EXAMINATION OF CHEMICAL, BIOLOGICAL, AND CULTURAL CONTROL MEASURES OF ROOT-KNOT NEMATODES IN LIMA BEANS

by

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PREFACE

General Background

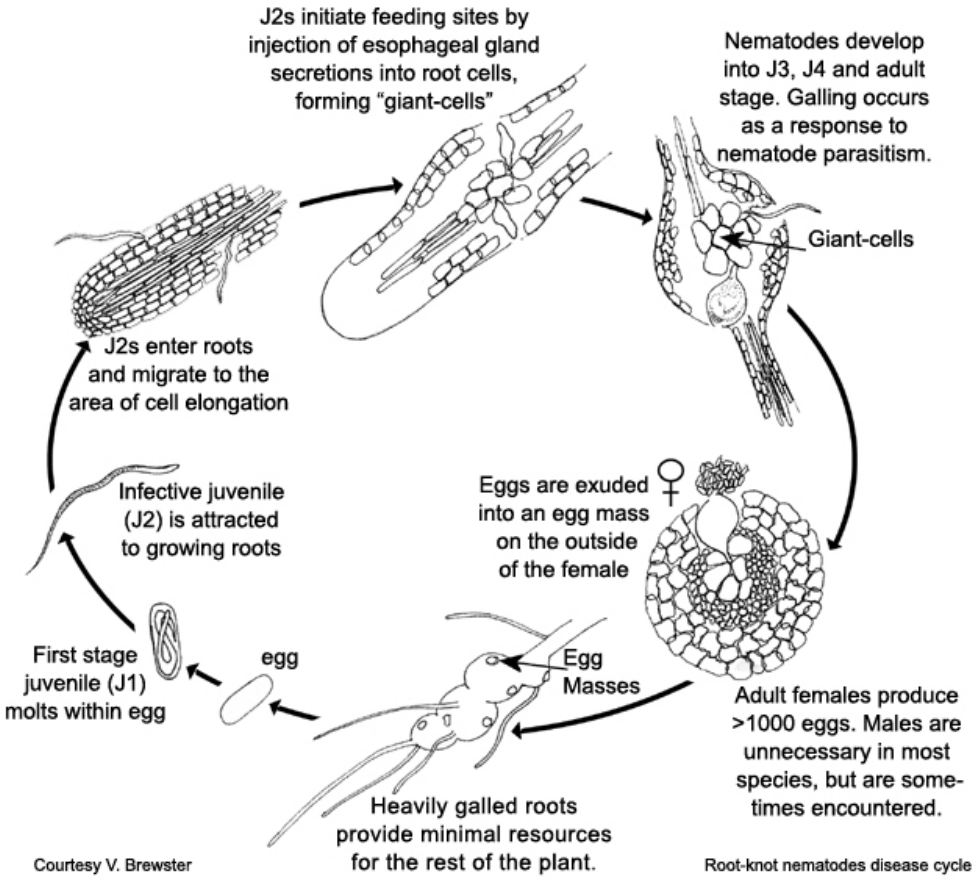
Nematodes are the most numerous metazoans on earth, accounting for approximately four out of every five multicellular animals (Bongers and Ferris 1999). The majority of species are free-living and feed on fungi or bacteria, but nematodes are more widely noted for their negative effects on animal and plant life. The estimated number of nematode species on earth ranges from 100,000-1,000,000, with only a small fraction (~15,000) of the species described (Bongers and Bongers 1998; Blumenthal and Davis 2004). Of this fraction, over 4,100 species of plant-parasitic nematode (PPN) species have been described (Decraemer and Hunt 2006). This very diverse and evolutionary successful group of nematodes is responsible for an estimated $78-125 billion of crop losses annually (Sasser and Freckman 1987) through decreased yields and the reduction in the quality of agricultural products, via secondary infection of the plant tissues by other plant pathogens (Moens et al. 2009).

Root knot nematodes (*Meloidogyne spp*; RKN) have a host range of over 1,700 plant species and are responsible for approximately 5% of crop losses worldwide (Barker 1998; McCarter 2008). The four major species of RKN causing the majority of crop loss are *M. arenaria, M. hapla, M. javanica* and the Southern root-knot nematode, *M. incognita* (Chitwood 1949; Eisenback et al. 1981; Taylor and Sasser 1978).

Lifecycle of Root Knot Nematode

Like all nematodes, RKN exist in six life stages: eggs, first-, second-, third-, and fourth-stage juveniles (J1, J2, J3, J4) and adults. The lifecycle starts with swollen, adult RKN females; which are fully or partially embedded in the surface of a host root

(see lifecycle image below) (Taylor and Sasser 1978). Females produce eggs within a gelatinous matrix, which holds the eggs together and forms an egg mass on the root surface and occasionally inside the tissue of galled roots (Taylor and Sasser 1978; Moens et al. 2009; Karssen et al. 2013). The gelatinous matrix is composed of glycoproteins and serves to protect the egg mass from environmental extremes and predation (Moens et al. 2009). The J1 and J2 are formed within the egg, and egg hatch is spontaneous, mostly influenced by temperature, aeration, and moisture conditions in the soil (Perry and Clarke 1982). After leaving the egg, the J2s move through the soil and are attracted to the root tips of their host plants (in the zone of elongation). After penetrating the root cell walls with their mouth stylet, the nematode releases cell-wall degrading enzymes and other effectors with roles in pathogenicity (Abad et al. 2003). Once inside the host root, the J2 migrate intercellularly and eventually reach the root vascular cylinder where they create specialized feeding sites called giant cells. (Hussey 1989; Williamson and Hussey 1996). The now sedentary RKN feeds in cells adjacent to its head, with a new feeding tube formed every time a nematode removes its stylet and reinserts it into a host cell to resume feeding (Rumpenhorst 1984). While feeding, the RKN continues its life-cycle and molts three more times (J2→J3→J4→Adult) in order to reach the reproductive mature adult stage (Abad et al. 2009). The characteristic root gall formation is due to hyperplasia, an increase in tissue, which is dependent on nematode species and host plant. Galls on lima bean roots can be quite large (see picture below). A single adult female produces 500-1000 eggs on average (De Guiran and Ritter 1979). In general, the entire life cycle usually takes 3-6 weeks from the time of initial infection to release of another generation of eggs, depending on environmental conditions (Williamson and Hussey 1996).



Root-knot nematodes disease cycle (from Brewster 2003).

Severe galling on lima bean roots (photo by Jake Jones).

Management Options for Plant Parasitic Nematodes

The rapid reproduction rate of RKN makes it an aggressive and economically damaging plant parasite. Management of RKN can include genetic host resistance, as well as biological, cultural, and chemical control measures. Genetic host resistance involves the use of plants with specific genes or sets of genes that protect the plant from nematode attack. Biological control is the utilization of living organisms, such as bacteria of fungi that reduce infection or populations of nematodes. Cultural controls include anything that makes the environment less favorable to the nematode, such as solarization, flooding and rotation to non-hosts crops. Chemical controls involve the use of synthetic and naturally produced chemicals to reduce RKN populations. Often integration of practices is needed to reduce nematode populations to the point that they do not cause significant economic damage to a susceptible crop.

Lima Bean Production in Delaware

The state of Delaware is a low-lying peninsula in the Mid-Atlantic Region. In Delaware the soil maps are dominated by sandy, sandy loam, and loamy sand soil types (with sand accounting for 50-100% of the particle composition in these soil types), covering approximately 43% of Kent County and over 87% of Sussex County, which make the soils ideal for RKN movement (USDA 1971; USDA 1974, Wallace 1968). With adequate drainage, Delaware soils are productive in growing grain crops, processing vegetables, and fresh market fruits and vegetables. Grain crops dominate the acreage of Delaware farmland but lima bean (*Phaseolus lunatus)* plays a key role in the agricultural industry in the state.

Delaware has more hectares of lima beans than any other state in the U.S.A., with a ten-year average of more than 5,261 hectares (13,000 acres) planted and harvested

annually. This generates an average value of $174 per hectare ($430 per acre) and a total value of production in excess of $5.65 million annually (DDA and NASS 2014). Green baby lima beans are often considered a cornerstone crop in Delaware vegetable production and serve as the backbone of the vegetable processing industry in the state and region (Kee et al. 2004). Lima bean accounts for 43% of total vegetable processing acreage in Delaware on average and 27% of the total value ($20.87 million seven-year average) produced from processing vegetables (DDA and NASS 2014). Lima bean yields must be protected in order to ensure the crop's survival in Delaware, along with the entire processing vegetable crop industry. RKN are known to be highly damaging to lima bean plant health and yield in the Delmarva Region and their management is key to maximizing grower profitability and production (Kee et al. 2004, McConnell 2016).

Research Objectives

The objectives of this thesis research were twofold. First, we sought to examine the efficacy of some new nematicides in a set of controlled, greenhouse and microplot studies, to ascertain their potential roles in RKN management and impact on lima bean production in Delaware. Second, we examined various cultural practices, including cover crops and soil amendments, for their potential to manage RKN.

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ABSTRACT

Southern root-knot nematodes (*Meloidogyne incognita*-RKN) are among the most destructive plant pathogens and are infectious on a range of crops, including lima bean (*Phaseolus lunatus*), a crop vital to the Mid-Atlantic Region vegetable industry. Control of root-knot nematode (RKN) cannot be limited to crop rotation or the fallowing of fields due to an extensive host range and survival characteristics that enable the pathogen to persist in the soil, even in non-ideal conditions. In relatively recent years, the most effective chemical nematode controls, such as methyl bromide, organophosphate and carbamate nematicides, were found to be harmful to the environment and to non-target soil biota, leading to a reduction or even prohibition of their usage, spurring a revival in research to control RKN.

In this research, the effects of new chemicals, biologicals, and cultural practices on RKN populations were examined. Chemical controls on RKN populations and lima bean plants were studied, in multiple greenhouse and microplot experiments. Emergence of lima bean seedlings was negatively affected by ethoprophos, but RKN galling and populations were significantly reduced, often as the best performing nematicide in the experiments. Two new nematicides, fluensulfone and fluopyram, reduced RKN galling compared to the untreated control. Spirotetramat, applied as a foliar treatment, performed equivalent to the control in every experiment (no significant effect). In addition, the use of biofumigant mustard and sorghum cover crops, which can reduce RKN levels by releasing compounds that react in the soil with an end-product resulting in natural fumigation, were investigated with findings that support the widespread use of ‘Dwarf Essex’ rapeseed in the region to control RKN. Cultural approaches also impacted RKN populations. Organic matter additions showed small but significant reductions in RKN galling with high rates of compost

and chicken manure. Winter survival rates of RKN on common cover crops in the region was similar for all crops tested.

