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For the degree of Master of Science

Is approved by the final examining committee:

Ricky E Foster

Virginia R Ferris

Stephen Weller

To the best of my knowledge and as understood by the student in the *Thesis/Dissertation Agreement. Publication Delay, and Certification/Disclaimer (Graduate School Form 32)*, this thesis/dissertation adheres to the provisions of Purdue University's "Policy on Integrity in Research" and the use of copyrighted material.

Approved by Major Professor(s): Ricky E Foster and Virginia R Ferris

Approved by: Stephen Yaninek 06/18/2014

Head of the Department Graduate Program Date

MANAGEMENT OF ROOT KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) IN INDIANA SOYBEANS

A Thesis

Submitted to the Faculty

of

Purdue University

by

David Edgardo Perla Martinez

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

August 2014

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West Lafayette, Indiana

To God, because everything is possible with God's will and man's determination.

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First I want to thank God because without Him and his many blessings I would not be here at Purdue and about to finalize this journey. A journey that even though has been full of challenges, it has also been filled with accomplishments and joy. Challenge is what drives me and my studies have presented me with that, a challenge so worthwhile trying.

I want to thank my committee members Dr. Ricky Foster, Dr. Virginia Ferris, Dr. Stephen Weller, for all their constant support, advise, feedback, and willingness to help while I completed my studies at Purdue. A special and much well deserved thank you to Dr. Jamal Faghihi whose continuous help and advice have made me grow as a nematologist and as a person. He has shown me that in nematology as in all sciences and disciplines there is always something new to learn, there is always something to amaze us. Thanks too to Min Chen for her valuable help in the laboratory. Dr. Ferris I do not know what I would have done without your valuable feedback and advice. You are not just an adviser but a mentor. The one who has helped me grow academically and professionally. I am really blessed to have you in my committee.

To my family I want to tell them thank you too for their support and encouragement.

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To the Angel who will always be with me, watching over me and guiding me. You will always remind me of God's plans for me.

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TABLE OF CONTENTS

Page

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

ABSTRACT

Perla, David E. M.S., Purdue University, August, 2014. Management of Root Knot Nematode (*Meloidogyne incognita*) in Indiana Soybeans. Major Professors: Ricky Foster and Virginia Ferris.

The aim of this project was to evaluate different strategies for management of Root Knot Nematode (RKN) on soybean and tomato in Indiana. Seed treatments were evaluated under field and greenhouse conditions, but no effect on RKN populations was observed. Soybean lines evaluated for resistance to RKN under greenhouse conditions showed that six lines may be resistant to RKN. Four different commercial mustard cover crops were evaluated for their bio-fumigant impact on RKN populations in the production of tomato. *Euruca sativa, Cv*. Nemat was a poor host of RKN. A positive impact on the vigor of the tomato plants followed the incorporation of the cover crops, suggested an increase of soil nutrition with the incorporation of the green manure.

INTRODUCTION

Root Knot Nematodes (RKN) belong to a relatively small but polyphagus and highly adapted genus of plant parasitic nematodes (*Meloidogyne*). RKN are taxonomically classified in the Order: Rhabditida; Sub-order: Tylenchina; Superfamily: Tylenchoidea; Family: Meloidogynidae; Genus: Melodogyne; and Species: e.g., *M. javanica, M. arenaria, M. hapla* and *M. incognita* (De Ley & Blaxter, 2002). These nematodes are worldwide in distribution and parasitize most plant species. RKN disrupt the plant physiology, and can drastically reduce plant yield and quality. Species of RKN are pests of high economic importance (Karssen & Moes, 2006). Over 90 species of *Meloidogyne* have been identified; however, four major species are recognized as problems especially for the production of vegetables. These are *M. javanica, M. arenaria, M. hapla* and *M. incognita* (Sikora & Fernandez, 2005).

RKN infestations reduce plant vigor, resulting in yield losses, and even death of the plants. The effects of RKN can vary according to the cultivar, season, soil conditions, and cultural practices.

Usually a direct diagnosis of RKN is possible because of the swollen plant root symptoms they manifest (Olsen, 2011). Several techniques have been developed for

identification of RKN and their species. Microscopic observation of the main characteristics, including body shape, and stylet length and shape, is probably the technique most often used (Hunt & Handoo, 2009). Other techniques used to identify RKN are type of perineal pattern and a variety of molecular techniques (for more details refer to Hunt & Handoo, 2009; Blok & Power, 2009; and Eisenback, 1985).

RKN are sedentary endo-parasitic nematodes with a migratory phase. After entering the root, nematodes migrate through the root tissue until they find and establish themselves in the phloem, where they induce physiological and morphological changes in the cells until these become specialized feeding sites (Karssen & Moens, 2006).

RKN lay their eggs at the posterior part of their body in a gelatinous mass to keep them together and to protect them from environmental damage. These egg masses are visible on the surface of galls and they stick to the root gall surface. The hatching process begins once the environmental conditions are suitable for nematode success and it is triggered by the diffusates released from the plant roots of susceptible hosts (Moens et al, 2009).

Some species of RKN are particularly adapted to specific conditions, including temperature, precipitation, texture and PH of the soil. Of the four best known species of RKN named previously, *M. hapla* is best adapted to cold weather, in contrast with the other three species that are more adapted to warm conditions. As for precipitation, M. incognita is most adapted to areas that receive over 1500 mm of precipitation a year; in contrast with *M. javanica* that is more adapted to more arid areas (Sasser et al, 1982).

Food searching by RKN juveniles is a complex process during which nematodes respond to compounds in the root zones (Moes & Perry, 2009). Living roots normally release large amounts of root exudates. These exudates are primarily carbon-based compounds that the plants produce during photosynthesis, as well as non-carbon compounds, inorganic compounds, water, and electrons. The amount of root exudates varies according to the variety of the plant, age, and stress factors (Uren cited by Bertin et al., 2003). These compounds encourage nematode hatching and enable the nematodes to orient toward the roots (Rovira, 1969; Bilgrami & Gaugler, 2004).

Nematodes combine two main responses to find a food source. First, all plant parasitic nematodes employ an exploratory behavior, during which they move randomly until they detect chemical gradients from roots. Once they identify their food source, they display a chemotactic response during which their movement follows the host cues or chemical gradient derived from the plant roots until they reach the source. During this process their uncoordinated movements become coordinated and they identify the food using their mechanoreceptors or tactile mechanisms (Bilgrami et al, 2004). The efficiency of nematodes' chemosensory responses depends on the composition and concentration of the attractants (kairomones), but other factors such as distance to host, temperature, and starvation of nematodes can affect the chemosensory response as well (Huettel, 1986; Pervez & Bilgrami, 2000).

The RKN have four different molts from the moment the female lays its eggs until juveniles become adults (J1, J2, J3, and J4,). The second stage juveniles penetrate behind the root tip in the elongation zone. After root penetration, intracellular movement is

displayed. This migration is performed by breaking down the cell walls with enzymes produced in their sub-ventral glands including cellulases, pectate-lyases, xylanase, and polygalacturonase (Perry & Moes, 2009).

Migration of Root Knot Nematodes through the plant cells continues until the nematodes become established in the perivascular cells. Then migration stops and the sedentary process begins with the induction of giant cells, for which the nematodes use proteins and enzymes from the sub-ventral glands. These proteins interrupt the normal differentiation of the cell wall (Jones & Goto, 2011). As a result, four or six cells become one giant cell or gall, which is the feeding site where the nematode establishes itself for the rest of its life (Jones & Goto, 2011). During this process the last three molts (J3, J4 and adult) rapidly occur within 4-6 days (Moens et al, 2009).

