Assessment of Dry Bean (*Phaseolus vulgaris* L.) Susceptibility to Soybean Cyst Nematode (*Heterodera glycines* Ichinohe) and the Effects of Biological and Chemical Control in a Controlled Environment

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ABSTRACT

Assessment of Dry Bean (*Phaseolus vulgaris* L.) Susceptibility to Soybean Cyst Nematode (*Heterodera glycines* Ichinohe) and the Effects of Biological and Chemical Control in a Controlled Environment

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Soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN) is a parasite that is a major pest of soybean (*Glycine max* (L.) Merr.) production worldwide and impacts dry bean (*Phaseolus vulgaris* L.) as well. Black (cv. Zorro) and kidney (cv. Red Hawk) bean seed were treated with two rates of biological (*Pasturia nishizawae* and *Bacillus firmus*) and chemical (fluopyram) nematicides in a controlled environment (27^{o}C and16:8 h light:dark) for 30 days. Kidney bean was more susceptible to SCN than black bean. *B. firmus* + fluopyram reduced cyst numbers on both black and kidney beans with a clear rate response. In a second study, *B. firmus* at one rate and fluopyram at three rates were tested alone and together on two kidney beans (cv. Red Hawk and Dynasty). The seed treatments impacted SCN at all life stages, but the response was inconsistent over products and rates.

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1 CHAPTER ONE: Literature Review

1.1 Introduction to Dry Bean

Dry or common bean (*Phaseolus vulgaris* L.) globally is planted on all continents except Antarctica (Gept 1998). It belongs to the Fabaceae family which can fix nitrogen using symbiotic bacteria in their roots (Graham and Ranalli 1997; AAFC 2000). This nitrogen-fixing ability has significant positive effects for the environment and agriculture by reducing the need for commercial nitrogen fertilizers (Uebersax 2006). Dry bean includes a wide range of market classes that vary in size, colour, and shape of seeds (Kelly and Cichy 2013). The pinto, small white (navy), and red kidney bean market classes have the largest production in the United States and Canada (Singh 2013). *Phaseolus vulgaris* is an important plant source of dietary fiber, starch, protein, minerals, and vitamins (Kutoš et al. 2003). It is grown widely around the world, especially by people with low income in developing countries (Broughton et al. 2002; Kutoš et al. 2003). *Phaseolus vulgaris* contains significantly more protein, dietary fiber and has a lower content of fat and carbohydrates than cereal grains (Osorio-Diaz et al. 2003; USDA 2012). In addition to being an excellent plant-based source of protein, consuming dry beans on a daily basis is believed to help prevent many human diseases like coronary heart disease, diabetes, and colon cancer (Tharanathan and Mahadevamma 2003; Wu et al. 2004). This could partially explain the increasing consumption of dry bean in developed countries in recent years (Singh 2013).

Pasteuria vulgaris is a self-pollinated diploid crop (2n=22) that prefers warm temperatures from 18 to 25°C (Fageria 2002). The seeds can germinate at a

temperature as low as 12°C, and have an optimum range of 22 to 30°C (Fageria 2002). Dry bean is a short season crop that can mature in 85 to 100 days after planting in the northern states in America (Kelly and Cichy 2013). Dry bean is a one season crop in temperate areas that have only one rainy season (Beebe et al. 2014). Flower abortion can happen with temperatures above 30°C, which frequently causes yield reduction (Fageria 2002). Dry bean can adapt to various soil types and cropping systems; however, higher yield and quality can be achieved in well drained loam soils with good soil moisture availability and a pH from 5.5 to 7.0 (Singh 1999). Dry bean plants do not have a high water requirement during the vegetative growth stages (first three to four weeks), but have higher water requirement with increasing canopy cover and leaf area index (Muñoz-Perea et al. 2007). The yield of dry bean also can be enhanced by planting at higher populations and narrow row spacing (Grafton et al. 1988).

Dry bean plant types are classified into types I, II, III, and IV, based on the plant architecture, fruiting patterns, type of terminal bud, and the plant's climbing ability (Singh 1999). Type I plants have determinate growth habit, which has terminal reproductive buds located at the end of branches and the main stem. After flowering initiates, there is no more production of vegetative tissue (Singh 1982). Type II plants have indeterminate growth habit, where vegetative tissue forms after the terminal flower bud has formed on branches (Singh 1982). This growth habit is similar to soybean, and is more suitable for direct harvest methods (Kelly and Cichy 2013). Type II may be divided into two sub-groups, where IIa type has long vines and type IIb has short vines and more upright architecture (Singh 1982). Type III plant has a relatively weak plant

architecture and open branches, and are non-climbing or semi-climbing, while Type IV plants are indeterminate, with a prostrate growth habit and a strong climbing ability (Singh 1982).

1.1.1 History and Development of Dry bean and Centres of Origin

In the recent years, both archaeological and botanical data supports the theory of the evolution of modern dry bean cultivars from the wild ancestors in Central America more than 7000 years ago (Kaplan 1981; Gepts and Debouck 1991). The remains of seeds, whole plants, and pod fragments were discovered in the late nineteenth century in South, Middle and North America supports the idea that dry bean has an America centre of origin (Kaplan and MacNeish 1960; Brooks et al. 1962; Kaplan 1967; Kaplan et al. 1973; Gepts and Debouck 1991). Other studies with biochemical markers, agronomic performance, and archaeology suggest that dry bean was domesticated at two different geographic origins (Gepts 1988; Singh et al. 1991).

Modern dry bean cultivars were derived from wild ancestors from central Mexico, which were dispersed and formed from two geographically distinct gene pools; the Mesoamerican and the Andean pools (Gepts 1998; Kelly and Cichy 2013). The Andean gene pool has been found in Chile, Bolivia, Peru, and Argentina, while the Mesoamerican gene pool was dispersed from central Mexico as far south as Venezuela (Mamidi et al. 2011). Recent studies suggest that mutation, migration, selection, and genetic drift during domestication impacted the whole genome of present day dry bean (Gepts and Debouck 1991; Wright et al. 2005). The genome-wide resequencing from domesticated and wild accessions and the genome of dry bean proved that the species

experienced two domestications in Mesoamerica and Andes (Gaut 2014). The domestic Mesoamerican population is about three fold more genetically diverse than the Andean gene pool, with more segregation sites and higher haplotype diversity (Mamidi et al. 2011, Schmutz et al 2014). The wild Andes population was derived from Mesoamerican ancestors (Schmutz et al 2014). This may be also linked to the number of domestication events, with the Mesoamerican having several, while the Andean gene pool had only one known event (Gepts 1998; Gepts et al 1988; Chacon et al. 2005).

The two gene pools are genetically distinct from each other, and have significant differences in morphological, molecular, and biochemical characterization (Gepts 1988). The major phenotypic differences include smaller seed size, a shorter fifth internode and straighter lead hairs in the Mesoamerican versus the Andean gene pool (Sigh et al. 1991). Other phenotypic differences include the position of seed within the pod, which is located on the placental structure in Mesoamerican cultivars, but it is located between the placental and ventral structures in Andean cultivars (Singh et al. 1991). The crossing of Mesoamerican and Andean cultivars sometimes results in a F1 generation with weakness or dwarf lethality from outbreeding depression that typically results in a yield reduction (Gepts and Bliss 1985; Koinange and Gepts 1992; Johnson and Gepts 1999; Broughton et al. 2002). The two gene pools have been utilized in different regions of the world. For example, cultivars in Africa, the north-eastern United States, and Europe are predominantly from the Andean gene pool, while cultivars grown in Brazil and the southwestern United States are predominantly from the Mesoamerican gene pool (Gepts and Debouck 1991).

1.1.2 Differences between Market Classes

The size, shape, and colour of seeds at maturity, abiotic and biotic stress susceptibility and growth habits are used to visually distinguish the market classes of dry bean (McClean et al. 1993). The major difference between the two gene pools is the variation in phaseolin seed proteins (Gepts 1988). Cultivars of the Mesoamerican have S (Sanilac) phaseolin types and have smaller seeds, while Andean cultivars have T (Tendergreen) phaseolin types and have larger seeds (Gepts 1988). The Andean and Mesoamerican gene pool can be further classified into three and four races based on growth habit (Singh 1982). Race Mesoamerica includes black and navy market classes, which were domesticated in the lowland regions of Latin America from Mexico south to Columbia and Venezuela (Mamidi et al 2011). Race Durango includes the medium red, pink, great northern and pinto market classes, which were domesticated in the semi-arid northern highlands of Mexico, while race Jalisco overlaps the Durango area at the southern end (Mamidi et al. 2011). The light and dark red kidney beans, most horticultural snap beans, and cranberry beans are classified into the Andean gene pool (Mamidi et al. 2011). The genetic differences between the two gene pools have different physiological mechanisms, which affect yield (Adams 1967).

1.1.3 Dry Bean Production in the World, in Canada, and Ontario

The major production area of the dry bean is between 52°N to 32°N latitude and from 0 to 3000 m above sea level (Schoonhoven and Voyset 1991). The five largest dry bean producing countries in the world are India (4.8 million metric tons (mmt)), Brazil (3.2 mmt), Myanmar (3.0 mmt), China (1.5 mmt), and United State (1.4 mmt), together

with Canada, Mexico, Argentina, and Uganda account about 75% of the total world dry bean production (Broughtone et al. 2003, FAO 2010). India has the largest dry bean production area at about 8.3 million ha (Nedumaran et al. 2015). Brazil is the largest consumer of dry bean in the world (Singh 2013). The consumption per person in Brazil was about 15 kg year^{-1} and a total consumption over 3 mmt between 2005 and 2010 (USDA 2010). World production of dry bean has increased in the last three decades in most regions of the world except Europe (FAO 2009) and reached 23.2 million metric tons in 2010 (Siddiq and Uebersax 2013). This increase is due to the improvement of genetics and the application of good agricultural practices. United States is the world's leading exporter of dry bean, with 20% of production exported (USDA 2016). Based on the data of Census of Agriculture in 2007, there were 6236 dry beans farms with 0.59 million hectares in bean production in the United States. North Dakota accounts for 38% of US production (USDA 2016), followed by Michigan, Nebraska, Minnesota and Idaho (Câmara et al 2013). The distribution of market classes in the USA is regional. Black and kidney beans are concentrated in the northeast states; pinto and navy beans are the major types in the upper Great Plains, while the far western states grow pink, kidney, pinto, and red Mexican beans (McClean et al. 1993). The most popular dry bean market class in the United States is pinto beans, followed by the navy and black beans (USAID 2012). Black bean is the only market class that expanded production dramatically (300%) from 1980 to 2010 (USDA-ERS 2011). From 2006 to 2008, the average farm-gate value of dry bean in the United States was US $759 million, and the consumer sales were approximately US $2 billion (USDA 2016).

Dry bean is Canada's fourth largest specialty crop after lentils, dry peas, and chick peas (AAFC 2000). The market classes grown in Canada are grouped into the white and coloured bean types. Both white and coloured beans are grown in Ontario and Manitoba, while Alberta only grows coloured beans (Goodwin 2003). The dry bean classes produced in Canada include white pea beans (navy) in Manitoba and Ontario, pinto beans in Manitoba and Alberta, black beans in Manitoba and Ontario, cranberry beans in Ontario, Quebec, and Manitoba, and great northern beans in Alberta and Manitoba (AAFC 2000). Dry bean production has increased from 0.15 mmt in 1966-1997 to 0.3 mmt in 1999-2000 (AAFC 2000). In 2014, the total harvested area of dry bean production in Canada was 122,000 ha with an average yield of 2.5 tons ha^{-1}(OMAFRA 2016). The production of coloured beans has increased from 5,500 metric tons (mt) in 1992 to 62,831 mt in 2015 (OMAFRA 2016). The farm cash receipts from dry beans increased from $40.6 million in 1981 to $150.4 million in 2010 (Bekkering 2011). Canada is one of the top five dry bean exporters in the world, and exports mostly unprocessed beans to about 70 countries (AAFC 2000; Goodwin 2003; FAO 2009). Canadian exports have expanded from $96.2 million in 1996 to $188.5 million in 2000 (AAFC 2000). Canada exported 0.28 mmt of dry beans in 2009 and 2010, primarily to North and South America and Europe (AAFC 2011). The value of Canadian dry bean exports was close to $180 million in 2001, and increased to $207.6 million by 2011 (Bekkering 2011).

Ontario accounts for 38.4% of the national production area, followed by Manitoba, Alberta, and Quebec, respectively (Goodwin 2003). In 2014, the total

coloured bean and white bean production in Ontario were about 71,000 mt and 65,000 mt, respectively (OMAFRA 2016).