Chapter 1

EVALUATION OF NEMATICIDES FOR RKN MANAGEMENT IN LIMA BEAN

Abstract

Southern root-knot nematodes (*Meloidogyne incognita;* RKN) significantly reduce lima bean (*Phaseolus lunatus*) yields. Chemical control options for RKN are limited. We evaluated the efficacy of new nematicidal products on RKN in lima bean experiments conducted in greenhouse (GH) and microplot (MP) settings. Treatments included fluensulfone at low and high labeled rates, fluopyram, spirotetramat, fluopyram +spirotetramat, oxamyl, ethoprophos, and an untreated RKN-infested control. GH treatments were arranged in a factorial design, with application of nematode eggs (0, 6,000, or 30,000 eggs pot^{-1}) crossed with nematicide treatment. MP treatments were arranged in a randomized complete block design and all plots were infested with RKN infected tomato root tissue and soil grown in the greenhouse. Root galling, RKN egg and juvenile numbers, and aboveground plant dry masses were determined and analyzed using Mixed Model ANOVA. In the GH studies, fluensulfone at both rates provided the greatest reduction in RKN galling compared to the untreated control, whereas spirotetramat treatments were not significantly different from the untreated control. In the MP 2 study, all treatments except spirotetramat significantly reduced RKN populations and had significantly greater yields relative to the untreated control. Oxamyl, ethoprophos, and fluensulfone (high and low rate) treatments had the highest yields in the microplot study. Based on these results, the

two new nematicides, fluensulfone and fluopyram, show good potential for managing RKN in lima beans.

Introduction

Root knot nematodes (*Meloidogyne* spp.) have a host range of over 1,700 plant species and are responsible for approximately 5% of crop losses worldwide (Barker 1998; McCarter 2008). *Meloidogyne spp.* are considered the most damaging plant-parasitic nematodes in the world, and include four major species: *M. arenaria, M. hapla, M. incognita,* and *M. javanica* (Jones et al. 2013, Moens et al. 2009). Southern root-knot nematode (RKN), *M. incognita* (Kofoid and White 1919), is commonly found in soils in the mid-Atlantic region and can cause extensive damage to crops via the formation of giant cells (galls), which impede nutrient and water uptake in the plant and consume plant nutrients, resulting in reduced plant growth, productivity, or even plant death (Karssen et al. 2013, Barker 1998).

The mid-Atlantic region is predisposed to successful RKN infections, as the predominate soil types have a large sand component, thereby facilitating RKN movement in the soil and infection of plant roots (Wallace 1968, UDSA NRCS). RKN are capable of severely damaging many vegetable crops, including lima beans (*Phaseolus lunatus).*

Delaware has more hectares of lima beans than any other state in the U.S., with a ten-year average of 5,261 hectares planted and harvested annually. Lima bean accounts for 43% of total vegetable processing hectares in Delaware on average and 27% of the total value produced from processing vegetables, thereby contributing approximately $5.65 million to state growers annually (DDA and NASS 2014). Consequently, lima bean yields must be protected in order to ensure the crop's continued production in Delaware and the mid-Atlantic region.

RKN on lima beans is of increasing importance and more effective management practices are needed (PMSP 2003). Chemical controls were for decades

an effective stand-alone tool in the control of RKN (Moens et al. 2009). Effective fumigants such as 1,2-dibromo-3-chloropropane (DBCP), ethylene dibromide (EDB), and methyl bromide, as well as non-fumigants including fenamiphos, carbofuran, and aldicarb, provided excellent control, but their environmental impacts, issues with human safety, and other concerns led to discontinuation (Johnson and Feldmesser 1987, Abdel-Rahman et al. 2008, Aspelin and Grube 1999, Giannakou et al. 2002, UNEP 1992, EPA 2008, EPA 2009, EPA 2012). Most non-fumigant nematicides fall into one of two classes: carbamates or organophosphates. These chemicals inhibit acetylcholinesterase which leads to nematode paralysis (Chitwood 2001, IRAC 2015). Both classes of chemicals are considered highly toxic to both humans and the environment and the majority of lack specificity in their toxicity (Gupta 2011). Phase-outs of synthetic nematicides have left a void in RKN control as the development of safer, non-fumigant chemical control options has not kept pace with the loss of the older nematicides (Nyczepir and Thomas 2009).

Because of the lack of available nematicides, and the high cost of using them, the main nematode control measures that growers utilize in the region are cultural controls, including crop rotation, avoidance of infected fields and biofumigation with rapeseed. The resulting RKN reduction from crop rotation and rapeseed is often transient and inconsistent (Everts et al. 2006). Recently, several new active ingredients have received EPA registration as nematicides. These include spirotetramat (2010), fluensulfone (2014) and fluopyram (2015) (Table 1.1).

Spirotetramat is a systemic insecticide labeled for use in certain tree, tropical fruits, vine and vegetable crops, including lima beans, with up to two foliar applications per growing season. There is evidence that spirotetramat may suppress

plant-parasitic nematode populations after foliar applications (McKenry et al. 2009, McKenry et al. 2010). Timing appears to be critical to the effectiveness of spirotetramat. Spirotetramat does not prevent the nematodes from invading roots, because the nematodes must ingest the translocated chemical for efficacy. Therefore, galls will still appear, but egg production, and therefore overall RKN populations, may be reduced (Perry et al. 2013). Fluensulfone is labeled for use in cucumbers, melons, squash, tomatoes, okra, eggplant, and peppers, and is marketed as a contact nematicide, but also has some systemic activity (Oka et al. 2011, Kearn et al. 2014, Oka et al. 2009). The efficacy of fluensulfone has been studied in recent years on vegetables such as cucurbits, carrots, and tomatoes, but not on lima bean crops (Oka et al. 2011, Morris et al. 2015, Morris et al. 2016). In a study by Oka et al. (2009), fluensulfone application reduced the fresh weights of tomato plants, albeit inconsistently, indicating a need for more research on its phytotoxicity among a variety of crops. Fluopyram is labeled as a nematicide seed treatment in soybean and for liquid application potatoes, cotton, and peanut. In addition, fluopyram is an effective fungicide and has been studied for its efficacy against RKN as well as its use in controlling Sudden Death Syndrome of soybean (Faske and Hurd 2015, Kandel et al. 2016). Fluopyram has been shown to cause some phytotoxicity in soybeans but its effects on lima beans are unknown (Kandel et al. 2016).

The objective of this research was to evaluate the efficacy of these new nematicides , as well as two older nematicides, for suppression of RKN in lima bean. Testing was done in greenhouse and microplot experiments.

Materials and Methods

Greenhouse Experiments

Two greenhouse trials (GH 1, GH 2) were conducted at the Fischer Greenhouses (531 South College Ave. Newark, DE) at the University of Delaware's main campus from November 6, 2015 through December 15, 2015 and from March 22, 2016 through April 28, 2016, respectively. A spatially balanced factorial design was used, consisting of three levels of RKN inoculum (0, 3,000, and 6,000 eggs pot^{-1}), and egg inoculum levels were crossed with each of seven chemical treatments and an untreated control (Kayani et al. 2016) (Table 1.1). Each experimental combination was replicated six times and the entire experiment was replicated twice (GH 1, GH 2), for a total of 144 experimental units. Experimental units were 10.2 cm square plastic pots filled ¾ full with screened, autoclaved Pepperbox loamy sand (70 to 80% sand), with organic matter (OM) of 1.0 and 1.1%, and pH of 5.2 and 4.8, respectively for GH 1 and GH 2 (USDA NRCS). The soil was acquired from the University of Delaware Carvel Research and Education Center in Georgetown, Delaware. Two untreated '242' Fordhook bush lima bean seeds were planted in each pot, and thinned to one seedling per pot 10 and 12 days after planting (DAP) for GH 1 and GH 2, respectively. Pots were arranged on a bench containing heat mats set at 27°C. Plants received supplemental lighting provided by high pressure sodium lamps set to maintain 12 hours of daylight when the outside light dropped below 400 Lux. The average temperature in the greenhouse was 22.8° C, with a low of 18.8° C and a high of 27.4° C for GH 1, while the average temperature was 23.2° C, with a low of 18.8° C and high of 32.3° C for GH 2. Pots were watered as needed.

For both greenhouse trials, RKN populations were increased on the susceptible tomato host *Lycopersicum esculentum* cv. Rutgers, grown in the greenhouse for 42 days (GH 1) and 60 days (GH 2) in a mixture of sand and field soil. RKN eggs were extracted with 0.5% NaOCl from infected roots and were adjusted with the aid of a hemocytometer to add concentrations of 6,000 and 30,000 eggs per pot (low and high rates). Next, 3 mL (GH 1) or 12 mL (GH 2) of the respective egg suspension was added into three holes, 4 cm deep, placed at equal distance around the circumference of each pot (Kayani et al. 2010).

Fluensulfone treatments were applied the same day as RKN egg inoculation, 7 days prior to lima bean planting, as recommended by the manufacturer. Fluopyram, oxamyl, and ethoprophos were all applied pre-plant on the day of planting. Spirotetramat was applied to appropriate treatments after sufficient growth of foliage to apply the foliar spray on 19 (GH 1) and 16 DAP (GH 2). See Table 1.1 for a complete list of chemical treatments, modes-of-action, rates, and application methods.

On 32 DAP (GH 1) and 30 DAP (GH 2) the experiments were harvested. Shoots were separated from the roots at the soil line and placed in paper bags before drying until constant drymass at 60^{o} C. Roots were rinsed in running tap water to remove soil and debris, and then stored in sealed plastic bags at 4.5^{o} C until galls were counted. RKN galls were stained red with 15 mg phloxine B/L of water and enumerated under a dissecting microscope (Dickson and Struble 1965). After counting, roots were placed in paper bags and dried as described above, to allow for calculations of egg masses per gram root dry weight. Dry weights (g) were recorded for the shoots and roots of each chemical treatment. The dry masses of the shoots and

roots were combined and used to calculate the carbon allocation and total biomass patterns for each chemical treatment.

