Evaluating Seed Treatments for the Management of Soybean Cyst Nematode
(*Heterodera glycines* Ichinohe) in Dry Bean (*Phaseolus vulgaris* L.)

by

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ABSTRACT

Evaluating Seed Treatments for the Management of Soybean Cyst Nematode (Heterodera glycines Ichinohe) in Dry Bean (Phaseolus vulgaris L.)

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Soybean cyst nematode (Heterodera glycines; SCN) infestation is a major cause of yield loss in soybean (Glycine max), and dry bean (Phaseolus vulgaris) is an alternative host. In soybean, genetic resistance and seed treatments are mainly used for SCN management however these options are not available in dry bean. Seven seed treatments were assessed for effects on SCN populations in black (cv. Zorro) and kidney (cv. Dynasty; Red Hawk) bean. Two field studies were conducted in 2018 on naturally infested soils near Highgate and Rodney, Ontario. In addition, two different controlled environment studies were completed. There was little treatment response in field studies. In the first controlled environment study, Bacillus amyloliquefaciens and Bacillus firmus reduced cysts in black and kidney bean while fluopyram reduced cysts in Red Hawk only. In second study, fluopyram reduced cysts by 50% and 88% in Dynasty and Red Hawk, respectively while other treatments were inconsistent.
DEDICATION

This dissertation is dedicated to my caring and loving parents Michael and Sarudzai Katsande, my brothers Austin and Omega for their endless support. You will forever be in my heart.
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Chapter One: Literature Review

1.1 Introduction to Dry Bean

Dry bean (*Phaseolus vulgaris* L.) is an important food legume, which belongs to the Leguminosae family (Gepts 2001). The Leguminosae family includes soybean (*Glycine max* (L.) Merr.), chickpea (*Cicer arietinum* L.), clover (*Trifolium* L.), pea (*Pisum sativum* L.), lentils (*Lens culinaris*), and peanut (*Arachis hypogaea* L.), among others. The genus *Phaseolus* also contains several domesticated species of importance such as tepary bean (*Phaseolus acutifolius* A. Gray), lima bean (*Phaseolus lunatus* L.) and runner bean (*Phaseolus coccineus* L.) (Gepts 2001). Dry bean plants are true diploids and most have 22 chromosomes (*2n = 2x = 22*) (Broughton et al. 2003). There are several dry bean market classes including navy, pinto, kidney, black, cranberry and great northern (McDonald et al. 2003). Dry bean is grown worldwide and several products are made from dry bean by different cultures (Prolla et al. 2010). In the tropics, beans are grown mainly for dry seeds alone, while in temperate countries they are grown for dry seeds, fresh pod consumption and processing (Lynch and van Beem 1993). Dry bean is consumed in several ways including chili dishes, soups, fresh salads; beans of low quality can be used to feed livestock (Hardman et al. 1990).

Dry bean is a short season crop, reaching maturity in 65 to 105 d after sowing (Kelly et al. 1999). High temperatures are required for both germination and growth of dry bean (Kooistra 1971; Scully and Waines 1987). The optimum temperature for dry bean germination is 20-30 °C (Scully and Waines 1987). Seedling emergence takes about 17 d when soil temperature is at 10–11°C and only 5 d at 15–16°C (Scarisbrick et al. 1976; Fageria 2002). Extremely high temperatures (32/27°C day/night) can cause male sterility, which results in reduced yields (Gross and Kigel...
There are two plant types found in dry beans namely, determinate bush or indeterminate vine types (Graham and Ranalli 1997; Helm et al. 1990). In addition, the identification of four plant growth habits in bean plants have led to further classification into type I – determinate bush; type II - upright short vine; type III - indeterminate with prostrate vine; type IV - indeterminate with climbing tendencies (Helm et al. 1990). The aforementioned classification of bean plants is based on plant architecture, fruiting patterns, and the plants’ climbing ability (Singh 1999).

Dry bean production in North America is greatly influenced by market prices, while in developing countries beans are used as a major source of dietary protein, especially among subsistence farmers (Hardman et al. 1990). In most parts of the world, there is an inverse relationship between income levels and legume consumption; as meat replaces legumes with increasing income (Robertson and Frazier 1978). On the contrary, there is an exception to the aforementioned relationship in North America, where both the consumption of meat and beans are relatively high.

1.1.1 History and Development of Dry Bean

The true origin of the term ‘bean’ is not known, though some languages have words which relate to the contemporary English word ‘bean' including bona in Old Saxon, bauno in German, boon in Dutch and bonne in Norwegian (Allaire and Brady 2010). The term Phaseolus was derived from the Greek word phaselua, referring to the canoe-shaped bean pods. Beans were among the first crops to be cultivated below the frost line in southern Mexico along with corn, squash and gourd and were part of the Tropical Agricultural Complex (Ford 1981).

Dry bean is believed to have been domesticated independently in South and Central America about 7000 years ago (Hardman et al. 1990). Dry bean’s origins are evident from the fossilized seed material found in Central America and Peru, which dates to that period (Hardman et al. 1990).
Other sources use the terms Andes and Mesoamerica to describe the centres of origin for dry bean (Marzooghian et al. 2013). These two centers of origin gave rise to two major gene pools known as Andean and Mesoamerican, distinguished mainly by morphological as well as genetic traits (Gepts 1998; Johnson and Gepts 1999; Klaedtke et al. 2017).

When the first European explorers arrived in America, the indigenous people were already growing dry bean and it was an important food staple (Robertson and Frazier 1978). The European settlers then introduced the cultivated bean to Europe after 1492 (Klaedtke 2017) and shared the crop with other nations (Wehmeier 2005). The hybridization of the Mesoamerican and Andean gene pools took place immediately after its introduction onto the Iberian Peninsula from the Americas, leading to the creation of novel genetic variation. Thus, Europe is now considered a secondary center of diversity for bean (Klaedtke 2017). The popularity of bean then grew rapidly in Europe, Africa, and Asia by the early 1700s (Wehmeier 2005).

The species in the *Phaseolus* L. genus are centered in the highlands of Mexico, southern and northern parts of Argentina, areas extending from northern to southern and central Arizona, New Mexico and the western part of Texas (Mercado-Ruaro and Delgado-Salinas 1998). Five major geographical groups for wild bean populations have been identified namely, Mexico, southern Peru, Argentina, Central America, and Colombia, by the use of allelic frequency differences of nine polymorphic allozyme loci (Singh et al. 1991). Of the 70-80 described wild species in the *Phaseolus* L. genus, five species have been domesticated in different eco-geographic zones: common bean (*P. vulgaris* L.); runner bean (*P. coccineus* L.); lima bean (*P. lunatus* L.); year bean (*P. dumosus* Macfady) and tepary bean (*P. acutifolius* A. Gray) (Acosta-Gallegos et al. 2007; Rendón-Anaya et al. 2017).
The origin of domestication for dry bean was assigned based on wild bean populations, some well-documented ancient archaeological remains, mentions of common bean in 16th-century Spanish texts, and native Indian language terms used to identify the common bean (Gepts 1988). Plant domestication led to the reduction in genetic diversity in crop gene pools during the initial domestication phase until the subsequent dispersal from the centers of domestication. The domestication of wild bean populations also led to major changes in physiological, biochemical, and morphological characteristics of the crop (Singh et al. 1991). Common bean was domesticated twice in western Mexico and the southern Andes, and the domestication took place in different geographic regions from its wild progenitors (Gepts 2014).

During the evolution and domestication process, the bean crop was exposed to long day photoperiods and warmer temperatures leading to the widening of the crop’s ecological range (Gepts 1998). Dry bean species also possess a wealth of genetic diversity, which facilitated in colonizing such diverse ecological niches (Acosta-Gallegos et al. 2007). A comparison between wild and domesticated forms of common bean showed that in Mesoamerican populations there is a higher variation as compared to Andean populations. The variation probably developed during the dispersal process from the ancestral form (Acosta-Gallegos et al. 2007).

1.1.2 Differences in Market Classes

Dry bean can be divided into two main types, white and coloured while the cultivars are grouped according to their growth habit; determinate cultivars (bush) or indeterminate cultivars (vining or trailing). Today, dry bean exist in several different classes varying in taste, color, and texture (Wehmeier 2005). These market classes are classified according to seed size, seed color, gene pool, and geographic origin (Soltani et al. 2011). The major market classes of beans grown globally are: navy, pinto, kidney (white, light red, and dark red), cranberry, black, small red (also
referred as small Mexican red), brown, yellow-eye and great northern beans (Agriculture and Agri-Food Canada (AAFC) 2005).

Originally, bean germplasm was classified into two centers of domestication; Mesoamerican and Andean (Acosta-Gallegos et al. 2007). These centers were then further classified into races according to their isozyme and molecular information (Singh et al. 1991), genetic lineage and geographic origin (Singh et al. 1991; Mensack et al. 2010). Andean was subdivided into three races: Chilean (northern Chile and Argentina), Peruvian (Peruvian highlands), and Nueva Granada (Columbia). The race Nueva Granada in the USA has the following market classes; white kidney, light red kidney, dark red kidney, and cranberry beans. On the other hand, Mesoamerican was subdivided into Durango, Jalisco, and Mesoamerican races. Market classes in the Race Durango include pink bean, great northern, small red, and pinto, and Mesoamerica landrace is represented by navy, black, and small white beans (Acosta-Gallegos et al. 2007; Mensack et al. 2010).

Physical appearances are used to classify commercial market classes of dry bean grown in North America. Adzuki bean (Vigna angularis (Willd.) Ohui & Ohashi) is small, deep red and oval shaped, black bean is small, oval-shaped with a shiny black coat and a small white hilum, cranberry bean is large, oval-shaped and creamy white with red speckles, great northern bean is large, oblong-shaped and plump with a white skin; red kidney bean (light and dark) is large, kidney-shaped with thicker skins, small red bean is small and round and burgundy red in color, navy bean is small, oval shaped and white, pinto bean is medium sized and oval shaped, with mottled beige and brown skin and yellow-eye bean is round and plump and white with reddish brown and black shades (Uebersax and Siddiq 2012).

Different market classes found in Canada have been categorized in terms of seed size and 100-seed weight; small bean including navy and black (< 25 g), medium sized bean including pinto,
pink, small red and great northern bean (25-40 g), and large bean including yellow, cranberry, and
dark red, light red and white kidney bean (> 40 g) (Balasubramanian 2011). Canadian dry bean
growers also produce some niche market classes on a smaller scale such as kintoki, otebo, Dutch
brown, Jacob's cattle and yellow eye bean (Balasubramanian 2011). In Ontario, a vast array of dry
bean market classes are grown such as cranberry, black, kidney and navy bean. In addition, Ontario
bean producers are now producing other market classes including otebo, pinto, pink and small red
Mexican bean in order to satisfy new global markets, especially those in Asia (Soltani et al. 2011).

1.1.3 Dry Bean Production: World, Canada and Ontario.

The world demand for dry bean is increasing, with an annual production of 20 million t (MMT),
worth approximately US$10 billion. Small subsistence farms in Africa, Mexico, Brazil, and
Central America account for approximately 80% of the global annual production (Goodwin 2003).
World bean production is mainly in developing countries and the distribution by region are Asia
(49.3%), Americas (25.2%), Africa (21.8%), Europe (3.5%) and Oceania (0.1%) (The Food and
Agriculture Organization of the United Nations (FAO) 2017). The top dry bean producers in
descending order are India, Myanmar, Brazil, USA, China, Mexico, Tanzania, Uganda, Kenya,
and Ethiopia (Food and Agriculture Organization of the United Nations (FAO) 2017). The top
exporters of dry bean in 2016 were Myanmar (604 713 t), China (589 857 t), USA (473 975 t),
Argentina (436 055 t), Canada (336 154 t), Ethiopia (184 276 t), Australia (151 601 t), Tanzania
(97 936 t), Kyrgyzstan (83 751 t) and Madagascar (58 510 t) (FAO 2017).

Eight of the world’s top ten bean producers are developing countries (Gepts et al. 2008). During
the past years, bean production has been expanding in some Asian countries such as China and
Myanmar, mainly for export. In most developing countries, dry bean is primarily produced for
consumption rather than for export. For instance, Brazil is the third leading producer but exports
less than 0.1% of the 2 million t produced annually (Gepts et al. 2008). Developing countries export only about 13% of their produce while developed countries exports about 31%. In addition, the average dry bean yield in developed countries is 1944 kg ha\(^{-1}\), almost twice the yield of 1035 kg ha\(^{-1}\) in developing countries (Gepts et al. 2008).

By 2009, more than 10 000 Canadian farms reported pulse crops production with a farm cash receipt of about $1.7 billion. Canada is a major exporter of pulse crops with approximately 75% of the production exported annually (Agriculture and Agri-Food Canada (AAFC) 2011). It is the fifth largest exporter of dry beans in the world (FAO 2017). In 2013, Canada produced 205 910 tonnes of dry beans which generated about $328 million from dry bean exports exceeding $306 million generated in 2012 (FAOSTAT, 2013). The economic importance of dry bean to Canada is shown by farm cash receipts, which were $40.6 million in 1981, and later increased to $150.4 million by 2010 (Bekkering 2014). The major export partners for Canada in 2013 were USA, China, Japan, Mexico and India (FAO 2017).

Southern Ontario has been producing dry beans since the 1800s. In this region, dry bean is produced as a niche high-value crop, and it is normally grown in rotation with field corn (\textit{Zea mays} L.), wheat (\textit{Triticum aestivum} L.), and soybean (Soltani et al. 2011). By the 1980s, the crops’ production area started to expand into western Canada, initially in the provinces of Manitoba and some irrigated areas in Alberta (AAFC 2005). Most Canadian dry bean production for the period, 2000-2002 was in the provinces of Manitoba (57%), Ontario (27%) and Alberta (12%). By 2011, Ontario had the largest dry production area in Canada again, accounting for 38.4% of the national production, followed by Manitoba and Alberta, with 32.1% and 18.8%, respectively (Bekkering 2014). In Ontario and Manitoba, both white and coloured beans are grown, while in Alberta bean producers focused mainly on coloured beans (Bekkering 2014).
In Ontario, dry bean is among the top produced field crops, which include wheat, corn, soybean, barley (*Hordeum vulgare* L.), and oat (*Avena sativa*). In 2014 in Ontario 53,216 ha of dry bean were produced; navy beans (30,351 ha), cranberry (5,666 ha), adzuki (5,261 ha), black (3,642 ha), dark red kidney (3,238 ha), otebo (3,035 ha) and light red kidney (2,023 ha) market classes (Ontario Bean Growers, 2015).

### 1.2 Soybean Cyst Nematode (SCN)

Soybean cyst nematodes are microscopic round worm-like parasites, which attack roots of host plants such as soybean and dry bean (Iowa State University (ISU) 2012; Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) 2017). SCN belongs to the phylum Nematoda, class Secernentea, order Tylenchida, suborder Hoplolaimina, superfamily Hoplolaimoidea, family Heteroderidae, genus *Heterodera*, and species *glycines* (Yu 2011). SCN is distinguished by sexual dimorphism in which the male is vermiform, and the female is lemon shaped (Yu 2011). *H. glycines* females are approximately 0.8 mm in length and they are visible with the naked eye (Smolik and Draper 2007).

#### 1.2.1 History and Worldwide Distribution

There is a current widespread belief that SCN originated in China, even though it was first recorded to have been discovered and described in Japan in 1952 (Yu 2011). Evidence to support this belief includes the fact that China is the centre of origin for soybean (Liu et al., 1997), and most of the genetic sources for resistance are from China (Yu 2011). However, some sources argue that *H. glycines* has been present in Japan since 1881 (European and Mediterranean Plant Protection Organization (EPPO) 2008). This is supported by evidence of SCN damage in Japanese soybean in 1915 (Hori 1915), which precedes discoveries in Korea, China and Columbia (Wrather et al. 1984). SCN was first discovered in North America, in New Hanover County, North Carolina,
USA in 1954 (Wrather et al. 1984). *H. glycines* was then discovered in Tennessee and Missouri in 1956, Mississippi, Kentucky and Arkansas in 1957, and Virginia in 1958. Since then, SCN spread to Alabama, Delaware, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Louisiana, Maryland, Minnesota, Michigan, Nebraska, New Jersey, North Dakota, Ohio, Oklahoma, Pennsylvania, South Carolina, South Dakota, Texas and Wisconsin (Riggs 1975; Yu 2011).

In Canada, SCN was first identified in Kent County, Ontario in 1987 (Tylka and Marett 2014). Since then it has been identified in 15 other counties including Lambton, Essex, Elgin, Haldimand-Norfolk, Huron, Middlesex, Glengarry, Prescott, Perth, Stormont, Oxford, Peel, Bruce and Brant (Tenuta et al. 2006; Yu 2011). Specifically, *H. glycines* was discovered in Peel County in 2003, then in Bruce and Brant County in 2005 (Tenuta et al. 2006), and by 2013 it was reported as far east as Quebec (Oud et al. 2013). Huron and southern Bruce counties were reported to have very high concentrations of 6000 SCN eggs 100 g⁻¹ soil (Bohner 2014). According to OMAFRA (2017) recent surveys indicate that SCN was found in 80% of the tested fields in southwestern Ontario. However, SCN is not reported in the Canadian soybean producing provinces of Manitoba and Prince Edward Island (Tylka and Marett 2014).