RKN use different mechanisms that let them survive adverse conditions with relatively minimal harm to their populations. Some of these mechanisms are dormancy, quiescence, and diapause (Evans & Perry, 2009). Although survival mechanisms are displayed in all of the stages of the life cycle, the eggs are the main stage of survival because the gelatinous matrix that covers the eggs is the first line of defense of RKN against negative environmental conditions. This matrix has low oxygen levels and protects the egg from desiccation. Eggs could remain viable 1-2 years, depending on the environmental conditions (Evans & Perry, 2009; Van Gundy, 1985; Sasser & Carter, 1985). In some other cases the nematodes could hatch only when the environmental conditions will permit the success of the population. In other cases, the mechanisms slow down the RKN metabolism to permit the RKN to overcome negative environmental conditions.

Sometimes RKN are able to change the sex ratio, increasing the number of males within a population. Males, which do not feed, are the mobile form of adult RKN, and this allows them to move out of unsuitable conditions (McSorley, 2003).

Root-Knot Nematodes (*Meloidogyne spp*.) are the most important plant parasitic nematodes in the world, considering the economic losses and the wide range of hosts that these nematodes utilize. RKN may be managed via biological control by bacteria, fungi, and predaceous nematodes, cultural practices such as soil amendments and rotational cover crops, host plant resistance, and synthetic pesticides (Sikora & Fernandez, 2005). Certain bacteria have negative effects on plant parasitic nematodes. A strain of *Bacillus firmus* reduced RKN in the production of greenhouse cucumber (Giannakou et al, 2003). *B. firmus* is registered as a nematode suppressor with low toxicity for handlers (Bacchus, 2008). Some commercial products with *B. firmus* as an active ingredient have been recommended for management of RKN, and have been used as seed treatments. An example of such seed treatments is Poncho®/VOTiVO®, a commercial seed treatment that has two components. Poncho® is a neonicotinoid insecticide targeting those insects that might cause severe damage during early stages of the plant growth. VOTiVO® is a strain of *B. firmus* that targets plant parasitic nematodes.

Use of resistance mechanisms is one of the most efficient methods for management of RKN. These mechanisms can vary from those in plants selected for toxins they release that affect directly the nematodes' performance to the use of plants that do not respond to signals sent by nematodes, and therefore do not establish giant cell feeding sites (Trudgill, 1991). Thies et al (1998) reported a reduction of RKN juveniles in a particular cultivar of

peppers, and recommended the use of such resistant cultivars as an effective alternative method for RKN suppression. The search for RKN resistance is ongoing for many crops species, including soybeans.

Cover crops can reduce the initial RKN population density and may serve as bio-fumigants (Barker & Koenning, 1998). Because of the wide range of hosts for RKN, suitable cover crops are hard to find. Cover crops that release toxins or secondary metabolites that negatively affect RKN populations have been used (Whitehead, 1998). These secondary metabolites (allelochemicals) or other products defined as plant metabolites may be released into the environment through volatilization, exudation from roots, leaching from plants or from decomposition of plant residues. Such compounds vary in their effect on nematodes. They may be anthelminthic (stunning or killing the nematodes) or nematostatic (affecting the movement of nematodes).

Some plants used as cover crops may reduce nematode populations actively, in contrast to others that are simply non-hosts for nematodes (Rodriguez-Kabana & Canullo, 1992). The action of allelopathic compounds as bio-pesticides may suppress nematodes by affecting their behavior. One effect of such compounds may be to alter the nematodes' chemotaxic response, one of the important cues used by nematodes to locate hosts (Kokalis-Burelle & Rodriguez-Kabana, 2006; Huettel, 1986). Marigold (*Taggetes spp*.) is one of the first plants reported with such effects on nematode populations. Several reports recommended use of *Taggetes spp* as a cover crop (Kokalis et al., 2006) because marigold produces nematotoxic compounds (e.g., Alpha tierthienyl). According to Chitwood (2002) and Ferraz and Grassi de Freitas (2004) after these chemicals are

delivered in the soil, they require peroxidase as an activator to release singlet oxygen into the soil to kill the nematodes by the oxidation of amino acid, proteins, and fatty acids resulting in damage of nematode membranes. Another possible way that cover crops interact with nematode populations includes a species of endo-root bacteria which interacts in a non-effective way with the plant, but produces bacterial endophytes that suppress nematode populations (Sturz & Kimpinski 2004).

RKN are obligatory parasites, and therefore, use of non-host cultivars for a considerable period of time may reduce drastically the population of RKN. As was motioned above, nematode hatching can be influenced by the environmental conditions. A combination of suitable environmental conditions that encourage RKN hatching with a presence of a non host crop may have a severe impact on RKN populations. Use of non-host crop rotation in combination with a fallow year may reduce the RKN population enough for production of a susceptible annual crop. However, the wide range of hosts permits RKN to use weeds as alternative hosts for survival (Perry & Ploeg, 2010; Sikora & Fernandes, 2005).

We report on experiments to evaluate the use of different strategies to develop an efficient RKN management program. These include the use of seed treatments, RKN resistant soybean lines, and cover crops as possible alternatives.

MATERIALS AND METHODS

Methods used repeatedly during the study are described below. These include methods to obtain inoculum, to weight fresh roots, and to calculate root gall indices, number of nematodes per gram of root, and nematodes per 100 cc of soil. Statistical methods are also described.

To obtain sufficient inoculum for the experiments, tomato (*Lycopersicum esculentum Mill*) plants (cv. Rutgers) were planted in infested soil to increase the nematode populations. The inoculum was then extracted from the tomato roots using the method of Hussey and Barker (1973), which requires the dissolution of the egg masses of Meloidogyne in a solution of 0.53% NaOCl (Clorox ®). The infested roots of the plants were washed under a gentle stream of water to remove all soil particles. The root system was submerged into a solution of 0.53% of NaOCl, and vigorously shaken manually for 5 minutes after which the solution was placed on a 500-mesh sieve. The number of eggs per ml was counted under a microscope to ensure an inoculum density of 5000 eggs per plant.

The evaluation of root gall indices was made using the root evaluation chart in Bridge and Page, 1980 (Figure 1). The roots were rinsed with tap water, placed on paper towels to eliminate excess water, and observed using magnifying goggles to determine the extent of gall formation on the roots (Figure 2).