All data were tested for normality using the Shapiro-Wilk Goodness of Fit test before analysis. Transformations for normality in both trials were performed on percent root weight data (Log transformed) and galls per root dry weight (g) (Log+1 transformed). A random effects mixed model was used with block as a random effect with treatment, and egg level combinations as fixed effects. Pots not receiving eggs (controls) were not included in assessments of treatments on RKN. However, controls were included in the analysis to test the effects of chemical treatments on overall plant growth and productivity. Means were separated using Student's Protected LSD (α=0.05). Preliminary analysis indicated a significant effect of experimental trial on data ($P<0.0001$) and studies were therefore analyzed separately (SAS Institute, Cary, N.C.).

Microplot Experiments

Microplot (MP) studies were conducted at the University of Delaware's Elbert N. and Ann V. Carvel Research and Education Center (16483 County Seat Hwy. Georgetown, DE 19947). The soil type in the microplot trials was a Pepperbox loamy sand (70 to 80% sand), with 1.0 and 0.8% OM, and pH of 4.2 and 4.8, respectively, for Microplot 1 trial (MP 1) and Microplot 2 trial (MP 2) (USDA NRCS). In 2014, a set of semi-permanent, 61 x 61 cm circular microplots made from High Density Polyethylene (HDPE) resin tree nursery pots with the bottoms removed were installed to a depth of 51 cm for MP 1. In 2015, a set of permanent, 61 x 122 cm circular microplots made from corrugated Polyethylene tile pipe were installed to a depth of 92 cm for MP 2. Microplots were spaced apart with aboveground lips to avoid cross-

contamination during both experiments. Experiments were arranged in random complete block designs, with 5 blocks and 3 and 7 chemical treatments plus untreated controls in 2014 and 2015, respectively (Table 1.1). Weeds were controlled mechanically and by hand for both trials. Microplots were fertilized with the recommended rates of 62 kg/ha nitrogen, 67 kg/ha phosphorus, and 112 kg/ha potassium. The microplots were irrigated by hand throughout the experiments to add the equivalent of 2.5 cm of rainfall per week.

RKN populations were increased on the susceptible tomato host *Lycopersicum esculentum* cv. Rutgers in the greenhouse. On July 9, 2014, each microplot in MP 1 was inoculated with a 2.72 kg mixture of infested tomato roots and soil. On July 22, 2015, each microplot in MP 2 was inoculated with a 4.80 kg mixture of infested tomato roots and soil, equivalent to 120,000 eggs and 3,450 second stage RKN juveniles (J2) per microplot (no population counts were taken in MP 1). The inoculum was chopped and mixed before application to each microplot and was immediately incorporated in the upper 30 cm of soil.

Nematicide treatments included: 1) fluopyram (0.220 L ai/ha [MP 1, MP 2]), 2) ethoprophos (4.10 kg ai/ha [MP 1, MP 2]), 3) fluensulfone high rate (2.34 L ai/ha [MP 1, MP 2]), 4) fluensulfone low rate (1.64 L ai/ha [MP 2]), 5) oxamyl (2.25 L ai/ha [MP 2]), 6) spirotetramat (2x) (0.37 L ai/ha each application [MP 2]), 7) a combination of fluopyram followed by spirotetramat (1.34 L ai/ha and 0.37 L ai/ha, respectively [MP 2]), and 8) an untreated control [MP 1, MP 2] (Table 1.1). Chemical treatments were applied on July 15, 2014 for MP 1 and July 23, 2015 for MP 2. In MP 2, spirotetramat was applied at 20 DAP to the spirotetramat (2x) and fluopyram + spirotetramat treatment plots, and again 27 DAP for the second treatment in the

spirotetramat (2x) treatments. Liquid treatments in both trials were applied using 2 L handheld pump sprayers. One sprayer was calibrated to spray in a 5-8 cm for band applications and the other was calibrated to spray a mist of ~30 cm broadcast over the entire soil/leaf surface. Mechanical incorporation of treatments into the upper 5-10 cm of soil and irrigation was performed as recommended (Table 1.1).

On July 15, 2014 (MP 1) and July 23, 2015 (MP 2) each microplot was planted with 20 seeds of the common commercial green baby lima bean cv. 'Cypress' in a circular row 36 cm in diameter. Seeds were treated with polymer coat containing 0.05 g a.i. of Fludioxonil per kg and 0.20 g a.i. of Metalaxyl M per kg, Lorsban, and a colorant.

Emergence was recorded 13 and 14 DAP for MP 1 and MP 2, respectively. Early season roots from MP 1 were collected 37 DAP, by removing 3 plants and rating them for root gall severity. Mid-season roots (3 root systems) were collected as described above 62 DAP in MP 1, and 42 DAP in MP 2 (5 root systems). At harvest, 5 root systems were collected 83 DAP (MP 1) and 10 root systems were collected 82 DAP (MP 2). Severity of root galling was rated from 0 to 10 using the following scale: 0 = 0-5%, 1 = 6-15%, 2 = 16-25%, 3 = 26-35%, 4 = 36-45%, 5= 46-55%, 6 = 56-65%, 7 = 66-75%, 8 = 76-85%, 9 = 86-95% and 10 = 96-100% of the root surface area galled (Bridge and Page 1980). Six soil samples were collected (0-30 cm) with a probe near the lima bean roots in each microplot at harvest and J2 were extracted from 100 cc subsamples of thoroughly mixed soil via decantation, sieving and sucrose centrifugation (Byrd et al. 1976, Jenkins 1964). RKN juveniles were identified morphologically and enumerated under a light microscope. RKN eggs were extracted from five lima bean roots at harvest with 0.5% NaOCl (Hussey and Barker 1973).

Aboveground biomass was recorded for each microplot. Yields were calculated as the numbers and weights (g) of full, flat, and dry lima bean pods, along with average shelled bean weight for each treatment.

Data from both MP trials were tested for normality using the Shapiro-Wilk, Goodness of Fit test, before mean comparisons. RKN populations, lima bean emergence, and yield data were subjected to analysis of variance (ANOVA) and following significant results, means were separated according to the Student's Protected LSD tests. In MP 1 and MP 2, lima bean emergence data was arc-sine transformed, and in MP 2, the number of eggs per root fresh weight (g) and the number of J2 per 100cc soil were LN and $\log_{10}+1$ transformed, respectively, before statistical analyses. Root ratings were subjected to Wilcoxon Rank Sums Tests (Mann-Whitney U-Tests) for comparison of nonparametric data via rankings and transformed to the midpoints of the percentage ranges (Kleczewski and Flory 2010). A significance level of $\alpha = 0.05$ was used in all analyses.

Table 1.1: Microplot (MP) and Greenhouse (GH) Trials: Chemical treatments, active ingredients, rates, modes of action, application methods, and experimental setting.

Trade Name	Active Ingredient	Rate (ai/ha)	Mode of Action	Application Method	Exp. Included
Luna Privilege SC	Fluopyram	0.22 L	Succinate dehydrogenase inhibitor (SDHI)	5-8cm band, below the seed in-furrow	MP 1, 2; GH 1, 2
Luna Privilege SC → Movento 240 SC	Fluopyram, Spirotetramat	0.22 L, 0.37 L	Succinate dehydrogenase inhibitor (SDHI), Inhibitor of acetyl CoA carboxylase	5-8cm band, below the seed in-furrow and a foliar broadcast spray	MP 2; GH 1, 2
Mocap 15G	Ethoprophos	4.10 kg	Acetylcholinesterase inhibitor (AChE)	30 cm band, incorporated	MP 1, 2; GH 1, 2
Movento 240 SC (2x)	Spirotetramat	0.37 L (2x)	Inhibitor of acetyl CoA carboxylase	Foliar broadcast sprays	MP 2; GH 1, 2[y]
Nimitz 480 EC	Fluensulfone	1.64 L	Nematode specific serotonin pathway[z]	Soil broadcast spray, irrigated	MP 2; GH 1, 2
Nimitz 480 EC	Fluensulfone	2.34 L	Nematode specific serotonin pathway	Soil broadcast spray, irrigated	MP 1, 2; GH 1, 2
Vydate L	Oxamyl	2.25 L	Acetylcholinesterase Inhibitor (AChE)	Soil broadcast spray, incorporated	MP 2; GH 1, 2
Untreated Control	N/A	N/A	N/A	N/A	MP 1, 2; GH 1, 2

[y]Movento only applied once in GH 1 and 2.
[z]Putative pathway (Holden-Dye et al. 2015)

Results

Greenhouse Experiments

Some replicates were lost in GH 2 due to poor emergence resulting from overwatering. Loss of replication was random across treatments, with each egg concentration x nematicide treatment achieving at least 3 replications.

Effects of Nematicides on Plant Growth and Biomass Allocation.

In non-inoculated treatments, none of the tested nematicides had significant effects on total plant biomass [$F_{12,35} = 0.42$, $p = 0.95$ (GH 1); $F_{12,23} = 1.44$, $p = 0.22$ (GH 2)] nor percent root weight of the total biomass [$F_{12,35} = 0.52$, $p = 0.88$ (GH 1); $F_{12,23} = 1.55$, $p = 0.18$ (GH 2)], in either greenhouse study (data not shown). No evidence of phytotoxicity (i.e., chlorosis, growth deformities, etc.) were observed in either study.

Effects of Nematicides on Root Galling.

Main effects of nematicide treatments and egg level, but not their interactions, were significant for root galling in both GH studies (Table 1.2). Root galling in the 30,000 egg pot^{-1} treatments was more than 264% and 144% greater than in the 6,000 egg pot^{-1} rate in GH 1 and GH 2 studies, respectively (data not shown).

Table 1.2: Results of ANOVA on LN transformed *Meloidogyne incognita* (RKN)-induced galls per gram lima bean root dry weight in Greenhouse (GH) studies.

Number of RKN galls per (g) dry root weight						
	GH 1			GH 2		
Factor	df[x]	*F*[y]	*P*[z]	df	*F*	*P*
Nematicide Treatment	7	12.38	<0.0001	7	5.36	0.0002
Egg Concentration	1	33.91	<0.0001	1	7.63	0.009
T x E	7	0.50	0.833	7	2.04	0.073
Error	79			44		

[x]Degrees of freedom.
[y]*F*-statistic.
[z]*P*-value.

In GH 1, all nematicide treatments except spirotetramat significantly reduced root galling relative to the untreated control (Table 1.3). Ethoprophos reduced galling by nearly 77% compared to the untreated control, but was not different from fluopyram, fluopyram + spirotetramat, and oxamyl. Fluensulfone at both rates (1.64L and 2.34L a.i. ha^{-1}) resulted in the greatest reduction of root galling (Table 1.3). In GH 2, spirotetramat and ethoprophos did not reduce root galling compared to the untreated control (Table 1.3). Root galling was reduced the most by both fluensulfone rates (1.64L and 2.34L a.i. ha^{-1}), fluopyram, and fluopyram + spirotetramat treatments. No correlations between root galls and plant biomass measurements were detected.