Riggs (1975) hypothesized that SCN spread to North Carolina, USA through narcissus bulbs imported from Japan, based on the similarity of disease symptoms on infected plants in both regions. Some publications in the late 1800s reported that soybean seed and soil were imported from eastern Asia to North America (Noel 1993), to obtain the nitrogen fixing bacteria *Bradyrhizobium japonicum* (Kirchner), which accelerated the spread of SCN. In addition, farmers also planted imported black seeded soybean, which was resistant to *H. glycines*. The black seed germplasm is believed to have caused the development of a range of SCN races by exerting selection pressure on the SCN population (Noel 1993). Fourteen SCN races have been identified
and are widely distributed in soybean producing countries (Yu 2011). To date, SCN has been found in Africa in Egypt (unconfirmed), in Asia including China, Indonesia (Java), Korean peninsula, Japan, Taiwan (unconfirmed) and Russia (Amur District in the Far East) in North America including Canada and USA and in South America including Argentina, Brazil, Chile, Columbia and Ecuador (Yu 2011).

1.2.2 Vectors for the Spread of SCN

SCN spreads rapidly through any means that involves soil movement including the movement of farm equipment, animals, contaminated seed and plant parts (Riggs 1975; Chen 2011). For instance, practices such as land leveling and shared use of equipment between farmers can increase the spread of SCN. In addition, practices that involve mixing of soil and seed during harvesting can accelerate SCN spread. Birds can spread SCN, since SCN eggs can pass through the bird’s digestive system without losing their viability (Riggs 1975; Chen 2011). Three black bird species namely the grackle (*Quiscalus quiscula*), the brown-headed cowbird (*Molothrus ater*) and the starling (*Sturnus vulgaris*) have been shown to transport SCN over long distances (Riggs 1975). SCN cysts can resist desiccation and are also spread by the wind. Thus, in some studies, SCN cysts were found mainly at the leeward side of the field (Riggs 1975). SCN is also carried by water especially when streams cross an infested soil or when flooding occurs in infested areas (Chen 2011).

1.2.3 Life Cycle of SCN

SCN is classified as an obligate parasite because it requires nutrients from a host plant to complete its life cycle (Goheen et al. 2013). The life cycle occurs in three main stages: egg, four juvenile or larval states (J1 to J4) and adult (Iowa State University (ISU) 2012; OMAFRA 2017). The highest population densities of SCN are found at a soil depth of 7.5-15 cm (Esser and Langdon...
1967). In contrast, in some studies, the cysts have been found 107 cm deep in the soil while juveniles have been detected on roots at about 168 cm deep (Esser and Langdon 1967). SCN eggs can survive within cysts for more than a decade, waiting for appropriate conditions to hatch (Niblack 2009; Giesler and Wilson 2011; Yu 2011). However, a few eggs can hatch when a non-host crop is cultivated (Niblack 2009). SCN eggs normally hatch in the spring season or during early summer. Hatching may be determined by temperature, host exudates, or time, signifying that some SCN eggs from the same batch hatch under an optimum temperature range, others hatch only when subjected to specific host plant exudates, and still others hatch only when subjected to a certain amount of time (Doyle and Lambert 2003).

The first juvenile stage (J1) occurs in the eggs. The eggs will hatch into second stage (J2) juveniles, which then locate host roots presumably by chemolocation. The thrusting effect of the SCN stylet used in combination with an arsenal of hydrolytic and cell modifying enzymes such as pectate lyases, polygalacturonases, endoglucanases and xylanase assists the J2 to enter the root and migrate intracellularly (Hussey and Grundler, 1998; Davis et al. 2000; Hussey et al. 2002; Jasmer et al. 2003; Davis 2004).

Upon entering the host roots, young nematodes (J2) move into the vascular tissue of the plant (Niblack 2009; Giesler and Wilson 2011; ISU 2012; OMAFRA 2017). SCN invasion results in soybean seedlings that are more susceptible to other pathogens such as *Fusarium virguliforme* (Sudden Death Syndrome (SDS)), *Phialophora gregata* (Brown Stem Rot (BSR)), *Sclerotium rolfsii* (southern stem blight) and root rots (*Phytophthora, Pythium, Rhizoctonia*) because SCN create wounds used as entry points by these pathogens (ISU 2011). Juveniles inject saliva-containing enzymes in the vascular tissues causing the cells to form a syncytium, or feeding site (Giesler and Wilson 2011). The syncytia will then affect the normal functioning of the roots and
divert plant nutrients to the juveniles (Niblack 2009). Juveniles remain at the feeding site where they will molt into J3 and J4 stages before turning into adults. Adult males leave the roots while females continue to feed, swell and protrude from the roots (Giesler and Wilson 2011). Initially females will appear as white spheres before turning yellow and then brown.

Before migrating out of roots, males will mate with and fertilize females, which will then produce eggs filling up their whole body (Giesler and Wilson 2011; ISU 2012). The cavity of a dead female filled with eggs is called the cyst and can contain up to 400 eggs (Giesler and Wilson 2011). *H. glycines* females also deposit about 50–100 eggs outside of their body, prior to forming a hardened body wall around the eggs in which the eggs will survive for a period of up to 10 or more years (ISU 2012). The period of the SCN’s life cycle varies with environmental conditions, for instance ISU (2012) noted that during the summer when soils are warm *H. glycines* completes its life cycle in about 24 to 30 days.

### 1.2.4 Host Crops of SCN

SCN has up to 100 host plant species worldwide (Yu 2011). Most are in the Leguminosae family including soybean, edible bean, bird’s-foot trefoil (*Lotus corniculatus*), cowpea (*Vigna unguiculata* L.), and lupine (*Lupinus albus* L.) (Giesler and Wilson 2011). The following edible bean types have been recorded as hosts of SCN: dry and green beans, snap beans, red beans, lima beans, bush beans, mung beans (*Phaseolus aureus* Roxb.) and adzuki beans (Noel 1993; Giesler and Wilson 2011; Goheen et al. 2013). Weed hosts of SCN include common chickweed (*Stellaria media* L.), wild mustard (*Sinapis arvensis* L.), common mullen (*Verbascum thapsus* L.), field pennycress (*Thlaspi arvense* L.), purslane (*Portulaca oleracea* L.), henbit (*Lamium amplexicaule* L.), pokeweed (*Phytolacca decandra* L.), as well as common (*Lespedeza striata*); service (*L. cuneate*) and shrub (*L. bicolor*) types of lespedeza (Giesler and Wilson 2011; Goheen et al 2013).

### 1.2.5 Symptoms of SCN

The feeding habit of SCN disrupts the normal functioning of the host roots by reducing host plant’s ability to translocate assimilates to the rest of the plant, hence, stunting of plant and yellowing of leaves is noticeable in severely damaged plants (Wrather et al. 1984; ISU 2012; OMAFRA 2017). SCN injury symptoms are most severe under hot or dry conditions when plants are water stressed (OMAFRA 2017). For instance, during summer, SCN-infested soybean plants suffer from premature defoliation caused by wilting (Wrather et al. 1984). Belowground SCN symptoms include stunted roots with a reduced number of nodules. Damage within fields first appears in small circular patterns, a few meters in diameter to huge areas covering the whole field (Wrather et al. 1984). However, SCN was reported to cause up to 30% yield loss without any noticeable above ground symptoms, such as yellowing and stunting (Niblack 2009; Jardine and Todd 2001; ISU 2012).
Symptoms caused by SCN in soybean are often misdiagnosed as nutrient deficiency, herbicide injury, root rots and drought (Wrather et al. 1984; Jardine and Todd 2001; ISU 2012). Appearance of symptoms differs with the level of infestation. For instance, when fields are heavily infested young plants will show symptoms while in lighter infestations, symptoms will become evident usually at the beginning of the podding stage (Wrather et al. 1984).

1.2.6 Economic Importance of SCN

Nematodes are serious parasites of plants worldwide, with economic losses reaching up to US$100 billion annually (Opperman and Bird 1998). SCN is the world’s major cause of economic loss in soybean (Opperman and Bird 1998; Wrather et al. 1998, ISU 2012). Several diseases have been noted to be suppressing soybean yields in Ontario and USA, thus, causing less than optimal returns from the crop (Wrather et al. 2003). In 1998, 10 countries including USA, Canada, India, China, Brazil, Indonesia, and Bolivia among others produced about 97.6% of the total soybean production in the world (Wrather et al. 1998). However, SCN caused higher yield loses than any other disease in these countries during the same period. In 2006, total yield loss caused by SCN in Canada and USA was estimated at 97 800 and 3 368 100 tonnes, respectively (Wrather et al. 2006). SCN losses in soybeans in the USA are approximately US$1.5 billion annually (Wrather et al. 1997). The yield losses in USA have been fluctuating over the years with 3 720 934 tonnes in 2004 to 3 368 658 and 2 557 723 tonnes in 2006 and 2007, respectively (Wrather and Koenning 2009). Wrather et al. (2010) confirmed that the major soybean yield limiting disease is SCN in Canada and it caused some yield loses of up to 40% in 2006. However, the use of resistant cultivars can reduce the economic losses caused by SCN. For instance, a study conducted in southern USA to evaluate the economic impact of SCN resistant cultivar ‘Forrest’ from 1975 to 1980, indicated that the cultivar prevented a yield loss worth US$401 million (Noel 1993). However, there is less
information available on the effect of SCN on dry bean yield, but some studies have shown that SCN can reproduce on several dry bean market classes hence a potential threat to dry bean production (Poromarto and Nelson 2009; Poromarto et al. 2010; Zhang 2018).

1.3 SCN Management

SCN is impossible to eradicate once established in a field, however, SCN population can be managed by lowering the densities below the economic damage threshold (Wrather et al. 1984; Jardine and Todd 2001). The initial step in SCN management is scouting for the presence of SCN, followed by implementing a management plan to reduce SCN populations while maintaining profitable crop yields (ISU 2012).

1.3.1 Crop Rotation

Practicing crop rotation can enhance plant health by reducing the SCN population density in the field. Even though SCN eggs can remain viable in the cysts for up to 12 years, practicing crop rotation with either a non-host crop, or SCN-resistant cultivars will reduce SCN’s impact on yield losses (Niblack 2009; ISU 2012). Planting non-host crops for 1-2 yrs. resulted in a decrease in SCN populations of 75% to 92%, respectively (Wrather et al. 1984). In addition, corn-soybean rotations are relatively effective since it usually takes 8–12 yrs. for the cyst populations to reach economically damaging levels and using resistant cultivars increases the effectiveness of the rotations (Smolik and Draper 2007).

On the other hand, SCN populations can resurge quickly when susceptible soybean cultivars are planted in a crop rotation system (Wrather et al. 1984; ISU 2012). This problem is resolved by rotating resistant soybean cultivars with different sources of resistance such as Peking and PI 88788 as well as rotating to a non-host crop, but the rate of decline is dependent on the initial number of eggs and environmental conditions (Jardine and Todd, 2001).
1.3.2 Host Crop Resistance in Soybean

Populations of SCN are classified into distinct ‘HG’ types, races or strains (Mathew et al. 2015). The ‘HG’ type classification indicates the sources of resistance on which SCN can develop and reproduce. HG stands for *Heterodera glycines*, and SCN populations are referred to as ‘HG types’ if they possess the ability to develop and reproduce on specific resistant cultivars (Dorrance et al. 2012). The most common HG type globally is HG type 0, formerly known as race 4 (Yu 2011).

After the discovery of SCN in the USA, plant breeders made efforts to develop resistant cultivars. The first developed included Plant Introduction (PI) 90763, PI 84751-1, Peking and Ilsoy (Wrather et al. 1984). Three cultivars named Dyer, Custer and Pickett were then released during the late 1960s. These cultivars had low productivity in the absence of diseases; hence, only serve as breeding material. Franklin, Centennial, Forrest and Mack were then released during the second cycle and were higher yielding and resistant to race 3 (HG type 7) of SCN (Wrather et al. 1984). On the contrary, these four cultivars were susceptible to race 4 (HG type 1.2.3.5.6.7) of SCN in Missouri and Tennessee. Plant breeders then used PI 88788 to develop Bradley, Bedford, Nathan and Fayette that were resistant to race 4 (Wrather et al. 1984). Currently, Peking and PI 88788 are the most utilized sources of SCN resistance (Noel 1993; Goheen et al. 2013). About 95% of all resistant commercial soybean cultivars are from one source, PI 88788 (Goheen et al. 2013). As a result, there was a shift in SCN populations and the PI 88788 no longer manages some SCN populations (Faghihi and Ferris 2017).

SCN-resistant soybean cultivars are beneficial to the farmer since they are high yielding in SCN-infested fields and inhibit increase in SCN population densities (ISU 2012). SCN resistant cultivars function by limiting the reproduction rate of SCN, even though they are still attacked by
SCN in the soil. SCN resistant cultivars can increase soybean yields in highly infested fields by more than 50% as compared to susceptible cultivars (EPPO 2008).

1.3.3 Chemical Control

Soil-applied nematicides were frequently used for SCN control before resistant cultivars became common (Noel 1993). Nematicides are categorized into two groups, fumigants and non-fumigants (Wrather et al. 1984). A number of factors such as chemical type, application method, temperature, rainfall, and soil type determines the efficacy of soil-applied nematicides. In the 1960s and 1970s, the most commonly used fumigant was dibromochloropropane commonly known as DBCP due to its effectiveness and affordability, but it was banned in 1979 in the USA (Noel 1993). Other formally used chemical nematicides include carbofuran, fenamiphos and aldicarb (Noel 1993). However, these chemical nematicides have been losing popularity among farmers for several reasons. First, they do not provide season-long control, although they can increase economic returns (ISU 2012). Second, their use raises the production costs (Smolik and Draper 2007; ISU 2012). Third, there is an inconsistent decline in SCN numbers when nematicides are applied (ISU 2012). Lastly, their efficacy is affected by the following environmental factors: rainfall, soil moisture, soil microbial activity, and soil pH (University of Illinois n.d).

On the other hand, both biological and chemical nematicide seed treatments are gaining popularity as SCN management products (ISU 2012). For SCN control in soybean the following seed treatments are being used: *Bacillus firmus* and fluopyram (Bayer Crop Science, Mississauga ON), abamectin and *Pasteuria nishizawai*ae (Syngenta Crop Protection, Guelph ON), and *Bacillus amyloliquefaciens* (Valent, Guelph ON). Chemical seed treatments provide early season protection to the crop, however the protection will decline later on in the season and SCN reproduction will resume (ISU 2012). Just like fumigants, these products also increase the cost of production and
their value depends on the following: effectiveness, impact of SCN induced yield loss, and value of the crop (ISU 2012). In addition, the effect of seed treatments in field studies has been noted to be inconsistent which might be due to environmental conditions or initial nematode densities (Wheeler et al. 2013; Tylka et al. 2015).

1.3.3.1 Fluopyram

Fluopyram is a systemic broad-spectrum fungicide registered on several horticultural and field crops in Canada (Pest Management Regulatory Agency (PMRA) 2016). The fungicide belongs to the chemical class pyridinyl-ethyl-benzamides. The compound can either be applied as a foliar spray or incorporated through drip irrigation (PMRA 2014). Fluopyram formulated as seed treatment has been reported to have an effect on *H. glycines* populations under field and controlled environment (Zaworski 2014; Faske and Hurd 2015; Beeman 2017; Beeman and Tylka 2018; Zhang 2018). It reduced SCN’s reproductive rate, root penetration, and motility in soybean compared to untreated control (Beeman 2017; Beeman and Tylka 2018; Zhang 2018). In addition, fluopyram reduced both egg and cyst numbers in dry bean (Zhang 2018).

Fluopyram belongs to the Group 7 fungicides (FRAC), which are succinate dehydrogenase inhibitors (Australian Pesticides and Veterinary Medicines Authority (APVMA) 2015; PMRA 2016). Fluopyram affects the Ascomycete class of fungi by inhibiting the cell respiration (PMRA 2014). Ascomycetes include various crop pathogens that cause various diseases of economic importance such as *Venturia inaequalis* (apple scab), *Apiosporina morbosa* (black knot) and *Claviceps purpurea* (ergot).

Reduction in root weight was observed in fluopyram treated soybeans under 30-d greenhouse experiments and this was possibly due to the phytotoxic effects of fluopyram (Wise et al. 2015; Beeman and Tylka 2018). Some studies done in Canada indicate that fluopyram is
persistent in soils and has a potential of residue carryover from one growing season to the other (PMRA 2014). Fluopyram is a stable compound and resists processes like photolysis, hydrolysis, and biotransformation (aerobic and anaerobic) in soils under Canadian field conditions (PMRA 2014). In addition, fluopyram has a potential to leach into groundwater in some soil types found in Canada.

1.3.3.2 BAS79800F

The BAS79800F experimental constitutes two components, a non-biological BAS576AAS and a biological BAS97474F. The treatment is still proprietary hence little is known about its components or impact on SCN in soybean and dry bean.

1.3.4 Biological Control

Giesler and Wilson (2011) noted that if non-hosts of SCN were planted for several seasons, some of the eggs would lose viability due to infections from natural bio-agents such as fungi and bacteria. In Alabama, some SCN eggs and cysts were found infected by the fungi Fusarium oxyporum, F. solani and Exophiala pisciphila while in Tennessee, some eggs and cysts were affected by Nematophthora gynophila and Catenona auxiliaris (Wrather et al. 1984). Additionally, natural bio-control agents like Hirsutella species of nematodes can control SCN, while plants in the Brassicae family can also function as bio-fumigants (EPPO 2008).

Several studies have indicated positive impact of nematode-protectant seed treatments on SCN populations (Beeman 2017; Bissonnette et al. 2017; Beeman and Tylka 2018; Zhang 2018). Regardless of the considerable advancement in seed treatment improvement, their performance, in terms of yield increase and SCN population reduction is highly variable under field conditions (Wheeler et al. 2013; Lund et al. 2016; Potter et al. 2016; Bissonnette et al. 2017; Beeman and Tylka 2018). The variability might be due to a number of reasons. First, the interaction between
environmental conditions, nematode populations and other pathogens in the field can potentially reduce the consistency of seed treatments (Beeman 2017). Second, the seed treatments tend not to provide season-long protection since, roots grow away from the treated seed (Beeman 2017). In addition, biological controls requires time to establish and develop hence has slow action as compared to chemical control (Soffar 2017; CABI 2019). Several biological controls have been registered for SCN suppression in soybean in Canada including *Pasteuria nishizawai*, *Bacillus amyloliquefaciens* and *Bacillus firmus* (PMRA 2011, PMRA 2015).