1.2 Soybean Cyst Nematode (SCN)

Nematodes, which constitute the phylum Nematoda, are small roundworms that include free-living species in various environments, as well as plant and animal parasites (Williamson and Gleason 2003). The phylum Nematoda accounts for 80-90% of all metazoan life forms and is mostly found in deep seas and tropical soils (Boucher and Lambshead 1995; Blanter et al. 1998). Soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN) is a plant-parasitic nematode that is considered to be one of the major pests of soybean (*Glycine max* (L.)) worldwide (Poromarto and Nelson 2009; Wrather et al. 2010; Liu et al. 2012). SCN feeds on the roots of host crops to get nutrients and water, which often results in a yield reduction (Liu et al. 2012). SCN can be found in most soybean producing states in the United States, due to the effects of continuous soybean planting, limitations in cultivar resistance, and the ability of SCN to adapt to new environments (Noel 1992; Avendaño et al. 2003; Niblack et al. 2006). Dry bean (*Phaseolus vulgaris*,) is an alternate host of SCN (Fujita and Miura 1934).

1.2.1 History and Worldwide Distribution

SCN can be found in the major soybean producing countries including China, Argentina, United States and Brazil, and SCN is spreading quickly in recent years due to the use of mono-cropping practices and expanding soybean production (Riggs 1977; Noel 1992). *Heterodera glycine* was first identified in a soybean field in Japan in 1915 (Hori 1916), followed by Korea in 1936 (Yokoo 1936), China (Heilongjiang province) in

1938 (Nakata and Asuyana 1938), and the United States in 1954 (Winstead et al. 1955). In the United States, SCN was first reported in New Hanover County in North Carolina (Winstead et al. 1955). It was hypothesized that SCN arrived in on flower bulbs from Japan (Winstead et al. 1955). The evidence of this hypothesis is that symptoms of the infected plants in Japan and North Carolina were similar (Riggs 1977). It is generally accepted that the importation of soil and seeds is responsible for the introduction of SCN in North Carolina, Missouri, Mississippi, and Kentucky in the US in the 1950s (Noel 1992).

SCN has been spreading rapidly since it was first detected in the US (Noel 1992).There was only a small area of one county in Arkansas that detected SCN in 1957, but 12 years later, there were 24 counties in Arkansas including the lower Arkansas and White River Delta that detected it (Riggs 1977). In 2006, SCN has been detected at 82 of 92 counties with soybean production in Indiana (Faghihi and Ferris 2006). By 2017, SCN was found in 17 states in USA (Tylka and Marett 2017). Both natural pathways like wind, water, and wildlife, as well as the movement on seed from infested areas have spread SCN into uninfected areas (CFIA 2013). SCN was first discovered in Kent County in Ontario in 1988 (Anderson et al. 1988), and then it dispersed along the St. Lawrence Seaway with a north and northeast direction into 12 other counties in Ontario (Mimee et al. 2014). Ontario was the only province in Canada with SCN until 2014, when second stage juveniles and cysts were detected in St. Anicet, Quebec (Mimee et al.2014). The soybean production area of Manitoba in the

Red River Valley also has the potential of SCN infection, as the infested areas in Minnesota and North Dakota are only 300 km away along the Red River (CFIA 2013).

1.2.2 Vectors for the Spread of SCN

Soil movement from an infested area is the primary pathway for the spread of SCN (CFIA 2013). SCN in soil can be spread with agents like wind, water, farm machinery, crop seed and wildlife (Riggs and Wrather 1992). Since the cysts are resistant to a dry environment, they can be transferred by wind (Epps 1968). The first detection of SCN in some fields was on the lee side of hill, which supports this idea (Riggs 1977). Water is the important agent for nematode to move in the soil, as they do not move through an empty pore space (Wallace 1964). The flowing of excess surface water across soil and into streams, rivers or drainage canals that transports infested soil can spread SCN both within a field and over long distances (Skotland et al. 1956; Spears 1956; Riggs 1977). SCN can be still viable after passage through the digestive system of three species of blackbirds (*Molothrus ater*, *Quiscalus quiscula*, and *Sturnis vulgaris*), which demonstrates that SCN can spread from infested to uninfected areas through fecal droppings of wildlife (Smart and Thomas 1969; Epps 1971). The distance of SCN spread with birds depends on the bird species and their feeding habits, and can be both short and long distance (Epps 1971). SCN can survive on crop seeds for months, which results in the spread of SCN to uninfected areas (Riggs and Wrather 1992). Peds of soil found within soybean seed may have cysts of SCN in them (Riggs 1977). Other agents like contaminated soil on farm machinery, and the movement of soil attached to farm workers clothing also assist the spreading of SCN (NCSRP 2017).

1.2.3 Life Cycle

SCN is an obligate endoparasitic pathogen that requires a host to complete its life cycle, and has an egg, four juvenile (J1 to J4), and an adult stage (Tylka 1994; Niblack et al. 2006). SCN usually takes 21 days to complete its' life cycle, at an average temperature of 23.3°C and an optimum temperature range between 19.2 to 26.5°C (Ross 1963). The optimum temperature for egg hatching is 24°C and the hatching rate decreases rapidly when the soil temperature decreases from 10 to 21°C (Ross 1963; Young 1992). In addition, some SCN only hatch after a certain period of time passes, but this delay is poorly understood (Niblack 2005). Typically, SCNs have two to five generations per year (Lauritis et al. 1983; Winter et al. 2006).

There is a linear relationship between soil temperature and the development of the second stage of the juvenile, for soil temperatures between 15 to 30°C (Alston and Schmitt 1988). SCN development and feeding activity stops if the soil temperature is lower than 14°C or above 35°C (Melton et al. 1986; Alston and Schmitt 1988). Soil moisture also influences hatching and development of SCN (Schmitt 1992). The abundance of SCN increases significantly when soil water potential is low (Heatherly et al.1982). The reproduction rate of SCN is enhanced with low soil water level (Barker and Koenning 1989), especially late in the growing season.

When temperature and moisture are adequate, the J1 develops and molts to form a J2 within the egg before it hatches (Ichinohe 1955; Niblack et al. 2006). At the J2 stage, SCN moves through the soil to establish a feeding site on the plant root, and utilizes its stylet to pierce the epidermis of the root, at a site near the vascular tissue

(Young 1992). It inserts its body into the root tissue and initiates a metabolic sink feeding site on the host root (Young 1992; Niblack 2005). After establishing a feeding site, the nematode becomes sedentary and swells, molting three times inside the root (Young 1992). Under suitable conditions, it takes 3 to 4 days to develop from the J2 to the third stage juvenile (J3), and it then takes 6 to 7 days to develop into fourth stage juvenile (J4) (Niblack 2005). Sex differentiation happens between the J3 and the J4 (Niblack 2005). From J4 to adult stage, the female needs 9 to 10 days, and while the male needs one day less (Niblack 2005). The adult females are larger than males and can be observed by the unaided eye on the root surface (Niblack et al. 2006). After the last molt, vermiform-shaped males leave the root and mate with sedentary females, which remain attached to the host root (Niblack et al. 2006). The majority of plant damage was caused by the females because they grow larger within plant roots, feed longer, and create syncytia that can be almost four times larger than the males (Caswell-chen and Thomason 1993). Males migrate to females for fertilization and after mating; they eventually die, while females continue feeding in the soybean roots, and start producing eggs (Lauritis et al. 1983; Williamson and Gleason 2003). Adult female initially produce 50 to 200 eggs and deposit them in an external gelatinous matrix (Riggs and Wrather 1992). Then, the female produces another 50 to 200 eggs, which are contained in an egg sack within her body (Young 1992; Williamson and Gleason 2003; Niblack et al. 2006). After the female dies, her body changes colour from white to yellow to brown and forms a protective cyst around the eggs (Young 1992; Williamson and Gleason 2003; Niblack et al. 2006). One female can produce up to a total of 600

eggs, with an average of 200 (Sipes et al. 1992; Niblack 2005). The eggs that are stored in the gelatinous matrix lack the protection from the cyst, and therefore these eggs hatch easier during the current crop season (Ishibashi et al. 1973).

1.2.4 Population Dynamic

The population of various life stages of SCN can fluctuate due to environmental factors like temperature and moisture, as well as the use of SCN control measures (Schmitt et al. 1992). The number of cysts and eggs changes gradually over time (Schmitt and Riggs 1989). Early in the growing season, the number of eggs decreases due to hatching, but increase by the end of season due to the completion of several life cycles (Bonner and Schmitt 1985). The population density of J2 fluctuates due to their ephemeral presence between soils and roots (Thefft and Bone 1985). Sex ratios of SCN that differs from 1:1 have been observed frequently (Sipes 1992). If higher male number were observed, this usually reflects a high death rate of females from stressful conditions, such as temperature, nutritional status and space (Christie 1929; Petersen 1971; Bull et al 1982).

1.2.5 Host Crops of SCN

Soybean is not the only host of *H. glycines* as other members of the Fabaceae family including dry bean are also attacked by SCN (Abawi and Jacobsen 1984). SCN also has other cultivated hosts including most of the other species of the genus Phaseolus, mung bean (*Vigna radiata*), green pea (*Pisum sativum*), sugar beet (*Beta vulgaris*), and tomatoes (*Solanum lycopersicum*) (Riggs and Hamblen 1962, 1966; Miller 1983; Abawi and Jacobsen 1984). Other common crops in North Dakota like

canola (*Brassica napus* L.), sunflower (*Helianthus annuus* L.), lentil (*Lens culinaris* Medik), and clover (*Trifolium* sp. and *Melilotus* sp) were non-hosts for SCN Type 0 in greenhouse studies; and therefore can be used as suitable rotation crops for soybean production in that region (Miller et al. 2006; Warnke et al. 2006; Poromarto and Nelson 2010). In the same study, camelina (*Camelina microcarpa* Andrz. ex DC.), chickpea (*Cicer arietinum* L.), borage (*Borago officinalis* L.), crambe (*Crambe maritima* L.), field pea (*Pisum sativum* L.), safflower (*Carthamus tinctorius* L.), and nyjer (*Guizotia abyssinica* (L.f.) Cass) were found to be poor hosts for SCN HG Type 0, while lupines (*Lupines albus* L.) were a suitable host (Poromarto and Nelson 2010). The host range of SCN also includes non-crop plant species (Venkatesh et al. 2000). A greenhouse study found that 22 weed species from 13 different families were potential alternate hosts to SCN (Venkatesh et al. 2000). In this study, purple deadnettle (*Lamium purpureum*) has the highest number of eggs/cyst of race 3 (HG Type 0) produced, followed by soybean, field pennycress (*Thlaspi arvense*), and henbit (*Lamium amplexicaule*) (Venkatesh et al. 2000). These weed species are not a major concern in Ontario, as they are not significant problem weeds (Frick and Thomas 1992).

1.2.6 Symptoms of SCN

SCN feeding disrupts the host root function as well as the growth and development of the host (Wang et al. 2003). SCN juveniles penetrate the host root epidermal cell layers and then move intracellularly to the root vasculature (Ithal et al. 2007). An individual cell in the host root vasculature is selected to initiate a feeding site, and it is referred to as syncytia (Ithal et al. 2007). SCN feeding reduces leaf water

potential and cause nutrient stress throughout the plant (Johnson et al. 1993). A high population of SCN in the soil can cause a reduction in pod number, plant height and biomass accumulation of the host (Wang et al. 2003). The population of SCN can fluctuate dramatically in a field due to variability in the environment like soil water, oxygen, soil pH, and essential plant nutrient availability (Wang et al. 2003; NCSRP 2017). Soil water distribution has a great effect on the movement of SCN juveniles and in well-watered soil the infectivity of SCN can be extended (Slack et al.1972). In a sandy loam soil, excess water can reduce the number of cysts developed, due to reduced O_2 content in the soil (Robbin and Barker 1974). A soil pH level greater than 7 will result in higher SCN populations than a field with pH 6.5 or 5.9 (NCSRP 2017). The visible plant symptoms of SCN include yellowing of the leaves, stunting, and early maturity (Tylka 1994). These symptoms are not unique to SCN and are difficult to distinguish from nitrogen and iron deficiency, soil compaction, drought stress, damage caused by other pests, and herbicide stress (Tylka 1994). Yield loss in soybean from SCN can be as high as 30% with no visible above ground symptoms (Chattopadhyay et al.1962; Noel 1992; Niblack and Edwards 1993).

1.2.7 Economic Importance of SCN

Soybean cyst nematode is one of the major pests for each of the top 10 soybean producing countries (Wrather et al. 1997). In 1994, soybean yield loss was estimated at 3 million metric tons (mmt) worldwide (Wrather et al. 1997). By 1998, the yield loss had increased to 9 mmt (Wrather et al. 2001). In the USA, SCN has infected most of the major soybean production areas, resulted in a yield reduction of 3.3 mmt in 2009 (Noel

1986; Wrather and Koenning 2010). Between 2003 and 2005, the economic loss in soybean caused by SCN was approximately $460 - 818 million per year, which was higher than any other soybean disease in the US (Wrather and Koenning 2006). Recently, the economic loss that caused by SCN in United States are valued at more than $1 billion annually (Liu et al. 2012; Koenning and Wrather 2010). SCN is also an important pathogen in Canada. Yield loss caused by SCN in Ontario was 65,397 metric tons in 2002, which is more than the losses that caused by other pests like seedling diseases, Phytophthora root and stem rot (*Phytophthora sojae* (Kaufman & Gerdemann)) (Wrather et al. 2003). Reliable surveys of economic losses caused by SCN are useful to define the severity of this disease and promote effective disease management (James et al. 1991; Wrather et al. 2003).