Table 1.3: *Meloidogyne incognita*-induced galls per gram lima bean root dry weight as affected by nematicide treatments in Greenhouse (GH) experiments.

	RKN galls per (gram) root dry weight	
Nematicide Treatment	GH 1	GH 2
Control	148.9 ± 38.2 a[z]	361.3 ± 49.1 a
Ethoprophos	34.6 ± 40.2 c	77.3 ± 54.9 abc
Fluensulfone (2.34L a.i. ha^{-1})	14.0 ± 38.2 d	67.6 ± 50.9 cd
Fluensulfone (1.64L a.i. ha^{-1})	6.4 ± 38.2 d	37.2 ± 49.6 d
Fluopyram	66.4 ± 38.2 c	63.9 ± 64.7 cd
Fluopyram + spirotetramat	65.0 ± 38.2 c	31.1 ± 53.9 cd
Oxamyl	134.3 ± 38.2 bc	108.2 ± 46.7 bc
Spirotetramat	183.0 ± 38.2 ab	204.3 ± 53.9 ab

[z]LS means ± the standard error. Any means within the same column not connected by the same letter are considered significantly different according to Protected LSD (α =0.05). Statistical analysis conducted on LN transformed data. Back transformed data depicted.

Microplot Experiments

Effects of Nematicides on Lima Bean Emergence.

In both MP experiments, nematicide treatment significantly affected lima bean emergence ($F_{3,16}$= 16.27, $p < 0.0001$ in MP 1; $F_{7,32}$= 2.51, $p = 0.036$ in MP 2). In MP 1, all nematicide treatments significantly reduced emergence compared to the control (Table 1.4). The greatest reduction of emergence occurred in ethoprophos treatments and fluensulfone (2.34L a.i. ha^{-1}) with reductions of 49% and 35% compared to the untreated control, respectively. In MP 2, a reduction in emergence was only detected in spirotetramat and ethoprophos and fluensulfone (2.34L a.i. ha^{-1}) treatments (Table 1.4).

Table 1.4 Mean emergence (%) of lima bean seedlings as affected by nematicide treatment in Microplot (MP) experiments.

	MP 1	MP 2
Nematicide Treatment	Emergence (%)	Emergence (%)
Control	94 a[x]	95 a
Fluopyram	73 b	84 abc
Ethoprophos	48 c	67 c
Fluensulfone (2.34L a.i. ha^{-1})	61 bc	82 bc
Fluensulfone (1.64L a.i. ha^{-1})	-[z]	83 ab
Fluopyram + spirotetramat	-	85 ab
Spirotetramat (2x)	-	79 bc
Oxamyl	-	91 ab

[x]LS Means ± standard error. Any means within the same column not connected by the same letter are considered significantly different according to Protected LSD ($\alpha = 0.05$). Statistical analysis conducted on ArcSin transformed data. Back transformed data depicted.
[z]Nematicides were not treatments in MP 1, only MP 2.

RKN Densities.

RKN J2 and eggs were not collected in MP 1. In MP 2, nematicide treatment affected RKN J2 densities per 100cc soil ($F_{7,32}$= 25.32, $p < 0.0001$). Only spirotetramat treatments did not significantly reduce RKN J2 abundance relative to the untreated control (Table 1.5). Ethoprophos reduced J2 abundance by over 99% compared to the untreated control and nearly 94% compared to the closest treatment, fluopyram (Table 1.5). J2 abundance was negatively correlated with lima bean pod yield (Spearman's ρ (38) = -0.47, $p = 0.0026$).

RKN egg numbers in lima bean roots were significantly affected by nematicide treatment ($F_{7,32}$= 3.90, $p = 0.004$), with only fluopyram and ethoprophos affecting RKN egg counts relative to the untreated control. Ethoprophos affected RKN egg abundance the most, increasing numbers compared to control by nearly 293% (Table

1.5). Egg abundance was negatively associated with J2 abundance [Spearman's ρ (38) = -0.41, p = 0.0083].

Table 1.5: Mean densities of *Meloidogyne incognita* (RKN) second-stage juveniles (J2) per 100cc soil and eggs per gram lima bean root fresh weight, as affected by nematicide treatment, at harvest in the Microplot (MP) 2 experiment.

Nematicide Treatment	RKN J2 (#/100cc soil)	RKN Eggs (#/gram root weight)
Control	16,669 a[z]	39.9 cde
Fluopyram	1,943 d	81.4 ab
Ethoprophos	121 e	116.9 a
Fluensulfone (2.34L a.i. ha^{-1})	2,603 d	44.7 bc
Fluensulfone (1.64L a.i. ha^{-1})	5,995 bc	38.1 cd
Fluopyram + spirotetramat	2,165 d	37.0 cd
Spirotetramat (2x)	11,780 ab	20.9 e
Oxamyl	3,522 cd	23.1 de

[z]LS means ± standard error. Means sharing the same letter within a capitalization scheme are considered significantly different according to Protected LSD (α = 0.05). Statistical analysis conducted on Log_{10} transformed data. Back transformed data depicted.

Effects of Nematicides on Root Galling.

In both MP experiments, nematicide treatments had significant effects on root galling at harvest according to Kruskal-Wallis tests (Chi-square = 16.39, p = 0.0009 inMP 1; Chi-square = 34.33, p = <0.0001 in MP 2). In MP 1, all nematicide treatments reduced root galling relative to control (Fig. 1.1). However, fluensulfone (2.34L a.i. ha^{-1}) reduced root galling to the greatest degree, 74% compared to control (Figure 1.1). In MP 2, all nematicide treatments except spirotetramat significantly reduced root galling compared to control. Ethoprophos reduced root galling the most, 81% compared to control (Figure 1.1). In both experiments, root galling at harvest was

negatively associated with lima bean pod yield, [Spearman's ρ (18) = -0.68, p = 0.001 (MP 1); Spearman's ρ (37) = -0.41, p = 0.0092 (MP 2)].

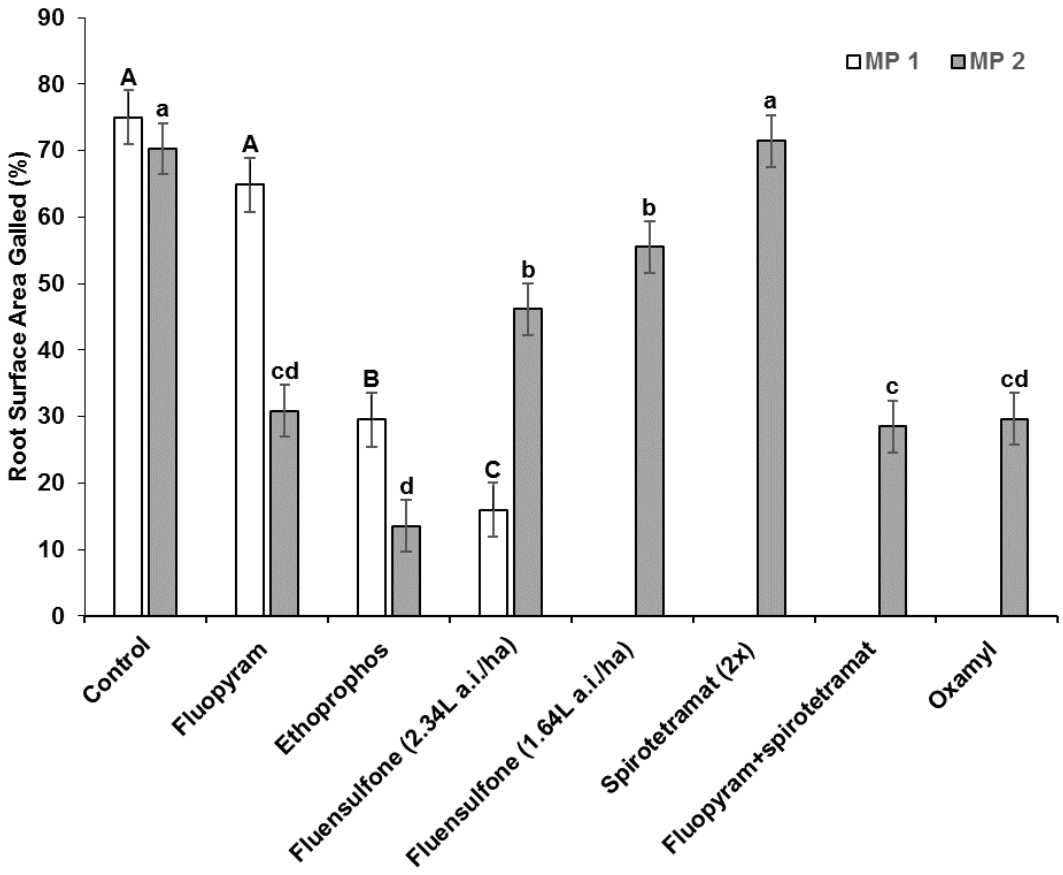

Figure 1.1: Root gall ratings from *Meloidogyne incognita* on lima bean as affected by nematicide treatments at harvest for Microplot (MP) experiments. Bar values represent Means ± standard error of five replicates. Treatments within the same experiment, connected by the same letter (capital letters for MP 1 and lowercase letters for MP 2) are not significantly different according to separate Wilcoxon Rank Sums Tests ($\alpha = 0.05$). Root galls were rated on a 0-10 scale, comparing the root surface area with galls to the total root surface area: 0 = 0-5%, 1 = 6-15%, 2 = 16-25%, 3 = 26-35%, 4 = 36-45%, 5= 46-55%, 6 = 56-65%, 7 = 66-75%, 8 = 76-85%, 9 = 86-95% and 10 = 96-100% of the root surface area galled.

Effects of Nematicides on Lima Bean Yields.

In both MP experiments, lima bean yields were significantly increased by nematicide treatment, [$F_{3,16}$= 5.92, p = 0.007 (MP 1); $F_{7,31}$= 6.87, p < 0.0001 (MP 2)]. In MP 1, ethoprophos and fluensulfone (2.34L a.i. ha^{-1}) increased yields by over 191% and 220% compared to control, respectively (Table 1.6). Fluopyram did not significantly increase yields. In MP 2, all treatments except spirotetramat increased yields compared to control (Table 1.6). Both fluensulfone rates (1.64L and 2.34L a.i. ha^{-1}), oxamyl, and ethoprophos treatments resulted in the greatest yields.