1.3.4.1 *Pasteuria nishizawai*

*Pasteuria nishizawai* is a gram positive, endospore-forming bacterium, found naturally in North American soils (United States Environmental Protection Agency (EPA) 2012, PMRA 2015). The genus *Pasteuria* is ubiquitous in several environments and it is found in nematodes in at least eighty different countries on five continents (EPA 2012). For instance, *P. nishizawai* was isolated from a soybean field located in Illinois, USA in the mid-2000s (EPA 2012). *Pasteuria nishizawai* was registered for SCN suppression in Canada in 2015 under product name Clariva (PMRA 2015).

*P. nishizawai* was found on three nematodes under genus *Heterodera* and one nematode under genus *Globodera* (EPA 2012). However, *P. nishizawai* is an obligate parasite known only to complete its lifecycle within females of *H. glycines* (EPA 2012; PMRA 2015). The endospores of *P. nishizawai* come into contact with the juveniles of SCN after egg hatch. Once the juvenile penetrates a soybean root, the non-motile endospores penetrate into the body of the juveniles (Wilson and Jackson 2013; PMRA 2015). The endospore will develop primary and secondary colonies, which will then fill the nematode body, causing the nematode’s death (PMRA 2015).
The parasitized SCN females normally produce very few or no eggs while other infected SCN females are weakened hence, they will not be able to enter soybean roots (PMRA 2015). More often, infected cysts will disintegrate upon death of the female, releasing endospores into the soil that will subsequently infect more nematodes, further reducing the population of SCN (Wilson and Jackson 2013; PMRA 2015). Toxicity tests for *P. nishizawai*e indicated the absence of adverse effects on both human health and/or the environment, if the nematicide was used accordance to the label directions (EPA 2012).

In some field trials *P. nishizawai*e reduced SCN egg numbers by 15-40% when used in combination with resistant or moderately resistant soybean cultivars. However, there was no significant yield increase in about 70-80% of the trials (Mueller and Kyveryga 2015). Other studies indicated that *P. nishizawai*e did not have an impact on cyst score and root rot within dry bean black and kidney market classes (Zhang 2016). In addition, *P. nishizawai*e had no effect on the yield or reproduction of *H. glycines* in soybean trials (Lund et al. 2016; Potter et al. 2016; Bissonnette et al. 2017; Jensen et al. 2018; Lund et al. 2018).

### 1.3.4.2 *Bacillus* spp.

During the last two decades, there has been increased global attention towards sustainable production systems leading to increased popularity of pest control products, which contain biological control organisms (Bissonnette and Tylka 2017). The most extensively used biocontrol organisms belong to the genus *Bacillus* (Khan et al. 2017). Microbes in the genus *Bacillus* are advantageous as compared to other microorganisms, owing to their long-term viability (Qiao et al. 2014), selective pathogenicity and tolerance to fluctuating temperature, pH, and osmotic conditions (Khan et al. 2017).
1.3.4.2.1 *Bacillus firmus*

*Bacillus firmus* is an aerobic, gram-positive, soil bacterium which degrades eggs of several plant pathogenic nematodes such as root knot nematode, SCN and stem nematode (*Ditylenchus dipsaci*) (PMRA 2011; Geng et al. 2016). *B. firmus* was first identified by Werner in 1933 and it has been isolated from several environments (Gordon et al. 1977; Geng et al. 2016). It is a biological nematode suppressant used on vegetable, fruit, and field crops (Geng et al. 2016). The *B. firmus* strains also have a wide range of uses including utilization of their extracellular enzymes in various industries and also the removal of heavy metals found in waste water (Geng et al. 2016). *Bacillus firmus* was registered in Canada for SCN suppression in 2011 under product name Votivo 240 FS (PMRA 2011).

There is a range of *B. firmus* formulations that were developed to protect plants from nematodes when applied to the soil directly, as seed treatments or as foliar treatments (Bacchus 2008). The mode of action of *B. firmus* include the utilization of effectors which inhibit nematode egg hatching and also the use of extracellular enzymes to degrade eggs of nematodes (PMRA 2011; Geng et al. 2016). In addition, *B. firmus* also exhibit paralytic activity, which is antagonistic to the J2 juvenile (Geng et al. 2016).

Some studies reported inconsistent effect of *B. firmus* on SCN (Beeman and Tylka 2018), others reported a reduction in SCN numbers (Schrimsher 2013), little effect was reported on SCN in black and kidney bean (Zhang 2016; Zhang 2018) and no effect on either yield or SCN population densities in soybean (Musil 2016).

1.3.4.2.2 *Bacillus amyloliquefaciens*

*Bacillus amyloliquefaciens* is a naturally occurring bacterium found in the soil root zone, commonly in close association with plant materials such as roots, leaf litter and also on fresh and
dried foods (PMRA 2015; Siemering et al. 2016). Several products containing *B. amyloliquefaciens* have been registered in U.S.A since 2011 for the control of diseases in horticultural, ornamental and field crops (EPA 2011). The first products containing *B. amyloliquefaciens* strain D747, Double Nickel 55 and Double Nickel LC were registered in 2015 in Canada for the management of fungal and bacterial diseases on various vegetables, pome fruit, strawberry, potato, and soybean (PMRA 2015).

*B. amyloliquefaciens* products are commonly used as foliar and soil applied treatments. In 2013, patent filings indicated that *B. amyloliquefaciens* may be effective in controlling soil nematodes such as SCN, root knot and lesion (Margolis et al. 2013). Aveo EZ nematicide, *B. amyloliquefaciens* containing biological seed treatment for the management of SCN, reniform nematode and others was registered under Valent U.S.A. LLC (Eckelkamp 2017). *B. amyloliquefaciens* can be used in combination with other organisms to form effective biocontrols, for instance, a fusant from *Lysinibacillus sphaericus* and *B. amyloliquefaciens* could be used against root-knot nematode (Abdel-Salam et al. 2018). Toxicity studies on the effect of *B. amyloliquefaciens* on aquatic organisms, plants, birds and other terrestrial arthropods indicated that *B. amyloliquefaciens* is non-toxic to these organisms (PMRA 2015). Little is known about *B. amyloliquefaciens* for SCN management in dry bean.

### 1.3.5 Other Control Measures

SCN spreads through soil movement, high levels of sanitation are required such as thorough cleaning of farm equipment and tools from infested fields, using different containers for marketing from the ones used for harvesting, and thoroughly washing root crops that are grown in infested soils (Giesler and Wilson 2011).
Good agronomic practices can decrease SCN induced yield loss by reducing stress on crops (Esser and Langdon 1967). These include providing optimum soil fertility for plant growth and development and effective weed and pest control measures. In addition, dipping the infested roots for 30 minutes in hot water at 48 – 49 °C is effective in eradicating the cysts on crops grown from bare root stocks (Esser and Langdon 1967).

1.4 Hypotheses and Objectives

It was hypothesized that:

1. the seed treatments will reduce the SCN population.
2. the seed treatments will increase growth and development of dry bean in presence of SCN.
3. the products will have a greater effect on a susceptible market class (kidney) than a resistant market class (black bean).
4. the products will be equally effective under field and controlled environments.
5. cv. Red Hawk and cv. Dynasty will have similar response to SCN pressure.

The objectives of this research are:

1. to evaluate the efficacy of experimental seed treatments (BAS576AAS, BAS79800F, BAS97474F) and *B. amyloliquefaciens* for SCN management in black (cv. Zorro) and kidney (cv. Red Hawk) bean and compare them to previously tested products: *P. nishizawai*, *B. firmus* and fluopyram under controlled environment and field conditions.
2. to assess the effects of BAS576AAS, BAS79800F, BAS97474F, *B. firmus*, *B. amyloliquefaciens*, and fluopyram seed treatments on SCN populations, plant growth and development in susceptible kidney bean (cv. Dynasty and Red Hawk) under controlled environment (growth cabinet).
3. to evaluate the susceptibility of two kidney bean cultivars (cv. Red Hawk and Dynasty) to soybean cyst nematode.
Chapter Two: Efficacy of seed treatments for *Heterodera glycines* management in dry bean under controlled environment and field conditions.

2.1 Introduction

Dry bean (*Phaseolus vulgaris* L.) is an important source of protein for human consumption. The crop is mainly produced and consumed in developing countries (Gepts et al. 2008); however, the USA and Canada are the third and fifth largest exporters of dry bean in the world, respectively (Food and Agriculture Organization of the United Nations (FAO) 2017). Dry bean is a source of the minerals manganese, magnesium, copper, and phosphorus and the vitamins thiamin, folic acid, riboflavin and vitamin B6 (Robertson and Frazier 1978). *P. vulgaris* is in the same family as soybean hence, they have common pests and diseases including soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN). Ontario is an important production area in Canada for both these crops. *H. glycines* has been detected in soybean and dry bean production areas across southern Ontario, and in some parts of central and eastern Ontario (Moran 2018).

Crop yield losses worldwide caused by all nematode species are approximately $100 billion annually (Opperman and Bird 1998). SCN is the most destructive pest of soybeans globally, and it has been detected in each of the top five soybean-producing countries namely the USA, Brazil, Argentina, China and India (Yu 2011; Centre for Agriculture and Bioscience International (CABI) 2018). Approximately $1.5 billion is lost in the USA from *H. glycines* infestation in soybean alone (Tylka 2012). It is impossible to eradicate SCN once it is established in a field; however, SCN can be managed by reducing population densities below the economic damage threshold (i.e. 2000 eggs 100 cm$^{-3}$ soil in soybean) mainly through crop rotation and the use of resistant cultivars (Jardine and Todd 2001; ISU 2012). SCN is hard to eradicate because of a high reproductive rate of 200 or more eggs cyst$^{-1}$ and a broad host range of up to 100 plant species (Yu
SCN populations can be influenced by several factors such as environmental conditions (Riggs and Wrather 1992), host plant resistance and feeding stress (Triantaphyllou and Koliopanos 1972; Lauritis et al. 1983). These factors can also affect SCN sex ratio (male/female). For instance, stressful conditions including host plant resistance, overcrowding and nutrient stress can cause higher male than female SCN populations (Triantaphyllou and Koliopanos 1972; Lauritis et al. 1983). A similar trend was noticed in other nematode species including *Reesimermis nielseni* (Petersen 1971), *Heterodera schachtii* (Steele 1974) and *Strongyloides ratti* (Harvey et al. 2000). Several studies have measured a significant difference in female numbers between resistant and susceptible soybean cultivars, while male numbers did not differ (Lauritis et al. 1982; Lauritis et al. 1983).

The impact of SCN on dry bean production is poorly understood. In contrast, some field studies indicate that SCN can reduce plant growth and seed yield of dry bean (Poromarto et al. 2010). Unfortunately, there are no registered biological and chemical control measures for SCN in dry bean. The key control measure in soybean is genetic resistance but the role of resistance genes in dry bean is poorly understood. A dry bean study has reported differences between four market classes of dry bean and showed that black bean is more resistant to SCN than kidney bean (Poromarto and Nelson 2009).

Considering the economic losses caused by SCN in soybean, there is a need to evaluate the effectiveness of potential controls of SCN on dry bean. Several seed treatments are registered for SCN suppression in soybean in Canada including *Pasteuria nishizawai* (Syngenta Crop Protection, Guelph, ON), *Bacillus firmus* and fluopyram (Bayer Crop Science, Mississauga ON). *P. nishizawai* is a gram-positive, endospore-forming bacterial parasite of SCN juveniles (Sayre et al. 1991; Pest Management Regulatory Agency (PMRA) 2015). In previous studies, *P. nishizawai*
reduced cyst numbers in kidney bean but not in black bean under controlled environment (Zhang 2018). On the contrary, it did not have an effect on SCN development and reproduction in soybean studies in a controlled environment (Jensen et al. 2018) or field conditions (Potter et al. 2016; Bissonnette et al. 2017; Lund et al. 2018). B. firmus is a soil bacterium registered in Canada in 2011, which degrades eggs produced by nematodes (PMRA 2011). B. firmus lowered cyst numbers in kidney bean while it increased cysts numbers in black bean (Zhang 2018). However, in a soybean study B. firmus reduced an SCN population by inhibiting egg hatch and paralyzing J2 juveniles (Schrimsher 2013). Fluopyram is a group 7 fungicide that has nematicidal properties (PMRA 2014; Zaworski 2014; Faske and Hurd 2015). In recent studies, a combination of fluopyram and B. firmus reduced cysts in black and kidney beans (Zhang 2018) while fluopyram alone reduced cyst numbers in soybean (Beeman and Tylka 2018). BAS79800F experimental contains two components, a biological BAS97474F and a non-biological BAS576AAS. It is a new experimental hence less is known about its efficacy in dry bean and soybean.

The objective of this experiment was to evaluate the effectiveness of the seed treatments BAS97474F, BAS576AAS, BAS79800F (BASF, Mississauga ON), Bacillus amyloliquefaciens (Valent, Guelph ON), P. nishizawai, B. firmus and fluopyram for the control of SCN in black and kidney bean in controlled environment and field experiments. B. amyloliquefaciens is a naturally occurring endospore-forming bacterium registered in soybean, potato (Solanum tuberosum L.), lettuce (Lactuca sativa L.), grape (Vitis vinifera L.), strawberry (Fragaria ananassa), as well as the cucurbits and pome fruits, which prevent the colonization of plant roots by nematodes (PMRA 2015; Siemering et al. 2016). Since BAS79800F experimental is still proprietary, nothing is known about its mode of action. This is the first study to test BAS79800F experimental along with each of its two components as well as B. amyloliquefaciens in dry bean. This experiment is expected
to enhance the knowledge and understanding on the effectiveness of potential SCN control products in dry bean.

2.2 Materials and Methods

The experiments were conducted under both field and controlled environments. The field studies were carried out at two sites near Rodney and Highgate, ON. The controlled environment study was done in a plant growth cabinet at the University of Guelph, Ridgetown Campus.

2.2.1 Controlled Environment Study

The experiment was conducted in a plant growth cabinet (Conviron® A1000) at the University of Guelph, Ridgetown Campus for 30 d at 27 ± 0.5°C with a photoperiod of 16/8 h (day/night). A randomized complete block design (RCBD) with six replications was used. The experiment consisted of 20 treatments (Table 2.0). The soybean controls were used to check if the SCN race (HG type 5.7) for this experiment had changed. Black (cv. Zorro) and kidney (cv. Red Hawk) bean were used with nine treatments applied to each market class, including non-inoculated and inoculated controls. The purpose of the non-inoculated control was to document any SCN cross-contamination between experimental units (e.u.) (Table 2.0). The remaining treatments included BAS79800F and its two components (BAS576AAS and BAS97474F), three bio-controls including *B. amyloliquefaciens*, *P. nishizawai* and *B. firmus* and one chemical fluopyram.

The seeds were pre-treated at BASF Canada Inc., Winkler, Manitoba with respective seed treatments at the recommended rate for soybean found on Canadian product labels. Seed germination was carried out at room temperature 3–4 d before transplanting. The seeds were germinated on 15 cm filter paper (Fisher Scientific Ltd, Pittsburgh, PA) moistened with distilled water and placed in petri dishes. Two industrial sand products namely ‘All Purpose Medium’ and ‘Body Shot’ (K&E Sand and Gravel, Wyoming ON) were mixed 1:1 and used as the growth
medium. The sand was autoclaved at 121°C for 25 min, followed by 10 min of dry time prior to use. The autoclaved sand was then transferred to SC10 Super Cell cone-tainers (3.8 x 21-cm; Stuewe & Sons Inc., Tangent, OR) for planting. Cone-tainers are cone shaped plastic tubes used for planting. The cone-tainers for each block were placed in turface (MVP Athletic™, Buffalo Grove, IL) filled 15 L polycarbonate containers (Cambro CamSquares® - Camwear®, Huntington Beach, CA) which supported the cone-tainers. A cotton ball was placed at the bottom of each cone-tainer to prevent loss of media through the bottom drain holes. The cone-tainers were filled with sand to about 2.5 cm below the top of the cone-tainer to allow for watering the plants. The sand was saturated with distilled water before each e.u. was inoculated with 4000 SCN eggs using a micropipette. Seedlings with root length above 2 cm were transplanted into the medium filled cone-tainers. The SCN eggs were collected from cysts gathered from soil in a naturally infested field near Rodney, ON. The population of SCN was previously identified as HG type 5.7 (A. Tenuta, per. comm). The methods of Poromarto and Nelson (2009) for cyst isolation, crushing and inoculation were followed. The cysts were collected from soil using a 30-mesh (600 -μm) sieve (Sargent-Welch Scientific, Buffalo, NY) nested over a 60-mesh (250-μm) sieve. Cysts were crushed using a 10" bench drill press (Mastercraft. Toronto, ON) and eggs were collected on a 230-mesh (63 -μm) sieve nested over a 500-mesh (25 -μm) sieve and washed into a 50-ml centrifuge tube. A solution of eggs and distilled water was prepared and the concentration was adjusted to 4000 eggs ml⁻¹.