1.3 SCN Management

Soybean cyst nematode is a top pest of soybean in North America due to its high reproductive rate and persistence in the soil without a host crop (Wrather et al. 1984). There are different tactics like genetic control, crop rotation, biological and chemical control that have the potential to manage SCN in soybean. Dry bean is an alternate host of SCN; however, aside from crop rotation there are few established controls for SCN in dry bean. Integrated pest management (IPM) is preferred to manage SCN in soybean. The four goals of nematode control in IPM include exclusion and avoidance, reduction of initial populations, suppression of reproduction, and prevention of current and future crop damage (Jordan 2017).

1.3.1 Crop Rotation

Proper cultural practices like crop rotation may reduce the yield losses caused by *H. glycines*, due to the host specificity of SCN (Sasser and Grover Uzzell 1991; Koenning et al. 1995; Koenning et al. 1996). Some studies have reported that the population of *H. glycines* in small plots could be reduced significantly with crop rotation (Sasser and Grover Uzzell 1991; Wheeler *et al* 1997). A two-year rotation with non-host crops like corn, wheat, oat, clover, and some other cover crops can reduce the population of SCN below the damage threshold level (Francl and Dropkin 1986). Other potential economically important non-host crops include peanut, tobacco, cotton, and sweet potatoes (Sasser and Grover Uzzell 1991). The population reduction of SCN was up to 75% for one year crop rotation and 92% for two years rotation (Wrather et al. 1984). A crop rotation can also include planting resistant cultivars and rotating resistant cultivars to increase the yield of infested field and reduce the chance that new races of nematode developing (Sasser et al. 1991; Niblack 2005). In Arkansas (Southern USA), a three year rotation that contained one year of a non- host crop, one year of a resistant cultivar, and one year of a susceptible cultivar was very effective (Winter et al. 2006). The northerly areas of the US, like Minnesota which have higher SCN populations, usually require rotations that are longer than three years (Chen et al. 2001).

1.3.2 Host Resistance

The development of resistant cultivars started after the discovery of SCN as separate species in 1962, in the USA (Wrather et al. 1984). On average, resistant cultivars have up to 18% higher yields than susceptible cultivars in a controlled

environment (fine textured soil, 463-1433 SCN eggs 100 cm^{-3}) (Wheeler et al. 1997). In coarse-textured soil infested with SCN, the yields of resistant cultivars were 21% to 56% more than susceptible cultivars (Wheeler et al. 1997). However, resistant cultivars offen had lower yields than susceptible cultivars on non-infested land (Wrather et al. 1984). As the SCN population increases, the difference in yield between resistant and susceptible cultivars increases (Chen et al. 2001). HG type, the first letter of the genus and species of *H. glycine*, is the term used to describe the genetic variation of SCN population (Niblack et al. 2002). Developing an understanding of the HG type of the pest and the genetic basis of host plant resistance is really helpful for breeders to develop pest resistant cultivars (Hartwig 1981).

1.3.2.1 Race and HG Type of SCN

A classification scheme of SCN that can separate the host compatibility within a crop species is the first step to breeding resistant cultivars (Niblack et al. 2002). A race test was developed in 1965 based on the development of females on four resistant differential lines compared to one susceptible soybean line (Golden 1970; Niblack et al. 2002). However, the number of SCN races increased to 25 over time as new resistance sources were discovered. Therefore, the methods developed by Golden in 1970 were no longer accurate and could not describe all of the variability in SCN/host compatibility (Riggs *et al* 1981; Niblack et al. 2002). HG type was developed to replace the race test to more accurately document individual genotypes of the *H. glycine* population present in the soil (Niblack et al. 2003). The calculation of HG type uses the Female Index (FI),

which compares the number of females on a resistant cultivar to the female number on the standard susceptible cultivar Lee 74 (Niblack et al. 2002).

$$FI = \frac{Ni\ (mean\ number\ of\ females\ on\ a\ test\ soybean\ line)}{Ns\ (mean\ number\ of\ females\ on\ the\ standard\ susceptible\ Lee\ 74)} \text{ X } 100$$

The seven indicator soybean lines that are used to test the HG type 1 to 7 are PI 548402 (Peking), PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, and PI 548316(Cloud), respectively (Niblack et al. 2002). When inoculated with same number of SCN eggs, an indicator line with a FI that is more than 10 is considered to be a suitable host (Niblack et al. 2002). For example, a SCN population that has a FI≥10 on PI 548402 (Peking) is HG type 1. A SCN population is HG Type 0 when the FI ≤ 10 for all seven indicator soybean lines (Niblack et al. 2002). There are some examples of overlap points between the race and HG type test. The examples include HG type 0 which was called race 3, HG type 1.2.3.5.7 was race 4, HG type 7 was race 3 or 6, HG type 2.7 was race 1 or 5, HG type 5.7 was race 3, HG type 1.3.7 was race 14, HG type 2.5.7 was race 1, and HG type 1.2.5.7 was race 2 (Table 1.1; Wang et al. 2014). An accurate classification of SCN populations is important to determine the population change over time and the HG type differences among field populations (Niblack et al. 2002).

Table 1 The overlap points between HG type and race

HG type	Race
0	3
1.2.3.5.7	4
7	3 or 6
2.7	1 or 5
5.7	3
1.3.7	14
2.5.7	1
1.2.5.7	2

1.3.2.2 Soybean Genetic Resistance SCN

The first cycle in the selection of SCN-resistant cultivars occurred in late 1960's and included the cultivars Pickett, Dyer, and Custer. These cultivars had better yield in infected fields but had relatively lower yields than susceptible cultivars in non-infected fields (Brim and Ross 1966; Hartwig and Epps 1968; Luedders et al. 1968). The second-cycle resistant cultivars like Forrest, Macj, Pickett 71, and Centennial, had the same Peking source of resistance as earlier cultivars, yet had higher yields even in soil without SCN (Hartwig 1981). 136 of total 306 soybean cultivars grow in Ontario are SCN resistant (OSSACC 2017). All six conventional varieties and 83% of the Round-up Ready varieties grown in Ontario have the PI 88788 source of resistance other than Peking (OSACC 2017). The continued use of cultivars with same resistance source is likely to increase the selection pressure on SCN, which may cause changes in the frequency of alleles and allow SCN to overcome the resistance source (Young and Hartwig 1988).

1.3.3 Chemical Control

Nematicides are effective for short term SCN control when resistant cultivars are not available, but they are usually reserved for high value cops due to their relatively high cost (Wrather et al. 1984; Chen et al. 2001). Nematicides usually decrease SCN population density right after application and cause a delay in nematode reproduction and population development (Riggs and Wrather 1992). Nematicides can be classified based on the mode of action, the chemical group, and the mode of application (Haydock et al. 2006). The two types of nematicides that are used to control *H. glycines* are fumigants, which have liquid formulations and move through soil as a gas, and the non-fumigants that are water soluble and move with water in the soil (Wrather et al. 1984). For SCN, which has limited mobility, a seed treatment is more economical for crops like soybean (Haydock et al. 2006; Grabau 2016).

1.3.3.1 Fluopyram

Fluopyram (Bayer CropScience, Guelph, ON) was registered in 2014 to manage sudden death syndrome in soybean production as ILeVO®. Fluopyram is a systemic fungicide from Fungicide Resistance Action Committee group 7 with a mode of action that restricts fungal respiration and blocks the electron transport of the respiratory chain as a succinate dehydrogenase inhibitor (Avenot and Michailides, 2010; Labourdette et al. 2010; Andersch et al. 2014). Fluopyram controls a broad spectrum of fungi in more than 20 crops (Andersch et al. 2014). In a recent greenhouse study, fluopyram reduced the reproduction, motility, root penetration, and hatching of SCN with an initial inoculation level at 4,000 eggs 100 cm^{-3} of soil (Beeman and Tylka 2017).

1.3.4 Biological Control

Many developed countries have restricted the use of chemical nematicides, due to increasing concerns over their toxicity and environmental effects (Scheider et al. 2003). This has led to an increase in interest in biological controls (Anderson and Lafuerza 1992). The first nematode parasite *Pasteuria penetrans* was discovered in 1940, and this led to the first biological control of plant-parasitic nematodes (Akhtar and Malik 2000). Other antagonists of nematodes include predatory nematodes, some other invertebrate organisms, and arthropods (Walter et al. 1987; Small 1987, Sayre and Walter 1991). The biocontrol of nematodes has been practiced for several decades and provides an additional SCN management tool (Chen et al. 1996).

1.3.4.1 *Bacillus firmus*

The *Bacillus firmus* strain GB-126, a rhizobacteria, was registered for use as a seed treatment (VOTiVO®; Bayer CropScience Research, Guelph, ON) in soybean and corn (Lamovsk et al. 2013; Wilson and Jackson, 2013) in Canada in 2011 (Health Canada 2017). *Bacillus firmus* belongs to Phylum *Firmicutes*, order *Bacilliales*, Family *Bacilliaceae* (Hoeschle-Zeledon et al. 2013). *B. firmus* produces toxins that can kill nematodes (Walia et al. 2000), which is different from *Pasteuria penetrans* that parasitizes the nematode directly. The *B. firmus* strain GB-126 was formulated to reduce the population of *H. glycines* by producing a toxic bioactive secondary compound that inhibits egg development (Schrimsher 2013), and inhibits egg hatch, causes paralysis to juveniles, and possesses lethal activity in multiple life cycle on plant-parasite nematodes like *Radopholus similis, Ditylenchus dipsaci*, and *H. glycine*

(Mendoza et al. 2008). *Bacillus firmus* produces endospores that have a relative long shelf life (two years) under dry and cool conditions (Wilson and Jackson 2013).

1.3.4.2 *Pasteuria* spp and *Pasteuria nishizawae*

Pasteuria nishizawae Pn1 is a gram-positive, endospore-forming, and mycelial bacterium (Sayre and Sayer 1988) that was registered (Clariva® Syngenta Crop Protection Inc., Mississauga ON) for use as a seed treatment to control SCN in soybean in Canada in 2015 (Health Canada 2015). *Pasteuria nishizawae* Pn1 is native to field soils throughout North America. It is a parasite of the adult female of SCN, and is a good candidate to manage other plant-parasitic nematodes like root-knot nematodes (Chen et al. 1996; Noel et al. 2005). The densities of second juvenile stage of *H. glycines* of second life cycle decreased when the number of *Pasteuria* spp attached to SCN females increased in the first life cycle in soybean (Atibalentja et al. 1998). These characteristics make *Pasteuria* spp a potential additional option for SCN control.

1.4 Hypotheses and Objectives

This study tested three hypotheses. First, Ontario dry bean market classes will have different susceptibility to SCN. Second, biological and chemical seed treatments will reduce SCN in dry bean in Ontario. Third, the efficacy of seed treatments will increase with increasing does. The objective of this study include to evaluate the susceptibility of dry bean market class Mesoamerican (black) and Andean (kidney) to soybean cyst nematode, and to evaluate the potential of the three seed treatments for the management of soybean cyst nematode in dry bean production. In the second experiment, the objective were to evaluate the susceptibility of two kidney bean cultivars

Red Hawk and Dynasty to soybean cyst nematode and to evaluate the impact of *B. firmus* at one rate, fluopyram with and without *B. firmus* at three rates for the management of soybean cyst nematode. The results from both experiments should offer important information for breeders for screening for resistant cultivars. In the first study (presented in chapter two) Kidney (cv. Red Hawk) and black (cv. Zorro) bean market classes were used to evaluate the SCN susceptibility to the biological seed treatments (*Bacillus firmus* and *Pasteuria nishizawa*) and the chemical fluopyram (label rate of Sudden Death Syndrome (SDS)). In the second study (presented in chapter three), *B. firmus* and fluopyram were applied alone and in combination at 1.0, 1.5 and 2.0 times of the high label rate to two kidney bean cultivars Red Hawk and Dynasty to determine the rate response in SCN cysts, eggs, juveniles and adult males. The cultivars being tested are the primary cultivars in Ontario.

2 CHAPTER TWO: Assessment of soybean cyst nematode susceptibility in dry bean under controlled environment

2.1 Introduction

Cultivated dry bean (*Phaseolus vulgaris* L.) is an important plant source of protein, especially for people in developing countries (Broughton et al. 2002; Kutoš et al. 2003). In 2013, Canada was the 20th largest dry bean producer in the world with 0.27 mmt (FAOSTAT 2016). However, Canada was the fourth largest exporter in the world, marketing mostly unprocessed beans to about 70 countries (AAFC 2000; FAOSTAT 2016). The dry bean market classes produced in Canada include white pea (navy), black, pinto, cranberry and kidney (dark red, light red and white) beans (AAFC 2000). The four provinces that grow dry bean are Ontario, Manitoba, Alberta, and Quebec, with Ontario accounting for 38% of the total production (Goodwin 2003; AAFC 2011).

Soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN) is a major pest of soybean (*Glycine max* (L.) Merr.) worldwide (Wrather et al. 2001; Poromarto and Nelson 2009; Liu et al. 2012). SCN extracts nutrients and water from the host by feeding on the roots, which often disrupts the host root function and results in a yield reduction (Wang et al. 2003; Liu et al. 2012). SCN can cause a 30% yield loss in soybean with no visible above-ground symptoms (Chattopadhyay et al.1962; Noel 1992; Niblack and Edwards 1993). The visible plant symptoms including leaf chlorosis, stunting, and advanced maturity are not unique to SCN infection (Tylka 1994). They are difficult to distinguish from nitrogen and iron deficiency, drought stress, soil compaction, and herbicide stress (Tylka 1994). SCN infection also interacts with other diseases. For

example, SCN can increase abiotic stress in soybean which can lead to greater symptoms of brown stem rot (*Phyalophora gregata*), as well as root and stem rot (*Phytophthora sojae*). SCN is frequently present in soil infected with sudden death syndrome (*Fusarium solani f. sp. glycines*), a serious disease of soybean (Tabor et al. 2003; Gao et al. 2006; Tabor et al. 2006).

Soybean cyst nematode is spread by the movement of soil infested with cysts, and it has infected soybean production regions in 17 states in the USA (Riggs and Wrather 1992; Gregory and Christopher, 2017) resulting in yield losses of more than USD $1 billion annually. SCN was first identified in Canada in 1988 in Kent County, which is located in southwestern Ontario (Anderson et al. 1988). It then dispersed north and east into at least 12 other counties in Ontario, and was identified in St. Anicet Quebec in 2014 (Mimee et al. 2014). The dry bean and soybean production areas in the Red River Valley of Manitoba are also at risk, as SCN has been identified in soils along the Red River in Minnesota and North Dakota, just 300 km south of the Canadian border (CFIA 2013).

Dry bean is an alternate host for SCN, with yield losses documented under both field and controlled environment experiments (Abawi and Jacobsen 1984; Poromarto and Nelson 2009; Poromorto et al. 2010). The first report of SCN in the USA occurred in a commercial dry bean field in Minnesota in 2017, where irregular patches of stunted chlorotic plants were observed in dark red kidney beans (Yan et al. 2017). SCN reproduces at a higher rate on kidney versus black bean, which suggests that the

Andean market classes are more susceptible than the Mesoamerican classes (Poromorto and Nelson 2009; Poromorto et al. 2010).

Resistant cultivars and crop rotation are the core management strategies used to reduce SCN populations in soybean (Wrather et al. 1984; Sasser and Grover Uzzell 1991; Koenning et al 1995; Koenning et al 1996; Niblack 2005 ;). The primary source of SCN resistance in soybean is from PI 88788, followed by the Peking source (Young and Hartwig 1988; OSACC 2017). However, the continuous use of cultivars with the same resistance source will increase the selection pressure of SCN to overcome this source (Young and Hartwig 1988). A two year rotation with non-host crops like corn, wheat, oat or clover can reduce SCN populations below the damage threshold (Francl and Dropkin 1986).

Recently, chemical and biological seed treatments were registered to manage SCN in soybean, including *Bacillus firmus* and fluopyram (Bayer Crop Science Inc., Guelph ON), and *Pasturia nishizawae* (Syngenta Crop Protection Inc., Mississauga ON). *B. firmus* is a rhizobacteria (Wilson and Jackson, 2013) that produces a toxic bioactive secondary compound that inhibits soil nematode activity at multiple life stages (Mendoza et al. 2008). *Pasteuria nishizawae* is a soil bacterium native in North America (Preston et al. 2003) that is parasitic on SCN females and can reduce the population densities of second stage juveniles (Chen et al. 1996; Atibalentja et al. 1998). Fluopyram is a systemic fungicide belonging to FRAC group 7 that restricts fungal respiration and blocks the electron transport of the respiratory chain as a succinate dehydrogenase inhibitor (Avenot and Michailides, 2010; Labourdette et al. 2010;

Andersch et al. 2014). It was registered in Canada to control sudden death syndrome in soybeans but in a recent study, fluopyram showed potential to manage SCN in soybean (Beeman and Tylka 2017).

Soybean cyst nematode may become a major dry bean pest in Canada, particularly for the Andean market classes. The genetic resistance to SCN in dry bean has not been well studied, and further research is needed to confirm any differences in genetic resistance between the two gene pools of dry bean. Rotation to non-host crops will be an important control strategy, particularly in regions growing both soybean and dry bean. However, other tactics like seed treatments need to be investigated to manage SCN populations in dry bean production, as part of an integrated pest management strategy. The objectives of this study were to evaluate the susceptibility of Mesoamerican and Andean market classes to SCN, and to evaluate the potential of *P. nishizawae*, *B. firmus* and fluopyram seed treatments for the management of SCN in dry bean.

2.2 Materials and Methods

The experiment was conducted in a plant growth chamber (Conviron A1000 with PG Kit, Winnipeg MB), and the design was a randomized complete block with six replications. Each block was arranged in a 15-L polycarbonate container (Cambro CamSquares® - Camwear®, Huntington Beach CA) filled with turface (MVP Athletic™ Buffalo Grove IL), which was used to support each experimental unit. An experimental unit was a single bean plant placed in a cone-tainer (Type SC10 Super Cell, Stuewe & Sons, Inc., Corvallis OR) filled with autoclaved sand. The six blocks were arranged in

two rows of three containers on the floor of the growth chamber. The experiment was repeated three times.

Methods for the preparation of the sand media were adopted from similar work conducted at the Agriculture and Agri-Food Canada research and development centre at Harrow, ON. A 1:1 mixture of the industrial sand products All Purpose-Medium and Body Shot (K&E Sand and Gravel, Wyoming ON) provided a similar particle size (Table 2.5) to the beach sand used at Harrow. The sand was autoclaved at 128°C for 45 minutes and then stored in the closed container to prevent any contact with SCN. A cotton ball was placed at the bottom of each cone-tainer to block the loss of sand from the bottom drainage holes.

The 19 treatments included two susceptible (Essex and Lee 74) and one resistant (P92Y55) soybean controls, all of which were untreated, to monitor for changes in the HG type in the SCN population selected for this study. For dry beans, one cultivar from each of black (cv. Zorro) and dark red kidney (cv. Red Hawk) market classes were used. There were a total of eight treatments applied to each dry bean cultivar, including the inoculated and non-inoculated controls. The non-inoculated control documented if cross-contamination with SCN occurred between experiment units. Seed treatments included the biologicals *Bacillus firmus* and *Pasteuria nishizawae* and the chemical fluopyram (Table 2). Each treatment was applied to 500 g of seed and water was added as required to bring each treatment to the same total liquid volume. Treated seeds were well mixed and dried, and then stored in paper bags in a cabinet at room temperature. Treatments were arranged using four rows of five experimental units within each block.

The final experimental unit in each block contained an untreated black bean plant, to minimize any border effects.

Table 2 Treatment list of experiment one of *B. firmus*. *B. firmus* + fluopyram. *P. nishizawae* seed treatments on black and kidney bean in a growth chamber study in Ridgetown, ON

Market Class	Treatment	Rate (mg ai seed $^{-1}$)
soybean	Resistant Pioneer P92Y55	--
	Susceptible Lee 74	--
Black Bean cv. Zorro	Inoculated Control	--
	Uninoculated Control	--
	B. firmus (low)	0.02
	B. firmus (high)	0.04
	B. firmus + fluopyram (low)	0.02+0.075
	B. firmus + fluopyram (high)	0.04+0.15
	P. nishizawae (low)	0.164
	P. nishizawae (high)	0.205
Kidney bean cv. Red Hawk	Inoculated Control	--
	Uninoculated Control	--
	B. firmus (low)	0.02
	B. firmus (high)	0.04
	B. firmus + fluopyram (low)	0.02+0.075
	B. firmus + fluopyram (high)	0.04+0.15
	P. nishizawae (low)	0.164
	P. nishizawae (high)	0.205

The soybean seed had no seed treatment but was soaked in 1% NaOCl solution for 60s to remove any surface contamination, and then rinsed with distilled water for 60s. Commercial seeds (ADM SeedWest, Twin Falls ID) treated with thiamethoxam, metalaxyl-M and S-isomer, fludioxonil, sedaxane + azoxystrobin (Cruiser Maxx Bean + Dynasty®, Syngenta Crop Protection, Mississauga ON) at 56.25 + 1 g a.i. 100 kg^{-1} were used for the dry bean treatments. The commercial seeds were treated at the point of purchasing; however, these treatments do not have SCN inhibition function. Seeds

were germinated at room temperature on moistened filter paper in the petri dish (150 mm x 15 mm) for three days before the start of the experiment.

Soybean cyst nematode cysts were collected from a soybean field near Rodney, ON. The population of SCN was identified previously as HG type 5.7 following the methods of Niblack et al. (2002). The general processes of cyst crushing and inoculation from Poromarto and Nelson (2009) were followed with some the modifications to the sieve size. The cysts were extracted from the field soil with a 30-mesh (600-µm) sieve (Sargent-Welch Scientific, Buffalo NY) nested over a 60-mesh sieve (250-µm). Cysts were crushed over a 60-mesh sieve with a rubber flask stopper mounted in a 10" bench drill press (Mastercraft. Toronto ON) and eggs were collected on a 230-mesh (63 -µm) screen nested over a 500-mesh (25 -µm) screen.

Healthy seedlings with roots > 2-cm were transplanted into the autoclaved sand in plastic cone-tainers. The sand in the cone-tainer was then saturated with distilled water, and inoculated with 4000 eggs of SCN. A 2.5-cm space was left at the top of each cone-tainer to facilitate watering. Another 5 ml of distill ed water was applied following transplanting. Plants were placed in the growth chamber for 30 days at 27°C ± 0.5°C under supplemental light (light intensity 700 µmol) for 16/8 h (day/night). A digital thermometer (Minder Research Inc. Stuart FL) was used to monitor the temperature daily within the growth chamber. Plants were watered daily with distilled water to keep plants above but near their wilting point, as excess water reduces the development of SCN (Heartherly et al. 1982). Plants were fertilized with 3 ml of a 6-11-31 fertilizer solution (Plant-Prod® Hydroponic, Master Plant-Prod Inc. Brampton ON) at 14, 17, 21

and 24 days after planting. The fertilizer solution was produced by mixing 10 ml of fertilizer in 490 ml of water. The concentrations of N-P-K are 3.6, 6.6, and 18.6 ppm, respectively.

2.2.1 Data Collection

At the completion of each repetition of the experiment, measurements included above-ground plant dry weight, plant developmental stage and cyst number. Above-ground plant parts were harvested, placed in a paper bag and dried in a tobacco kiln (De Cloet, Simcoe ON) at 38°C for 15 days. The dried plants were removed from the kiln when the same weight was recorded from two consecutive measurements. Plant developmental stage was assessed according to the extended BBCH scale (German Federal Biological Research Centre for Agriculture and Forestry 2001), which has a uniform code for all mono- and dicotyledonous plant species that have phonologically similar growth stages. Roots were harvested and stored in a plastic bag at 4°C until SCN analysis. Adult females were gently washed off of the roots with water and collected using a 30-mesh sieve nested over a 60-mesh sieve. Plant roots were observed under a compound microscope (Olympus model SZX2-ILLT, Tokyo Japan) at 15 x magnification to ensure that all of the cysts were removed. Cysts were collected in a 50-ml centrifuge tube (Corning® CentriStarTM, Corning NY), mixed with water and stored at 4°C for a maximum of seven days prior to counting. Cysts were removed from the centrifuge tube and placed on lined red filter paper (d=15 cm. Fisher Scientific Ltd, Pittsburgh PA) within a Buchner funnel, so that excess water could be removed using a

vacuum pump. Cysts were counted using the compound microscope at 15 x magnification.

2.2.2 Statistical Analysis

The dependent variables were the cyst number (adult female), the above-ground plant dry weight and the plant developmental stage. All analyses were performed in SAS 9.4 (SAS Institute 2002-2012) using PROC GLIMMIX. Normality of the residuals was tested using a Shapiro-Wilk test for the dependent variables. An examination of the residuals confirmed the independence of errors and homogeneity of variances plotted against the predicted values. A Gaussian distribution was employed, except for cyst number which used a Poisson distribution. The mean cyst numbers were back-transformed for presentation in the tables. Environment was used to represent the three repetitions of the experiment over time. The fixed effect was treatment, and the random effects were the environment and the blocks within the environment. The significance of the fixed effects was tested using an F-test at a confidence level of 0.05. Tukey's HSD test was used to compare the means for cyst number and plant dry weight, but the Tukey's HSD test was not applicable for plant developmental stage, so a series of contrast tests were applied to compare the treatment effects at a confidence level of 0.05. According to the multiple mean comparisons, there were potential interactions between the factors market class and seed treatments on the independent variables. Therefore, a variance analysis conducted to test factorial interaction.

2.3 Results

Plant dry weight was influenced by dry bean market class only (Table 2.1). For cyst count and plant development stage, there was an interaction between market class and seed treatment (Table 2.1). There were more cysts collected from the susceptible soybean control Lee 74 than the resistant control P92Y55 in each repetition of the experiment (Table 2.4), which suggests the HG type of SCN did not change from the race previously documented from the field site. There were no cysts found on the non-inoculated dry bean treatments (data not shown), which suggests that there was no cross contamination between experiment units.