Table 1.6 Mean lima bean pod weights (grams) as affected by nematicide treatments in Microplot (MP) experiments.

	MP 1	MP 2
Nematicide Treatment	Pod Weight (g)	Pod Weight (g)
Control	256 c[x]	30 d
Fluopyram	342 bc	99 bc
Ethoprophos	488 ab	128 ab
Fluensulfone (2.34L a.i. ha^{-1})	562 a	127 ab
Fluensulfone (1.64L a.i.ha^{-1})	-[z]	130 ab
Fluopyram + spirotetramat	-	94 bc
Spirotetramat (2x)	-	50 cd
Oxamyl	-	196 a

[x]LS means ± standard error of five replicates. Any means within the same column not connected by the same letter are considered significantly different according to Protected LSD (α = 0.05). Analysis performed on square root transformed data. Back transformed data shown.
[z]Nematicides were not treatments in MP 1, only MP 2.

Discussion

In this study, we identified several new nematicide chemicals, currently unlabeled on lima beans that suppressed RKN populations and promoted lima bean growth. To our knowledge, this is the first time these products have been assessed in

lima beans. Data generated here may be useful in promoting label additions to include these newer products on lima beans.

The nematicides tested varied in their efficacy against RKN in lima bean. Fluensulfone application resulted in the greatest reduction of RKN in the greenhouse, while ethoprophos did so in the microplots. Spirotetramat did not appear to have efficacy against RKN in lima beans in either experimental setting. The interaction between egg level and nematicide treatment in the greenhouse was not significant, suggesting that the nematicides suppressed RKN at both low (6,000 eggs/pot) and high (30,000 eggs/pot) population levels in pots, without breakdowns in efficacy. No phytotoxic or beneficial effects on plant growth were found following nematicide application in the greenhouse in nematode free controls. However, fluensulfone and ethoprophos reduced seedling emergence significantly compared to the untreated control in the microplot trials.

Results from the greenhouse and microplot trials suggest spirotetramat has limited efficacy against RKN in lima bean. Spirotetramat did not reduce root galling compared to the untreated control when applied 19 DAP (GH 1), 16 DAP (GH 2), and 20 DAP (MP 2). The timing of foliar spirotetramat application is critical to its success as a nematicide. In a study on tomato by Vang et al. (2016), application of spirotetramat prior to RKN inoculation and 3 weeks post RKN inoculation did not consistently reduce the number of RKN egg masses, similar to our findings; conversely, application to foliage 1 or 2 weeks post inoculation did suppress egg mass production. In order to be effective, spirotetramat must be ingested by the target organism, and thus RKN gall formation will not be reduced in the first population cycle, helping to explain our findings in the single-generation time greenhouse trials

(Nauen et al. 2008, Vang et al. 2016). Fecundity, which was measured via egg extraction and enumeration is a more valuable metric than number of galls to determine the efficacy of spirotetramat (Vang et al. 2016). RKN egg density, therefore fecundity of RKN females, in lima bean roots in MP 2, was not reduced significantly following spirotetramat application. Smiley et al. (2011) called for the examination of spirotetramat as a companion treatment in combination with an early-season nematicide treatment for season-long nematode control and protection of crop plants, since spirotetramat cannot be applied until adequate leaf surface has emerged. In our study, fluopyram pre-plant plus spirotetramat at 19 (GH 1), 16 (GH 2) and 20 DAP (MP 2) did not improve RKN control when compared to fluopyram alone, except in one measure in MP 2, where a reduction in egg density in the lima bean roots was recorded. This suggests there was a decrease in fecundity due to spirotetramat, similar to Vang et al. (2016).

Fluopyram performed well in all trials, except in MP 1, where root galling was similar to the untreated control. Fluopyram application can result in a characteristic "halo effect" or damage to the cotyledons of emerging seedlings (Kendal et al. 2016). We recorded a 22% reduction in emergence due to fluopyram application in MP 1, similar to the findings of Kandel et al. (2016), who observed a reduction in soybean stand following fluopyram seed treatment in their study. In MP 2, fluopyram outperformed fluensulfone in reducing RKN galling and J2 populations in the soil, and was only outperformed in reducing RKN galling by fluensulfone in one of the greenhouse trials (GH 1). Overall, fluopyram performed well in our study warranting further research on potential registration for RKN control in lima beans as a seed

treatment or in-furrow application. It is already labeled as a seed treatment nematicide in soybeans (Velum Total ®).

Fluensulfone has been shown to suppress RKN populations in multiple vegetable cropping systems and is currently registered on cucumbers, melons, squash, tomatoes, okra, peppers, and eggplant as Nimitz ® (Morris et al. 2016, Oka et al. 2011, Kearn et al. 2014, Adama Ag Solutions, Raleigh, N.C.). Fluensulfone significantly reduced RKN galling in GH 1 and MP 1 more than any other nematicide tested. Our greenhouse results were similar to those obtained by Oka et al. (2011) where fluensulfone outperformed oxamyl. Fluensulfone can be applied in a broadcast/band spray that is followed by mechanical incorporation and irrigation (Oka et al. 2009; Nimitz Label). One limitation of fluensulfone is the 7-day pre-plant interval for seedling safety (Nimitz Label). We observed phytotoxicity following fluensulfone application in MP 1 and MP 2, similar to Morris et al. (2016) and Oka et al. (2009). Lima bean seedling emergence was reduced, when the 7-day pre-plant interval was not followed in our trials. In MP 2, both fluensulfone rates (1.64 and 2.34L a.i./ha) reduced root gall ratings similarly, but were outperformed by oxamyl, fluopyram, and ethoprophos treatments. Better control of RKN was recorded in GH 1 and GH 2 following the 7-day waiting period. Examination of the more economical band-application method of fluensulfone in lima bean is warranted as well as studying the duration of control.

In addition to the newly registered nematicides, ethoprophos (an organophosphate) and oxamyl (a carbamate) were also studied. Ethoprophos, was introduced in the 1960s and the granular formulation has long been labeled for use in lima bean as Mocap ® (Chitwood 2001, Amvac Chemical Corp. Los Angeles, C.A.).

One concern is the phytotoxicity associated with the chemical, which in our microplot trials, reduced lima bean seedling emergence almost 40% when compared to the untreated controls. This is similar to research by Sinclair et al. (1992) who found that even when applied correctly, ethoprophos can cause phytotoxicity on seedlings of carrot. Ethoprophos, performed well in our trials, with among the best RKN control in all but the GH 2 trial. In MP 2, ethoprophos had the lowest root gall ratings of all nematicides tested and the fewest number of J2 in the soil, a reduction of nearly 94% compared to the next lowest number of J2 following fluopyram application. The low number of J2 in the soil could be somewhat attributed to the limited root surface area resulting from poor emergence following ethoprophos application. This suggests lima bean growers' long-used tool is still highly effective at controlling RKN using the current label rates and application methods.

In summary, ethoprophos continues to be an effective chemical for controlling RKN in lima beans, although the phytotoxicity remains concerning and additional research on this should be considered. Fluopyram and fluensulfone show promise as forthcoming tools. We are the first to demonstrate that fluopyram and fluensulfone can reduce RKN galling on lima bean and provide support that a seed treatment application of fluopyram in lima bean should be evaluated. If research supports the efficacy of this product and a label can be obtained, it would benefit lima bean production in the mid-Atlantic, where nematodes are becoming a greater threat to production (PMSP 2003). Further research into the yield effects of the nematicides are needed, along with determination of an economic threshold for RKN in lima bean cropping systems to ascertain when nematicide application is warranted.

Chapter 2

CULTURAL MANAGEMENT PRACTICES AFFECTING RKN IN LIMA BEAN AND CUCUMBER ROTATIONS

Abstract

The Mid-Atlantic coastal plain soils are a favorable habitat for RKN. Chemical controls are costly and limited, so cultural controls, including common cover crops grown in the region, and organic soil amendments were examined for suppression of RKN. Common cover crop species (barley, wheat, and rye) examined did not result in significant differences in RKN populations in the cover crops or the subsequent cucumber crop. The species of sorghum appeared to play an important role in RKN control, with 'Trudan 8' *sorghum x sudangrass* reducing root galling in the subsequent lima bean crop 51% compared with 'Sordan 79' *sorghum x sudangrass,* in Biofumigant Trial 1. . Host status varied in Biofumigant Trial 2, with 'Image' radish having the fewest RKN eggs per gram root weight (2.2), while 'Dwarf Essex' rapeseed reduced root galling in the following lima bean crop by 74% compared with the highest galled treatment 'Kodiak' mustard. Yard-waste based compost applied at a rate of 13.5 t ha^{-1} and crust-out poultry litter applied at a rate of 6.7 t ha^{-1} both marginally lowered root galling compared with the untreated control in the Soil Amendment Trial 1 (11% and 18%, respectively) while no amendments were effective in Trial 2. The results from cultural control measures of RKN are often variable and dependent on many factors, as this study shows.

Introduction

Root knot nematodes (*Meloidogyne* spp.) have a host range of over 1,700 plant species and are responsible for approximately 5% of crop losses worldwide (Barker 1998; McCarter 2008). *Meloidogyne spp.* are considered the most damaging plant-parasitic nematodes in the world according to a 2012 survey, with four major species: *M. arenaria, M. hapla, M. incognita,* and *M. javanica* (Jones et al. 2013, Moens et al. 2009). Southern root-knot nematode [*M. incognita* (Kofoid and White 1919; RKN)] is commonly found in soils in the mid-Atlantic region and causes extensive damage to crops via the formation of giant cells, which impede nutrient and water uptake in the plant and serve as a specialized nutrient source for the nematodes (Karssen et al. 2013, Barker et al. 1998).

The mid-Atlantic region is predisposed to successful RKN infections, as the predominate sandy loam and loamy sand soil textures have large percentages of macropores, resulting in maximum RKN movement and infection (Wallace 1968, UDSA NRCS). RKN is capable of severely damaging many vegetable crops, including lima bean (*Phaseolus lunatus*) and cucumber (*Cucumis sativus*), thereby affecting plant health and yield in the Delmarva Region (Delaware, Maryland, and Virginia). Management of RKN is important to maximizing grower profitability and production (Kee et al. 2004, McConnell 2016, PMSP 2003, Kratochvil et al. 2004). Delaware has more hectares of lima beans than any other state in the United States, with a ten-year average in excess of 5,261 hectares planted and harvested annually. This generates an average value of $1,074 per hectare and a total value of production in excess of $5.65 million annually (DDA and NASS 2014). Green baby lima beans are often considered a cornerstone crop in Delaware vegetable production and a major component of the vegetable processing industry in the state and region (Kee et al.