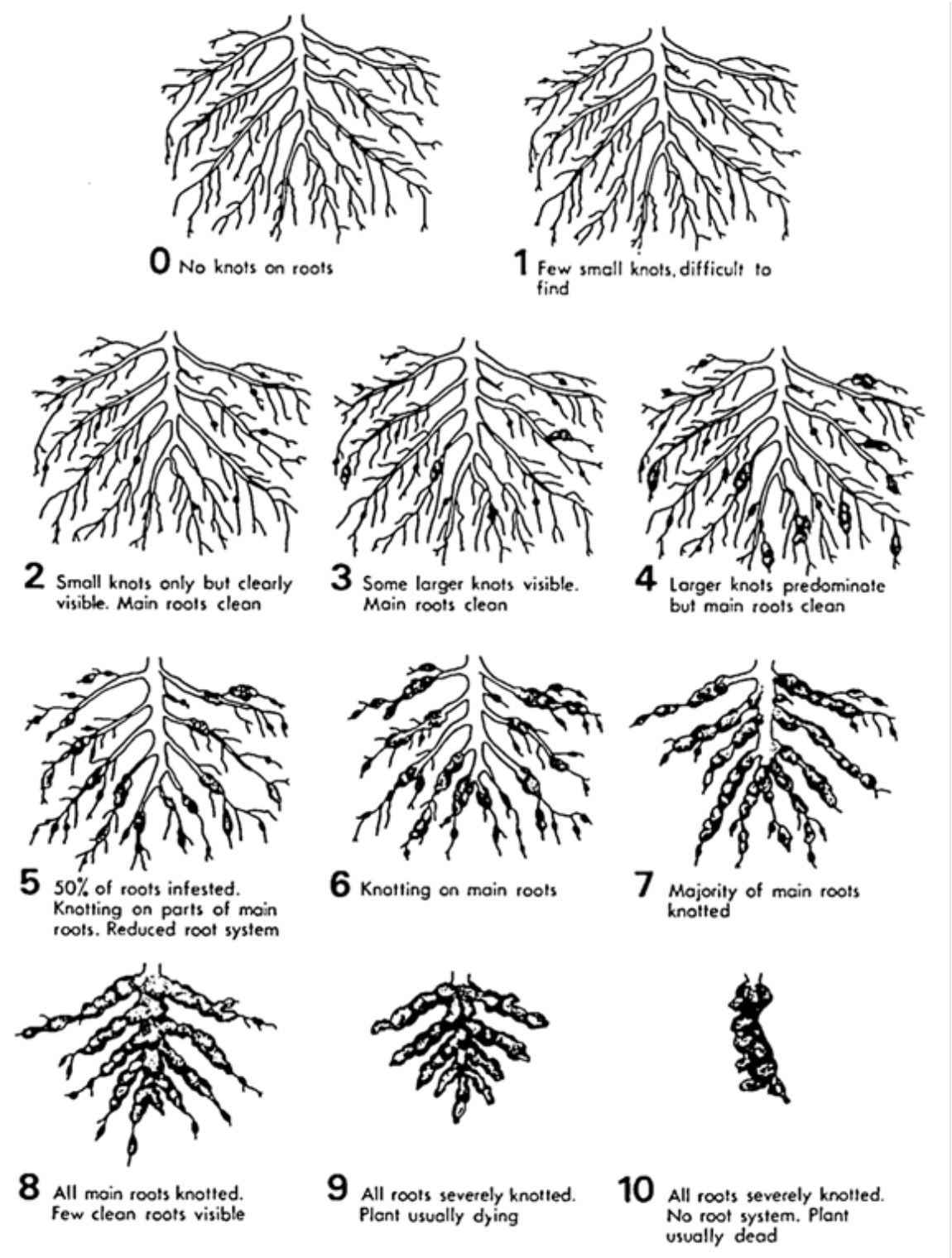

Figure 1. Root Evaluation Chart, Bridge and Page (1980)

Figure 2. Magnifying Goggles to Obtain Root Gall Indices

Fresh weight was obtained using a digital scale (Ohaus Galaxy 4000D). The roots were rinsed and placed over paper towels for 10 minutes to eliminate excess water, and then weighed.

To obtain the number of nematodes per gram of root, nematodes were extracted from the washed roots using the maceration centrifugal flotation technique (Coolen & D'Herde, 1972; Coolen, 1979). Roots were chopped into 2 mm length pieces and placed in a blender (Black & Decker 10-Speed Die-Cast Blender). The blender was run for 10 seconds (5 seconds at slow speed and 5 at high speed), the mixture poured into nested 10-mesh over 500-mesh sieves, and the sample collected from the 500-mesh sieve. This sample was placed in centrifuge tubes, centrifuged for 3 minutes at 7000 rpm, the water was discarded from the tubes, and the tubes refilled with a mixture of sugar and water

(1:1 ratio) and centrifuged once again for 30 seconds. The sample was placed in a 500-mesh sieve and rinsed using tap water until the sugar was completely rinsed away and the nematodes collected. The sample was placed in a beaker and filled to 20 ml. From this 20 ml, a sample of 2 ml was observed under the microscope for identification and counting of the nematodes present. After counting, the number of nematodes/gram of root was determined based on the root weight previously obtained.

To obtain the number of root knot nematodes per 100 cc of soil**,** soil samples were analyzed using the sugar flotation method (Jenkins, 1964). For this, 100 cc of soil were placed in a bucket, and tap water added. After stirring, the suspension was decanted through two sieves with different pore apertures (sizes10-mesh, and 500- mesh) and the sample was collected from the 500-mesh sieve. This sample was placed in 50 cc centrifuge tubes and centrifuged for 3 minutes at 7000 rpm. Water was discarded from the tubes and the tubes were filled with a sugar water solution (1:1 ratio), and then centrifuged again for 30 seconds. After centrifugation, each sample was placed in a 500-mesh sieve and rinsed using tap water until the sugar was completely eliminated. Each sample was placed in a beaker and filled to 20 ml. From this 20 ml sample, 2 ml were observed under a microscope for identification of the nematodes present. The data were analyzed using the statistical software Infostat (2008). As part of the analyses, data were tested for the normality assumption in order to establish the normality of the data. All data were tested with the Saphiro-Wilks test. When it was found that the data did not have a normal distribution, the data were transformed using the formula log(X+10),

Analysis of variance (ANOVA), and Duncan's multiple range tests were run to determine differences between treatments.

CHAPTER 1. SEED TREATMENTS TESTED FOR MANAGEMENT OF ROOT KNOT NEMATODES (*MELOIDOGYNE INCOGNITA*) IN SOYBEAN

In this study different combinations of soybean (*Glycine max* (L.) Merr.) seed treatments were used with and without VOTiVO® as the main component. The experiments were carried out under field and greenhouse conditions with either natural field populations of nematodes or populations of nematodes increased in the greenhouse.

1.1 Material and Methods

1.1.1 Greenhouse Experiment with Naturally Infested Soil

Five treatments were evaluated (Table 1) in a completely randomized block design with four replications and each experimental unit was completed by four cups per treatment per block for 16 cups were tested per treatment (Figure 3). Infested soil was collected from a field located near Vincennes, IN, with a history of severe RKN damage. This soil also contained Soybean Cyst Nematode (SCN).

Table 1. Treatments evaluated in the Greenhouse in Poncho® VOTiVO® Experiments

#	Treatment	**Active Ingredient**	**Type of Pesticide**	**Dosage of a.i (mg/seed)**
1	EverGold Xtend	Penflufen	Fungicide	0.015
	Allegiance FL	Metalaxyl	Fungicide	0.027
2	EverGold Xtend	Penflufen	Fungicide	0.015
	Allegiance FL	Metalaxyl	Fungicide	0.027
	Gaucho 600 FS	Imidacloprid	Insecticide	62.5 g/100 Kg seed
3	EverGold Xtend	Penflufen	Fungicide	0.015
	Allegiance FL	Metalaxyl	Fungicide	0.027
	Poncho®-VOTiVO®	Clothianidin-*Bacillus firmus*	Insecticide-Nematicide	0.13
4	EverGold Xtend	Penflufen	Fungicide	0.015
	Allegiance FL	Metalaxyl	Fungicide	0.027
	Gaucho 600 FS	Imidacloprid	Insecticide	78.1 g/Kg seed
	Poncho®-VOTiVO®	Clothianidin-*Bacillus firmus*	Insecticide-Nematicide	0.13
5	Untreated			

Three treated soybean seeds were planted in each of 80 6-ounce foam cups with 150 cc of soil (Figure 3). One week after germination, the most vigorous plant was selected in each cup and the other two were pulled out. All treatments were handled under the same conditions of irrigation (once a day), weed control (hand removal), and temperature.

Figure 3. Arrangement of Poncho®/VOTiVO® Experiment in the Greenhouse

Three months later data were taken for three variables (root gall indices, root knot nematodes per 100 cc of soil, and number of Soybean Cyst Nematodes per plant). Root gall indices and number of root knot nematodes per 100 cc of soil were obtained as it was explained previously.

To determine the number of cyst nematodes per plant, nematodes were extracted using a modification of the method proposed by Krusberg et al (1994) whereby the roots were rinsed in a plastic container using tap water, the water stirred, and the suspension decanted through a nested 20-mesh sieve over a 60-mesh sieve. Nematodes were collected from the 60-mesh sieve and placed in 10 x 10 cm counting dish, which was placed on a stereoscope for cyst identification and counting.

These greenhouse experiments were repeated three times: Experiment 1 (June-September 2012), Experiment 2 (February to May 2013), and Experiment 3 (March- June 2013) the three experiments were evaluated under greenhouse conditions. Environmental conditions within the greenhouse were regulated to ensure uniform conditions. During summer, the temperature in the greenhouse was regulated by fans and cooling wet pads.