2.3.1 Cyst Number

All of the black bean treatments had lower cyst number than the corresponding kidney bean treatments (Table 2.2). The cyst number of black bean treated with the low rate of *B. firmus* was 17% less than the high rate of *B. firmus*, but both treatments had higher numbers than the inoculated control. The kidney bean treated with the low rate of *B. firmus* had a higher number of cysts than the high rate, while both treatments had lower cyst numbers than the inoculated kidney bean control. There were no differences in cyst numbers on black bean for *B. firmus* + fluopyram at low versus high rate, but both treatment numbers were lower than the inoculated control. The kidney bean treated with the high rate of *B. firmus* + fluopyram had a lower cyst number than the low rate, and both treatments were lower than the inoculated control. The cyst number of black beans treated with the low rate *P. nishizawae* was similar to the control, but the high rate of *P. nishizawae* had higher cyst numbers than the control. There were more

cysts collected from the kidney bean treated with *P. nishizawae* at the low rate compared to the high rate. The low rate *P. nishizawae* was higher than the kidney control, while the high rate had lower cyst numbers than the control. In a comparison of mean cyst numbers across crop species (Tables 2.2 and 2.4), the black bean inoculated control was similar to the resistant soybean P92Y55 and the kidney bean inoculated control was similar to the susceptible soybean Lee 74. Overall for black beans, *B. firmus* + fluopyram were the only effective treatment. For kidney bean, the most effective seed treatment to reduce cyst numbers was *B. firmus* + fluopyram, followed by *B. firmus* alone. The *P. nishizawae* treatment was only effective at the high rate on kidney bean.

2.3.2 Plant Developmental Stage and Dry Weight

The black bean treatments had lower above-ground plant dry weights than the kidney bean treatments, but there were no differences between the treatments within a market class (Table 2.2). The black bean treatments consistently had less plant development ($P<0.0001$) than the kidney bean treatments (Table 2.3). There were no differences in plant development in contrasts comparing the seed treatments individually, except the black bean treated with *B. firmus* + fluopyram at the low rate, which had less plant development than the high rate (Table 2.3).

2.4 Discussion

The cyst number on the roots of the inoculated kidney bean controls was 342% higher than on the roots of black bean control. A previous study tested the susceptibility of four black and kidney beans cultivars and found that the kidney bean cultivars were more susceptible to SCN (Poromarto and Nelson 2009). In the study of Poromarto and

Nelson (2009), the four kidney bean cultivar include Red Hawk were have same susceptibility as susceptible soybean cultivar Lee 74 and all had more female number/plant than all black bean cultivars. The black bean cultivar Zorro was not included in that study; but the four black bean cultivars shown same susceptibility to SCN (Poromarto and Nelson 2009). In this experiment, by comparing the cyst number of dry bean and soybean, the SCN susceptibility of the black bean (Table 2.2) appears to be similar to the PI88788 resistance source (Table 2.4), while the susceptibility of the kidney bean (Table 2.2) appears to be similar to a susceptible soybean cultivar (Table 2.4). This is consistent with the findings of Poromarto and Nelson (2009) as well. Data from the dry bean and the soybean were not combined for analysis as a comparison in SCN susceptibility between two species is not applicable.

Bacillus firmus was consistently reduced the cysts number on the roots of kidney bean, but it increased the cyst number in black bean. The toxin produced by *B. firmus* possesses lethal activity and reduces the population of *H. glycines* by inhibiting the development and hatching of eggs, and causing paralysis in juveniles (Mendoza et al. 2008; Schrimsher 2013). In another study, a susceptible soybean cultivar treated with *B. firmus* had a greater reduction in cyst numbers than a resistant soybean cultivar (Beeman and Tylka 2018). This agrees with the difference in treatment effects between black and kidney beans in this study.

The most effective treatment in this study was the high rate of *B. firmus* + fluopyram, which reduced cyst number up to 52% in black bean and 83% in kidney bean, with a clear rate response in kidney bean. In a soybean study using four cultivars

with a range in SCN susceptibility, *B. firmus* + fluopyram had a greater reduction in cyst numbers than either product applied alone (Zaworski 2014). However, this study had a larger treatment differences because of higher SCN cyst numbers, ranging from 38 to 302 per plant vs 41 to 63 per plant in the previous study (Zaworski 2014).

Pasteuria nishizawae was the least effective treatment tested, and was only effective at the high rate in kidney bean. *Pasteuria* spp. can penetrate the cuticle of SCN J2 and parasitize them throughout their life cycle and over multiple life cycles (Noel and Stanger 1994). However, a nematode parasitized by *P. nishizawae* remains viable but has lower fecundity (Wilson and Jackson 2013). It is difficult to evaluate the effects of *P. nishizawae* on SCN fecundity in this study, as only cyst numbers were collected from one life cycle.

The treatment effects for plant developmental stage and plant dry weight were primarily due to market class, which was expected due to the differences in cultivar characteristics such as plant type and cultivar maturity. Only *B. firmus* + fluopyram in black bean impacted plant development, with the high rate providing slower plant development than the low rate. SCN infection can cause early mature of the hosts (Riggs and Wrather 1992), which explains the treatment response above. However, a similar treatment response was not observed in kidney beans. Therefore, there were no other interactions observed between plant development and SCN management in this study. In a dry bean study in Brazil (Becker and Ferraz, 2004) seed yield and root dry weight were reduced by SCN, following inoculation with 5,600 to 12,600 eggs per plant. Poromarto and Nelson (2010) found dry bean plant height; pod numbers, total plant dry

weight and seed weight were lower in a pot study under field conditions that was inoculated with 5,000 and 10,000 SCN eggs per plant versus a non-inoculated control. The conflicting results between the first experiment and the other studies may due to the short duration of this study and the lower inoculation rate used.

The limitations to this study include the fact that the experiment was conducted in a growth chamber only, and field experiments will be required to confirm the season long effects of the seed treatments. Fluctuations in temperature and moisture in the field will have different effects on the seed treatments particularly the biological treatments. The efficacy of the seed treatments on other HG types of SCN will need to be determined to have a better understanding of the benefits and/or limitations of the seed treatments. The treatment effect of fluopyram applied alone will need to be evaluated as another study has found that fluopyram can reduce the female number compared to an untreated control (Beeman and Tylka 2018). There was some evidence of a rate response in this study but the results were not consistent and further work is needed. Finally, the seed treatments had a greater efficacy in the kidney versus the black bean, so further work across cultivars and market classes is required.

Table2. 1 Variance analysis for the effect of *B. firmus*, *B. firmus* + fluopyram, and *P. nishizawae* seed treatments on soybean cyst nematode female numbers and the plant dry weight of black and kidney bean with artificial soybean cyst nematode inoculation in a growth chamber study at Ridgetown, ON in 2016-2017

Category	Random effects	Estimate	Standard error	Chi Square	Pr > ChiSq
Cyst number	Block (env)	0.0507	0.0188	1012.3	< 0.0001
	Env	0.9965	1.0050	36.13	< 0.0001
Plant dry weight	Block (env)	-0.0001	0.0004	0.05	0.8148
	Env	0.0043	0.0045	15.49	< 0.0001
	Fixed effects	***Numerator df***	***Denominator df***	***F Value***	***Pr> F***
Cyst number	Treatment	6	221	897.39	<0.0001
Plant dry weight	Treatment	6	221	0.94	0.4704
Cyst number	Market class	1	221	5963.15	<0.0001
Plant dry weight	Market class	1	221	243.36	<0.0001
Cyst number	Market class*treatment	6	221	203.39	<0.0001
Plant dry weight	Market class*treatment	6	221	1.56	0.1600

Table2. 2 Soybean cyst nematode cyst number, plant dry weight, and plant development stage of black and kidney bean for *B. firmus*, *P. nishizawae*, and fluopyram seed treatments with artificial SCN inoculation in a growth chamber study at Ridgetown, ON in 2016-2017

Market Class	Treatment	Rate (mg ai seed^{-1})	Cysts (per plant) [2]	Plant dry weight (g)	Plant development stage (BBCH)
Black Bean cv. Zorro	Inoculated Control	--	80.6 *h*[1]	0.47 *a*[1]	31
	B. firmus(low)	0.02	105.9 *g*	0.47 *a*	29
	B. firmus(high)	0.04	128.7 *e*	0.44 *a*	30
	B .firmus (low) + fluopyram (low)	0.02 + 0.075	43.2 *ij*	0.43 *a*	26
	B .firmus (high) + fluopyram (high)	0.04 + 0.15	38.4 *j*	0.51 *a*	38
	P. nishizawae (low)	0.164	74.3 *h*	0.49 *a*	27
	P. nishizawae (high)	0.205	116.1 *f*	0.53 *a*	31
Kidney Bean cv. Red Hawk	Inoculated Control	--	275.8 *b*	0.78 *b*	66
	B. firmus(low)	0.02	248.3 *c*	0.76 *b*	67
	B. firmus(high)	0.04	207.9 *d*	0.70 *b*	66
	B .firmus (low) + fluopyram (low)	0.02 + 0.075	115.3 *fg*	0.78 *b*	66
	B .firmus (high) + fluopyram (high)	0.04 + 0.15	46.3 *i*	0.72 *b*	67
	P. nishizawae (low)	0.164	302.0 *a*	0.70 *b*	61
	P. nishizawae (high)	0.205	257.1 *c*	0.75 *b*	67

[1]Means that share the same lower case letter are not significantly different cross the market class using multiple mean comparison Tukey's test (P<0.05)

[2] Cyst mean values were log transformed for statistical analyses and the data were back transformed for presentation

Table2. 3 Analysis of variance and contrast analysis for the effect of *B. firmus*, *B. firmus* + fluopyram, and *P. nishizawae* seed treatments on the plant developmental stage of black and kidney bean with artificial soybean cyst nematode inoculation in a growth chamber study at Ridgetown, ON in 2016-2017

Random effects	***Estimate***	***Standard error***	***Chi Square***	***Pr> ChiSq***
Block (env)	0.0005	0.0002	2.71	0.0996
Env	0.0022	0.0022	17.83	<0.0001
Fixed effects	***Numerator df***	***Denominator df***	***F Value***	***Pr> F***
Treatments	13	219	52.86	<0.0001
Contrast				
Black control vs kidney bean control	1	219	93.95	<0.0001
Soybean P92Y55 vs Lee 74	1	17	14.2	0.0015
B. firmus +fluopyram vs control	1	219	0.40	0.5302
B. firmus +fluopyram black vs kidney bean	1	219	178.21	<0.0001
B. firmus + fluopyram low rate VS high rate, black bean	1	219	9.53	0.0023
B. firmus + fluopyram low rate VS high rate kidney bean	1	219	0.10	0.7503
B. firmus vs control	1	219	0.11	0.7396
B. firmus Black vs kidney bean	1	219	217.15	<0.0001
B. firmus low vs high rate black bean	1	219	0.08	0.7751
B. firmus low vs high rate kidney bean	1	219	0.19	0.663
P. nishizawa vs control	1	219	0.11	0.7425
P. nishizawae low vs high rate	1	219	1.52	0.2193
P. nishizawae low vs high rate	1	219	0.37	0.5442
B. firmus vs *B. firmus* + fluopyram	1	219	1.38	0.2412

Fixed effects	***Numerator df***	***Denominator df***	***F Value***	***Pr> F***
B. firmus vs *P. nishizawae*	1	219	0.00	0.9970
B. firmus +fluopyram vs *P. nishizawae*	1	219	1.36	0.2444

Table2. 4 Soybean cyst nematode cyst number of resistant soybean and susceptible soybean with artificial SCN inoculation in a growth chamber study at Ridgetown, ON in 2016-2017

Soybean cultivar	Cyst (per plant)		Plant Dry weight (g)	
P92Y55	53.67	*b*[1]	0.86	*a*
Lee74	257.28	*a*	0.75	*b*

[1] Means within a column that share the same lower case letter are not significantly different using multiple mean comparison Tukey's test (P<0.05)

Table2. 5 Typical sieve analysis of industrial sand All Purpose- Medium and Body Shot compared to the beach sand used for SCN experiment at the Agriculture and Agri-Food research and development centre at Harrow, ON

Sieve size	Diameter (mm)	Percent of sand in the sieve			
		All Purpose - Medium	Body Shot	Experimental mixture	Beach sand
30	0.6	3.5		1.8	2
40	0.425	10.5	7	8.8	12
50	0.3	21	42	31.5	33
70	0.212	34.5	38	36.3	31
100	0.15	25	12	18.5	17
140	0.106	4.5	1	2.8	3
200	0.075	1		0.5	2

3 CHAPTER THREE: Efficacy of biological and chemical seed treatments to soybean cyst nematode in dry bean under controlled environment

3.1 Introduction

Dry bean (*Phaseolus vulgaris* L.) is an important plant source of dietary fibre, starch, protein, minerals and vitamins around the world, especially for people with low incomes in developing countries (Broughton et al. 2002; Kutoš et al. 2003). The health benefits of consuming dry bean is one of the reasons for the increase in market demand in developed countries in recent years (Singh 2013). The primary dry bean market classes produced in Canada are white pea (navy), black, pinto, cranberry, and kidney (dark red, light red and white) beans (AAFC 2000). Dry bean is the fourth largest specialty crop in Canada, and it is grown primarily for export (AAFC 2000; FAO 2009). Ontario accounts for 38.4% of the national production area, followed by Manitoba, Alberta, and Quebec, respectively (Goodwin 2003).