Each plant was fertilized with 3 ml of NPK (6-11-31) Plant-Prod® Hydroponic fertilizer (Master Plant-Prod Inc. Brampton, ON) at 14, 17, 21 and 24 d after planting. A total of 10 g of fertilizer was mixed with 490 ml distilled water. Harvesting was done 30 d after planting. The BBCH scale was used to measure the phenological development stage of the plants at harvest.
(Hack et al. 1993). The BBCH scale uses a coding system ranging from 0–9, to identify and distinguish the entire developmental cycle of plants into 10 developmental stages. For dry bean, these stages include germination, leaf development, side shoots formation, inflorescence emergence, flowering, fruit development, seed ripening and senescence (Feller et al. 1995). Plant growth was measured by cutting the plants at the soil line, placing the above-ground plant into brown paper bags and drying the plants in a tobacco kiln (De Cloet Greenhouse Manufacturing Ltd. Simcoe, ON) at approx. 38°C for 14 d until a constant weight was recorded for two consecutive measurements.

After the biomass harvest, the sand and roots in the cone-tainers were collected into 16.5 cm x 14.9 cm self-sealing sandwich bags (S.C Johnson and Son Ltd, Brantford, ON) and stored in a cooler at 4°C. The sand and roots were transferred to a lab for SCN egg, cyst, juvenile and male counts. First, the sand was washed off the root masses into a plastic bucket through a 30-mesh (600 µm) sieve (Sargent-Welch Scientific, Buffalo, NY). The collected solution was left to settle for about five seconds before being poured gently through a 500-mesh (25 µm) sieve (Sargent-Welch Scientific, Buffalo, NY) to collect juveniles, eggs and adult male nematodes. The collected nematodes were transferred into 50 ml centrifuge tubes (Corning® CentriStar™ Corning, NY). A 10x dilution was made, and mixed thoroughly using a vortex mixer. A milliliter of the diluted solution was spread over a counting pad (1*1 cm with 100 grids) using a micropipette and the total number of eggs, juveniles and adult males were counted with the aid of a dissecting microscope at 45x magnification. The process was repeated three times for each subsample, to increase the accuracy of the readings.

The cysts were washed off the plant roots and sieved using a 30-mesh (600 µm) sieve nested over a 60-mesh (250 µm) sieve (Sargent-Welch Scientific, Buffalo, NY). Following this,
roots were inspected under a dissecting microscope to confirm that all cysts were removed. The collected cysts were washed off the 60-mesh sieve gently into 50-ml centrifuge tubes using a 500-ml wash bottle (Fisher Scientific, Ottawa, ON) filled with distilled water. The collected cysts were placed on 15 cm lined red filter paper (Fisher Scientific Ltd, Pittsburgh, PA) in a Büchner funnel, which removed excess water prior to counting. The dry filter paper containing cysts was placed into a petri dish for counting. The number of cysts plant\(^{-1}\) were counted using a dissecting microscope at 15x magnification. The SCN sex ratio was calculated using the formula male number / female number. The samples containing nematodes were collected in a 1-L beaker and bleached overnight using didecyldimethylammonium chloride (DDAC) solution (Sanidate 5.0 KleenGrow™. Burnaby, B.C) at the rate of 30 ml L\(^{-1}\) of distilled water to kill the nematodes and avoid any potential spread of the pest.
Table 2.0 Treatment list for dry bean SCN control study of seed treatments applied on black and kidney bean in a controlled environment study at Ridgetown, ON and field studies at Highgate and Rodney, ON in 2018.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cultivar</th>
<th>Controlled environment Study</th>
<th>Field Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Inoculated Resistant Soybean</td>
<td>P92Y55</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Inoculated Resistant Soybean</td>
<td>Pioneer 92Y12</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>2 Inoculated Susceptible Soybean</td>
<td>Lee 74</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3 Inoculated Black Control</td>
<td>Zorro</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>4 Inoculated Kidney Control</td>
<td>Red Hawk</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>5 Inoculated BAS576AAS Black</td>
<td>Zorro</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>6 Inoculated BAS576AAS Kidney</td>
<td>Red Hawk</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>7 Inoculated BAS97474F Black</td>
<td>Zorro</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>8 Inoculated BAS97474F Kidney</td>
<td>Red Hawk</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>9 Inoculated BAS79800F Black</td>
<td>Zorro</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>10 Inoculated BAS79800F Kidney</td>
<td>Red Hawk</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>11 Inoculated <em>P. nishizawae</em> Black</td>
<td>Zorro</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>12 Inoculated <em>P. nishizawae</em> Kidney</td>
<td>Red Hawk</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>13 Inoculated fluopyram Black</td>
<td>Zorro</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>14 Inoculated fluopyram Kidney</td>
<td>Red Hawk</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>15 Inoculated <em>B. amyloliquefaciens</em> Black</td>
<td>Zorro</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>16 Inoculated <em>B. amyloliquefaciens</em> Kidney</td>
<td>Red Hawk</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>17 Inoculated <em>B. firmus</em> Black</td>
<td>Zorro</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>18 Inoculated <em>B. firmus</em> Kidney</td>
<td>Red Hawk</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>19 Non-inoculated black control</td>
<td>Zorro</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>20 Non-inoculated kidney control</td>
<td>Red Hawk</td>
<td>X</td>
<td>-</td>
</tr>
</tbody>
</table>

2.2.2 Field Studies

Seed treatment efficacy in fields naturally infested with SCN was evaluated at two sites near Highgate and Rodney, ON. Soil texture at Highgate was 86% sand, 9% silt and 5% clay while at Rodney was 92% sand, 6% silt and 2% clay. Each study was arranged in a RCBD with six replications using black bean (cv. Zorro) and kidney bean (cv. Red Hawk) since there is evidence that these market classes differ in their genetic background (Johnson and Gepts 1999; Klaedtke et al. 2017) and this contributes to differences in SCN resistance (Poromarto and Nelson 2009). The cyst population was determined prior to planting using methods described above in the controlled environment study. In each replication, five soil cores plot\(^{-1}\) to a depth of 15 cm were collected,
bulked and thoroughly mixed before samples were removed for counting. Each e.u consisted of two rows spaced 76 cm apart and 6 m long. Planting was done on 12 June 2018 at both sites using an Almaco plot planter at a seeding rate of 20 seeds m\(^{-1}\). The field experiments consisted of 18 treatments (Table 2.0) as it was not possible to have non-inoculated controls for the dry bean treatments. The soybean controls served the same purpose as in controlled environment study.

Emergence counts and plant vigour were determined at 1, 2, 3 and 4 wks after planting (WAP) on the middle 2 m portion of each row for each e.u. A 0-10 scale was devised for plant vigour ratings, where 0 and 10 represented the lowest and highest plant vigour respectively. Initially, the control for each market class was rated for vigour in each replication and then the vigour of all other treatments were compared to it. Fertilizer and herbicides were applied following normal production practises for dry bean (Ontario Ministry of Agriculture, Food & Rural Affairs (OMAFRA) 2017). Additional weed escapes were removed by hand hoeing. Herbicide injury rating was conducted 3 WAP at Rodney and deer feeding damage was documented on 26 Aug. 2018.

Ratings for cyst number, root rot, plant development and fresh aboveground plant and root weight were conducted at 6 WAP, using five plants from the front of each e.u. Root rot rating was assessed using a percentage root rot scale (Albert Tenuta, per. comm.) where 10 and 100 represented the lowest and highest percentage of root mass damaged respectively. Plant development stage was assessed using the BBCH scale as described above. The roots were placed in 27 cm x 28.5 cm double zipper freezer bags (Conglom Inc. St-Laurent, QC) and stored at 4\(^\circ\)C in a cooler. The cysts were extracted from the roots and counted using the same procedure as the controlled environment study. After cyst extraction, the dry weight of roots was determined as
outlined above for plant dry weight. The dry root weight and cysts count data were used to compute the cysts g\(^{-1}\) root.

Plants were cut at the soil line at harvest, and threshed using a Hege 140 plot combine. Harvest data included pick, 100-seed weight, yield and yield after pick was removed. Pick refers to whole seeds which are discolored or deformed. The 100-seed weight and yield was converted to the standard storage moisture of 18%.

2.2.3 Statistical Analysis

Data were analyzed with SAS 9.4 (SAS Institute Inc., Cary, NC) using PROC GLIMMIX. The blocks and environment (each experiment repetition) were considered random effects and the treatments were fixed effects. An F-test at a 5% confidence level was used to test for the significance of fixed effects and mean comparisons were computed using Tukey’s HSD test at \(\alpha=0.05\). Shapiro Wilk’s test was used to test for normality of the residuals of the dependent variables. The non-inoculated dry bean controls as well as the resistant and susceptible soybean controls were not included in the analysis. The soybean controls were compared in a separate analysis. The controlled environment experiment was repeated once – each study was considered a separate environment for statistical analysis. Dependent variables included cyst, male and juvenile numbers, sex ratio, aboveground plant dry weight and the plant development stage. The number of cysts, males, and juveniles followed a Poisson distribution, while sex ratio and plant dry weight followed a Lognormal distribution. The two field environments differed so data from each site were analyzed separately for all the dependent variables, which included cyst number, root rot, root fresh and dry weight, plant fresh weight and the plant developmental stage. Cyst number followed a Poisson distribution, root rot, plant fresh weight, root dry and fresh weight followed a Lognormal distribution while plant growth stage followed a Multinomial distribution.
A log transformation was used for data that followed a Lognormal distribution and an ‘ilink’ statement was used to transform the other distributions to meet the assumptions of normality. The transformed means were back-transformed for presentation in tables. Only significant differences (P ≤ 0.05) are reported.

2.3 Results

2.3.1 Controlled Environment Study

There was a significant effect for market class but there was an environment by market class interaction for all parameters tested so the means for the two market classes were presented separately (Table 2.3). No SCN population was found on the non-inoculated controls in both experiments (Data not shown).

2.3.1.1 Cyst Number

The resistant soybean P92Y55 had 73% fewer cysts than the susceptible soybean Lee 74 (79 vs 294 cysts plant⁻¹; SE =36) indicating that the intended HG type 5.7 for the experiment did not change.

In black bean, *B. amyloliquefaciens* and *B. firmus* had cyst numbers that were 24% and 10% lower than the control, respectively (Table 2.3). BAS57AAS had similar cyst numbers to the control and *B. firmus*, while fluopyram was similar to the control only. BAS79800F, BAS97474F and *P. nishizawae* had more cysts plant⁻¹ than the control.

In kidney bean, fluopyram and *B. amyloliquefaciens* had cyst numbers that were 25% and 24% lower than the control respectively followed by *P. nishizawae* (14%), BAS576AAS (11%) and *B. firmus* (6%). BAS79800F and BAS97474F were similar to the inoculated control.
2.3.1.2 Male Number

In black bean, *B. firmus*, *P. nishizawae* and fluopyram had the largest increase in males plant$^{-1}$ of 36%, 31% and 31%, respectively, compared to the control, followed by *B. amyloliquefaciens* (14%), BAS97474F (14%) and BAS79800F (6%) (Table 2.3). BAS576AAS had similar males plant$^{-1}$ as the control. For kidney bean, *B. firmus*, BAS576AAS, BAS79800F and *P. nishizawae* had similar males plant$^{-1}$ as the control (Table 2.3). Fluopyram, BAS97474F and *B. amyloliquefaciens* treatments reduced males plant$^{-1}$ by 9%, 9% and 8%, respectively.

2.3.1.3 Other Parameters

There was no treatment effect on juvenile numbers, sex ratio, aboveground plant dry weight and the plant development stage in both black and kidney bean treatments (Table 2.3).

2.3.2 Field Study

2.3.2.1 Cyst Number

*Highgate*

The average cyst count at Highgate at planting was 153 cysts 100 g$^{-1}$ of soil (data not shown) and samples ranged from 129 – 254 cysts 100 g$^{-1}$ of soil. There was an interaction between market class and seed treatment (Table 2.4) for cyst numbers. In black bean, BAS79800F, BAS97474F, BAS576AAS and *B. amyloliquefaciens* reduced cysts g$^{-1}$ root by 57%, 39%, 27% and 26%, respectively, compared to the control (Table 2.5). *P. nishizawae*, *B. firmus* and fluopyram were similar to the control. In kidney bean, *B. amyloliquefaciens*, BAS576AAS, *P. nishizawae* and BAS79800F lowered cysts g$^{-1}$ root by 31%, 23%, 19% and 18%, respectively, compared to the control. The cyst numbers for *B. firmus* and BAS97474F were similar to the control while fluopyram increased cyst numbers by 56%.
Rodney

At Rodney, the initial cyst number was 120 cysts 100 g\(^{-1}\) of soil (range: 93 – 155 cysts 100 g\(^{-1}\) of soil) (data not shown). In black bean, fluopyram reduced cysts g\(^{-1}\) root by 28%, BAS97474F increased cysts g\(^{-1}\) root by 39% and the other treatments were similar to the control (Table 2.5). In kidney bean, fluopyram, *P. nishizawae*, BAS79800F and BAS97474F increased cyst numbers by 73%, 62%, 44% and 29% respectively (Table 2.5). There was no effect of *B. amyloliquefaciens*, *B. firmus* or BAS576AAS compared to the control.

2.3.2.2 Root Rot, Root and Plant Weight

There was no seed treatment effect on root and aboveground plant weight at both Highgate and Rodney however, there was a market class difference (Table 2.4). Kidney bean had a higher mean aboveground plant weight (371.9 ± 16.48 g) as compared to black bean (293.5 ± 13.00 g) at Rodney. At Highgate, black bean had a higher mean root fresh weight than kidney bean (23.4 ± 1.03 g vs 18.0 ± 0.80 g) and root dry weight (3.6 ± 0.21 g vs 2.7 ± 0.16 g). At both Highgate and Rodney, kidney bean showed higher root rot scores than black bean. Black bean had lower average root-rot scores than kidney bean at Rodney (13.1 ± 0.83 vs 18.6 ± 1.19; P < 0.0001) and Highgate (17.3 ± 1.33 vs 33.5 ± 2.57; P < 0.0001).

2.3.2.3 Yield Parameters

Rodney

There was a seed treatment effect for yield and yield-pick at Rodney (Table 2.4). All seed treatments were similar to the control for yield and yield-pick except BAS76AAS, which had a lower yield than *B. firmus* (Table 2.6). There was a market class effect for pick and root rot (Table 2.4). Kidney beans had a higher pick (2.4 ± 0.09%) compared to black bean (1.4 ± 0.05%). There was a market class difference for 100-seed weight at Rodney, as expected (Table 2.4).
**Highgate**

At Highgate, only a market class effect was measured for each yield parameter (Table 2.4). Black bean had a higher yield and yield-pick than kidney bean (Table 2.7). Similar to Rodney trial, kidney bean treatments had a higher 100-seed weight as compared to black bean at Highgate. Additionally, the pick for black bean was lower than kidney bean (2.0 ± 0.11% vs 4.3 ± 0.24%).

### 2.4 Discussion

The kidney bean control had 67%, 87% and 182% more cysts than black bean control at Highgate, Rodney and under the two controlled environment studies respectively. These large differences in cyst numbers between these two market classes have been documented in other studies (Poromarto and Nelson 2009; Zhang 2018). In the current study, the Red Hawk control treatment tended to have higher SCN numbers than the susceptible control Lee 74, while in previous studies, Red Hawk was similar to Lee 74 for cyst numbers. In addition, the resistant soybean P92Y55 had less cysts plant^{-1} than Lee 74, which confirmed the HG type did not shift at the cyst collection site.

In the controlled environment study, *B. amyloliquefaciens* reduced cyst numbers in black bean by 24% and by 25% in kidney bean. However, in field studies, *B. amyloliquefaciens* reduced cyst numbers in both black and kidney bean at the Highgate site only. It is our understanding that, this was the first study globally to evaluate the efficacy of *B. amyloliquefaciens* in dry bean. *B. amyloliquefaciens* functions by rapidly colonizing root surfaces (PMRA 2015; Siemering et al. 2016), and by preventing the establishment of nematodes through competition for food and space (PMRA 2015) thus reducing the number of J2 juveniles feeding on the roots and ultimately decreasing cyst numbers.
Bacillus firmus reduced cyst numbers in black bean by 10% and kidney bean by 24% in the controlled environment study, but in the field studies it had no effect at either site. In previous work, B. firmus reduced cyst numbers in kidney bean while increasing cyst numbers in black bean (Zhang 2018). In a soybean study, B. firmus was reported to have inconsistent effects on SCN populations, which agrees with the current study (Beeman and Tylka 2018). Other studies indicated that B. firmus can effectively inhibit SCN egg hatching and can cause paralysis of J2 juveniles (Schrimsher 2013). The inconsistency on the effect of B. firmus may be due to a number of factors such as interaction with other soil microbes and environmental conditions (Beeman and Tylka 2018).

Fluopyram reduced SCN cyst numbers in kidney bean in the controlled environment study, which is similar to the results from a similar study (Zhang 2018). However, in the field fluopyram reduced cysts only in black bean at Rodney but increased cyst numbers in kidney bean at Highgate and Rodney. The nematicidal properties of the fungicide fluopyram have been reported in previous studies (Zaworski 2014; Faske and Hurd 2015). In a recent soybean study, fluopyram reduced egg hatching, reproduction, juvenile motility and root penetration of H. glycines (Beeman and Tylka 2018). Additionally, there was a reduction in H. glycines reproduction in soybeans when a combination of fluopyram with other products containing metalaxyl and B. firmus were used as compared to the other products applied in the absence of fluopyram (Zaworski 2014).