1.2 Field Experiment

The five previously described seed treatments (Table 1) were evaluated for their effect on the two plant parasitic nematodes under field conditions. In addition to RKN, Soybean Cyst Nmeatodes (SCN) was also present. The plots were set up in a field located near Vincennes, IN. The soil in this area is classified as Bloomfield loamy fine sand soil with 2-10 percent slope, and is used for the production of corn, soybean, and other vegetables. The experiment was arranged in a completely randomized block design with four replications. Each experimental unit measured 100 feet long and 15 feet wide where six rows of soybean were planted and data were taken from the two middle rows (Figure 4). Soil samples were taken at planting time to determine the initial nematode populations, and again just before harvest to measure the final nematode populations at the end of the season. The samples were taken using a cone sampler that collects about 1738 cc of soil. Nematodes were extracted from soil using the sugar flotation method (Jenkins, 1964) and the plants were evaluated using the rating chart of root infestation given by Bridge and Page, 1980 (Figure 1).

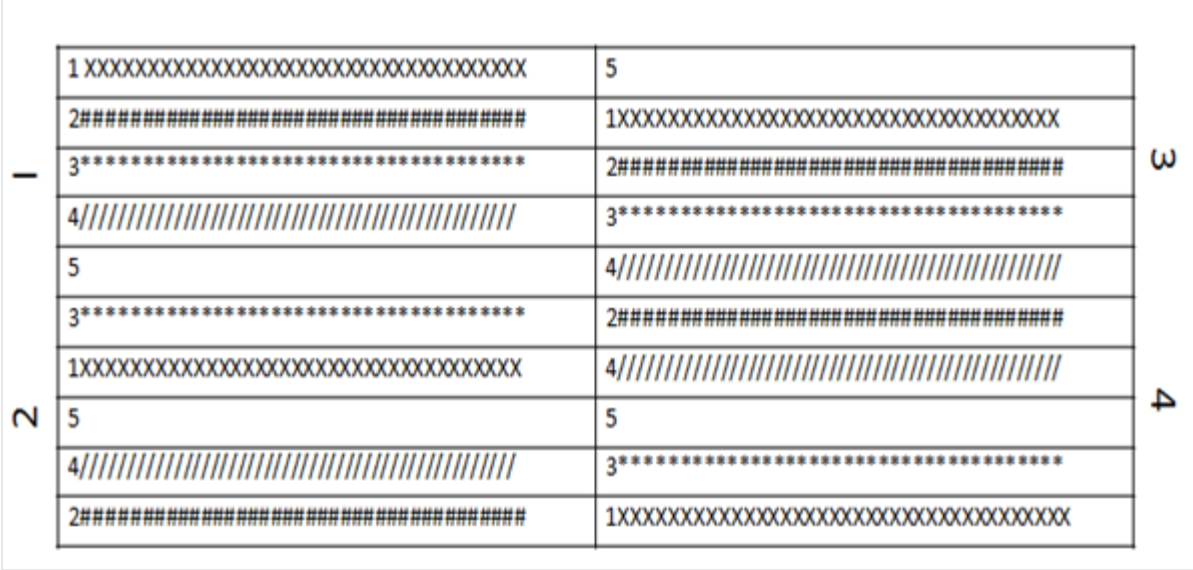

Figure 4. Field Experiment Design (Treatment numbers correspond to the treatments listed on Table 1)

1.3 Greenhouse Experiment with Greenhouse Raised Populations

The greenhouse experiment with artificial populations (RKN populations increased as it was explained previously) used a completely randomized block design with four replications and four experimental units per treatment per block (Figure 5). A tray was filled with sterile soil and three soybean seeds were placed in each tray cell. A week later, the most vigorous plant was selected and the other two were eliminated. All treatments were maintained under the same conditions of irrigation, weed control and temperature. Two weeks after germination, each plant was inoculated with 5,000 J2.

Figure 5. Randomized Block Design Treatment

Three months later data for fresh weight, root galling, and nematodes per gram of root were taken as described previously.

1.4 Results

1.4.1 Natural Populations Tested in the Greenhouse (Experiment 1)

No statistical differences were found among treatments for RKN in Experiment 1 (Table 2). The treatments with either Gaucho (2) or Poncho®/VOTiVO®(3) had significantly fewer SCN than the untreated control (5) and the fungicide only control (1), but surprisingly, the treatment with both Gaucho and Poncho®/VOTiVO®(4) did not.

In Experiment 2, no statistical differences were found among treatments in the root gall index, RKN/100 cc, or SCN numbers (Table 3).

Experiment 3 had much lower populations of RKN than did Experiments 1 and 2, and no statistical differences were observed for SCN numbers (Table 4).

As indicated in Table 5, no statistical differences were found for variables evaluated in the field study.

For the evaluation of seed treatments using the greenhouse-raised population of RKN, Treatment 2 (EverGold, Xtend, Allegiance FL and Gaucho 600 FS) had statistically significant higher fresh weights than did Treatment 4 or the untreated control. No statistical differences were observed for the RKN per gram of root (Table 6).

Table 2. Experiment 1. Greenhouse Test of Seed Treatment Combinations for Control of Root Knot Nematode and Soybean Cyst Nematode in Soil Taken from the Field (June-September 2012)

No.	Treatment	Root Gall Index	Rkn/100 cc of soil	SCN
1	**EverGold, Xtend Allegiance FL**	1.18 a	3.25 a	173.5 a
2	**EverGold, Xtend Allegiance FL Gaucho 600 FS**	1.81 a	8.56 a	101.16 c
3	**EverGold, Xtend Allegiance FL Poncho®-VOTiVO®**	1.43 a	3.5 a	107.5 bc
4	**EverGold, Xtend Allegiance FL Gaucho 600 FS Poncho®-VOTiVO®**	1.37 a	0.25 a	117.42 abc
5	**Untreated**	1.25 a	0.18 a	162.5 ab
	Cv	3.62	12.96	5.05
	R^2	0.1	0.4	0.7

Means followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test. Root Gall Index based on 0-10 scale (Bridge & Page, 1980). RKN/100 cc of soil = number of juveniles /100 cc of soil. SCN=Number of cysts found / plant.

Table 3. Table 3. Experiment 2. Greenhouse Test of Seed Treatment Combinations for Control Of Root Knot Nematode and Soybean Cyst Nematode in Soil Taken From the Field, February to May 2013, West Lafayette, IN

No	Treatment	Root Gall Index	Rkn/100 cc of soil	SCN
1	**EverGold, Xtend Allegiance FL**	0.42 a	0.76 a	104 a
2	**EverGold, Xtend Allegiance FL Gaucho 600 FS**	0.41 a	0.63 a	84.8 a
3	**EverGold, Xtend Allegiance FL Poncho®- VOTiVO®**	0.51 a	0.44 a	92.7 a
4	**EverGold, Xtend Allegiance FL Gaucho 600 FS Poncho®-VOTiVO®**	0.56 a	0.45 a	80.67 a
5	**Untreated**	0.55 a	0.10 a	72.5 a
	CV	1.59	2.28	3.62
	R^2	0.46	0.31	0.65

Means followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test. Root Gall Index based on 0-10 scale (Bridge & Page, 1980). RKN/100 cc of soil = number of juveniles /100 cc of soil. SCN = Number of cysts found / plant.

Table 4. Experiment 3. Greenhouse Test of Seed Treatment Combinations for Control of Root Knot Nematode and Soybean Cyst Nematode in Soil Taken From the Field, March- June 2013, West Lafayette, IN

No.	Treatment	Root Gall Index	Rkn/100 cc soil	SCN
1	**EverGold, Xtend Allegiance FL**	0 a	0 b	94.5 a
2	**EverGold, Xtend Allegiance FL Gaucho 600 FS**	0 a	0 b	104.43 a
3	**EverGold, Xtend Allegiance FL Poncho®- VOTiVO®**	0 a	0 b	83.62 a
4	**EverGold, Xtend Allegiance FL Gaucho 600 FS Poncho®-VOTiVO®**	0.2 a	0.375 a	110.43 a
5	**Untreated**	0 a	0 b	71.78 a
	CV	0.98	1.27	15.86
	R^2	0.2	0.3	0.2

Means followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test. Root Gall Index based on 0-10 scale (Bridge and Page, 1980). RKN/100 cc of soil = number of juveniles /100 cc of soil. SCN= Number of cysts found / plant.