Soybean cyst nematode (SCN) is one of the major pests of soybean worldwide (Poromarto and Nelson 2009). SCN feeding disrupts the host root function, as well as the development and growth of the host (Wang et al. 2003). SCN infection can cause up to a 30% yield loss without any noticeable above-ground symptoms (Niblack and Edwards 1993). Soybean production areas infected with SCN has expanded from New Hanover County in North Carolina in 1954 to 17 states in the USA by 2017 (Winstead et al. 1955; Tylka and Marett 2017). The annual economic loss from SCN is more than USD $1 billion (Riggs and Wrather 1992). SCN was first discovered in Kent County in Ontario in 1988 (Anderson et al. 1988). It then dispersed north and east into at least 12

other counties in Ontario, and was identified in St. Anicet Quebec in 2014 (Mimee et al. 2014). Dry bean is also infected by SCN with yield losses documented under both greenhouse and field conditions (Abawi and Jacobsen 1984; Poromarto and Nelson 2009; Poromorto et al. 2010). Manitoba dry bean and soybean production areas in the Red River Valley are under threat of SCN infection, as the SCN infected areas in Minnesota and North Dakota are just 300 km south of the Canadian border (CFIA 2013).

Crop rotation and planting cultivars with resistance to SCN are effective tactics to inhibit SCN in soybean production systems (Wrather et al. 1984; Sasser and Grover Uzzell 1991). However, genetic resistance to SCN in dry bean may be less effective, as it has not been well studied. Therefore, SCN may become a significant pest of dry bean production in Canada. Nematicides are a valid option for the short-term inhibition of SCN, particularly when other tactics are not available (Wrather et al. 1984; Chen et al. 2001). *Bacillus firmus* and fluopyram (Bayer Crop Science Inc., Guelph ON) seed treatments were registered to manage SCN in soybean, and have the potential to manage SCN in dry bean as well (Zhang, 2018). *B. firmus* is a rhizobacteria (Wilson and Jackson, 2013) that produces a toxic bioactive secondary compound that inhibits soil nematode activity at multiple life stages (Mendoza et al. 2008). The systemic fungicide fluopyram is a succinate dehydrogenase inhibitor that belongs to FRAC group 7. It restricts respiration by blocking electron transport (Avenot and Michailides, 2010; Labourdette et al. 2010; Andersch et al. 2014). It was registered in Canada to control

sudden death syndrome in soybean production in 2014. In a recent study, fluopyram was shown to potentially manage SCN in soybean (Beeman and Tylka 2017).

In the study presented in chapter two, *B. firmus* reduced the cyst counts on kidney bean roots, and a clear rate response was observed (Chapter 2). The combination of *B. firmus* + fluopyram was the most effective seed treatment evaluated for both black and kidney bean, with the largest reduction in cyst numbers occurring in kidney bean. A high rate of *B. firmus* + fluopyram resulted in a greater reduction in cyst numbers than the low rate (Chapter 2). However, *B. firmus* and fluopyram need to be evaluated individually to develop a better understanding of their ability to manage SCN. Low and high label rates should be tested to confirm the rate response observed in earlier work. Determining the susceptibility of other kidney bean cultivars to SCN can offer more information for SCN control in dry bean.

The results presented in chapter two demonstrated that *B. firmus* + fluopyram and *B. firmus* reduced the number of cysts, but it is equally important to measure treatment effects on the other life stages of SCN including egg, juvenile, and adult male. A reduction in the number of juveniles would lead to a reduction in feeding damage, adult numbers, and reproduction, which in turn would lead to a reduction in egg numbers. The reduction on the number of egg and juvenile has the potential to reduce the total SCN population in the next life cycle. Previous studies found that more males develop than females when the SCN population is under stress (Colgrove and Niblack 2005). This is due to the environmental sex determination and a higher survival rate for males (Bridgeman and Kerry, 1980; Leudders, 1987; Grundler et al., 1991). A change in sex

ratio is necessary to develop and improve nematode control methods (Evans and Fox 1977). Sex ratio is calculated as a ratio of male to female nematodes (cyst).

In this study, the SCN susceptibility of two kidney bean cultivars was evaluated. The efficacy of fluopyram and *B. firmus* applied alone and in combination was determined by measuring the number of SCN eggs, juvenile larvae, adult males and female cysts. Sex ratio is calculated to evaluate whether the treatment stressed the SCN population. The objectives of this study were to evaluate the cultivar differences in their response to soybean cyst nematode, and to evaluate the potential of the seed treatments *B. firmus* with and without fluopyram for the management of SCN in dry bean.

3.2 Materials and Methods

The experiment was conducted in a plant growth chamber (Conviron A1000, Winnipeg MB), using a randomized complete block design with six replications. An experimental unit was one bean plant grown in a Type SC10 super cell cone-tainer (Stuewe & Sons Inc., Corvallis OR) filled with autoclaved sand. A 15-L polycarbonate container (Cambro CamSquares® - Camwear, Huntington Beach CA) was used to house one block. The container was filled with turface (MVP Athletic™ Buffalo Grove IL) to support the experiment units. Each block used an arrangement of four rows of five experimental units, and blocks were arranged in two rows of containers on the floor of the growth chamber. The experiment was repeated two times.

Methods to prepare the sand media were developed to mimic the sand media used by staff at the Agriculture and Agri-Food Canada research and development centre at Harrow, ON. The industrial sand products All Purpose-Medium and Body Shot (K&E Sand and Gravel, Wyoming ON) were mixed at 1:1, which provided a similar particle size and density (Table 2.5) to the beach sand used at Harrow. The sand was autoclaved at 128°C for 45 min and then stored in a closed container to prevent any contact with SCN. The bottom holes of cone-tainers were blocked with cotton balls to prevent sand from escaping.

There were a total of 20 treatments tested, including a susceptible (cv. Lee 74) and a resistant (cv. P92Y55) soybean control. Soybean controls were used to monitor for changes in the HG type in the SCN population selected for the study. For dry beans, the dark red kidney bean cultivars Red Hawk and Dynasty were used. There were a total of nine treatments applied to each dry bean market class, including inoculated and non-inoculated controls. The non-inoculated control documented if any cross-contamination with SCN occurred (Table 3). Two SCN seed treatments were evaluated including a biocontrol *B. firmus* (Votivo® Bayer Crop Science, Guelph ON), and a chemical control fluopyram (Ilevo® Bayer Crop Science, Guelph ON). One bag of 500 g seeds for each treatment was treated and water was added if needed to bring each treatment to the same total liquid volume. Treated seeds were well mixed and stored in paper bags after drying, and placed in a cabinet at room temperature. Soybean seed had no seed treatment but were soaked in a 1% NaOCl solution for 60 s to remove any potential surface contamination, and then rinsed with distilled water for another 60 s. All

commercial seeds (ADM SeedWest, Twin Falls ID) with thiamethoxam, metalaxyl-M and S-isomer, fludioxonil, sedaxane + azoxystrobin (Cruiser Maxx Bean + Dynasty®, Syngenta Crop Protection, Guelph ON) at 56.25 + 1 g a.i. 100 kg^{-1} before treated with fluopyram or *B. firmus*.

Table 3 Treatment list of experiment two of *B. firmus*. *B. firmus* + fluopyram. fluopyram seed treatments on kidney bean cv. Red Hawk and Dynasty in a growth chamber study in Ridgetown, ON

Market Class	Treatment	Rate (mg ai seed $^{-1}$)
soybean	Resistant Pioneer P92Y55	--
	Susceptible Lee 74	--
Black Bean cv. Zorro	Inoculated Control	--
	Uninoculated Control	--
	B. firmus (high)	0.04
	B. firmus + fluopyram (low)	0.04 + 0.15
	B. firmus + fluopyram (medium)	0.04 + 0.225
	B. firmus + fluopyram (high)	0.04 + 0.30
	fluopyram (low)	0.15
	fluopyram (medium)	0.225
	fluopyram (high)	0.3
Kidney bean cv. Red Hawk	Inoculated Control	--
	Uninoculated Control	--
	B. firmus (high)	0.04
	B. firmus + fluopyram (low)	0.04 + 0.15
	B. firmus + fluopyram (medium)	0.04 + 0.225
	B. firmus + fluopyram (high)	0.04 + 0.30
	fluopyram (low)	0.15
	fluopyram (medium)	0.225
	fluopyram (high)	0.3

The SCN cysts used for inoculation were collected from a soybean field near Rodney, ON. The population of SCN was identified as HG type 5.7 according to the method of Niblack et al. (2002). The methods for cyst crushing and inoculation were

followed with modifications on the sieve size from Poromarto and Nelson (2009). The cysts were extracted from the field soil with a 30-mesh (600 -µm) sieve (Sargent-Welch Scientific, Buffalo, NY) nested over a 60-mesh (250-µm) sieve. Cysts were crushed in the 60-mesh sieve using a rubber flask stopper mounted in a 10'' bench drill press (Mastercraft. Toronto, ON) and eggs were collected on a 230-mesh (63 -µm) screen nested over a 500-mesh (25 -µm) screen and stored in a 50-ml centrifuge tube. The eggs numbers were counted from the 1 ml sample of the collected solution to calculate the total number of egg of in one centrifuge tube. This progress was repeated three times. Distilled water was added when the solution needs to be diluted to 4000 egg per ml.

Seed was germinated at room temperature for three days on moistened filter paper in the petri dish (150mm x 15mm). Healthy seedlings with > 2-cm root length were transplanted into a cone-tainer filled with autoclaved sand that was saturated with distilled water, and then inoculated with 4000 eggs of SCN. A 2.5-cm space between the top of the con-tainer and the sand surface was left to facilitate watering of the plants. Another 5 ml of distilled water was added after transplanting. The experiment went for 30 days at 27°C ± 0.5°C, with supplemental light (light intensity of 700 µmol) to provide 16/8 h (day/night). Daily watering with distilled water was done to keep plants near their wilting point, as excess water reduces the development of SCN (Heartherly et al. 1982). Plants were fertilized with three ml of a solution of 6-11-31 (Plant-Prod® Hydroponic, Master Plant-Prod Inc. Brampton, ON) at 17, 17, 21 and 24 days after planting. The

fertilizer solution was the mixture of 10 ml of fertilizer in 490 ml of water. The concentrations of N-P-K are 3.6, 6.6, and 18.6 ppm,

3.2.1 Data Collection

At the completion of each repetition of the experiment, measurements were made that included the above-ground plant dry weight, plant developmental stage, the number of cysts, juveniles, adult males and eggs. Above-ground plant parts were harvested and dried in paper bags in a tobacco kiln (De Cloet, Simcoe ON) at 38°C for 15 d. The dried plants were removed from the kiln when the same weight was recorded from two consecutive measurements. The BBCH scale, which is a uniform code for all mono- and dicotyledonous plant species with similar phenological growth stages, was used to assess the plant developmental stage (German Federal Biological Research Centre for Agriculture and Forestry 2001).

Harvested roots were stored in plastic bags at 4°C for future analysis. Adult females were washed off the roots gently with water and collected between 30-mesh and 60-mesh sieves. To ensure all of the cysts were removed, the washed plant roots were observed under a compound microscope (Olympus model SZX2-ILLT, Tokyo) at 15 x magnifications. The cysts collected were placed in a 50 ml centrifuge tube (Corning® CentriStarTM Corning, NY), mixed with 45 ml of water and stored at 4°C for no longer than seven days prior to counting. The mixture of water and cysts were pulled from the centrifuge tube and placed on a lined red filter paper (Fisher Scientific Ltd, Pittsburgh, PA) within a Buchner funnel. The excess water was removed using a

vacuum pump, and the cysts were counted under a compound microscope at 15 x magnification.

The number of juveniles, adult males, and eggs were counted following extraction from the plant roots and the sand. The harvested plant root together with the sand from the cone-tainer was washed through a 30-mesh sieve into a plastic bucket using 3 L of water. The solution was allowed to settle in the bucket for five seconds and then the liquid fraction was passed through a 500-mesh sieve. A mixture of juveniles, adult males, and eggs was collected from the 500-mesh sieve into a 50-ml centrifuge tube and stored at 3°C for a maximum of 3 d. After thorough mixing, a 1 ml sample of the solution was pipetted onto a counting grid pattern (1x1 cm with 100 grids). Juveniles, adult males, and eggs were counted using a microscope at 45x magnification. This progress was repeated three times for each experimental unit, and the average number per ml was recorded and multiplied by the total sample volume to estimate the total number of the juveniles, eggs, and adult males.