2004). Pickling cucumber is another important processing vegetable in the region. Combined annual production in Delaware and Maryland varies between 1,800 and 2,500 hectares, with an average value per hectare of $2,600 (Johnson personal communication). Lima bean and cucumber yields must be protected from RKN in order to continue profitable production of both crops on Delmarva.

Chemical controls were for decades an effective stand-alone tool in the control of RKN, but their environmental impacts, issues with human safety, and other concerns led to discontinuation in use (Moens et al. 2009, Johnson and Feldmesser 1987, Abdel-Rahman et al. 2008, Aspelin and Grube 1999, Giannakou et al. 2002, UNEP 1992, EPA 2008, EPA 2009, EPA 2012). These phase-outs have left a void in RKN control, and the development of safer, non-fumigant chemical control options has not kept pace with the loss of the older nematicides (Nyczepir and Thomas 2009). Currently, the main nematode control measures that growers utilize in the region are non-chemical, cultural controls, including crop rotation, avoidance of infected fields, and biofumigation with rapeseed (Everts et al. 2006). However, RKN on lima beans and cucumber is of increasing importance and more effective management practices are needed (PMSP 2003). This has created a need for research into the efficacy of cultural techniques commonly used to manage RKN. Consequently, a series of studies were conducted between 2014-2016 to investigate cultural practices, including common cover crops grown in the region, fallow periods, fall and spring planted biofumigants, and organic soil amendments for suppression of RKN populations in lima beans and cucumbers.

Crop rotation for control of RKN is the practice of rotating non-hosts, poor hosts, or fallow prior to planting RKN-susceptible hosts. The greatest advantage of

crop rotation is the cash return from growing a non/poor host (Nyczepir and Thomas 2009). On Delmarva, a portion of the wheat and barley crops are enrolled in cover crop cost-share incentive programs to reduce soil erosion, recycle nitrogen, and reduce nutrient runoff in the region (Sussex Conservation District 2016). Other common winter cover crop species grown regionally include rye, rapeseed, and daikon radishes. The major practices are often to fallow a field over winter, plant wheat or barley for grain harvest or prevention of soil erosion, or plant cover crops such as rye, rapeseed, and radishes for nutrient management, forage, nematode suppression, or compaction reduction. To determine whether selected cover crops could contribute to RKN management, this study focused on the effects of commonly available cover crops in the region, including small grains, arugula, radish, and rapeseed that are planted in the fall and either harvested or killed in the spring.

A potential limitation to the use of small grains for RKN management, is that *M. incognita* has the capability to reproduce on the roots of wheat, barley, and rye when the soil temperature is above 18^{o} C (Johnson and Motsinger 1989, Roberts et al. 1981). In a greenhouse study, all three cover crops allowed similar levels of nematode egg production (Johnson and Motsinger 1989). In field studies, a clean fallow reduced midseason galling in a subsequent crop compared with wheat and rye, although another study showed no differences in mid-season galling following rye and weedy fallow (Wheeler et al. 2008, Timper et al. 2006). *Poaceae* spp., such as rye, wheat, and barley produce secondary metabolites, benzoxazinoids, that are toxic to nematodes, but despite similar levels of these compounds in various cultivars, cultivars vary in host status to RKN (Johnson and Motsinger 1990, McSorley and Dickson 1995, Barnes and Putnam 1987, Rice et al. 2005, Zasada et al. 2005, Zasada et al. 2007,

Grun et al. 2005, Wu et al. 2001). The variable results suggest that type and variety of the cover crop grown and the overwintering temperature are important factors for the survival of RKN on cover crop roots. Interpretation of field results can also be confounded because much of the cover crop rye, wheat, and barley is planted were the variety is not known ("variety not stated").

Another cultural control for nematodes is planting of biofumigant crops. *Brassicaceae* spp. crops produce glucosinolate compounds, which degrade into biocidal isothiocyanates and have been shown to reduce nematode numbers (Brown et al. 1991, Ku et al. 2016, Edwards and Ploeg 2014, Rudolph et al. 2015, Fourie et al. 2016). Multiple commercially available Brassica species were therefore evaluated for RKN control potential including: Kodiak' and 'Pacific Gold' mustards (*Brassica juncea*), and 'Caliente 199' mustard (*Brassica juncea* + *Sinapis alba* blend). 'Dwarf Essex' rapeseed (*Brassica napa*) is a commonly grown biofumigant crop in fields with known RKN problems; it can be planted in the fall or spring and requires chopping and incorporation for RKN suppression (Mid-Atlantic Commercial Vegetable Recommendations Guide 2016). 'Dwarf Essex' has high foliage production and concentration of glucosinolates (Johnson et al. 1992). 'Nemat' arugula (*Eruca sativa*), which also has biofumigant activity against RKN, is a possible alternative to rapeseed (Riga 2011, Edwards and Ploeg 2014). The use of forage, daikon and oilseed radishes (*Raphanus sativus*) as winter-killed cover crops has been widely adopted in the Delmarva region. Several of these radishes have been bred specifically for nematode control (Bunte et al. 1997, Gardner and Caswell-Chen 1994). Both 'Image' radish (*Raphanus sativus* var. *oliefera*) and 'Nemat' arugula are purported to perform as a trap crops, causing nematode egg hatch in some plant parasitic nematode species but

as a non-host, preventing nematode reproduction on their roots, in addition to their biofumigant activity when incorporated (Poindexter 2013, Edwards and Ploeg 2014, Riga and Collins 2004, Riga et al. 2004, Curto et al. 2006, Melakeberhan et al. 2006).

Other biofumigant cover crops included in this study were sorghum and sunnhemp. *Sorghum spp*. contain a different class of biofumigant chemicals; cyanogenic glycosides. Chewing or cutting results in the production of toxic hydrogen cyanide, which controls nematodes via the inhibition of cytochrome oxidase, preventing oxygen use (Chitwood 2002, Ntalli and Caboni 2012). *Crotalaria spp.* (sunnhemp) are leguminous plants that contain pyrolizdidine alkaloids and monocrataline, which are toxic to plant-parasitic nematodes (Rich and Rahi 1995, Wang et al. 2002). *Crotalaria spp.* are also well-known non/poor hosts of RKN, with possible trap crop activity by allowing RKN J2 infection but very limited or no egg production (Rich and Rahi 1995, McSorley 1999). *Crotalaria spp.* are best used as pre-plant cover crops, and have outperformed nematicides through continued suppression of nematode populations even after a host was planted (Huang et al. 1991, Sharma and Scolari 1984, Widmer and Abawi 1998).

Compost soil amendments can also be used to manage RKN populations in susceptible crops (Everts et al. 2006, Akhtar and Malik 2000). With the public readily accepting composting practices as an environmentally responsible way of handling yard, food and animal wastes, multiple companies have facilities in the region, although large scale adoption of compost application on agricultural fields has been slow, perhaps due the availability of more nutrient rich poultry litter as a soil amendment in the region. As a major poultry processing region, poultry manure mixed with bedding (poultry litter) is commonly applied to agricultural fields. Poultry litter

reduced the population densities of RKN, including in the Mid-Atlantic region (Badra et al. 1979, Riegel et al. 1996, Riegel and Noe 2000). However, results are variable, with some studies demonstrating increased nematode numbers due to increased carrying capacity of the more prolific root systems (Everts et al. 2006, Oka 2010, Thoden et al. 2011).

The main objective of this research was to examine the effects of cultural practices for potential use in regional processing vegetable crop rotations for RKN suppression. Two field experiments examined fall planted wheat, barley, rye, 'Dwarf Essex' rapeseed, and the alternatives 'Nemat' arugula and 'Image' radish, and a fallow control. Two field experiments examined the effects of late-spring planted biofumigant 'Piper' sudangrass, 'Sordan 79' *sorghum x sudangrass*, 'Trudan 8' *sorghum x sudangrass*, 'Tillage Sunn' sunnhemp, 'Kodiak' mustard and early-spring planted 'Kodiak' mustard, 'Pacific Gold' mustard, 'Caliente 199' mustard, 'Southern Curled' mustard, 'Dwarf Siberian' kale, 'Dwarf Essex' rapeseed, 'Bonar' rapeseed, 'Nemat' arugula, and 'Image' radish as tools for managing RKN immediately prior to a susceptible crop. In addition, we investigated the efficacy of the regionally plentiful poultry litter and compost, each from multiple sources, against RKN in lima beans and cucumbers.

Materials and Methods

Winter Cover Crop Trials with Cucumber

The locations of the winter cover crop trials overwintered from October 2014 to May 2015 and October 2015 to April 2016 (CC 1 and CC 2, respectively) were chosen because of existing natural RKN pressure in two grower's fields in Milford,

DE. An Ingleside sandy loam, with a pH of 5.2 and organic matter (OM) of 0.6% was the predominant soil type in CC 1, while Downer loamy sand, with a pH of 4.9 and OM of 0.8%, was the predominant soil type in CC 2 (USDA, NRCS).

In CC 1, the research plot was tilled with a disc harrow before cover crop planting but no tillage occurred in CC 2. Cover crop treatments for CC1 and/or CC2 included: 1) wheat (135 kg ha^{-1} [CC 1, CC 2]), 2) barley (134.5 kg ha^{-1} [CC 1, CC 2]), 3) rye (125.5 kg ha^{-1} [CC 1, CC 2]), 4) 'Nemat' arugula (9.0 kg ha^{-1} [CC 1, CC 2]), 5) 'Dwarf Essex' rapeseed (9.0 kg ha^{-1} [CC 1, CC 2]), 6) 'Image' radish (11.3 kg ha^{-1} [CC 2]), 7) weedy fallow control (naturally emerged [CC 1, CC 2]), and 8) clean fallow control [CC 1]. The cover crops were broadcast by hand at recommended rates and incorporated with a rake on October 1, 2014 (CC 1) and October 27, 2015 (CC 2). Treatments were applied to the field in a randomized complete block design (RCBD), with seven treatments and five blocks in both experiments.