Table 5. Field Test of Seed Treatment Combinations for Control of Root Knot Nematode and Soybean Cyst Nematode, June- September 2012, Vincennes, IN

No.	Treatment	Root Gall Index	Rkn/100cc Soil	SCN
1	**EverGold, Xtend Allegiance FL**	3.63 a	8.62 a	59.75 a
2	**EverGold, Xtend Allegiance FL Gaucho 600 FS**	2.75 a	4.75 a	55.12 a
3	**EverGold, Xtend Allegiance FL Poncho®- VOTiVO®**	2.94 a	4.86 a	52.34 a
4	**EverGold, Xtend Allegiance FL Gaucho 600 FS Poncho®-VOTiVO®**	3.34 a	6.69 a	148.63 a
5	**Untreated**	3.34 a	4.67 a	49.61 a
	CV	3.48	6.60	5.43
	R^2	0.73	0.58	0.44

Means followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test. Root Gall Index based on 0-10 scale (Bridge and Page, 1980). RKN/100 cc of soil = number of juveniles /100 cc of soil. SCN = Number of cyst found / plant.

Table 6. Greenhouse Test of Seed Treatment Combination for Control of Root Knot Nematode Using Sterile Soil Infested Artificially, May-July 2013, West Lafayette, IN

No	Treatment	Fresh Weight (g)	Root Gall Index	Rkn/g root
1	**EverGold, Xtend Allegiance FL**	3.4 ab	4.25 a	5.3 a
2	**EverGold, Xtend Allegiance FL Gaucho 600 FS**	5.28 a	5.25 a	4.12 a
	EverGold, Xtend Allegiance FL Poncho®-VOTiVO®	3.57 ab	4.5 a	7.6 a
4	**EverGold Xtend Allegiance FL Gaucho 600 FS Poncho®-VOTiVO®**	2.71 b	4.25 a	5.4 a
5	**Untreated**	2.56 b	3.75 a	7.4 a
	CV	3.79	4.79	8.56
	R^2	0.55	0.45	0.46

Means followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test. Fresh weigh t= Weight in grams of the roots after been cleaned. Root Gall Index based on 0-10 scale (Bridge & Page, 1980). RKN/g of root = number of root knot nematodes found per gram of fresh weight of the root.

1.5 Discussion

Although seed treatments could be efficient alternatives for the management of root knot nematodes, none of the seed treatments in these experiments showed consistent differences in nematode control. The greenhouse experiments using natural populations

had low initial populations of root knot nematodes as did the field plots. Weather conditions were hot and dry during the year (2012) and this may have contributed to the population levels of the nematodes in the greenhouse experiment using field soil and in the field.

According to Dickerson et al (2000), levels of root knot nematodes ranking from 1-99 nematodes per 100 cc of sandy soil are not a direct threat to soybean production. Additionally, no seed treatments were effective in reducing the number of soybean cyst nematodes, although the numbers of SCN nematodes exceeded in most cases the critical level recommended for commercial soybean production, which is 70 nematodes per 100 cc of sandy soil in South Carolina (Dickerson et al, 2000).

The data collected showed that these seed treatments did not reduce these plant parasitic nematodes in soybean. Even in those treatments where Poncho®/VOTiVO® (a bio nematicide) was used, no evidence of an effect on plant parasitic nematodes could be identified. Our results concur with Da Silva and Tylka (2013) who reported little or no effect of seed treatments on the number of plant parasitic nematodes present in the production of corn.

Datta et al (1982) in a discussion of beneficial effect of bacteria on cash crops suggested that some strains of *Bacillus firmus* enhance the plant's phosphorous uptake improving the plant health. However, we could not evaluate that theory, because yield data were not collected. In summary, we could not demonstrate beneficial results from the use of Poncho®/VOTiVO® as a seed treatment for control of root knot nematodes in soybean.

CHAPTER 2. GREENHOUSE TESTS OF RESISTANCE SOYBEAN LINES

Greenhouse screening for Root Knot Nematodes is the most useful way to evaluate lines of soybean with potential resistance to RKN. The process permits evaluating the lines with known nematodes populations and avoids variability that may be present under field conditions (Saichuk et al, 1976). The objective of these experiments was to screen a group of soybean lines thought to have resistance to RKN.

2.1 Materials and Methods

Lines evaluated were obtained from the soybean breeding program at Southern Illinois University at Carbondale (SIUC) (Table 7) and were arranged in a completely randomized design with four replications where a nursery tray cell acted as an experimental unit (Figure 6). Therefore, at the end of the experiment, four experimental units were evaluated for each treatment. The lines were compared with Williams-82, a line thought to have a high degree of susceptibility to RKN (Davis, 20013).

Table 7. Soybean Lines Tested for Resistance to Root Knot Nematodes

Treatments	Genotype	Treatments	Genotype	Treatments	Genotype
1	LS05-3229	10	LS05-6442	19	LS05-0107
2	LS05-3110	11	LS05-6513	20	LS05-0202
3	LS05-0242	12	LS05-6521	21	LS05-0216
4	LS05-2610	13	LS05-4007	22	LS05-0220
5	LS04-27138	14	LS05-2202	23	LS90-1920
6	LS04--30080	15	LS05-2658	24	LS97-1610
7	LS05-8130	16	LS05-2705	25	Williams-82
8	LS04-49077	17	LS03-4294		
9	LS05-3915	18	LS05-1065		

The seeds were planted in sterilized soil and inoculated 10 days after germination with 5000 eggs of RKN. All the plants were grown under the same conditions of water, temperature, fertilization, and weed control. The inoculum was obtained from RKN-infested tomato plants grown in the greenhouse. The inoculum was prepared using the Hussey and Barker method (1973).

Two soybean seeds were placed in each cell of a nursery tray (Figure 6), and a week after germination, the most vigorous plant was selected and the other seedling was removed by hand. Three months after planting, the lines were evaluated for resistance to RKN based on the variables: Gall index, nematodes per gram of roots, and reproductive factor (R=Pf/pi).To determine host suitability (Table 9), gall indices were obtained using the rating chart of root infestation in Bridge and Page, 1980 (Figure 1), and the number of nematodes/gram of root was determined. The roots were rinsed and placed over paper towels for 10 minutes to eliminate excess water, and then weighed. Nematodes were

extracted from the washed root using the maceration centrifugal flotation technique (Coolen & D'Herde, 1972; Coolen, 1979). After the number of nematodes from the plants was counted, the number of nematodes/gram of root was determined.

Figure 6. Nursery Tray Used for Testing Soybean Lines

The reproductive factor (R) was calculated using the formula R= Pf/Pi, where Pf is the final population of Root Knot Nematodes collected from the roots, and Pi is the initial population or the number of nematodes inoculated at the beginning (5000 nematodes per plant).

To determine the host suitability, the lines were categorized as proposed by Canto-Saenz (1983) (Table 8), using the following criteria. If the gall index was lower than or equal to 2 and the R factor lower than or equal to 1, the line was considered to be resistant. If the gall index was between 2-4, and the R factor lower than or equal to 1, the line was considered to be tolerant or moderately resistant. If the gall index was higher than or equal to 4, and the R factor lower than or equal to 1, the line was considered to be

susceptible; and if the gall index was higher than 4 and the R factor higher than 1, the line was considered to be very susceptible . The data were analyzed using the statistical software Infostat (2008).