Pasteuria nishizawai reduced cyst numbers only in kidney bean in the controlled environment study, which is consistent with a previous study (Zhang 2018), where it was more active at the high label rate. In the field, P. nishizawai had similar cyst numbers as the control in black bean at both sites, however in kidney bean it reduced cyst numbers at Highgate and increased cyst numbers at Rodney. There is evidence that P. nishizawai spores attach and penetrate J2...
nematodes after egg hatching, weakening the nematodes (Wilson and Jackson 2013; PMRA 2015). The parasitized nematodes remain viable but the adult females will produce little or no eggs ultimately reducing the population of *H. glycines* (Wilson and Jackson 2013; PMRA 2015). Some soybean studies have documented that *P. nishizawai* had no effect on the development rate of SCN (Jensen et al. 2018), SCN reproduction (Potter et al. 2016; Bissonnette et al. 2017; Jensen et al. 2018; Lund et al. 2018) and soybean yield (Potter et al. 2016; Lund et al. 2018) as compared to the untreated control. In contrast, a yield increase was noticed in a field scale soybean study when *P. nishizawai* treated seeds were tested (Bissonnette et al. 2017).

The BAS79800F experimental was not effective in reducing cyst numbers in black or kidney bean in the controlled environment study. However, this treatment had lower cyst numbers in both black and kidney beans at Highgate, but higher cyst numbers in kidney bean at Rodney. When tested separately only one of the components, BAS57AAS reduced cyst numbers in kidney bean in the controlled environment study. Therefore, the two components of the experimental were inconsistent in reducing cyst numbers, which likely affected the overall efficacy of the two components combined in both controlled environment and field studies.

The sex ratio of males to females in SCN populations is typically 1:1 (Schmitt et al. 2004), and a deviation in the sex ratio could be the result of food and water stress, overcrowding and host immunity status (Triantaphyllou and Koliopanos 1972; Steele 1974; Lauritis et al. 1982; Lauritis et al. 1983; Harvey et al. 2000). Various stresses can inhibit the growth of females since they have a longer development rate, resulting in a higher male to female ratio (Lauritis et al. 1983; Schmitt et al. 2004). Studies with other nematode species, such as *Strongyloides ratti* (Harvey et al. 2000), *Heterodera schachtii* (Steele 1974) and *Reesimeris nielseni* (Petersen 1971) have indicated similar results. In black bean, all the treatments except BAS576AAS had higher male numbers
than the control, which might be mainly due to the efficacy of seed treatments. The higher male numbers might also be due to the additive effect of the host plant’s resistance to SCN and the efficacy of seed treatments. Male numbers in the black bean treatments tended to be higher than in kidney bean treatments, which is likely due to kidney bean susceptibility causing less stress on the SCN population. However, kidney beans had much higher female numbers than black bean treatments. These results are consistent with previous studies in soybeans (Lauritis et al. 1982; Lauritis et al. 1983).

There was no yield difference between seed treatments and the control in field studies except in the case of *B. firmus*, which resulted in a higher yield than BAS57AAS at Rodney. Few studies have been carried out on the effects of SCN on dry bean yield, however recently Zhang (2016) found less pronounced yield differences between dry bean market classes. This is consistent with Poromarto and Nelson (2010), who tested different SCN inoculation levels on several dry bean market classes including kidney bean in the field, and reported no treatment differences on pod number, pod weight, seed number, and seed weight in North Dakota. However, black bean (cv. Zorro) yielded higher than kidney bean (cv. Red Hawk) when planted in SCN infested field (Zhang 2016), which is consistent with the results from this study at Highgate. Yield differences in the current study might be due to high SCN cysts in kidney bean.

Root rot pathogens were present in the field trials, with kidney bean having higher root rot scores than black bean at both sites. High SCN infestation and differential host plant resistance to root rot might have caused the differences and root rot scores. The root rot pathogens were not isolated and identified in this study; however, in Ontario the major root rot fungi include *Fusarium*, *Rhizoctonia* and *Pythium* (OMAFRA 2017). In general, Mesoamerican market classes such as black bean are more resistant to root rot pathogens such as *Fusarium*, as compared to Andean
market classes such as kidney bean (Beebe et al. 1981). In another study, the resistance of 11 dry bean genotypes to *Fusarium* root rot was tested using three evaluation methods, and found that black bean (cv. Eclipse) was more resistant than kidney bean (cv. Red Hawk) across all evaluation methods (Bilgi et al. 2008). In the current study, the root rot pathogens might have affected the efficacy of the seed treatments, which resulted in inconsistent treatment response. SCN feeding creates entry points for root rot pathogens, which can lead to increased incidence and severity of root rot diseases, which might have caused slightly more treatment effect in resistant black bean as compared to the susceptible kidney bean.

There were some limitations to this study. The plants under controlled environment study was conducted for only 30 d, which was not enough to assess the effect of SCN on parameters such as yield. The field studies assessed yield but were conducted for only one cropping season. Further field studies are needed to better understand the effect of the seed treatments on SCN and crop yield, in response to variations in climate which occur each year. Additionally, root rot was documented in the field studies, which might have influenced the efficacy of the seed treatments by increasing the incidence and severity of root rot diseases thus influencing the effect of seed treatments. The assessment of the products on SCN population dynamics under field conditions is required to establish and enhance our understanding of the relationship between naturally occurring soil microorganisms such as root-rot pathogens and SCN at each life stage. In addition, SCN population dynamic studies will help understand the impact of abiotic stresses such as soil characteristics and climate on SCN population. The cultivars seem to have responded differently between the two sites, which might have been influenced by environmental conditions (Koenning et al. 1988) and differences in initial SCN populations. Additionally, differences in cultivar
response might have been influenced to a lesser extent by soil characteristics such as texture (Koenning et al. 1988) since the soil texture from both sites were slightly different (Table 2.8).

In conclusion, all the tested products provided some nematicidal effect for at least one of the variables measured, but some products were more consistent than others. The effect of the products in field studies was less consistent; thus, the variability in field studies must be managed more closely. An interaction between initial nematode populations, environmental conditions and other microbes in the field might have reduced the efficaciousness of seed treatments (Beeman 2017). The inability of the bio-controls to reduce SCN populations might be due to the slow action of the treatments. Bio-controls require time to establish and develop before they can affect the nematode populations in the soil (Soffar 2017; CABI 2019). As the plant develops, the root mass grow larger, explores a larger volume of soil, and encounters a larger number of nematodes. In an infested field, it is unlikely that a seed treatment, particularly a chemical seed treatment, will be able to provide consistent pest protection for the entire growing season (ISU 2012; Beeman 2017). There were greater treatment differences in the susceptible kidney market class, hence the need to assess the products on other cultivars in this market class under a controlled environment.
Table 2.1 Combined analysis of variance for the effect of BAS576AAS, BAS79800F, BAS97474F, *B. amyloliquefaciens*, *B. firmus*, *P. nishizawai* and fluopyram seed treatments on juvenile, cyst and male numbers per plant, sex ratio and plant dry weight of black (cv. Zorro) and kidney bean (cv. Red Hawk) under controlled environment at Ridgetown, ON in 2018.

<table>
<thead>
<tr>
<th>Main Effect</th>
<th>Cysts plant&lt;sup&gt;−1&lt;/sup&gt;</th>
<th>Males plant&lt;sup&gt;−1&lt;/sup&gt;</th>
<th>Juveniles plant&lt;sup&gt;−1&lt;/sup&gt;</th>
<th>Sex Ratio&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Aboveground Dry Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pr &gt; F&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
</tr>
<tr>
<td>Environment (ENV)</td>
<td>0.5550</td>
<td>0.2979</td>
<td>0.3261</td>
<td>0.1022</td>
<td>0.4232</td>
</tr>
<tr>
<td>Market Class (MC)</td>
<td>&lt;.0001</td>
<td>0.0007</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>ENV * MC</td>
<td>0.0303</td>
<td>0.0447</td>
<td>0.0045</td>
<td>0.0005</td>
<td>0.0081</td>
</tr>
<tr>
<td>Seed Treatment (ST)</td>
<td>0.1784</td>
<td>0.5621</td>
<td>0.5001</td>
<td>0.1509</td>
<td>0.9538</td>
</tr>
<tr>
<td>ENV * ST</td>
<td>0.0759</td>
<td>0.3571</td>
<td>0.2560</td>
<td>0.1235</td>
<td>0.5706</td>
</tr>
<tr>
<td>MC * ST</td>
<td>0.4629</td>
<td>0.3297</td>
<td>0.0679</td>
<td>0.8911</td>
<td>0.7562</td>
</tr>
<tr>
<td>ENV * MC * ST</td>
<td>0.4453</td>
<td>0.1131</td>
<td>0.3306</td>
<td>0.1147</td>
<td>0.7929</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant effects (P<0.05) in each column are indicated in bold.

<sup>b</sup> Sex ratio= males: females
Table 2.2 Effect of BAS576AAS, BAS79800F, BAS97474F, B. amyloliquefaciens, B. firmus, P. nishizawai and fluopyram seed treatments on juvenile, cyst, and male numbers per plant, sex ratio and plant dry weight of black bean (cv. Zorro) and kidney bean (cv. Red Hawk) under controlled environment at Ridgetown, ON in 2018.

<table>
<thead>
<tr>
<th>Market Class</th>
<th>Cysts plant(^{-1}) (\text{Pr} &gt; F)(^{ac})</th>
<th>Males plant(^{-1}) (\text{Pr} &gt; F)</th>
<th>Juveniles plant(^{-1}) (\text{Pr} &gt; F)</th>
<th>Sex Ratio(^b) (\text{Pr} &gt; F)</th>
<th>Aboveground Dry Weight (g) (\text{Pr} &gt; F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black bean</td>
<td>(&lt;.0001)</td>
<td>(&lt;.0001)</td>
<td>0.3277</td>
<td>0.3315</td>
<td>0.9794</td>
</tr>
<tr>
<td>Kidney bean</td>
<td>(&lt;.0001)</td>
<td>(&lt;.0001)</td>
<td>0.2056</td>
<td>0.7211</td>
<td>0.4391</td>
</tr>
</tbody>
</table>

\(^a\) Significant effects (P<0.05) in each column are indicated in bold.

\(^b\) Sex ratio: males: females

\(^c\) Data pooled from two experiments with two replications per experiment
Table 2.3 SCN population numbers, sex ratio and aboveground plant dry weight (g) of black bean (cv. Zorro) and kidney bean (cv. Red Hawk) with seed treatments applied across controlled environments at Ridgetown, ON in 2018.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cysts plant(^{-1})</th>
<th>Males plant(^{-1})</th>
<th>Juveniles plant(^{-1})</th>
<th>Sex Ratio(^b)</th>
<th>Aboveground Dry Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black(^a)</td>
<td>Kidney</td>
<td>Black</td>
<td>Kidney</td>
<td>Black</td>
</tr>
<tr>
<td>Inoculated Control</td>
<td>194 b</td>
<td>548 a</td>
<td>574 d</td>
<td>571 ab</td>
<td>62 a</td>
</tr>
<tr>
<td>B. amyloliquefaciens</td>
<td>167 d</td>
<td>418 d</td>
<td>651 b</td>
<td>527 c</td>
<td>74 a</td>
</tr>
<tr>
<td>B. firmus</td>
<td>175 cd</td>
<td>517 b</td>
<td>782 a</td>
<td>572 ab</td>
<td>53 a</td>
</tr>
<tr>
<td>BAS576AAS</td>
<td>188 bc</td>
<td>487 c</td>
<td>584 cd</td>
<td>544 bc</td>
<td>80 a</td>
</tr>
<tr>
<td>BAS79800F</td>
<td>218 a</td>
<td>552 a</td>
<td>609 c</td>
<td>564 ab</td>
<td>109 a</td>
</tr>
<tr>
<td>BAS97474F</td>
<td>232 a</td>
<td>525 ab</td>
<td>655 b</td>
<td>518 c</td>
<td>81 a</td>
</tr>
<tr>
<td>P. nishizawae</td>
<td>237 a</td>
<td>472 c</td>
<td>750 a</td>
<td>578 a</td>
<td>95 a</td>
</tr>
<tr>
<td>Fluopyram</td>
<td>198 b</td>
<td>410 d</td>
<td>751 a</td>
<td>519 c</td>
<td>76 a</td>
</tr>
</tbody>
</table>

\(^a\) Means within a column followed by the same letter are not significantly different according to Tukey’s multiple means comparison (\(\alpha=0.05\)).

\(^b\) Sex ratio = males: females
Table 2.4 Variance analysis on the effect of seed treatments on cyst numbers, root rot, root and plant fresh weight, 100-seed weight, pick, yield and yield – pick of black and kidney bean with 4000 eggs applied per experimental unit at naturally infested field studies at Rodney and Highgate, ON in 2018.

<table>
<thead>
<tr>
<th>Highgate</th>
<th>Cysts plant$^{-1}$</th>
<th>Root Weight (g)</th>
<th>Cysts g$^{-1}$ root</th>
<th>Yield (kg ha$^{-1}$)</th>
<th>Yield-Pick (kg ha$^{-1}$)</th>
<th>100-Seed Weight (g)</th>
<th>Pick$^b$ (%)</th>
<th>Root Rot (%)</th>
<th>Plant Fresh Weight (g)</th>
<th>Root Fresh Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Market Class (MC)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Seed Treatment (ST)</td>
<td>0.5761</td>
<td>0.7034</td>
<td>&lt;.0001</td>
<td>0.4038</td>
<td>0.3740</td>
<td>0.7445</td>
<td>0.6840</td>
<td>0.2385</td>
<td>0.2385</td>
<td>0.9777</td>
</tr>
<tr>
<td>MC*ST</td>
<td>0.9201</td>
<td>0.6122</td>
<td>&lt;.0001</td>
<td>0.1110</td>
<td>0.1054</td>
<td>0.8606</td>
<td>0.9185</td>
<td>0.4945</td>
<td>0.4945</td>
<td>0.1044</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rodney</th>
<th>Cysts plant$^{-1}$</th>
<th>Root Weight (g)</th>
<th>Cysts g$^{-1}$ root</th>
<th>Yield (kg ha$^{-1}$)</th>
<th>Yield-Pick (kg ha$^{-1}$)</th>
<th>100-Seed Weight (g)</th>
<th>Pick$^b$ (%)</th>
<th>Root Rot (%)</th>
<th>Plant Fresh Weight (g)</th>
<th>Root Fresh Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Market Class (MC)</td>
<td>&lt;.0001</td>
<td>0.8206</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.1631</td>
</tr>
<tr>
<td>Seed Treatment (ST)</td>
<td>0.3621</td>
<td>0.8897</td>
<td>0.3878</td>
<td>0.0076</td>
<td>0.0070</td>
<td>0.4419</td>
<td>0.8237</td>
<td>0.7388</td>
<td>0.6511</td>
<td>0.9152</td>
</tr>
<tr>
<td>MC*ST</td>
<td><strong>0.0147</strong></td>
<td>0.6389</td>
<td><strong>0.0322</strong></td>
<td>0.3574</td>
<td>0.3377</td>
<td><strong>0.0187</strong></td>
<td>0.3620</td>
<td>0.2134</td>
<td>0.2504</td>
<td>0.6386</td>
</tr>
</tbody>
</table>

$^a$ Significant effects (P<0.05) in each column are indicated in bold.

$^b$ Pick refers to whole seeds, which are discolored or deformed.
Table 2.5 SCN cyst numbers on black bean (cv. Zorro) and kidney bean (cv. Red Hawk) with seed treatments applied in naturally infested field studies at Rodney and Highgate ON in 2018.

<table>
<thead>
<tr>
<th>Market Class</th>
<th>Treatment</th>
<th>Cysts (g⁻¹ root)</th>
<th>Highgate⁹</th>
<th>Rodney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black bean</td>
<td>Inoculated Control</td>
<td>69 ef</td>
<td>49 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>B. amyloliquefaciens</em></td>
<td>51 gh</td>
<td>52 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>B. firmus</em></td>
<td>56 fg</td>
<td>47 gh</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BAS576AAS</td>
<td>50 gh</td>
<td>44 gh</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BAS79800F</td>
<td>30 i</td>
<td>44 gh</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BAS97474F</td>
<td>42 h</td>
<td>69 f</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>P. nishizawae</em></td>
<td>57 fg</td>
<td>48 gh</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluopyram</td>
<td>70 ef</td>
<td>36 h</td>
<td></td>
</tr>
<tr>
<td>Kidney bean</td>
<td>Inoculated Control</td>
<td>115 b</td>
<td>92 e</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>B. amyloliquefaciens</em></td>
<td>79 de</td>
<td>110 de</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>B. firmus</em></td>
<td>110 bc</td>
<td>101 de</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BAS576AAS</td>
<td>89 d</td>
<td>108 de</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BAS79800F</td>
<td>94 cd</td>
<td>133 bc</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BAS97474F</td>
<td>111 bc</td>
<td>119 cd</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>P. nishizawae</em></td>
<td>93 cd</td>
<td>150 ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluopyram</td>
<td>180 a</td>
<td>160 a</td>
<td></td>
</tr>
</tbody>
</table>

⁹ Means within a column followed by the same letter are not significantly different according to Tukey’s multiple means comparison (α=0.05).
Table 2.6 Yield and yield-pick of black (cv. Zorro) and kidney (cv. Red Hawk) beans with seed treatments applied grown in a naturally SCN infested field study at Rodney, ON in 2018.

<table>
<thead>
<tr>
<th>Main Effect</th>
<th>Treatment</th>
<th>Yield (kg ha(^{-1}))(^a)</th>
<th>Yield-Pick(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed Treatment</td>
<td>Inoculated Control</td>
<td>2152 ab</td>
<td>2112 ab</td>
</tr>
<tr>
<td></td>
<td>B. amyloliquefaciens</td>
<td>2176 ab</td>
<td>2135 ab</td>
</tr>
<tr>
<td></td>
<td>B. firmus</td>
<td>2268 a</td>
<td>2228 a</td>
</tr>
<tr>
<td></td>
<td>BAS576AAS</td>
<td>1684 b</td>
<td>1648 b</td>
</tr>
<tr>
<td></td>
<td>BAS79800F</td>
<td>1891 ab</td>
<td>1854 ab</td>
</tr>
<tr>
<td></td>
<td>BAS97474F</td>
<td>2086 ab</td>
<td>2044 ab</td>
</tr>
<tr>
<td></td>
<td>P. nishizawae</td>
<td>1991 ab</td>
<td>1955 ab</td>
</tr>
<tr>
<td></td>
<td>Fluopyram</td>
<td>1728 ab</td>
<td>1694 ab</td>
</tr>
</tbody>
</table>

\(^a\) Means within a column followed by the same letter are not significantly different according to Tukey’s multiple means comparison (\(\alpha=0.05\)).