The clean fallow control in CC1 was sprayed with glyphosate 192 days after planting, when weeds emerged in the spring. In CC 1, the plots were side-dressed with 67 kg ha^{-1} N via urea (46-0-0) on the same day as planting while plots were not fertilized in CC 2. The individual plot dimensions were 3 m x 3.66 m in both experiments. The cover crops overwintered in the plots and were allowed to grow until 218 (May 9, 2015) and 177 (April 21, 2016) days after planting (DAP), when the rapeseed and arugula were flowering in CC 1 and the grower was preparing to till the field in CC 2. In CC 1, all treatments were chopped from top-to-bottom with a handheld gas-powered string-trimmer, to simulate mechanical chopping with a flail chopper. A tractor-mounted rotary tiller was then used to incorporate the cover crop

residue 20 cm into the soil. The entire plot was then compacted by driving the tractor over it to seal the soil surface.

In CC 1, the plots remained fallow until the planting of cucumbers on July 27, 2015 with a tractor and mechanical planter in 76.2 cm rows. The plot was managed by the grower, including fertilization, irrigation, and weed control. A heavy downpour of rain that caused flooding in the field before cucumber emergence severely stunted the growth of the cucumber plants.

Ten (2.54 cm x 2.54 cm) soil cores were randomly taken from each plot in CC 1 and CC 2 on the day of cover crop planting, the day of cover crop destruction [218 (May 9, 2015) and 177 DAP (April 21, 2016) the cover crops for CC 1 and CC 2, respectively] and for a third time near the cucumber rows at harvest in CC 1 [43 DAP cucumber (September 8, 2015)]. RKN J2 were extracted via decantation (Byrd et al. 1976) and sucrose centrifugation (Jenkins 1964) for morphological identification and enumeration under a light microscope.

In CC 1 only, aboveground biomass samples were taken by cutting 1 m^2 sections in each plot at ground level and fresh weights were recorded. These samples were dried at 0% humidity and 60^o C for 14 days and then weighed for dry matter content. The root systems of eight cover crop plants in each plot were collected and the RKN eggs extracted via 0.5% NaOCl for enumeration (Hussey and Barker 1973). Seven cucumber root systems were collected at harvest per treatment plot [43 DAP cucumber (September 8, 2015)] for an enumeration of galls on each root system under a magnifying lamp. Yield was not determined in CC 1, as the cucumber plants were still stunted from the flooding before emergence and no data was collected from the subsequent crop in CC 2.

In CC 1 and CC 2, RKN J2 abundance was subjected to analysis of variance (ANOVA), and means were separated according to the Student's T LSD test. In CC 1, the number of eggs per (g) cover crop root fresh weight and the number of eggs per (g) cucumber root fresh weight were $Log_{10}+1$ and Log+1 transformed, respectively, before statistical analyses. A significance level of $\alpha = 0.10$ was used in all analyses.

Biofumigant Trials with Lima Bean

A location was chosen at the University of Delaware's Elbert N. and Ann V. Carvel Research and Education Center (UD REC), a research farm in Georgetown, DE for the 2014 late spring planted biofumigant trial (BIO 1). The field had been inoculated annually for four years with RKN to create an infestation. A second biofumigant trial (BIO 2) was conducted in a grower's field with a natural infestation of RKN, near Harbeson, DE in 2015. The soil type in the BIO 1 research plot was Hurlock loamy sand, with a pH of 5.3 and OM of 1.4%, and in BIO 2 was a Pepperbox-Rosedale complex, with a pH of 5.0 and OM of 1.0% (USDA, NRCS).

The RKN inoculum for Bio 1 was increased on the susceptible tomato host, *Lycopersicum esculentum* 'Marglobe', in a greenhouse at DuPont Crop Protection®, Wilmington, DE. On June 12, 2014, the same day as cover crop planting, each plot (with the exception of un-inoculated control plots) was inoculated with a 6.82 kg mixture of infested tomato roots and soil. The inoculum was chopped and mixed before application to each plot, providing approximately 2 million eggs per plot, in addition to the existing RKN population in the soil. The inoculum was spread by hand in each plot and immediately incorporated in the upper 20 cm of soil with a tractor-mounted disc harrow. BIO 2 received no additional RKN inoculum. Both experiments were tilled using a disk harrow to prepare for planting.

Treatments varied between the two trials, with BIO 1 focusing on previously studied *Sorghum* spp. for late-spring planting and BIO 2 focusing on commercially available *Brassicaceae* spp. biofumigants, many with reported biofumigant activity for early-spring planting. Treatments in BIO 1 were: 1) 'Sordan 79' (*Sorghum* x *Sudangrass* hybrid), 2) 'Trudan 8' (*Sorghum* x *Sudangrass* hybrid), 3) 'Piper' (*Sudangrass*), 4) 'Tillage Sunn' (*Crotalaria juncea*) sunnhemp, and 5) 'Kodiak' (*Brassica juncea*) and the controls 6) RKN-inoculated clean fallow, 7) clean fallow that was not inoculated with additional RKN, and 8) a RKN-susceptible host, 'Caprice' (*Phaseolus vulgaris*). Treatments in BIO 2 included: 1) 'Bonar' (*Brassica napus*), 2) 'Dwarf Essex' (*Brassica napus*), 3) 'Caliente 199' (*Brassica juncea* + *Sinapis alba*), 4) 'Kodiak' (*Brassica juncea*), 5) 'Pacific Gold' (*Brassica juncea*), 6) 'Southern Curled' (*Brassica juncea*), 7) 'Dwarf Siberian' (*Brassica oleracea*), 8) 'Nemat' (*Eruca sativa*), 9) *Raphanus sativus* var. *oliefera*), and 10) weedy fallow control (naturally emerged).

In BIO 1, the sudangrass and Sorghum x Sudangrass hybrids were planted with a single row push planter in 38.1 cm rows, the sunnhemp was planted in 76.2 cm rows, and the mustard was broadcast by hand at a rate of 11.2 kg ha^{-1} and incorporated with a hand rake on June 12, 2014. In BIO 2, all treatments were broadcast by hand and incorporated with a hand rake at recommended rates of 11.2 kg ha^{-1} for *B. juncea, B. oleracea,* and *R. sativus* treatments and 9.0 kg ha^{-1} for *B. napus* and *E. sativa* treatments on April 17, 2015. Treatments were applied in an RCBD with eight (BIO 1) and ten (BIO 2) treatments and five blocks. Plot dimensions were 3 m x 3 m, with 1 m non-treated alleys between plots within a block in BIO 1, and 3.7 m x 4.6 m with no any alleys in BIO 2. Plots were side-dressed with 90 kg ha^{-1} N with urea, 27 days after

cover crop planting (BIO 1), or with 67 kg ha^{-1} N with ammonium sulfate, 4 days after planting (BIO 2).

In BIO 1, cover crops were incorporated 41 DAP (July 23, 2014), at which point the 'Kodiak' mustard was in bloom stage and the *sorghum x sudangrass* hybrids and the sudangrass were approximately 1.5 meters tall. Plants in each plot were chopped from top to bottom with a handheld gas-powered string-trimmer, to simulate mechanical chopping with a flail chopper. Each block was chopped and then immediately incorporated into the upper 20 cm of soil with a tractor-mounted rotary tiller. Following incorporation, the entire field was compacted with a tractor mounted cultipacker to seal the soil. Control plots were tilled and compacted as well. The plots were sealed by 1.26 cm of natural rainfall six hours after incorporation. In BIO 2, the biofumigant cover crops were chopped and incorporated 53 DAP (June 9, 2015), as previously described. To mimic common farm practices, the soil surface was not irrigated or compacted to seal the plant volatiles in the soil in this experiment.

On July 30, 2014 (BIO 1) and June 11, 2015 (BIO 2) seeds of the common commercial green baby lima bean cultivar 'C-Elite' were mechanically planted with a tractor-mounted planter in 76.2 cm rows, resulting in a stand of approximately 13 plants per m^2 and 12 plants per m^2, respectively. Plots were not irrigated in BIO 1, but were irrigated by the grower with overhead irrigation in BIO 2. Summer weeds were controlled by mechanical cultivation and the lima beans were side-dressed with 22 kg ha^{-1} N in the form of urea ammonium nitrate in both experiments.

Ninety-three DAP lima bean (October 31, 2014), 2.45 m were harvested from each of the inner two rows per plot in BIO 1, while 82 DAP lima bean (September 1, 2015), 5.3 m were harvested from 3 inner rows of lima beans in each plot in BIO 2.

Aboveground plant biomass fresh weights (kg) and the number of plants harvested were recorded for both experiments. Yield was recorded after lima beans were shelled with a stationary-viner (mechanical sheller) as the combined weight of the shelled dry and succulent beans for both experiments. Ten roots were collected at harvest from each plot in both experiments and root galling was rated on a 0-10 scale, comparing the root surface area with galls to the total root surface area: 0 = 0-5%, 1 = 6-15%, 2 = 16-25%, 3 = 26-35%, 4 = 36-45%, 5= 46-55%, 6 = 56-65%, 7 = 66-75%, 8 = 76-85%, 9 = 86-95% and 10 = 96-100% of the root surface area galled (Bridge and Page 1980).

In BIO 2 only, ten (2.54 cm x 2.54 cm) soil cores were randomly taken from each plot on three dates: the day of cover crop planting (April 17, 2015), 53 days after cover crop planting (June 9, 2015, directly preceding the destruction of the biofumigants), and near the lima bean rows at harvest on September 1, 2015. RKN J2 abundance in the soil was enumerated as previously described. The amount of aboveground biomass grown per 1 m^2 and incorporated in each plot and the percentage of dry matter of the biofumigants was recorded, as previously described. RKN eggs were counted as described in the cover crop trials from: a) the root systems of eight biofumigant plants in each plot, and b) five root systems of lima beans from each plot. The weeds in the weedy fallow were sparse and no roots or biomass were collected from those plots.

Lima bean yield data (BIO 1 and BIO 2), RKN J2 and egg abundance (BIO 2), and cover crop biomasses (BIO 2) were subjected to analysis of variance (ANOVA), and means were separated according to the Students T LSD test. In BIO 2, the number of J2 per 100cc of soil was log transformed for normality before statistical analyses. For both experiments, the root rating data was subjected to Kruskal-Wallis tests to

permit mean separation via Wilcoxon Rank Sums Tests for the comparison of nonparametric data via rankings. A significance level of $\alpha = 0.10$ was used in all analyses.

Soil Amendment Trials with Lima Bean and Cucumber

The locations of the soil amendment trials (SAT), which were conducted in 2014 and 2015 (SAT 1 and SAT 2, respectively), were chosen because of existing natural RKN pressure in two grower's fields in Milford, DE. The predominant soil type in SAT 1 was a Downer loamy sand, with a pH of 5.1 and OM of 0.8%, and in SAT 2 was an Ingleside sandy loam, with a pH of 5.1 and OM of 0.7% (USDA, NRCS).