Table 8. Host Suitability Chart modified (Canto-Saenz, 1983)

	Damage to plants	
Nematode Reproduction on Host	**Significant**	**Insignificant**
Efficient	Very susceptible	Tolerant
Inefficient	Susceptible	Resistant

Table 9. Modified Quantitative Chart of Host Suitability, based on Canto-Saenz's, (1983)

Gall Index	**R= PI/PF**	**Degree of resistance**
≤ 2	≤1	Resistant
2-4	≤1	Tolerant
≥4	≤1	Susceptible
>4	>1	Very susceptible

Gall Index= Root Gall Index based on 0-10 scale (Bridge & Page, 1980). R = Reproductive Factor. Pf= Final Population. Pi= Initial Population. Resistance = response of the soybean to RKN attack based on Sasser et al (1984). Degree of resistance based on modified Canto-Saenz (1983).

2.2 Results

In Experiment 1, 17 soybean lines were screened to determine their resistance to RKN, using Williams-82 as the susceptible control. The lowest root weight was found for the control (Table 10). Six of the lines evaluated had statistically higher fresh weights than

did the control. These lines were LS05-3110, LS05-8130, LS04-40077, LS05-3915, LS03-4294, and LS05-0216. However, the weight differences were independent of gall indices or resistance.

With respect to gall indices, statistical differences were found. LS05-6513, LS05-6521, and LS05-2658 had the lowest gall indices, whereas LS05-8130 and LS04-49077 had the highest gall indices. Additionally, LS05-6513 and LS05-6521 had the fewest nematodes per gram of root. The susceptible control, Williams-82, had the most RKN per gram of root. Statistical differences were also found for the reproductive factor (R). The R value for LS05-3229 was statistically higher than for the rest of the lines (Table 10). Based on the values shown on the rating chart (Table 9) six lines could be categorized as resistant, LS05-6513, LS05-6521, LS05-2658, LS05-1065, LS05-0216, and LS97-1610. The rest of the lines evaluated were categorized as very susceptible and susceptible.

Table 10. Experiment 1. Greenhouse Test of Soybean Lines for Root Knot Nematode Resistance (May- August, 2013)

Lines	**FW(g)**		**Gall Index**		**Nem/g-root**		**R=PF/PI**	
LS05-3229	9.3	ab	5.8	cdef	423.4	bc	1.59	b
LS05-3110	14.2	a	5.3	bcdef	46.8	abc	0.20	a
LS05-2610	10.5	ab	6	def	171.9	bc	0.24	a
LS05-8130	13.2	a	7.3	ef	215.8	bc	0.64	a
LS04-49077	14.4	a	7.8	f	146.9	abc	0.75	a
LS05-3915	15.2	a	4	abcde	51	abc	0.23	a
LS05-6513	4.5	ab	1	a	3.6	a	0.02	a
LS05-6521	7.0	ab	1	a	2.5	a	0.01	a
LS05-4007	12.0	ab	3.8	abcd	125.0	abc	0.33	a

Table 10 (Continued). Experiment 1. Greenhouse Test of Soybean Lines for Root Knot Nematode Resistance (May- August, 2013)

LS05-2202	5.4	ab	3.8	abcd	65.9	abc	0.22	a
LS05-2658	8.9	ab	1.3	a	129.3	abc	0.13	a
LS05-2705	7.5	ab	2.3	abc	74.8	abc	0.11	a
LS03-4294	14.2	a	4	abcde	43.6	ab	0.18	a
LS05-1065	9.6	ab	2	ab	25.5	ab	0.06	a
LS05-0107	8.4	ab	4.3	abcde	76.1	abc	0.18	a
LS05-0216	6.8	a	1.8	ab	19.2	ab	0.04	a
LS97-1610	5.9	ab	2	ab	102.0	ab	0.03	a
Williams - 82	1.8	b	3.8	abcd	724.51	c	0.44	a

Means followed by the same letter are not significantly different at ($p<0.05$) according to Duncan's multiple range test. FW= Fresh Weight of the root. Gall Index = Root gall based on 0-10 scale (Bridge and Page, 1980). Nemas/g = Number of Root Knot Nematodes found per gram of fresh weight of the root. R = Reproductive Factor; Pf= Final Population, Pi= Initial Population, Resistance = response of the soybean to RKN attack (R = Resistance, T = Tolerant, Vs = Very Susceptible, and S = Susceptible) based on Sasser et al (1984).

2.3 Experiment 2, Greenhouse Test of Resistant Soybean Lines

In Experiment 2, data were collected from 24 lines of soybeans with potential resistance to RKN plus a susceptible control (William-82). Statistical differences were found for the fresh weight of the roots. LS03-4294 had a statistically higher fresh weight, as compared with LS05-0220, the line with the lowest fresh weight; whereas LS04-27138 had the highest RKN per gram of root. Statistical differences were found for the reproductive factor (R=FP/IP). Line LS05-3915 had the lowest R factor, whereas LS04-49077 had the highest R (Table 11). The gall indices and the R value obtained were too low to permit establishing resistance categories for these lines.

In Experiment 3, 14 lines of soybeans were screened for their potential resistance to RKN using William-82 as the susceptible control. Statistical differences were found for the fresh weight of the roots. LS05-2610 had the highest fresh weight, as compared with LS05-0107 and LS05-0220. No statistical differences were found for the Root Galling Index, Nematodes per gram of root, or R. Due to the low Gall Indices and low R values obtained from the lines evaluated no resistance categories could be established for the lines (Table 12).

Table 11. Experiment 2. Greenhouse Test of Soybean Lines for Root Knot Nematode Resistance (August -November, 2013)

Lines	**FW (gr)**		**Gall Index**		**RKN/g root**		**R=FP/IP**	
LS05-3229	0.9	bc	0.8	a	1.4	ab	0.05	ab
LS05-3110	0.4	b	0.3	a	2.1	ab	0.01	ab
LS05-0242	1.0	bcd	0.3	a	3.5	ab	0.03	ab
LS05-2610	0.9	bcd	0	a	2.0	a	0.06	abc
LS04-27138	1.4	cde	1	a	6.7	b	0.03	ab
LS04-30080	0.2	b	0	a	0.5	ab	0.01	ab
LS05-8130	0.9	bcd	1	a	3.4	ab	0.001	b
LS04-49077	1.6	de	0.5	a	1.3	ab	0.23	c
LS05-3915	0.6	abc	0.3	a	5.1	ab	0.01	a
LS05-6442	0.9	abcd	0.7	a	4.7	ab	0.03	ab
LS05-6513	0.7	abc	0.5	a	2.0	ab	0.07	bc

Table 11 (Continued). Experiment 2. Greenhouse Test of Soybean Lines for Root Knot Nematode Resistance (August -November, 2013)

LS05-6521	0.5	ab	0.3	a	3.9	ab	0.01	ab
LS05-4007	0.8	abc	0.5	a	2.0	ab	0.04	ab
LS05-2202	0.6	ab	0.25	a	5.3	ab	0.04	ab
LS05-2658	0.8	abc	0.25	a	7.0	ab	0.02	ab
LS05-2705	0.9	bcd	1	a	4.3	ab	0.01	ab
LS03-4294	2.1	e	1	a	10.0	ab	0.03	ab
LS05-1065	0.4	ab	0	a	2.9	ab	0.02	ab
LS05-0107	0.5	ab	0.5	a	5.0	ab	0.01	ab
LS05-0202	0.3	ab	0.5	a	1.6	ab	0.03	ab
LS05-0216	0.5	ab	0.3	a	1.4	ab	0.02	ab
LS90-1920	0.4	ab	0.3	a	1.0	ab	0.01	ab
LS97-1610	0.5	ab	0.3	a	4.0	ab	0.01	ab
LS05-0220	0.03	a	1	a	0.5	abc	0.001	ab
Williams -82	1.0	bcd	0.5	a	3.2	ab	0.03	ab

Means followed by the same letter are not significantly different at ($p<0.05$) according to Duncan's multiple range test. FW= Fresh Weight of the root. Gall Index = Root gall based on 0-10 scale (Bridge and Page, 1980). Nemas/g = Number of Root Knot Nematodes found per gram of fresh weight of the root. R = Reproductive Factor; Pf= Final Population, Pi= Initial Population.