\(^b\) Pick refers to whole seeds, which are discolored or deformed
### Table 2.7 Yield and 100-seed weight of black (cv. Zorro) and kidney (cv. Red Hawk) bean with seed treatments applied grown in a naturally SCN infested field at Highgate, ON in 2018.

<table>
<thead>
<tr>
<th>Main Effect</th>
<th>Treatment</th>
<th>Yield (kg ha(^{-1}))(^a)</th>
<th>Yield-Pick</th>
<th>100-Seed Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Market class</td>
<td>Black</td>
<td>1891 a</td>
<td>1853 a</td>
<td>23.14 b</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>465 b</td>
<td>441 b</td>
<td>41.92 a</td>
</tr>
</tbody>
</table>

\(^{a}\) Means within a column followed by the same letter are not significantly different according to Tukey’s multiple means comparison (\(\alpha=0.05\)).

\(^{b}\) Pick refers to whole seeds, which are discolored or deformed.
Table 2.8 Site information including soil texture, and planting and harvest dates at Rodney and Highgate, ON in 2018.

<table>
<thead>
<tr>
<th>Location</th>
<th>% Sand</th>
<th>% Silt</th>
<th>% Clay</th>
<th>Soil Texture</th>
<th>Planting Date</th>
<th>Harvest Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodney</td>
<td>92</td>
<td>6</td>
<td>2</td>
<td>Sand</td>
<td>June 12</td>
<td>Sept. 13, Oct. 2</td>
</tr>
<tr>
<td>Highgate</td>
<td>86</td>
<td>9</td>
<td>5</td>
<td>Loamy Sand</td>
<td>June 12</td>
<td>Sept. 5, 24</td>
</tr>
</tbody>
</table>
Chapter Three: Effect of six seed treatments on SCN populations, growth and development in two kidney bean cultivars under controlled environment.

3.1 Introduction

Dry bean (*Phaseolus vulgaris* L.) is an important legume crop, cultivated and consumed worldwide (Gepts et al. 2008; Prolla et al. 2010). Dry bean is mainly produced in developing countries as a cheap source of protein (Gepts et al. 2008). The crop is also used to reduce the risk of cancer and other chronic diseases since it is a natural source of phytochemicals and antioxidants (The Bean Institute 2019). Canada is the fifth largest dry bean exporter globally, making it an important producer worldwide (Food and Agriculture Organization of the United Nations (FAO) 2017). Dry bean yield is constrained in both developed and developing countries due to several biotic and abiotic constraints (Fageria 2002). Biotic constraints include pests and diseases such as soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN).

Soybean cyst nematode is the most yield-limiting pest of soybean, causing billions of dollars in losses each year (Tylka 2012). It was first detected in North America in 1954 in New Hanover County, North Carolina and by 1987, it had reached Kent County, Ontario (Wrather et al. 1984; Tylka and Marett 2014). SCN feeding habits affect normal root function by disrupting the translocation process hence affecting plant growth and development (Wrather et al. 1984; Niblack 2009). *H. glycines* has about 100 alternate hosts including dry bean (Yu 2011). The economic impact of SCN in dry bean is poorly understood, however several studies have shown its impact on growth and development of dry bean (Poromarto and Nelson 2009; Poromarto et al. 2010; Zhang 2018). Since SCN has been detected in several dry bean and soybean production
areas in Ontario, there is a need to develop SCN management options to assist growers (Moran 2018).

Soybean cyst nematode is difficult to manage since it cannot be eradicated once established in a field (Jardine and Todd 2001). However, SCN can be managed by reducing the population density below an economic threshold through crop rotation with non-hosts and resistant varieties (Wrather et al. 1984; Jardine and Todd 2001). The discovery of sources of SCN genetic resistance such as Peking and PI 88788 has improved the management options in soybean with the majority of resistant cultivars using PI 88788 (Goheen et al. 2013). In addition, numerous seed treatments are registered for SCN management in soybean in Canada including Bacillus firmus, fluopyram (Bayer Crop Science, Mississauga ON), Pasteuria nishizawae (Syngenta Crop Protection, Guelph, ON) and Bacillus amyloliquefaciens (Valent Canada Inc., Guelph ON). However, there are no seed treatments currently available in dry bean, while the genetics of host resistance is poorly understood.

In controlled environment studies in Chapter Two, B. amyloliquefaciens and B. firmus had lower cyst (cysts plant$^{-1}$) numbers in both black (cv. Zorro) and kidney (cv. Red Hawk) bean while fluopyram, P. nishizawae and BAS576AAS only reduced cysts plant$^{-1}$ in kidney bean. In field studies, B. amyloliquefaciens, BAS576AAS and BAS79800F lowered cysts g$^{-1}$ root in black and kidney bean at Highgate, while there was little treatment effect at Rodney. In a previous study, only the low rate of fluopyram (0.15 mg ai seed$^{-1}$) reduced cysts plant$^{-1}$ in two kidney bean cultivars (Zhang 2018). In controlled environment studies, all the seed treatments increased the number of males plant$^{-1}$ in black bean except BAS576AAS, while the same treatments had similar or lower male numbers than the control in kidney bean. The number of juveniles was highly variable across treatments and studies, which is similar to previous studies (Zhang 2018). Kidney bean had more
cysts than black bean suggesting that kidney bean is more susceptible to SCN than black bean, which agrees with Poromarto and Nelson (2009), and Zhang (2018).

Each seed treatment evaluated in Chapter Two had some nematicidal effect on SCN populations, although some of these effects were inconsistent. The first objective of Chapter Three was to assess the effect of BAS576AAS, BAS97474F, BAS79800F (BASF, Mississauga ON), *B. firmus*, fluopyram, and *B. amyloliquefaciens* seed treatments on SCN populations in a controlled environment study. The seed treatments were applied to two kidney beans (cv. Red Hawk and Dynasty), as the treatment response was greater in kidney versus black bean in Chapter Two. The second objective was to compare the kidney bean cultivars for SCN infection, as well as plant growth and development. *P. nishizawai* was excluded from chapter three since it did not perform well in reducing SCN populations in Chapter Two, as well as in previous dry bean studies (Zhang 2018). Results from this study will improve the industry’s knowledge on the efficacy of SCN management options for dry bean in Canada.

### 3.2 Materials and Methods

#### 3.2.1 Controlled Environment Study

The experiment was conducted at the University of Guelph, Ridgetown Campus using a RCBD with six replications. The plants were grown in a plant growth cabinet (Conviron® A1000) for 30 d at 27°C ± 0.5°C and were exposed to a photoperiod of 16/8 h (day/night). The seed treatments were pre-applied to the seeds at BASF Canada Inc., Winkler, Manitoba using recommended rates for soybean found on Canadian product labels (Table 3.0). This experiment consisted of 18 treatments (Table 3.0). The resistant (P19T39R2) and susceptible (Lee 74) soybean controls were used to monitor for any potential change in SCN race (HG type 5.7) over time. Two kidney bean cultivars (Dynasty and Red Hawk) were used and eight treatments were applied to
each cultivar, including an inoculated and non-inoculated control (Table 3.0). The non-inoculated control were used to monitor for cross-contamination between treatments. The seed treatments included BAS79800F, BAS576AAS and BAS97474F, a chemical fluopyram and the biologicals *B. amyloliquefaciens* and *B. firmus*. The BAS79800F experimental was evaluated together with its two components BAS97474F (biological) and BAS576AAS (non-biological). The seed treatments were applied at the soybean crop rate listed in the Canadian label for each product.

Seeds were germinated for 4 d at room temperature before transplanting, in a 15 cm petri dish on filter paper (Fisher Scientific Ltd, Pittsburgh, PA) moistened with distilled water. The resistant soybean cultivar P92Y55 used in chapter two was replaced with P19T39R2 due to poor germination. The growth medium was prepared by mixing two industrial sands ‘Body Shot’ and ‘All Purpose Medium’ (K&E Sand and Gravel, Wyoming ON) using a 1:1 ratio. The sand medium was autoclaved at 121°C for 25 min and exposed to 10 min of dry time before use. SC10 Super Cell cone-tainers (Stuewe & Sons Inc., Tangent OR) were used for planting. Each cone-tainer was filled with the medium to about 2.5 cm below the surface to provide space for watering of the plants. Six 15 L polycarbonate containers (Cambro CamSquares® - Camwear®, Huntington Beach, CA) were filled with turface (MVP Athletic™, Buffalo Grove, IL) and used to support the cone-tainers for each block. The bottom drain holes on each cone-tainer were blocked using a cotton ball to prevent loss of sand medium.

Cysts were gathered from infested soil near Rodney, ON and were crushed to obtained eggs for inoculation on the day before planting. The SCN population was identified as HG type 5.7 (A. Tenuta, per. comm). The general methods for cyst extraction and inoculation by Poromarto and Nelson (2009) were followed. Cysts extraction from the soil was done using a 30-mesh (600-μm) (Sargent-Welch Scientific, Buffalo, NY) nested over a 60-mesh (250-μm). A 10" bench drill
press (Mastercraft, Toronto, ON) was used to crush the cysts and a 230-mesh (63 -μm) nested over a 500-mesh (25 -μm) was used to collect the eggs. The eggs were washed into a 50-ml centrifuge tube using distilled water and the egg suspension was adjusted to 4000 eggs ml⁻¹ using distilled water. The medium in each cone-tainer was saturated to field capacity with distilled water, followed by inoculation with 4000 SCN eggs using a micropipette. A healthy seedling (root length >2 cm) was then transplanted into each cone-tainer. Due to the germination issue mentioned above, three replications of the resistant soybean treatment did not emerge. Thus, seedlings of the replacement cultivar were transplanted 4 d after the initial planting.

Three ml of NPK (6-11-31) fertilizer plant⁻¹ (Master Plant-Prod Inc. Brampton, ON) was applied at 14, 17, 21 and 24 d after planting. The solution was formed by mixing 10 g of fertilizer into 490 ml of distilled water. The plants were grown for 30 d before harvesting. The phenological development stage of the plants was measured using the BBCH scale (Hack et al. 1993). The scale has 10 developmental phases including germination, inflorescence emergence, flowering, fruit development and senescence, among others.

The plants were cut at soil line, placed in brown paper bags for drying in a tobacco kiln (De Cloet Greenhouse Manufacturing Ltd. Simcoe, ON) at about 38°C for 14 d. Three plants were randomly selected and weighted until a constant weight was recorded on two consecutive measurements and then the dry weight of all plants was recorded. The sand and roots in the cone-tainers were transferred into 16.5 x 14.9-cm sandwich bags (S.C Johnson and Son Ltd, Brantford, ON). They were stored at 4°C before counting juveniles, cysts and males at the laboratory. Initially the sand was washed off the plant roots through a 30-mesh (600 μm) (Sargent-Welch Scientific, Buffalo, NY) sieve into a plastic bucket. The solution was left to settle in the plastic bucket for about five seconds before the solution was poured through a 500-mesh (25 μm) sieve.
(Sargent-Welch Scientific, Buffalo, NY) to collect the juvenile and male nematodes. The collected mixture of juvenile and male nematodes was transferred from the 500-mesh sieve into a 50-ml centrifuge tube (Corning® CentriStar™ Corning, NY) and was stored at 4°C. The solution was diluted 10x with distilled water, and a vortex mixer was used to mix the solution. A micropipette was used to spread 1 ml of the solution over a 1 x 1-cm counting pad. A dissecting microscope (Olympus model SZX2-ILLT, Tokyo, Japan) at 45x magnification was used to count the number of juveniles and males. To ensure greater accuracy, three subsamples was counted for each experimental unit (e.u), and an average value was calculated for juveniles and males.

The cysts (females) were gently washed off the roots with tap water and the solution was passed through two sieves (Sargent-Welch Scientific, Buffalo, NY) nested over each other with a 30-mesh (600 µm) sieve on top of a 60-mesh (250 µm). The roots were observed under a dissecting microscope (Olympus model SZX2-ILLT, Tokyo, Japan) to ensure all the cysts were removed. Cysts were collected in the 60-mesh sieve and a 500-ml wash bottle (Fisher Scientific, Ottawa, ON) filled with distilled water was used to wash cysts into a 50-ml centrifuge tube, which were stored at 4°C. The cyst solution was poured onto a 15-cm lined filter paper (Fisher Scientific Ltd, Pittsburgh, PA) placed in a Büchner funnel, which was used to drain excess water before counting. The same dissecting microscope at 15x magnification was then used to count the total number of females.

After counting, nematodes were placed in a 1-L beaker along with didecyldimethylammonium chloride (DDAC) (Sanidate 5.0 KleenGrow™. Burnaby, B.C) at the rate of 30 ml L⁻¹ of distilled water. DDAC was used to kill the nematodes, as a measure of preventing the spread of the nematodes from discarded materials.
Table 3.0 Treatment list for dry bean SCN management controlled environment study at Ridgetown, ON in 2019.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cultivar</th>
<th>AI Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Inoculated Resistant Soybean</td>
<td>P19T39R2</td>
<td>--</td>
</tr>
<tr>
<td>2. Inoculated Susceptible Soybean</td>
<td>Lee 74</td>
<td>--</td>
</tr>
<tr>
<td>3. Inoculated Dynasty</td>
<td>Red Hawk</td>
<td>--</td>
</tr>
<tr>
<td>4. Inoculated Red Hawk</td>
<td>Red Hawk</td>
<td>--</td>
</tr>
<tr>
<td>5. Non-inoculated Dynasty</td>
<td>Dynasty</td>
<td>--</td>
</tr>
<tr>
<td>6. Non-inoculated Red Hawk</td>
<td>Red Hawk</td>
<td>--</td>
</tr>
<tr>
<td>Seed Treatments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Inoculated BAS576AAS Dynasty</td>
<td>0.2 g ai/100 kg</td>
<td></td>
</tr>
<tr>
<td>8. Inoculated BAS576AAS Red Hawk</td>
<td>0.2 g ai/100 kg</td>
<td></td>
</tr>
<tr>
<td>9. Inoculated BAS97474F Dynasty</td>
<td>0.11 m-cfu seed$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>10. Inoculated BAS97474F Red Hawk</td>
<td>0.11 m-cfu seed$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>11. Inoculated BAS79800F Dynasty</td>
<td>0.4 g ai/100 kg</td>
<td></td>
</tr>
<tr>
<td>12. Inoculated BAS79800F Red Hawk</td>
<td>0.4 g ai/100 kg</td>
<td></td>
</tr>
<tr>
<td>13. Inoculated Fluopyram Dynasty</td>
<td>0.15 mg ai seed$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>14. Inoculated Fluopyram Red Hawk</td>
<td>0.15 mg ai seed$^{-1}$</td>
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<tr>
<td>15. Inoculated B. amyloliquefaciens Dynasty</td>
<td>0.35 g ai/100 000 seed</td>
<td></td>
</tr>
<tr>
<td>16. Inoculated B. amyloliquefaciens Red Hawk</td>
<td>0.35 g ai/100 000 seed</td>
<td></td>
</tr>
<tr>
<td>17. Inoculated B. firmus Dynasty</td>
<td>0.04 mg ai seed$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>18. Inoculated B. firmus Red Hawk</td>
<td>0.04 mg ai seed$^{-1}$</td>
<td></td>
</tr>
</tbody>
</table>

3.2.2 Statistical Analysis

Data were analyzed with SAS 9.4 (SAS Institute Inc., Cary, NC) using PROC GLIMMIX. The experiment was repeated once and the two studies were considered separate environments for the statistical analysis. Environments and blocks were treated as random effects, while treatment was considered a fixed effect. The significance of fixed effects was tested using an F-test at a 5% confidence level and Tukey’s HSD test was used to compute mean comparisons. A test for normality of the residuals of dependent variables was performed using Shapiro Wilk’s test. No differences were observed between the environments for aboveground plant weight (Table 3.1), so data were pooled over environments. Data for the other variables were analyzed separately for each environment, which will be identified as experiment 2.1 and 2.2 for the remainder of this chapter. Dependent variables were juvenile, male and cyst numbers, root dry weight, aboveground...
plant dry weight, root/shoot weight ratio and plant development stage. The number of males, cysts, and juveniles plant\(^{-1}\) and g\(^{-1}\) root followed a Poisson distribution, while root dry weight, plant dry weight, root/shoot dry weight ratio and plant development stage followed a Lognormal distribution after transformation to normalize the data for ANOVA by GLIMMIX. Back transformation was done for log-transformed data for the presentation of the means. The soybean controls were compared in a separate analysis. Data for the non-inoculated dry bean control were not included for the dependent variables that described SCN populations, but were included for plant growth and development variables. Only significant differences according to (P ≤ 0.05) are reported.

3.3 Results

3.3.1 Cyst Number

The susceptible soybean control Lee 74 had 40% higher cysts plant\(^{-1}\) and 37% higher cysts g\(^{-1}\) root than the resistant cultivar P19T39R2 in experiment 2.1, and 50% higher cyst numbers than P19T39R2 in experiment 2.2, which confirms that the SCN HG type did not shift from previous surveys (Table 3.9).

