should be SCN-infested soil from the specific field site of interest. After 90 days, the soil should be processed for SCN cysts, eggs, and eggs per cysts population densities. The field Type Test should be conducted during an appropriate window for cover crop planting. If possible, root staining should also be conducted, or possibly substituted for the soil sample depending on available time and labor.

The data recovered from the susceptible soybean cultivars and PI 88788 cultivars confirm the most recent HG Type Tests for each field location used in this research. There is also potential that the dual PI 437654 x PI 88788 resistance cultivars may have potential as a Category 2 Trap Crops, as indicated from the results of INA (PI 437654 x PI 88788) soybean cultivar, as well as the TCJS001 blend. It is recommended that this be evaluated in future SCN research. However, as the PI 88788 source of resistance becomes increasingly compromised, it may be best to switch to a pure PI 437654 cultivar.

Conclusion

The results show that while greenhouse experiments can provide insight into organismal interactions. While there was more variability observed in the field, cover crop cultivars did not behave differently from the greenhouse experiments. Further experimentation is necessary to determine if the site or populations cause this variability. The field locations demonstrated that SCN populations present at those sites are highly aggressive on PI 88788 resistant soybean cultivars, with higher reproduction observed than what has been reported in past studies. The PI 437654 x PI 88788 most often had the lowest densities of the soybeans in all parameters, however it was not always significantly different from the other two soybean cultivars. Additionally, at Dundee it had the highest eggs counts and at Edwardsburg had the greatest eggs per cyst. Oilseed radish and white mustard cultivars appear to be safe for use as cover crops in SCN infested soybean fields. While they resulted in less SCN than the soybean cultivars, their effects on SCN were still variable among locations. In the future, a simple SCN cover crop test may aid in the selection of cultivars. The results also indicate that HG types may differ in the way they interact with cover crops. If this is the case then it is necessary to conduct testing that is both field and cultivars specific before selecting cover crops.

While a promising trap crop was not found from this research, the studies provide important information to soybean growers. All Brassica and Raphanus cultivars evaluated resulted in fewer eggs than what was recovered from the soybeans cultivars. This indicates that the cover crop cultivars and blends are safe for use in soybean production in order to provide other ecosystem services. Oilseed and daikon radishes can be used for weeds suppression and to reduce soil compaction (Curran *et al.,* 2006: Clark, 2015). Mustards can be used for biofumigation (Fourie *et al.,* 2015). The TCJS001 blend is designed to provide several benefits. The Wheeler Rye is to provide biomass and build soil organic matter, the oilseed radish is to break up hardpan, and the PI 437654 resistant soybean will reduce nematode confirmation, although further testing is needed to confirm this. If found to reduce SCN populations, this blend could be interseeded with corn, opening a window for use in corn-soybean rotations.

APPENDICES

APPENDIX A. Results from preliminary greenhouse trials.

These experiments were not considered successes but were used to refine methods for future experiments. The methods were the same as described in the thesis with two exceptions. Cultivars were run for 30 days instead of 45 days and root staining did not occur.

Table 18: Results of preliminary greenhouse experiments.

Treatment	Mean[1] Cysts	Mean[1] Eggs	Mean[1] Eggs per Cyst	N
Action® white mustard	2.00	40.00	10.00	3
Biofum® summer blend	1.18	65.45	24.55	11
Braco® white mustard	0.29	45.71	40.00	7
Cappuchino® oilseed radish	1.45	116.36	33.82	11
Defender® oilseed radish	0.00	22.22	22.22	9
Frostmaster® winter pea	0.33	33.33	33.33	6
Groundhog® daikon radish	2.83	153.33	40.00	6
Maximus® oilseed radish	0.83	63.33	36.67	6
DF242N® PI 88788 soybean	0.63	32.50	26.88	16
DF5242R2Y® susceptible soybean	2.00	243.75	86.25	16
Terranova® oilseed radish	0.00	20.00	20.00	6

1: Based on two greenhouse experiments after 30 days.
2: 50% Brassica carinata, 40% Raphanus sativus var. oleiferac, 10% Sinapis alba.

APPENDIX B. Results from 2015 preliminary East Lansing field trial.

Planting: The field location for the 2015 summer trial was at the MSU Department of Entomology Research Farm in East Lansing (East Lansing, Mi. 42°41'25.8"N 84°29'53.0"W). Plot size was 1.8m x 3.7m. Each plot contained six rows of plants. Each treatment (cultivar) was replicated six times in a randomized complete block design. The blocks were separated by seventeen feet while there was three feet between plots within the same block. Seven Brassica and Raphanus cultivars were planted in addition to SCN resistant and SCN susceptible soybean controls. Planting of cover crops and soybeans occurred on August 18th. Nematode populations were determined using the methods outlines in the thesis.

Table 19: Results from the 2015 preliminary field trial at East Lansing, MI.

Treatment	Mean[1] Cysts	Mean[1] Eggs	Mean[1] Eggs/cyst	Mean[1] RF	N
Biofum® summer blend	0.83	33.33	26.67	0.1	6
Braco® white mustard	0.33	13.33	13.33	0.02	6
Cappuchino® oilseed radish	1.17	53.33	38.33	0.08	6
Defender® oilseed radish	0.83	30.00	25.00	0.1	6
Frostmaster® winter pea	1.17	43.33	25.50	1.17	6
Groundhog® daikon radish	1.83	40.00	19.17	0.15	6
INA® PI 437654 x PI 88788	0.50	36.67	14.50	0.09	6
DF5242R2Y® susceptible soybean	1.83	110.00	39.50	0.26	6
Terranova® oilseed radish	1.50	103.67	33.33	0.2	6

1: Based on six field experiments after 90 days.
2: 50% Brassica carinata, 40% Raphanus sativus var. oleiferac, 10% Sinapis alba.

APPENDIX C. Record of deposition of voucher specimens.

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher specimens were submitted as photographs.

Voucher Number: 2018-08

Author and Title of thesis: Jeffrey J. Shoemaker, IMPACT OF *RAPHANUS* AND *BRASSICA* CULTIVARS ON *HETERODERA GLYCINES* (Nematoda) POPULATION DEVELOPMENT

Museum(s) where deposited: Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens: *Heterodera glycines*

Table 20: Voucher Specimens.

Family	Genus/Species	Life Stage	Quantity	Preservation
Heteroderidae	*Heterodera glycines*	Juvenile	3	Photograph
Heteroderidae	*Heterodera glycines*	Adult	2	Photograph

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