Table 12. Experiment 3. Greenhouse Test of Soybean Lines for Root Knot Nematode Resistance (November, 2013-February, 2014)

Lines	FW (g)	Root Gall Index	Nemas/g	R=pf/pi
LS05-3229	0.1 ab	0 a	8.7 a	0.002 a
LS05-3110	0.2 ab	0.3 a	1.6 a	0.01 a
LS05-0242	0.2 ab	0.3 a	5.2 a	0.001 a
LS05-2610	0.4 a	1 a	4.0 a	0.003 a
LS05-8130	0.2 ab	0 a	21 a	0.002 a
LS05-6521	0.1 ab	0.3 a	4.3 a	0.001 a
LS05-2202	0.2 ab	0.3 a	9.0 a	0.002 a
LS05-2658	0.2 ab	0.3 a	3.6 a	0.002 a
LS05-2705	0.2 ab	0.3 a	8.0 a	0.001 a
LS05-0107	0.1 b	0 a	18.1 a	0.003 a
LS05-0202	0.1 ab	0.3 a	7.4 a	0.002 a
LS05-0220	0.1 b	0 a	1 a	0.0002 a
LS90-1920	0.4 ab	0 a	1 a	0.001 a
LS97-1610	0.2 ab	0.5 a	16.5 a	0.002 a
Williams-82	0.1 ab	0.3 a	6.6 a	0.003 a

Means followed by the same letter are not significantly different at ($p<0.05$) according to Duncan's multiple range test. FW= Fresh Weight of the root. Gall Index = Root gall based on 0-10 scale (Bridge and Page, 1980). Nemas/g = Number of Root Knot Nematodes found per gram of fresh weight of the root. R = Reproductive Factor; Pf= Final Population, Pi= Initial Population.

2.4 Discussion

The use of resistant varieties is the most economic and efficient method for the control of RKN. Some of the lines evaluated in these experiments showed resistance to RKN in the first experiment. Based on the results obtained from Experiment 1, evaluated in summer months, six lines indicated strong potential to be considered resistant. These were LS05-6513, LS05-6521, LS05-2658, LS05-1065, LS05-0216, and LS97-1610. Allen et al

(2005) and Wright (2011) reported that LS97-1610 has been released as resistant to SCN and Sudden Death Syndrome (SDS). Both LS05-6513 and LS05-6521 have LS97-1610 in their backgrounds, which could provide the resistance to RKN. For LS05-0216, which showed resistance in Experiment 1, no published evidence of previous RKN tests of this line could be found.

The differences shown in Experiment 1 were not evident in Experiments 2 or 3 (Table 11, Table 12). The experiments were carried out at different times of the year, which cause differences in soil temperatures. Such differences might have affected the tests. Soil temperatures have been shown to directly influence the degree of susceptibility of the host to Root Knot Nematode attack (Carter, 1982). Dusembery (1988) also demonstrated that *Meloidogyne incognita*, our RKN species, changes its behavior as it is exposed to small temperature changes. Based on the first experiment, the lines evaluated under greenhouse conditions that might be categorized as resistant are LS05-6513, LS05-6521, LS05-2658, LS05-1065, LS05-0216, and LS97-1610.

CHAPTER 3. TEST OF THE USE OF COVER CROPS FOR MANAGEMENT OF ROOT KNOT NEMATODES

Chitwood, 2002 reported that mustard plants produce chemical compounds that affect nematode development. The main compounds present in mustard plants are glucosinolates and isothiocyanates, which have been related to inhibition of nematode egg hatch. Therefore, such plants have been used in some pest management programs as bio-fumigants and for crop rotation. The objective of these experiments was to evaluate the efficiency of four commercial mustard cover crops (selected for their potential in the management of plant parasitic nematodes) under greenhouse conditions for the management of RKN in the production of tomato.

3.1 Materials and Methods

Two experiments were carried out in the greenhouse: Experiment 1 - evaluation of the usefulness of each mustard cover crop species as measured by the final biomass of the cover crop plants and the nematodes per gram of root; and Experiment 2 - evaluation of tomato plant development after incorporation of the cover crops into the soil. Treatments were set up in a completely randomized block design with four replications, where every 2000 cm^2 tray was an experimental unit.

Data of both experiments were analyzed and tested for the normality assumption using the statistical software INFOSTAT (2008).

Susceptible tomatoes (var. Rutgers) were planted in trays of sterilized soil. A week after germination, each tomato plant was inoculated with 5000 RKN eggs and infective stage larvae (J2). After 120 days the inoculum was obtained from these infested tomato plants. The inoculum was extracted using the Hussey and Barker (1973) method.

The infested tomato plants were grown until they showed signs of wilting, leaves yellowing and gall development, after which the plant roots were chopped and incorporated into the soil; then all the infested soil was homogenized in order to obtain uniform distribution of the nematode population throughout the trays.

Experiment 1 (August-November 2013). Four commercial mustard cover crops were planted in the RKN-infested soil to determine the susceptibility of each cover crops species planted as compared with a susceptible control, melon (Table 13). These mustard cover crops were planted at a seeding rate of 8 to 9 Kg/ Ha as commercially recommended. The plants were fertilized with nitrogen at a rate of 134 to 145 kg/Ha and sulfur applied at a rate of 28 to 33 Kg/Ha.

Table 13. Cover Crops Treatments Tested

.	Cover crops	
Treatment	Common name	Scientific name
1	Nemat & Caliente 199	*Brassica hirta/Euruca sativa* (blend)
2	Indian Mustard	*Brassica juncea Cv.* Pacific Gold
3	Rocket salad	*Euruca sativa. Cv.* Nemat
4	white mustard	*Brassica hirta Cv.* Caliente 199
5	Melon (Control)	*Cucumis melo* var. Athena

The mustard cover crops were grown in the greenhouse for three months until flowering. Once they started to flower, the mustard plants were trimmed (Figure 7) the amount of biomass determined, expressed as Kg/Ha as well as the number of nematodes per gram of cover crop root, and the number of nematodes per 100 cc of soil.

Figure 7. Trimming of Mustard Cover Crops Before Incorporation

To obtain the production of biomass (Kg/ha), the above ground tissue was separated from the roots and weighed using a digital scale. These data were obtained from 2000 cm^2 (area of each tray used) and were extrapolated to Hectares.

To obtain the number of nematodes per gram of root, the roots of the cover crops were weighed and nematodes were extracted from the roots using maceration-centrifugal flotation technique (Coolen & D'Herde, 1972; Coolen, 1979).

To obtain the number of nematodes per 100 cc of soil, the soil samples were processed using the sugar flotation method (Jenkins, 1964). After evaluation, cover crop plants were chopped and incorporated into the soil and left there for two weeks (Figure 8).

Figure 8. Process of Incorporation of the Cover Crops

Experiment 2 (December 2013-March 2014). A tomato variety susceptible to RKN was planted to measure the bio-fumigation impact of the cover crops on the population of RKN and also the physiological development of the tomatoes. Data collected were root weight, weight of the tissue above the ground, plant height, gall indices, number of nematodes per gram of root, and number of nematodes per 100 cc of soil. Tomato plants were trimmed to measure the root weight and the weight of the tissue above the ground. After trimming, the height of the tissue above the ground was measured (in centimeters).

Data of gall indices, nematodes per gram of roots and nematodes per 100 cc of soil were taken by methods previously described (root rating chart in Bridge & Page, 1980; Coolen & D'Herde, 1972; Coolen, 1979; Jenkins, 1964).

3.2 Results

3.2.1 Experiment 1, Evaluation of the Susceptibility of the Mustard Cover Crops to RKN

All of the mustard crops had a higher biomass for incorporation into the soil than did the melon control (Table 14). Of the mustard species tested, the Nemat-Caliente 199 combination produced the most biomass for incorporation into the soil, 10 kg/ha of organic matter more than Caliente 199 alone, which produced the least amount of organic matter.