There was a cultivar by seed treatment interaction for cyst numbers in all experiments (Table 3.2; Table 3.3); hence, the data were analyzed separately for each market class (Table 3.5). In experiment 2.1, fluopyram reduced cysts plant\(^{-1}\) in Dynasty and Red Hawk by 57% and 84%, respectively. \textit{B. amyloliquefaciens} and \textit{B. firmus} also lowered cysts plant\(^{-1}\) in both Dynasty and Red Hawk. In addition, BAS79800F and BAS97474F reduced cysts plant\(^{-1}\) in Dynasty only, while BAS576AAS reduced cysts plant\(^{-1}\) in Red Hawk only. All other treatments had cyst counts similar to their corresponding inoculated control (Table 3.5).

In experiment 2.2, fluopyram reduced cysts plant\(^{-1}\) by 43% and 89%, in Dynasty and Red Hawk respectively. \textit{B. amyloliquefaciens} and BAS79800F reduced the cysts plant\(^{-1}\) in Red Hawk by 19% and 33%, respectively, while \textit{B. firmus}, BAS576AAS and BAS97474F had cyst counts
similar to the control (Table 3.7). All of the seed treatments except fluopyram had higher cysts plant\(^{-1}\) and higher cysts g\(^{-1}\) root than the control in Dynasty (Table 3.7). In Red Hawk, fluopyram, BAS79800F and \textit{B. amyloliquefaciens} reduced cysts g\(^{-1}\) root by 89\%, 31\% and 19\%, respectively, compared to the control. BAS576AAS and BAS97474F were similar to the control, while \textit{B. firmus} had higher cysts g\(^{-1}\) root compared to the control (Table 3.7).

### 3.3.2 Male Number

In experiment 2.1, a seed treatment effect was observed for male numbers (Table 3.2), across the two kidney bean cultivars. All the seed treatments were similar in male counts to the control except fluopyram, which lowered males plant\(^{-1}\) and males g\(^{-1}\) root by 78\% and 72\%, respectively (Table 3.6).

There was a cultivar by seed treatment interaction for male numbers in experiment 2.2 (Table 3.3) so data was analyzed by kidney bean cultivar and presented separately (Table 3.7). In Dynasty, \textit{B. amyloliquefaciens} increased males plant\(^{-1}\) by 16\%, BAS79800F and BAS97474F were similar to the control, while \textit{B. firmus}, BAS576AAS and fluopyram reduced male numbers by 18\%, 22\% and 74\%, respectively, compared to the control (Table 3.8). \textit{Bacillus firmus}, BAS576AAS, BAS79800F and fluopyram reduced male g\(^{-1}\) root by 22\%, 25\%, 11\% and 75\%, respectively. \textit{Bacillus amyloliquefaciens} increased males g\(^{-1}\) root, while BAS97474F was similar to the control (Table 3.7).

In Red Hawk, fluopyram reduced both males plant\(^{-1}\) and males g\(^{-1}\) root by 82\% (Table 3.7). The other treatments increased male plant\(^{-1}\) by 36-75\% and males g\(^{-1}\) root by 36-74\%.

### 3.3.3 Juvenile Number

There was no treatment effect for juveniles plant\(^{-1}\) in experiment 2.1 (Table 3.2). There was a cultivar by seed treatment interaction for juveniles g\(^{-1}\) root in experiment 2.1 (Table 3.2). In Red
Hawk, all of the seed treatments increased the number of juveniles g\(^{-1}\) root, while in Dynasty, \textit{B. firmus}, and BAS576AAS increased juveniles g\(^{-1}\) root, \textit{B. amyloliquefaciens}, BAS79800F and fluopyram decreased juveniles g\(^{-1}\) root by 37%, 24% and 14%, respectively, while BAS97474F was similar to the control (Table 3.5).

There was a cultivar by seed treatment interaction for juveniles plant and juveniles g\(^{-1}\) root for experiment 2.2 (Table 3.3); hence the data were analyzed separately for each kidney cultivar. Fluopyram reduced juveniles plant\(^{-1}\) and juveniles g\(^{-1}\) root in Dynasty by 25% and 28%, respectively, while BAS79800F reduced juveniles plant\(^{-1}\) and juveniles g\(^{-1}\) root in Red Hawk by 28% and 26%, respectively (Table 3.7). \textit{Bacillus firmus} and BAS576AAS increased juvenile numbers in Dynasty (Table 3.7), while \textit{B. firmus} and BAS576AAS increased juvenile numbers in Red Hawk (Table 3.7). The remaining treatments had juvenile counts that were similar to the control.

#### 3.3.4 Plant Growth and Development Parameters

There was a seed treatment effect for aboveground plant weight, combined across kidney bean cultivars and across environments (Table 3.1). The non-inoculated control had lower plant weight than all the treatments except the inoculated control and BAS97474 (Table 3.4).

There was a seed treatment effect for root weight and root/shoot weight ratio, combined across kidney bean cultivars in both experiment 2.1 (Table 3.2) and experiment 2.2 (Table 3.3). All the treatments including the inoculated control had lower root weight than the non-inoculated control (Tables 3.6; 3.8). In experiment 2.1, all of the treatments except BAS97474F had a lower root/shoot weight ratio than the non-inoculated control (Table 3.6), while in experiment 2.2, all the treatments had lower root/shoot weight ratio than the non-inoculated control (Tables 3.8).
Plant development stage indicated a cultivar effect in experiment 2.1 (Table 3.2) with Red Hawk at a lower developmental stage than Dynasty (68 ± 0.72 vs 63 ± 0.66). In experiment 2.2, there was a seed treatment effect in plant development stage (Table 3.3) combined over kidney bean cultivars. *B. amyloliquefaciens, B. firmus, BAS576AAS, BAS79800F* and fluopyram had a lower developmental stage than the non-inoculated control (Table 3.8). However, all the treatments were similar to the inoculated control for plant development in experiment 2.2.

### 3.4 Discussion

Cultivar susceptibility studies are an important component of pest management. A susceptibility study for dry bean market classes to SCN documented no differences in cyst numbers across four kidney bean cultivars (Poromarto and Nelson 2009). In Chapter Three, frequently there was an interaction between the two kidney cultivars for cyst, male and juvenile counts, and therefore comparisons cannot be made between the two cultivars for SCN infection.

Fluopyram was the most effective treatment at reducing cyst counts, reducing cysts plant⁻¹ and cysts g⁻¹ root in both cultivars, which agrees with previous work (Zhang 2018). In the current study, fluopyram reduced male numbers (g⁻¹ root; plant⁻¹) for both cultivars, which contradicts Zhang (2018). In soybean studies, fluopyram reduced SCN reproduction, J2 hatching, and juvenile motility (Beeman 2017; Beeman and Tylka 2018). In addition, a reduction in SCN reproduction was observed when fluopyram was combined with metalaxyl and *B. firmus*, while no effect was measured in the absence of fluopyram (Zaworski 2014). In the current study, juvenile counts were similar or lower for both cultivars with the application of fluopyram, with the exception of Red Hawk in experiment 2.1. This response is difficult to explain, as little is known about shifts in SCN life stages in response to seed treatments in soybean and dry bean (Riggs and Wrather 1992; Zhang 2018).
Bacillus amyloliquefaciens functions by colonizing roots and preventing the establishment of root nematodes (PMRA 2015; Siemering et al. 2016). There have only been a few soybean studies that evaluated B. amyloliquefaciens for SCN management, and we believe this is the first study to evaluate B. amyloliquefaciens for SCN management in dry bean. Bacillus amyloliquefaciens was less consistent at reducing SCN numbers than fluopyram in the current study. In experiment 2.1, it reduced cysts plant\(^{-1}\) and cysts g\(^{-1}\) root in both cultivars. In experiment 2.2, B. amyloliquefaciens reduced cysts plant\(^{-1}\) and cysts g\(^{-1}\) root in Red Hawk only. B. amyloliquefaciens had male numbers that were similar or higher than the control for both Dynasty and Red Hawk. Compared to the inoculated control in experiment 2.1, B. amyloliquefaciens increased juvenile numbers for Red Hawk, but it reduced juveniles g\(^{-1}\) root by 37% for Dynasty. In experiment 2.2, it had similar or lower juvenile numbers for both Dynasty and Red Hawk. It is important to note that the number of juveniles has been reported to be highly variable (Riggs and Wrather 1992; Zhang 2018).

Bacillus firmus reduced cysts plant\(^{-1}\) and cysts g\(^{-1}\) root in both cultivars in experiment 2.1 while in experiment 2.2, it increased cysts plant\(^{-1}\) by 36% for Dynasty, compared to the control. Results from previous studies in dry bean (Zhang 2018) and soybean (Beeman and Tylka 2018) also demonstrated some inconsistency with B. firmus. Beeman and Tylka (2018) suggested that this inconsistency may be due to inadequate environmental conditions for growth and development of the bio-control as well as competition with other microbes in the soil. In the current study, B. firmus had an inconsistent response on male numbers (g\(^{-1}\) root; plant\(^{-1}\)), but increased juvenile numbers, which is similar to results from a previous study (Zhang 2018).

The BAS79800F experimental had an inconsistent effect on SCN populations for both cultivars. The two individual components of BAS79800F were also inconsistent, which might have
reduced the efficacy of the combined treatment. The experimental material is still unidentified, hence little is known about its effect in both dry bean and soybean.

In the current study, the non-inoculated control had a higher root/shoot weight ratio due to higher root dry weight and lower plant dry weight compared to other treatments. Olthof and Potter (1972) measured an increase in above ground fresh weight in cauliflower and cabbage when nematode densities were increased. It has been suggested that plants increase the rate of nutrient uptake in response to the stress of nematode feeding, which leads to higher above ground plant growth during initial growth stages (T. Blauel per. comm). Viglierchio and Yu (1965) suggested that plant nematode feeding could reduce plant tissue growth by inactivating auxins, which may in turn lead to a stunted root system. This effect was also observed with a stem nematode (Ditylenchus dipsaci), which reduced the elongation of the stem resulting in stunting in alfalfa (Viglierchio and Yu 1965). It is possible that these two factors may have resulted in lower root weight and root/shoot weight ratio in the inoculated versus the non-inoculated control.

There were some limitations to this experiment. First, the study was only conducted in a controlled environment, thus the response of the treatments may be different under field conditions due to fluctuations in temperature and moisture and interactions with other soil microorganisms (Riggs and Wrather 1992). Second, each experiment was done for 30 d, which is only enough for the completion of one SCN life cycle, as compared to the 2-3+ life cycles that generally occur under field conditions. As a result, this study was not long enough to measure any interaction of the seed treatments across multiple life cycles of SCN. This is particularly important for chemical treatments such as fluopyram, which are typically broken down by microorganisms in the soil and become less efficacious over time (ISU 2012). Theoretically, biological treatments have the potential to grow and multiply, protecting the root system as the plant grows (Soffar 2017; CABI
2019). Third, some root tissue was lost through the process of collecting the cysts, which might have resulted in variation between treatments in cysts g\textsuperscript{-1} root, root weight and root/shoot weight ratio. There was an interaction between the kidney cultivars and the seed treatments for most of the SCN population variables measured; hence more study is needed to understand the susceptibility of dry bean cultivars and market classes to SCN, as well as the performance of seed treatments across cultivars in a market class, under both field and controlled environment conditions. Other limitations include the inoculation level, which may have been too high for the biologicals to establish and grow before the J2 juveniles begin feeding on root vascular tissues (Soffar 2017; CABI 2019). For instance, \textit{B. firmus} primary mode of action is through degrading eggs before they hatch thus a high SCN inoculation level may undermine its efficacy. \textit{P. nishizawai}e’s performance may have been similarly affected as it controls SCN by penetrating J2 juveniles before they enter the roots. Results from this research has identified seed treatments that provide new SCN management options for dry bean farmers in Canada.
Table 3.1 Combined analysis of variance for the effect of BAS576AAS, BAS79800F, BAS97474F, *B. amyloliquefaciens*, *B. firmus* and fluopyram on juvenile, cyst and male numbers, sex ratio, aboveground plant weight, root dry weight, root/shoot dry weight ratio and plant developmental stage (BBCH) of kidney bean (cv. Red Hawk and Dynasty) across controlled environments at Ridgetown, ON in 2019.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Cysts plant⁻¹</th>
<th>Males plant⁻¹</th>
<th>Juveniles plant⁻¹</th>
<th>Cysts g⁻¹ root</th>
<th>Males g⁻¹ root</th>
<th>Juveniles g⁻¹ root</th>
<th>Aboveground Plant Weight (g)</th>
<th>Root Weight (g)</th>
<th>Root/shoot Weight Ratio b</th>
<th>Plant Development Stage (BBCH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pr &gt; F a</td>
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<td>0.4519</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.8113</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.6774</td>
</tr>
<tr>
<td>Environment (ENV)</td>
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<td></td>
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<tr>
<td>Cultivar (C)</td>
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<td>&lt;.0001</td>
<td>&lt;.0001</td>
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a Significant effects (P<0.05) in each column are indicated in bold.
b Root/shoot weight ratio: root dry weight/shoot dry weight
Table 3.2 Analysis of variance for the effect of BAS576AAS, BAS79800F, BAS97474F, *B. amyloliquefaciens, B. firmus* and fluopyram seed treatments on juvenile, cyst and male numbers, root dry weight, root/shoot dry weight ratio and plant developmental stage (BBCH) of kidney bean (cv. Red Hawk and Dynasty) under controlled environment at Ridgetown, ON in 2019 (Experiment 2.1).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Cysts plant$^{-1}$</th>
<th>Males plant$^{-1}$</th>
<th>Juveniles plant$^{-1}$</th>
<th>Cysts g$^{-1}$ root</th>
<th>Males g$^{-1}$ root</th>
<th>Juveniles g$^{-1}$ root</th>
<th>Root Weight (g)</th>
<th>Root/Shoot Weight Ratio$^b$</th>
<th>Plant Developmental Stage (BBCH)</th>
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<tr>
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$^a$ Significant effects (P<0.05) in each column are indicated in bold.

$^b$ Root/shoot weight ratio: root dry weight/shoot dry weight
Table 3.3 Analysis of variance for the effect of BAS576AAS, BAS79800F, BAS97474F, *B. amyloliquefaciens*, *B. firmus* and fluopyram seed treatments on juvenile, cyst and male numbers, root dry weight, root/shoot dry weight ratio and plant developmental stage (BBCH) of kidney bean (cv. Red Hawk and Dynasty) under controlled environment at Ridgetown, ON in 2019 (Experiment 2.2).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Cysts plant(^{-1})</th>
<th>Males plant(^{-1})</th>
<th>Juveniles plant(^{-1})</th>
<th>Cysts g(^{-1}) root</th>
<th>Males g(^{-1}) root</th>
<th>Juveniles g(^{-1}) root</th>
<th>Root weight (g)</th>
<th>Root/shoot Weight Ratio(^b)</th>
<th>Plant Developmental Stage (BBCH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pr &gt; F(^a)</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
</tr>
<tr>
<td>Cultivar (C)</td>
<td>0.0017</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.2937</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.5965</td>
<td>0.0257</td>
<td>0.5507</td>
</tr>
<tr>
<td>Seed Treatment (ST)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.0013</td>
<td>&lt;.0001</td>
<td>0.0110</td>
<td></td>
</tr>
<tr>
<td>C*ST</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.0004</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.8814</td>
<td>0.3543</td>
<td>0.1492</td>
</tr>
</tbody>
</table>

\(^a\)Significant effects (P<0.05) in each column are indicated in bold.

\(^b\) Root/shoot weight ratio: root dry weight/shoot dry weight
Table 3.4 Effect of BAS576AAS, BAS79800F, BAS97474F, *B. amyloliquefaciens*, *B. firmus* and fluopyram on plant dry weight across kidney bean (cv. Red Hawk and Dynasty) and across two controlled environments at Ridgetown, ON in 2018.

<table>
<thead>
<tr>
<th>Seed Treatment</th>
<th>Aboveground Plant Weight (g)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated Control</td>
<td>0.58 ab</td>
</tr>
<tr>
<td>Non-inoculated control</td>
<td>0.52 b</td>
</tr>
<tr>
<td><em>B. amyloliquefaciens</em></td>
<td>0.69 a</td>
</tr>
<tr>
<td><em>B. firmus</em></td>
<td>0.69 a</td>
</tr>
<tr>
<td>BAS576AAS</td>
<td>0.66 a</td>
</tr>
<tr>
<td>BAS79800F</td>
<td>0.66 a</td>
</tr>
<tr>
<td>BAS97474F</td>
<td>0.58 ab</td>
</tr>
<tr>
<td>Fluopyram</td>
<td>0.67 a</td>
</tr>
</tbody>
</table>

\(^a\) Means within a column followed by the same letter are not significantly different according to Tukey's multiple means comparison (\(\alpha=0.05\)).
**Table 3.5** Effect of BAS576AAS, BAS79800F, BAS97474F, B. amyloliquefaciens, B. firmus and fluopyram seed treatments on juvenile and cyst numbers for two kidney bean (cv. Red Hawk and Dynasty) in one environment at Ridgetown, ON in 2019 (Experiment 2.1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cysts plant(^{-1})</th>
<th>Cysts g(^{-1}) root</th>
<th>Juveniles g(^{-1}) root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dynasty(^a)</td>
<td>Red Hawk</td>
<td>Dynasty</td>
</tr>
<tr>
<td>Inoculated Control</td>
<td>235 a</td>
<td>251 a</td>
<td>281 a</td>
</tr>
<tr>
<td><em>B. amyloliquefaciens</em></td>
<td>159 c</td>
<td>214 b</td>
<td>185 c</td>
</tr>
<tr>
<td><em>B. firmus</em></td>
<td>204 b</td>
<td>182 c</td>
<td>224 b</td>
</tr>
<tr>
<td>BAS576AAS</td>
<td>213 ab</td>
<td>190 bc</td>
<td>255 a</td>
</tr>
<tr>
<td>BAS79800F</td>
<td>203 b</td>
<td>243 a</td>
<td>216 b</td>
</tr>
<tr>
<td>BAS97474F</td>
<td>145 c</td>
<td>245 a</td>
<td>158 b</td>
</tr>
<tr>
<td>Fluopyram</td>
<td>100 d</td>
<td>36 d</td>
<td>120 e</td>
</tr>
</tbody>
</table>

\(^a\) Means within a column followed by the same letter are not significantly different according to Tukey’s multiple means comparison (\(\alpha=0.05\)).
Table 3.6 Effect of BAS576AAS, BAS79800F, BAS97474F, *B. amyloliquefaciens*, *B. firmus* and fluopyram seed treatments on male numbers, root dry weight and plant/root dry weight ratio across kidney bean (cv. Red Hawk and Dynasty) in one controlled environment at Ridgetown, ON in 2018 (Experiment 2.1).