3.2.2 Statistical Analysis

All analyses were performed with SAS 9.4 (SAS Institute 2002-2012) using PROC GLIMMIX. Random effects were the environment (i.e. the repetition of the experiment) and block within the environment and the fixed effect was treatment. The significance of the fixed effects was tested using an F-test at a confidence level of 0.05. The dependent variables were the cyst number (adult female), the number of eggs, juveniles, adult males, sex ratio, total population, the above-ground plant dry weight and the plant developmental stage scale. Tukey's HSD test was used to compare the means for the

dependent variables except for plant developmental stage to determine the treatment effects. When data were not normally distributed, they were transformed to meet the assumptions of normality based on the highest Shapiro-Wilk's statistic. The number of cysts, eggs, juveniles and adult males followed a Poisson distribution following a log transformation, while plant dry weight followed a Gaussian distribution. Sex ratio followed a lognormal distribution and data were not available for back transformation. The original data for sex ratio were presented. Log transformed data were back transformed for presentation in the tables. Examination of the residuals confirmed the independence of errors and homogeneity of variances plotted against predicted values.

3.3 Results

Block was added to the model as a random effect, to partition the variation in the SCN numbers observed between the two repetitions of the experiment. Table 3.1 identified the significance of block and environment (or repetitions) as random effects and shows the dry bean treatment effect on the number of cysts, eggs, juveniles and adult males and total population, as well as plant dry weight. Blocks were significant for all of the parameters measured except the plant development stage. Environment had no effect on all of the parameters except the number of juveniles (Table 3.1). Seed treatment impacted each parameter except plant development stage. There were more cysts collected from the susceptible soybean control Lee 74 than the resistant control P92Y55 in both repetitions (Table 3.4). SCN was identified based on cyst, egg and juvenile morphology, and the HG type of SCN did not change from the HG type previously documented from the field site, according to the methods of Niblack et al.

(2002). As seen in the study presented in chapter two, the treatments did not have an effect on plant developmental stage and therefore no further statistical analysis was performed (Table 3.1).

3.3.1 Cysts, Adult Males, and Sex Ratio

The Red Hawk inoculated control had fewer cysts than Dynasty (Table 3.2) as a whole. *Bacillus firmus* did not reduce cyst counts on Red Hawk, compared to the inoculated control. *Bacillus firmus* + fluopyram did not differ from the control in cysts counts, but the medium rate had fewer cysts than the low rate. The low rate of fluopyram had lower cyst numbers than the control and all the other treatments except for the medium rate of *B. firmus* + fluopyram. The medium or high rate of fluopyram on Red Hawk had similar cyst counts as the control, with the medium rate having the highest cyst count of all of the treatments for Red Hawk. *B. firmus* had the lowest number of cysts collected for the cultivar Dynasty (Table 3.2). The low and high rate of *B. firmus* + fluopyram had fewer cysts than the Dynasty control, while the medium rate of *B. firmus* + fluopyram was higher than the control. The low rate fluopyram had lower cyst numbers than the medium rate, and both were lower than the Dynasty control. The cyst number of Dynasty treated with the high rate fluopyram was not different than the control. Overall, fluopyram at the low rate reduced cyst numbers for both Red Hawk and Dynasty, while the treatments *B. firmus* and the *B. firmus* + fluopyram were only efficacious on the cultivar Dynasty.

Adult male counts collected from the inoculated control for Dynasty were higher than those for the Red Hawk control. *Bacillus firmus* increased the adult male counts on

Red Hawk, compared to the control. The low and high rate of the *B. firmus* + fluopyram increased the male counts as well, while the medium rate of the *B. firmus* + fluopyram had no effect. The Red Hawk control and the low rate of fluopyram had a similar number of adult males. Both the medium and high rates of fluopyram increased the number of males, compared to the Red Hawk control, with the medium rate having higher numbers than the high rate. *Bacillus firmus* did not reduce the adult male number for Dynasty. The three rates of *B. firmus* +fluopyram decreased the male numbers compared to the control. Male numbers for Dynasty treated with both low and medium rate of fluopyram were not different than the control, while the high rate of fluopyram was the only treatment with higher adult male numbers than the control. The treatment effects over cultivars were inconsistent for the number of males, and there was no clear treatment rate response.

There was no difference between treatments for sex ratio identified in this study (Table 3.2).

3.3.2 Plant Dry Weight

The F-test showed that the treatments had an effect on the plant dry weight (Table 3.1), but differences were not observed when Tukey's HSD test was applied to the means. After the pairwise comparisons adjusted by Tukey-Kramer, it is apparent that the differences in means were too small to make the treatment effects significant.

3.3.3 Eggs

Dynasty had lower egg numbers than Red Hawk for the control treatments (Table 3.3). *B. firmus* decreased egg numbers on Red Hawk versus the control. The number of eggs obtained from Red Hawk treated with *B. firmus* + fluopyram at the low rate was similar to the control, while the medium and high rates had lower egg numbers than the control. A similar response was observed for the low and medium fluopyram rates, but egg numbers increased for the high rate. The treatment with the largest reduction of egg number on Red Hawk was fluopyram at the medium rate. The egg number of Dynasty treated with *B. firmus* was not different from the control. *B. firmus* + fluopyram at the low rate had a higher number of eggs than the control, while the medium rate decreased the egg number. The egg numbers increased slightly at the high rate of *B. firmus* + fluopyram to levels similar to the control. The eggs collected from fluopyram at the low rate were higher than the control but similar to *B. firmus* + fluopyram at the low rate. Higher rates of fluopyram were similar to the low rate. Fluopyram was the most efficacious treatment for Red Hawk but there was no rate response observed. Dynasty had less consistency in treatment response.

3.3.4 Juveniles

Dynasty had a higher number of juveniles than Red Hawk for the untreated control (Table 3.3). Juvenile numbers increased more than 50% when the Red Hawk was treated with *B. firmus*. The low rate of *B. firmus* + fluopyram had lower juvenile numbers than the control and the high rate had even lower numbers, while the medium rate was similar to the control. The juvenile numbers for the low and medium rate of

fluopyram were higher than the Red Hawk control, while the high rate was similar to the control. For the cultivar Dynasty, *B. firmus* did not reduce the number of juveniles. The low rate of *B. firmus* + fluopyram had less juveniles compared to the control, while the medium rate gave a further reduction. However juvenile numbers for the high rate were the highest recorded. The low rate of fluopyram for Dynasty had similar juvenile numbers as the control, but juvenile numbers declined dramatically for the medium rate of fluopyram. The high rate fluopyram had similar numbers as the high rate of the *B. firmus* + fluopyram, and higher than the control. *B. firmus* + fluopyram were the most effective treatment for both the Red Hawk and Dynasty for juvenile numbers, but results were not consistent over the three rates tested.

3.3.5 Total Population

Dynasty had a higher total population of SCN than Red Hawk, for the inoculated control treatments (Table 3.3). Red Hawk treated with *B. firmus* and *B. firmus* + fluopyram at the low rate had a higher total population than the control. The medium and high rate of *B. firmus* + fluopyram had a lower SCN population than the control, but no rate response was observed. Red Hawk treated with the low rate of fluopyram had a smaller total population compared to the control, but both the medium and high rates of fluopyram had a larger population. Dynasty treated with *B. firmus* had a lower population than the control. The SCN population of Dynasty treated with *B. firmus* + fluopyram at all three rates was lower than the inoculated control. There was a rate response between the low and medium rate of *B. firmus* + fluopyram, but populations increased at the high rate. Similarly, the medium rate of fluopyram had the lowest SCN

population, followed by the low rate. The high rate of fluopyram did not reduce the total SCN population for Dynasty, compared to the control. Overall, at least one rate of *B. fimus* + fluopyram or fluopyram applied alone were effective in reducing total populations in both cultivars, but the response was not consistent over the rates tested. *B. firmus* applied alone was effective on Dynasty only.

3.4 Discussion

This was the first study to report the effects of seed treatments containing *B. firmus* and fluopyram on the number of SCN juveniles and adult males. *B. firmus* and fluopyram impacted the SCN population at all life stages including cyst, egg, juvenile, and adult male. However, the treatment effects were not consistent between the two kidney bean cultivars tested, and there was no logical rate response, which is inconsistent with the results of the study presented in chapter two. The susceptibility of Red Hawk and Dynasty were hypothesized to be similar as a previous study (Poromarto and Nelson 2009) found no differences in SCN cyst numbers between four kidney bean cultivars. However, in this experiment, Dynasty had higher SCN number than Red Hawk for all life stages, except the number of eggs. Dynasty was not involved in the previous study (Poromarto and Nelson 2009); therefore, there is no evidence in literature on the response in Dynasty to SCN. However, in that study, differences in cultivar susceptibility within the market class were observed for eight navy bean cultivars (Poromarto and Nelson 2009).

Bacillus firmus produces a toxin, which can reduce SCN populations by inhibiting the development and hatching of eggs (Mendoza et al. 2008; Schrimsher 2013). In this

study, *B. firmus* reduced the number of eggs in Red Hawk, but had no effect on cyst numbers, which contradicts the results of the study presented in chapter two (Zhang 2018). *B. firmus* increased the number of males, juveniles, and total population of SCN in Red Hawk, compared to the control. There is no explanation for these variations. However, *B. firmus* reduced the number of cysts and the total population in the cultivar Dynasty in this study. A previous study tested the effects of *B. firmus* on the reproduction, hatching, root penetration and mortality of SCN in both susceptible and resistant soybean cultivars in a controlled environment (Beeman and Tylka 2018). At the same inoculation level of SCN as this study, *B. firmus* had no effect on the number of cysts and eggs in resistant and susceptible soybean cultivars (Beeman and Tylka 2018).

Fluopyram consistently reduced egg number in Red Hawk but not Dynasty. The cyst number was reduced with the low rate of fluopyram in both cultivars and at the medium rate in Dynasty, but the high rate of fluopyram did not influence cyst numbers. This response is similar to a previous study (Beeman 2017), where fluopyram reduced the number of cysts and eggs on susceptible soybean cultivars that were inoculated at the similar level as this study. But in another study (Zaworski 2014), there were no effects of fluopyram on the cyst and egg numbers on four soybean cultivars with different SCN susceptibility. The male number of both Red Hawk and Dynasty were either increased or remained the same as the control. The juvenile numbers generally increased in response to fluopyram. Total population was increased in two of three rates of fluopyram in Red Hawk, but decreased with two of three rates in Dynasty. There is

evidence of a shift in numbers between the life stage of SCN in soybeans due to abiotic and biotic stress such as infection level, temperature, and host resistance (Bridgeman and Kerry 1980; Melton et al. 1986; Colgrove and Niblack 2005), however, the interaction between SCN population dynamic and seed treatment in dry bean is unknown.

Bacillus firmus+ fluopyram was the most effective treatment in the first study presented in this thesis, and a clear rate response was observed (Zhang 2018). *B. firmus* + fluopyam also were the most effective treatment in this study but the response different between the cultivars. The application of *B. firmus* +fluopyram at three rates to Dynasty decreased the SCN numbers in 11 of 15 cases across the five life stages. This is greater than the SCN response to *B. firmus* or fluopyram applied alone. The treatment effects were less clear in Red Hawk, but *B. firmus* + fluopyram had more consistent effect than either component applied alone. In a study that tested four cultivars of soybean with a range in SCN susceptibility, *B. firmus* + fluopyram caused a greater reduction in cyst number than either product applied alone (Zaworski 2014). However, this response was observed in only one of the two experiments in this study (Zaworski 2014). In another study, *B. firmus* and fluopyram were tested separately under controlled environment in soybean (Beeman and Tylka 2018). *B. firmus* has no effect on the SCN cyst numbers while fluopyram had negative effects, but the efficacy decreased over time as the root system grew larger (Beeman and Tylka 2018). The experimental results were unclear due to the variability across cultivars and treatment rates.

The first limitation of this study is that it was conducted in a controlled environment. The treatments may cause a different response under field conditions, where temperature and moisture fluctuate. Suboptimum conditions can inhibit the life cycle and the extreme conditions may cause mortality of SCN juveniles and adults (Ross 1963). Also, the study duration of 30 days provides the time to complete only one life cycle compare to two to five life cycle per crop season in the field. Therefore, it is not long enough to observe the changes of SCN population dynamics over multiple life cycles. Due to a lack of consistent results, this study should be repeated to further refine the effects of SCN seed treatments in dry bean. Multiple kidney bean cultivars should be tested to determine if cultivars differences exit. Dry bean cultivars from different market classes should be tested to identify if cultivar differences exist within other market classes. The treatment effects on sex ratio were not significant in the study presented in chapter three, but there were unique trends identified. Future study of sex ratio will help to document the impact of stress from seed treatment on SCN populations. This is the first study to measure the number of each life stage of SCN. The effects of the seed treatments on the development of various life stages of the SCN are not well understood. This study provides the first evidence of the seed treatments effects on SCN population dynamics.