The cover crops were all infested by RKN. However, all of them had statistically lower number of RKN per gram of root than the susceptible control. No statistical differences were found for the number of nematodes per 100 cc of soil.

Table 14. Susceptibility of the Treatments to Root Knot Nematodes

Treatments	Organic matter Kg/Ha		Nem/gr of Root		Nem/100 CC	
Nemat & Caliente 199	176.4	a	144.0	b	120	a
Indian Mustard	173.1	a	106.9	ab	135	a
Nemat	177.7	a	15.7	a	15	a
Caliente 199	163.4	a	114.3	ab	60	a
Melon (Control)	77.4	b	1271.9	c	285	a

Organic matter Kg/Ha = Amount of tissue above ground produced by the treatments per Hectare. Nem/gr of root = Numbers of nematodes in the root per gram root fresh weigh. Nem/100 CC= Number of nematodes per 100 cc of soil.

3.2.2 Experiment 2, Evaluation of Tomatoes Development after Cover Crops Incorporation

Data were obtained from tomato plants planted in each greenhouse tray after cover crops were incorporated. No statistical differences were found for plant root weight (Table 15). Incorporation of the Nemat & Caliente 199 combination resulted in higher fresh weight of above ground tissue than the melon control. The tomato plants in the Nemat treated soils were statistically taller than the control plants. No statistical differences in incidence and severity of RKN were observed (Table 16).

Table 15. Tomato Plants' Vigor Planted after Cover Crops Incorporation

Treatments	Root weight(g)	weight of tissue above ground (g)	Plant height (cm)
Nemat & Caliente 199	5.25 a	25.60 a	38.4 ab
Indian Mustard	5.58 a	18.18 ab	35.1 ab
Nemat	4.82 a	23.48 ab	45.5 a
Caliente 199	3.39 a	19.04 ab	35.3 ab
Melon (Control)	3.08 a	13.64 b	31.2 b

Root weight= Root weight of the tomato plants. Weight of tissue above ground = Weight of the stem plus leaves and flowers but not fruits of the tomato plants. Plant height= Height in inches without roots.

Table 16. Incidence and Severity of Root Knot Nematodes Attack on Tomatoes Planted after Cover Crops Incorporation

Treatments	Gall Index	Nem/ g of Root	Soil/100 cc
Nemat & Caliente 199	3.2 a	340.65 a	135 a
Indian Mustard	2.15 a	57.30 a	75 a
Nemat	1.95 a	31.19 a	187.5 a
Caliente 199	2.9375 a	90.34 a	172.5 a
Melon (Control)	4.4 a	148.51 a	240 a

Gall Index= Root Gall Index based on 0-10 scale (Bridge and Page, 1980). Nem/gr of root = Numbers of nematodes in the root per gram root fresh weigh. Nem/100 CC= Number of nematodes per 100 cc of soil.

3.3 Discussion

Based on the data, the mustard cover crops tested appeared to support RKN populations, although at statistically lower numbers than for the control, melon. Root Knot Nematode can use these mustards as a survival host even though they are not a preferred host. These results concur with results obtained by Liébanas and Castillo (2004) and Kokalis-Burelle, et al (2013) where they explained that some crucifers used as cover crops are susceptible to RKN infestation. Therefore, based on the results of this experiment, most of the mustard cover crops evaluated cannot be used in a management program for RKN as a non-host except for Nemat (*Euruca sativa*), the species which proved to be the poorest RKN host. These results concur with Curto et al (2005) who recommended Nemat (*Euruca sativa*) as non-host for management of RKN.

According to Barker and Koenning (1998), cover crops can also be used in a RKN management program as suppressors of nematode reproduction. Specifically, use of cover crops as green manures because of the bio-fumigation properties offered by plants in the Cruciferae, including mustards, that release lethal amounts of glucosinolates into the soil during plant tissue decomposition. Our experiments did not provide support for the use of mustard cover crop amendments for control of RKN. However, the number of RKN per 100 cc of soil before and after cover crop incorporation showed that Indian mustard (*Brassica juncea* cv. Pacific Gold) had a negative effect on RKN populations (tables 14, table16 and graph 9). The other mustard cover crops did not have a negative effect on the number of nematodes collected per 100 cc of soil. The negative impact of Indian mustard on RKN might be explained by the results of Antonious et al (2009), who found that

Indian mustard is one of the highest glucosinolates releasers of the mustards. They also found that the cultivar Pacific Gold is one of the best cultivars to use as green manure because of the high amount of biomass it produces and its above average production of glucosinolates.

The absence of a negative effect on the number of RKN per 100 cc of soil was not reflected in tomato plants vigor. In fact, there was a positive relationship between the amount of biomass of the cover crops and the response of the tomato plants planted after incorporation of the cover crops. Those cover crops with higher amounts of biomass incorporated resulted in taller tomato plants as compared with the control. Additionally, there was a relationship between the amount of biomass incorporated and the gall indices of the tomato plants planted after cover crop incorporation. The use of cover crops with the higher amount of biomass incorporated resulted in tomato plants with slightly lower gall indices. This phenomenon is explained by Omirou et al (2011) and Friberg (2009) who found that by incorporation of the biomass, mustard cover crops increase the microbiology activity of the soil, including ammonia oxidizing bacteria; and as a result of the biomass decomposed, different volatiles are released that can have a negative effect over the nematode population, and also enrich the fertility by increasing the nitrogen available for plants.

In summary, mustard cover crops used as part of a RKN management program might have some negative effect on RKN populations. Although, based on our results, it is a weak negative effect. Nevertheless, use of cover crops as green manure affects the plant growth positively and encourages the crop vigor. Therefore, based on the results of these

experiments, use of mustard as cover crops is not very useful for the management of RKN, but they could benefit crop health.

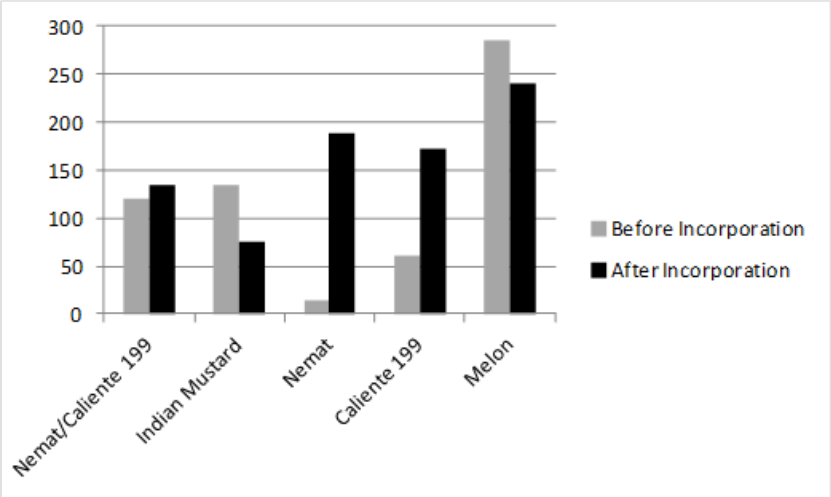

Figure 9. Graph of the Number of Nematodes per 100 cc of Soil Before and After Cover Crops Incorporation

3.4 Conclusions

Based on the results obtained, there is no evidence that supports the effectiveness of VOTiVO® seed treatments in a management program for RKN. Promising results were obtained from the tests of resistant lines of soybeans and six lines were identified that could be considered to have resistance. None of the mustard cover crops could be identified as a non-host of root knot nematode, but Nemat appeared to be a poor host of RKN, and therefore this species could be used in an integrated RKN management program. It was also found that the incorporation of the cover crops biomass resulted in increasing the vigor of the tomato crop.

Evaluation of these experiments under field conditions could be useful, especially for testing the resistant soybean lines and for evaluation of the use of cover crops to mitigate RKN populations.

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