<table>
<thead>
<tr>
<th>Seed Treatment</th>
<th>Males plant(^{1a})</th>
<th>Males root(^{1a})</th>
<th>Root Weight (g)</th>
<th>Root/Shoot Weight Ratio(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated Control</td>
<td>323 a</td>
<td>416 a</td>
<td>0.862 b</td>
<td>1.489 b</td>
</tr>
<tr>
<td>Non-inoculated control</td>
<td>--</td>
<td>--</td>
<td>1.137 a</td>
<td>2.102 a</td>
</tr>
<tr>
<td><em>B. amyloliquefaciens</em></td>
<td>259 a</td>
<td>316 a</td>
<td>0.861 b</td>
<td>1.331 b</td>
</tr>
<tr>
<td><em>B. firmus</em></td>
<td>235 a</td>
<td>330 a</td>
<td>0.855 b</td>
<td>1.280 b</td>
</tr>
<tr>
<td>BAS576AAS</td>
<td>327 a</td>
<td>402 a</td>
<td>0.853 b</td>
<td>1.281 b</td>
</tr>
<tr>
<td>BAS79800F</td>
<td>237 a</td>
<td>290 a</td>
<td>0.891 b</td>
<td>1.237 b</td>
</tr>
<tr>
<td>BAS97474F</td>
<td>299 a</td>
<td>352 a</td>
<td>0.869 b</td>
<td>1.645 ab</td>
</tr>
<tr>
<td>Fluopyram</td>
<td>72 b</td>
<td>116 b</td>
<td>0.868 b</td>
<td>1.307 b</td>
</tr>
</tbody>
</table>

\(^{a}\) Means within a column followed by the same letter are not significantly different according to Tukey’s multiple means comparison (\(\alpha=0.05\)).

\(^{b}\) Root/shoot weight ratio: root dry weight/shoot dry weight
Table 3.7 Effect of BAS576AAS, BAS79800F, BAS97474F, *B. amyloliquefaciens*, *B. firmus* and fluopyram seed treatments on juvenile, cyst and male numbers for two kidney bean (cv. Red Hawk and Dynasty) in one controlled environment at Ridgetown, ON in 2019 (**Experiment 2.2**).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Seed Treatment</th>
<th>Cysts plant(^{-1})^a</th>
<th>Males plant(^{-1})</th>
<th>Juveniles plant(^{-1})</th>
<th>Cysts g(^{-1}) root</th>
<th>Males g(^{-1}) root</th>
<th>Juveniles g(^{-1}) root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynasty</td>
<td>Inoculated Control</td>
<td>87 e</td>
<td>316 bc</td>
<td>134 c</td>
<td>130 e</td>
<td>475 b</td>
<td>198 c</td>
</tr>
<tr>
<td></td>
<td><em>B. amyloliquefaciens</em></td>
<td>196 a</td>
<td>365 a</td>
<td>145 c</td>
<td>278 a</td>
<td>522 a</td>
<td>206 c</td>
</tr>
<tr>
<td></td>
<td><em>B. firmus</em></td>
<td>137 d</td>
<td>260 d</td>
<td>187 b</td>
<td>193 d</td>
<td>368 d</td>
<td>263 b</td>
</tr>
<tr>
<td></td>
<td>BAS576AAS</td>
<td>151 cd</td>
<td>248 d</td>
<td>136 c</td>
<td>213 cd</td>
<td>357 d</td>
<td>196 c</td>
</tr>
<tr>
<td></td>
<td>BAS79800F</td>
<td>182 ab</td>
<td>297 c</td>
<td>134 c</td>
<td>259 ab</td>
<td>423 c</td>
<td>192 c</td>
</tr>
<tr>
<td></td>
<td>BAS97474F</td>
<td>165 bc</td>
<td>331 ab</td>
<td>215 a</td>
<td>231 bc</td>
<td>464 b</td>
<td>304 a</td>
</tr>
<tr>
<td></td>
<td>Fluopyram</td>
<td>50 f</td>
<td>83 e</td>
<td>100 d</td>
<td>72 f</td>
<td>120 e</td>
<td>143 d</td>
</tr>
<tr>
<td>Red Hawk</td>
<td>Inoculated Control</td>
<td>176 AB</td>
<td>200 E</td>
<td>100 C</td>
<td>252 B</td>
<td>295 C</td>
<td>141 C</td>
</tr>
<tr>
<td></td>
<td><em>B. amyloliquefaciens</em></td>
<td>143 C</td>
<td>290 CD</td>
<td>102 C</td>
<td>205 C</td>
<td>414 B</td>
<td>146 C</td>
</tr>
<tr>
<td></td>
<td><em>B. firmus</em></td>
<td>198 A</td>
<td>306 BC</td>
<td>147 B</td>
<td>289 A</td>
<td>476 A</td>
<td>201 B</td>
</tr>
<tr>
<td></td>
<td>BAS576AAS</td>
<td>156 BC</td>
<td>349 A</td>
<td>227 A</td>
<td>227 BC</td>
<td>513 A</td>
<td>328 A</td>
</tr>
<tr>
<td></td>
<td>BAS79800F</td>
<td>118 D</td>
<td>326 AB</td>
<td>72 D</td>
<td>173 D</td>
<td>481 A</td>
<td>104 D</td>
</tr>
<tr>
<td></td>
<td>BAS97474F</td>
<td>156 BC</td>
<td>271 D</td>
<td>105 C</td>
<td>225 BC</td>
<td>401 B</td>
<td>153 C</td>
</tr>
<tr>
<td></td>
<td>Fluopyram</td>
<td>19 E</td>
<td>37 F</td>
<td>113 C</td>
<td>28 E</td>
<td>53 D</td>
<td>162 C</td>
</tr>
</tbody>
</table>

\(^a\) Means within a column and kidney bean cultivar followed by the same letter are not significantly different according to Tukey’s multiple means comparison (\(\alpha=0.05\)).
Table 3.8 Effect of BAS576AAS, BAS79800F, BAS97474F, *B. amyloliquefaciens*, *B. firmus* and fluopyram seed treatments on plant/root dry weight ratio and plant developmental stage (BBCH) across two kidney bean (cv. Red Hawk and Dynasty) in one controlled environment at Ridgetown, ON in 2018 (Experiment 2.2).

<table>
<thead>
<tr>
<th>Seed Treatment</th>
<th>Root Weight (g)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Root/Shoot Weight Ratio&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Plant Development Stage (BBCH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated Control</td>
<td>0.682 b</td>
<td>1.194 b</td>
<td>64 ab</td>
</tr>
<tr>
<td>Non-inoculated Control</td>
<td>0.783 a</td>
<td>1.575 a</td>
<td>54 b</td>
</tr>
<tr>
<td><em>B. amyloliquefaciens</em></td>
<td>0.700 b</td>
<td>0.953 b</td>
<td>69 a</td>
</tr>
<tr>
<td><em>B. firmus</em></td>
<td>0.704 b</td>
<td>1.011 b</td>
<td>68 a</td>
</tr>
<tr>
<td>BAS576AAS</td>
<td>0.698 b</td>
<td>1.050 b</td>
<td>67 a</td>
</tr>
<tr>
<td>BAS79800F</td>
<td>0.690 b</td>
<td>1.135 b</td>
<td>66 a</td>
</tr>
<tr>
<td>BAS97474F</td>
<td>0.699 b</td>
<td>1.110 b</td>
<td>66 ab</td>
</tr>
<tr>
<td>Fluopyram</td>
<td>0.699 b</td>
<td>1.027 b</td>
<td>68 a</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means within a column followed by the same letter are not significantly different according to Tukey’s multiple means comparison (α=0.05).

<sup>b</sup> Root/shoot weight ratio = root dry weight/shoot dry weight
Table 3.9 Cyst numbers of a resistant soybean (cv. P19T39R2) and a susceptible soybean (cv. Lee 74) across two controlled environments at Ridgetown, ON in 2018.

<table>
<thead>
<tr>
<th>Soybean Cultivar</th>
<th>Cysts plant$^\dagger$</th>
<th>Cysts g$^{-1}$ root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. 2.1$^a$</td>
<td>Exp. 2.2</td>
</tr>
<tr>
<td>Lee 74</td>
<td>25 a</td>
<td>28 a</td>
</tr>
<tr>
<td>Pioneer 19T39R2</td>
<td>15 b</td>
<td>14 b</td>
</tr>
</tbody>
</table>

$^a$ Means within a column followed by the same letter are not significantly different according to Tukey’s multiple means comparison ($\alpha=0.05$).
Chapter Four: General Discussion

4.1 Research Contributions

Pulse crop production is a major component of Canadian agriculture, and 75% of the production is exported each year (Agriculture and Agri-Food Canada (AAFC) 2011). Canada is the fifth largest exporter of dry bean; which makes it a producer of global significance (FAO 2017). Ontario has the largest dry bean production area (38.4%) in Canada (Bekkering 2014). SCN is the most devastating pest of soybean, while dry bean is an alternative host (Poromarto and Nelson 2009). The current study demonstrates that SCN can reproduce and develop on dry bean, which agrees with previous work (Poromarto and Nelson 2009; Zhang 2018). SCN has been detected in most of the dry bean and soybean production areas in Ontario, and some parts of Quebec (Tenuta et al. 2006; Oud et al. 2013; Moran 2018). The management of SCN is difficult; however, the population density in soybeans can be reduced to levels below an economic threshold using crop rotation and resistant varieties (Wrather et al. 1984; Jardine and Todd 2001). In soybean, the use of genetic resistance is the primary mode of SCN control, with most of the commercial resistant cultivars containing PI88788 and Peking sources of resistance. To date, genetic resistance has not been identified in dry bean (Noel 1993; Goheen et al. 2013). In addition, several seed treatments have been registered for SCN control in soybean in Canada including *Pasteuria nishizawai*ae, *Bacillus firmus*, *Bacillus amyloliquefaciens* and fluopyram. Seed treatments vary in their efficacy in soybean (Schrismsher 2013; Zaworski 2014; Potter et al. 2016; Bissonnette et al. 2017; Jensen et al. 2018; Lund et al. 2018; Beeman and Tylka 2018). Only one study has been done in dry bean (Zhang 2018); hence further research is needed to evaluate the effect of the seed treatments for SCN management in dry bean.
In Chapter Two, seed treatments were evaluated for their efficacy on SCN populations, as well as the growth and development of two dry bean cultivars in controlled environment and field experiments. Comparisons between the black bean (cv. Zorro) and the kidney bean (cv. Red Hawk) could not be made, as there was an interaction between cultivar and seed treatment for all of the SCN population variables measured. Previous studies (Poromarto and Nelson 2009; Zhang 2018) have shown that black bean cultivars support less SCN cysts than kidney bean cultivars. There was little treatment effect on the number of cysts plant$^{-1}$ and dry bean seed yield in the field studies, which might be due to variation in SCN populations across each site, variation in environmental conditions between sites, or the root rot infection observed on the bean plants. It is possible that SCN feeding led to higher root rot infection however a link between SCN feeding and root rot scores was not determined. *B. amyloliquefaciens* and *B. firmus* reduced SCN cysts plant$^{-1}$ in both black and kidney bean in the controlled environment study. It is our understanding that, this was the first study to evaluate *B. amyloliquefaciens* on dry bean. It also reduced cyst numbers in the Highgate field experiment. It functions by preventing root penetration by J2 juveniles through colonization of root surfaces (PMRA 2015; Siemering et al. 2016). *B. firmus* showed no effect on cysts plant$^{-1}$ in field studies. Previous studies also noted this inconsistency with *B. firmus* (Beeman and Tylka 2018; Zhang 2018) however it was reported to successfully reduce J2 juvenile and egg numbers (Schrismsher 2013). Other treatments such as BAS576AAS, fluopyram and *P. nishizawai* only reduced cysts plant$^{-1}$ in kidney bean in the controlled environment study. *P. nishizawai* also performed poorly in past dry bean studies (Zhang 2018). Fluopyram has been reported to be effective for SCN management in dry bean (Zhang 2018) and soybean (Zaworski 2014; Beeman 2017; Beeman and Tylka 2018). Male numbers were impacted differently by seed treatments across market classes with black bean treatments having higher male numbers than
kidney bean. Since a higher male population is attributed to stressful conditions (Lauritis et al. 1983; Schmitt et al. 2004), the higher numbers in black bean may be a response to the seed treatments as well as the host resistance.

Chapter Three evaluated a subset of the seed treatments from Chapter Two on the growth and development of SCN on kidney beans in a controlled environment experiment. Two kidney bean cultivars (cv. Dynasty and Red Hawk) were compared for plant growth and development under SCN pressure. There was an interaction between the kidney bean cultivars and seed treatments tested, for most of the SCN population variables measured. Therefore differences in cultivar susceptibility could not be determined. Fluopyram reduced cyst and male numbers in both cultivars, which agrees with Chapter Two, while each of the other seed treatments were inconsistent. *B. amyloliquefaciens* and *B. firmus* had a greater effect on cyst numbers in second chapter. Little is known about BAS576AAS, BAS79800F and BAS97474F as they are experimental compounds, however they did not have consistent effect in either chapter. The response of treatments to juvenile populations was variable in both chapters, which agrees with previous soybean and dry bean studies (Riggs and Wrather 1992; Zhang 2018). For plant growth and development, the non-inoculated control had a higher root dry weight and lower aboveground plant dry weight that all of the other treatments, which resulted in a higher root/shoot dry weight ratio than most of the treatments. This might be due to the plant increasing growth in response to nematode stress, nematode feeding damage on the root system, and the inactivation of plant growth auxins at nematode feeding sites (Viglierchio and Yu 1965; T. Blauel per. comm.).

All of the treatments were inconsistent in reducing cyst populations, and no treatment provided a season long effect in field studies. However, research has documented that a reduction in SCN infection and reproduction early in the growing season can result in less crop damage and
higher yield (Beeman 2017). Seed treatments may assist in reducing SCN populations, in combination with other management tools such as resistant cultivars and crop rotation. The chemical seed treatment fluopyram provided more consistent early season protection than the bio-controls, which suggests bio-controls need time to multiply and establish (Soffar 2017; CABI 2019).

4.2 Research Limitations

This research used only the HG type 5.7 of SCN in all the experiments, therefore the treatment effect on other HG types is unknown. The seed treatments were only evaluated on three cultivars within two market classes, which was not enough to understand the effect of the treatments on other cultivars within these market classes, or cultivars from other market classes. The duration of the controlled environment experiments was 30 d, which was not sufficient to assess crop yield, or the treatments effect over multiple SCN life cycles, which typically occurs in field studies in Ontario. In addition, the impact of some seed treatments might be different in the field since chemicals (i.e. fluopyram) typically break down over time. Controlled environment studies minimized the variation that can occur in field studies, including pest populations, environmental conditions and interactions with other soil organisms such as root rot. However, a significant effect was measured between repeated controlled environment studies, which suggests that not all variation was accounted for. In the controlled environment studies, the response of dry bean cultivars and seed treatments to a range of SCN populations is unknown, as only one level of SCN inoculation (4000 eggs plant\(^{-1}\)) was evaluated. The economic threshold for SCN in soybean is 2000 eggs 100\(^{-1}\) g soil; however, a threshold has not been established in dry bean. There was some loss of root tissue during the process of collecting cysts from the roots of dry bean plants in
the controlled environment studies, which may have resulted in some variability in the calculation of cysts, males and juveniles g⁻¹ root.

**4.3 Future Research**

An alternative to controlled environment studies is to conduct a study in larger containers under field or greenhouse conditions to take plants through to maturity and measure the effect of the seed treatments on multiple generations of SCN, while maintaining uniform SCN populations in the study. Further evaluation of the effect of the seed treatments on *H. glycines* population dynamics under field environment is needed to better understand how environmental conditions, soil characteristics and soil microorganisms interact with multiple generations of SCN and the host plant. Greater effort is needed to minimize the variability found in field studies. The seed treatments were only tested on three cultivars hence there is a need to evaluate the seed treatments on more cultivars across the market classes of economic importance in Ontario including navy, cranberry and adzuki. Finally, the seed treatments should be tested under various inoculation levels of SCN to determine the interaction between seed treatment and inoculation level and to establish an economic threshold in dry bean.
References


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Kelly, J.D. 2010. The story of bean breeding. Michigan State University


Schrimsher, D. W. 2013. The Studies of Plant Host Resistance to the Reniform Nematode in Upland Cotton and the Effects of Bacillus firmus GB-126 on Plant-Parasitic Nematodes. Graduate Theses and Dissertations. Auburn University, Auburn, AL 36849, USA


University of Illinois. (n.d). The Soybean Cyst Nematode Problem. Urbana