Table3. 1 Analysis of variance for the effect of *B. firmu*, *B. firmus* + *fluopyram*, and *P. nishizawae* seed treatments at two rates on the cyst, egg, juvenile, adult male number, plant development stage, and plant dry weight of kidney bean cultivar Red Hawk and Dynasty with artificial soybean cyst nematode inoculation in growth chamber study at Ridgetown, ON 2017

Category	***Random effects***	***Estimate***	***Standard error***	***Chi Square***	***Pr > ChiSq***
Cyst	Block (env)	0.0947	0.0425	6.06	0.0139 [1]
	Env	-0.0057	0.0160	0.08	0.7788
Egg	Block (env)	0.1101	0.0495	2526.00	<0.0001
	Env	0.1424	0.2276	3.69	0.0548
Juvenile	Block (env)	0.1938	0.0869	5071.00	<0.0001
	Env	0.6534	0.9700	8.41	0.0037
Adult male	Block (env)	0.1311	0.0587	5346	<0.0001
	Env	0.0592	0.1151	1.11	0.2921
Plant dry weight	Block (env)	0.0062	0.0033	26.35	<0.0001
	Env	-0.001	0.0007	0.76	0.3838
Plant development stage	Block (env)	1.961	1.7950	2.65	0.1035
	Env	2.191	4.0700	1.41	0.2355
Total population	Block (env)	0.0909	0.0407	13027.00	<0.0001
	Env	0.0234	0.0550	0.51	0.4742
	Fixed effects	***Numerator df***	***Denominator df***	***F Value***	***Pr> F***
Cyst	Seed Treatments	15	164	81.65	<0.0001
Egg	Seed Treatments	15	158	25.10	<0.0001
Juvenile	Seed Treatments	15	159	281.76	<0.0001

Category	*Fixed effects*	*Numerator df*	*Denominator df*	*F Value*	*Pr> F*
Adult male	Seed Treatments	15	159	103.52	<0.0001
Plant dry weight	Seed Treatments	15	148	1.86	0.0312
Plant development stage	Seed Treatments	15	148	1.03	0.4278
Total population	Seed Treatments	15	165	134.13	<0.0001

[1]The *P* values of the contrast test that bolded are significantly different at confidence level of 0.05

Table3. 2 Cyst, adult male number, and sex ratio of kidney bean cultivar Red Hawk and Dynasty for *B. firmus*, and fluopyram seed treatments with artificial soybean cyst nematode inoculation in growth chamber study at Ridgetown, ON in 2017

Kidney bean cultivar	Treatment	Rate (mg ai seed $^{-1}$)	Cyst number [2]		Adult male		Sex Ratio	
Red Hawk	Inoculated Control	--	284.9	*de* [1]	210.9	*ij*	0.81	*a*
	B. firmus	0.04	289.0	*de*	243.8	*fg*	0.77	*a*
	B. firmus + Fluopyram	0.04 +0.15	293.8	*cd*	317.7	*b*	1.02	*a*
	B. firmus + Fluopyram	0.04 +0.225	270.3	*efg*	198.6	*j*	0.84	*a*
	B. firmus + Fluopyram	0.04 +0.3	277.0	*def*	231.6	*gh*	0.80	*a*
	Fluopyram	0.15	250.2	*gh*	221.5	*hi*	1.13	*a*
	Fluopyram	0.225	336.9	*ab*	322.8	*b*	0.89	*a*
	Fluopyram	0.3	298.7	*cd*	283.3	*cd*	0.72	*a*
Dynasty	Inoculated Control	--	316.5	*bc*	301.6	*bc*	1.09	*a*
	B. firmus	0.04	187.8	*j*	305.5	*bc*	1.86	*a*
	B. firmus + Fluopyram	0.04 +0.15	249.6	*gh*	269.9	*de*	1.25	*a*
	B. firmus + Fluopyram	0.04 +0.225	357.2	*a*	240.7	*fgh*	0.69	*a*
	B. firmus + Fluopyram	0.04 +0.3	256.6	*fgh*	253.2	*ef*	1.54	*a*
	Fluopyram	0.15	217.5	*i*	292.8	*cd*	1.18	*a*
	Fluopyram	0.225	245.5	*h*	319.3	*b*	1.15	*a*
	Fluopyram	0.3	300.5	*cd*	378.1	*a*	1.22	*a*

[1]Means that share the same lower case letter are not significantly different cross the market class using multiple mean comparison Tukey's test (P<0.05)

[2] Values were log transformed for statistical analyses and back transformed for presentation in the table

Table3. 3 Egg, juvenile, adult male number of kidney bean cultivar Red Hawk and Dynasty for *B. firmus*, and fluopyram seed treatments with artificial soybean cyst nematode inoculation in growth chamber study at Ridgetown, ON in 2017

Kidney bean cultivar	Treatment	Rate (mg ai seed $^{-1}$)	Egg Number [2]		Juvenile Number		Total population [2]	
Red Hawk	Inoculated Control	--	178.6	*a* [1]	128.4	*d*	841.3	*fg*[1]
	B. firmus	0.04	156.5	*bdc*	200.9	*b*	945.3	*bc*
	B. firmus + Fluopyram	0.04 +0.15	164.9	*abc*	85.6	*fg*	893.0	*de*
	B. firmus + Fluopyram	0.04 +0.225	157.2	*bdc*	131.5	*d*	736.1	*hi*
	B. firmus + Fluopyram	0.04 +0.30	136.1	*fg*	72.9	*h*	742.8	*hi*
	Fluopyram	0.15	136.8	*efg*	149.8	*c*	728.0	*i*
	Fluopyram	0.225	112.1	*h*	189.7	*b*	964.2	*b*
	Fluopyram	0.3	147.7	*def*	136.6	*cd*	907.3	*cd*
Dynasty	Inoculated Control	--	153.1	*cde*	202.3	*b*	1019.9	*a*
	B. firmus	0.04	154.1	*cd*	194.0	*b*	896.9	*de*
	B. firmus + Fluopyram	0.04 +0.15	172.6	*ab*	95.3	*ef*	820.1	*g*
	B. firmus + Fluopyram	0.04 +0.225	128.0	*g*	77.9	*gh*	767.4	*h*
	B. firmus + Fluopyram	0.04 +0.30	144.5	*def*	236.6	*a*	954.0	*b*
	Fluopyram	0.15	172.3	*ab*	186.1	*b*	922.5	*bcd*
	Fluopyram	0.225	158.2	*bdc*	103.3	*e*	861.5	*ef*
	Fluopyram	0.3	156.4	*bdc*	235.9	*a*	1023.8	*a*

Table3. 4 The cyst number of resistant soybean cultivar P92Y55 and the susceptible soybean cultivar Lee 74 with artificial soybean cyst nematode inoculation in growth chamber study at Ridgetown, ON in 2017

Soybean cultivar	Cyst (per plant)		Plant dry weight (g)	
P92455	27.4	*b*[1]	0.7	*a*
Lee74	107.5	*a*	0.8	*a*

[1] Means that share the same lower case letter are not significantly different cross the market class using multiple mean comparison Tukey's test (P<0.05)

4 CHAPTER FOUR: General Discussion

4.1 Research Contributions

Ontario is the top dry bean producing province in Canada (AAFC 2000; Goodwin 2003). SCN is a major pest of soybean, and dry bean is an alternate host (Abawi and Jacobsen 1984; Riggs and Wrather 1992). In a comparison of mean cyst numbers across crop species, the number of cysts that were collected from black bean cv. Zorro was close to that for resistant soybean cv. P92Y55 and the number of cysts collected from kidney bean cv. Red Hawk was close to the susceptible soybean cv. Lee 74. These results indicate dry bean is a good host of SCN, which is risky for future dry bean production. The wide spread infestation of SCN has the potential to impact dry bean yield in Ontario, especially for the Andean market classes (Poromorto and Nelson 2009). The use of resistant cultivars is a major management tool to control SCN in soybean, but genetic resistance in dry bean is not well understood (Wrather et al. 1994). The recent registered biological and chemical seed treatments *B. firmus*, *P. nishizawae*, and fluoyram may use as management tools when other tactics like resistant cultivars and crop rotation are not available. They had inconsistence performance in managing SCN reproduction under field conditions (Barham et al. 2005; Tylka et al. 2015; Zhang 2016). Therefore, controlled environment studies are necessary to provide the detailed information on crop response, and the inhibition of various developmental stages of SCN.

Chapter two reported the first study that tested the efficacy of *B. firmus*, fluoyram, and *P. nishizawae* to control SCN in Ontario one cultivar of each dry beans market

class in controlled environment. Here it was shown that kidney bean of Andean gene pool, is more susceptible to SCN than black bean of Mesoamerican gene pool. Documenting the range of susceptibility of dry bean market classes is an important first step in breeding for pest resistance. The experiment one also found that the treatment with *B. firmus* + fluopyram at a high rate has greater reduction on cysts counts for both market classes. *Bacillus firmus* has the potential to inhibit SCN in kidney bean, and *P. nishizawae* was only effective at a high rate on kidney bean. All the three seed treatments are not registered for dry bean production. The results of the first experiment build up the feasibility of use these seed treatment in dry bean. Chapter three reports the first study to test the effects of *B. firmus* and fluopyram with and without *B. firmus* in on soybean cyst nematodes in Ontario kidney bean (cv. Red Hawk and Dynasty) at all life stages in controlled environment. According to the experimental results, treatment effects have better expression on the cultivar of kidney bean, which has weaker susceptibility to SCN. The experiment two tested two cultivars of kidney bean, which were found to have different susceptibility to SCN from each other at all life stages. According to the study of Poromarto and Nelson (2009), the susceptibility to SCN of kidney bean cv. Red Hawk and cv. Dynasty were hypothesized to be similar. Poromarto and Nelson tested the susceptibility of four kidney bean cultivars and found all four cultivars had same susceptibility to SCN. In the second experiment, the seed treatment of *B. firmus* and fluopyram with and without *B. firmus* were tested and they did not inhibit the SCN in two kidney bean cultivars consistently, however, treatment effects were observed in some treatments. The data like the reduction of juveniles, increase of

male adults can be a reference for the researchers on evaluating the efficacy of the two seed treatments. This study offers important information to dry bean breeders, including a description of a method for screening for resistant cultivars.

4.2 Research Limitations

The SCN inoculation used in both experiment were HG type 5.7. The treatment effects and the cultivar susceptibility were unknown with other HG types. Poromarto and Nelson (2009) tested the susceptibility of the four cultivars of black and kidney bean to HG type 0. The use of indicator lines suggests that resistant soybean cultivars with different resistant genes have susceptibility to the specific SCN HG type. Dry bean cultivars potentially have similar responses to SCN. Also, the experiments were conducted in controlled environment for 30 d. The seed treatments may need a longer time to be effective. The efficacy of biological and chemical seed treatments may need to be applied over an extended period, since the *B. firmus* and *P. nishizawae* do not just cause immediate lethal effects, but weaken the population by inhibiting egg development and affect adult females. The treatment effects in the field can be affected by unpredictable environmental changes in temperature, moisture and soil components. Only three dry bean cultivars of two market class were tested in the experiment, which does not represent the diversity available in the dry bean crop.

The initial inoculation of SCN was 4000 eggs per plant. The seed treatment's efficacy with other levels of initial inoculation is unknown. For instance, the study of Beeman and Tylka (2018) found that in the controlled environment fluopyram was not effective in reducing cyst number when inoculated with 4,000 eggs 100 cm^{-3} but was

effective when inoculated 1,000 eggs 100 cm^{-3} in soybean. Niblack et al. (2008) showed that the egg population of 2,700 eggs 100 cm^{-3} is high enough to reduce soybean yield. Poromarto et al. (2010) tested the effects of SCN on the growth of different dry bean market class with SCN inoculation at 0, 5,000, or 10,000 eggs 100 cm^{-3}. In the study of Poromarto et al. (2010), there was greater increase in SCN population at lower egg densities than the high egg densities. The effects of seed treatment on the population increase were unknown. In soybean studies, female index is a common index used to measuring the susceptibility of a cultivar. So far, there is no published standard to evaluate the susceptibility of dry bean cultivars to SCN. Therefore, the number of cysts, juveniles, eggs, and adult males of treated plants can only be compared to their inoculated control.

4.3 Future Research

The rate response of *B. firmus* + fluopyram was consistent in the first study only. Therefore, more repetitions of experiment two can help to prove the hypothesis that there is a rate response to this seed treatment combination. Since the first study offered consistent results of the efficacy of seed treatments, it may be reasonable to conduct further field tests on the efficacy of *B. firmus* + fluopyram, even though the field studies of Zhang (2016) were not able to measure an effect. Treatment effects are influenced by temperature and moisture, which can vary with each field season. The susceptibility of other dry bean market classes to SCN should be tested under both controlled and field conditions. Several dry bean cultivars from market classes should be tested. Currently, there is no information about the density development mortality of SCN in dry

bean. Seed treatment efficacy and SCN survival rate at various inoculation levels (e.g. 1000, 2000, 6,000, and 8,000 eggs 100 g^{-1} soil) can also offer further information to growers and researchers. Experiment two provided the first evidence of seed treatments effects on the number of SCN at different life stages. Further evidence is required but future tests could determine the seed treatment effects on SCN mortality, hatching rate, and root penetration to offer more information to understand the biology of SCN to various seed treatments.

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