The research plots were tilled with a disk harrow before application of the soil amendments in both trials. Treatments included: 1) Jones poultry litter, low rate (3.4 t ha^{-1} [SAT 1, SAT 2]), 2) Jones poultry litter, high rate (6.7 t ha^{-1} [SAT 1, SAT 2]), 3) Peninsula compost, low rate (6.7 t ha^{-1} [SAT 1, SAT 2]), 4) Peninsula compost, high rate (13.5 t ha^{-1} [SAT 1, SAT 2]), 5) Blue Hen compost, low rate (6.7 t ha^{-1} [SAT 1]), 6) Blue Hen compost, high rate (13.5 t ha^{-1} [SAT 1]), 7) Perdue poultry litter, low rate (3.4 t ha^{-1} [SAT 2]), 8) Perdue poultry litter, high rate (6.7 t ha^{-1} [SAT 2]), and 9) untreated control [SAT 1, SAT 2].

The treatments differed in sources and composition: Blue Hen compost was composed mainly of food wastes, Peninsula compost was composed mainly of yard wastes, Jones poultry litter was from a "crust out" (where a thin layer of manure, known as the crust, is removed from the poultry house between flocks) and Perdue poultry litter was composed of manure and litter from a "clean-out" (where all the manure and sawdust bedding that has accumulated over 3-5 years of time is removed

from a poultry house before new litter is added). The application rates were determined based on realistic application rates of poultry manure in the region (Lichtenberg et al. 2002, DE Nutrient Management Commission 2014, Everts 2006). Average fertility benefits of each of the treatments was accounted for and the remaining nutrient requirements of lima beans were applied using inorganic fertilizer, following nutrient recommendations from the Commercial Vegetable Recommendations Guide for Delaware (Zhang et al. 2013, Sánchez and Richard 2009). The soil amendments were applied by hand, on June 12, 2014 (SAT 1) and July 20, 2015 (SAT 2), followed by incorporation with a disk harrow. The experimental design in both trials was a RCBD with seven treatments and five blocks. Individual plots were 3.0 m x 6.1 m, with 1.5 m alleys between treatments in each block in SAT 1, and 3.0 m x 4.3 m with no alleys in SAT 2.

On June 16, 2014 the common commercial green baby lima bean cultivar 'C-Elite' was planted in rows 76.2 cm apart with a mechanical planter in SAT 1, resulting in a final stand of approximately 12 plants per m^2. SAT 1 was not irrigated and had significant weed pressure. On July 27, 2015, pickling cucumbers were planted in rows 76.2 cm apart with a mechanical planter in SAT 2. A heavy downpour of rain and flooding in the field before emergence of the cucumbers severely stunted the growth of the cucumber plants in SAT 2. Weeds were controlled with mechanical cultivation and the research plot was irrigated in SAT 2.

Eighty-five DAP (September 9, 2014), a complete harvest of SAT 1 was conducted with 3.6 m harvested from the inner two rows of lima beans in each plot. Aboveground plant biomass fresh weights (kg) were recorded for each plot as a group, along with the number of plants harvested. Yield was recorded after lima beans were

shelled as previously described. Ten lima bean roots were collected at harvest per treatment plot and rated for root galling as previously described. At harvest in SAT 2, 43 DAP (September 8, 2015), 8 cucumber root systems were collected per treatment plot, for an enumeration of galls on each root system under a magnifying lamp. RKN eggs were extracted and enumerated as previously described, from the 8 cucumber root systems.

Lima bean plant weights and yields in SAT 1, and the number of RKN galls and eggs in SAT 2 were subjected to analysis of variance (ANOVA) and following significant results, means were separated according to the Student's Protected LSD tests. The root rating data in SAT 1, was subjected to Kruskal-Wallis tests to permit mean separation via Wilcoxon Rank Sums Tests (Mann-Whitney U-Tests) for the comparison of nonparametric data via rankings. The root rating data was subjected to Wilcoxon Rank Sums Tests for comparison of nonparametric data via rankings. A significance level of $\alpha = 0.10$ was used in all analyses.

Results

Winter Cover Crop Trials with Cucumber

Biomass and RKN Egg Production, CC1. In the CC 1 trial, the cover crops produced different amounts of biomass for incorporation ($F_{5,24}= 2.79$, $p = 0.0400$; Table 2.1). All cover crops served as hosts for RKN, permitting reproduction (Table 2.1). Cover crop treatment did not have a significant effect on the number of galls per (g) cover crop root fresh weight (data not shown), RKN eggs per (g) cover crop root fresh weight ($F_{5,24}= 1.97$, $p = 0.1198$; Table 2.1), or eggs per (g) root fresh weight in the subsequent cucumber crop ($F_{6,28}= 0.15$, $p = 0.9871$ and $F_{6,28}= 0.06$, $p = 0.99$; Table

2.1). RKN galling on cucumber roots had a strong positive correlation with egg density in the cucumber roots [Spearman's ρ (33) = 0.94, $p < 0.0001$]. RKN eggs could not be collected from cover crop roots in CC 2 because 40% of the research plot was killed by herbicide two weeks before the trial ended.

RKN J2 Soil Populations, CC2. In CC 1, the soil populations of RKN J2 were too low for accurate comparison between treatments. J2 densities in soil were enumerated in CC2, but the cover crop treatments did not affect the numbers of RKN J2 found in the soil ($F_{6,33}$= 1.01, p = 0.4367; Table 2.1).

Table 2.1: Cover Crop (CC) trials CC 1 (2014) and CC 2 (2015) with cucumber in two Milford, DE locations. Mean aboveground dry biomass produced by each cover crop in CC 1 and mean number of *Meloidogyne incognita* (RKN) eggs per gram cover crop root fresh weight and per gram cucumber root fresh weight. Mean densities of RKN J2 in CC 2

	Cover Crop 1			Cover Crop 2
Treatment	Cover crop dry biomass (g/m^2)	Eggs/g cover crop root fresh weight	Eggs/g cucumber root fresh weight	J2/100 cc soil
Control (Clean Fallow)	N/A	N/A	576	N/A
Control (Weedy Fallow)	1,437 c	7.34	312	1,341
Arugula	1,514 bc[x]	3.90	601	1,067
Barley	2,252 a	12.06	367	1,283
Radish[y]	N/A	N/A	N/A	1,071
Rapeseed	2,294 a	11.11	483	1,769
Rye	2,734 a	5.38	849	1,827
Wheat	2,181ab	10.95	452	1,494

[x]LS means within the same column not connected by the same letter are considered significantly different according to Protected LSD ($\alpha = 0.05$). Five replicates were used per treatment.
[y]Radish was not a treatment in CC 1.

Biofumigant Trials with Lima Bean

Biomass Production. Aboveground biomass production was not recorded for Bio 1, but in Bio 2 there were no significant differences in the amount of dry biomass produced per m^2 by the various biofumigant crops ($F_{8,36}= 1.10$, $p = 0.3873$; Table 2.2).

RKN Egg Production. In BIO 2, biofumigant crops had a significant effect on number of eggs per (g) biofumigant crop root fresh weight, but no significant effect on the density of RKN eggs in the subsequent lima bean crop roots ($F_{8,36}= 4.03$, $p = 0.0017$ and $F_{9,40}= 1.09$, $p = 0.3892$, respectively; Table 2.2). 'Image' radish had the fewest number of eggs per (g) biofumigant crop root fresh weight, a decrease of 90% compared to the highest egg density in 'Pacific Gold' mustard. All four mustard

varieties were among the highest levels of egg density in biofumigant roots. ‘Dwarf Essex’ rapeseed resulted in the fewest amount of eggs per (g) lima bean root fresh weight, a decrease of 67% compared to the lima beans following ‘Bonar’ rapeseed.

Table 2.2: Biofumigant (BIO) 2 field trial with lima bean in Harbeson, DE, 2015. Mean aboveground dry biomass produced by and incorporated for each cover crop, mean number of *Meloidogyne incognita* eggs per gram cover crop root fresh weight and lima bean root fresh weight, and mean lima bean seed yield harvested from 5.33 meters of row per plot.

Biofumigant Cover Crop	Dry biomass (g per m^2)	Egg per (g) biofumigant crop root fresh weight	Eggs per (g) lima bean root weight	Lima bean seed yield
Control (Weedy Fallow)	N/A	N/A	111.4	2,137 a
‘Bonar’ Rapeseed	130.8	11.0 b[z]	139.4	1,908 ab
‘Caliente’ Mustard	145.9	19.6 a	110.1	1,375 c
‘Dwarf Essex’ Rapeseed	121.7	14.1 ab	46.4	1,991 a
‘Dwarf Siberian’ Kale	113.3	9.4 bc	97.0	1,964 a
‘Image’ Radish	163.7	2.2 c	96.4	1,927 ab
‘Kodiak’ Mustard	131.9	13.8 ab	67.6	1,479 bc
‘Nemat’ Arugula	103.8	15.8 ab	114.5	1,853 ab
‘Pacific Gold’ Mustard	137.8	21.0 a	123.6	1,761 abc
‘Southern Curled’ Mustard	127.0	15.6 ab	116.4	1,741 abc

[z]LS means within the same column not connected by the same letter are considered significantly different according to Protected LSD (α = 0.05). Five replicates were used per treatment.

Lima Bean Yield. In BIO 1, the preceding biofumigant crop had a significant effect on lima bean seed yield ($F_{7,29}$= 2.01, p = 0.0887; Fig. 2.1). The non-inoculated control treatment had the highest lima bean seed yield in BIO 1, similar to sunnhemp, the inoculated control, and ‘Trudan 8’ treatments. ‘Kodiak’ mustard resulted in the lowest yielding lima bean plots, a decrease of 40% compared to the non-inoculated control. ‘Piper’ sudangrass, ‘Sordan 79’, and snap beans resulted in lima bean yields

that were all nearly identical, with only 0.56% separating the highest yielding treatment from the lowest yielding in that group. In BIO 2, the effect of cover crop treatment on lima bean yield was significant ($F_{9,40}$= 1.92, p = 0.0767; Table 2.2). The untreated (weedy fallow) control had the highest yielding lima bean plants and was statistically similar to all but two treatments, ‘Kodiak’ mustard and ‘Caliente’ mustard (Table 2.2). The latter two treatments yielded 69% and 66% of the weedy fallow, respectively.

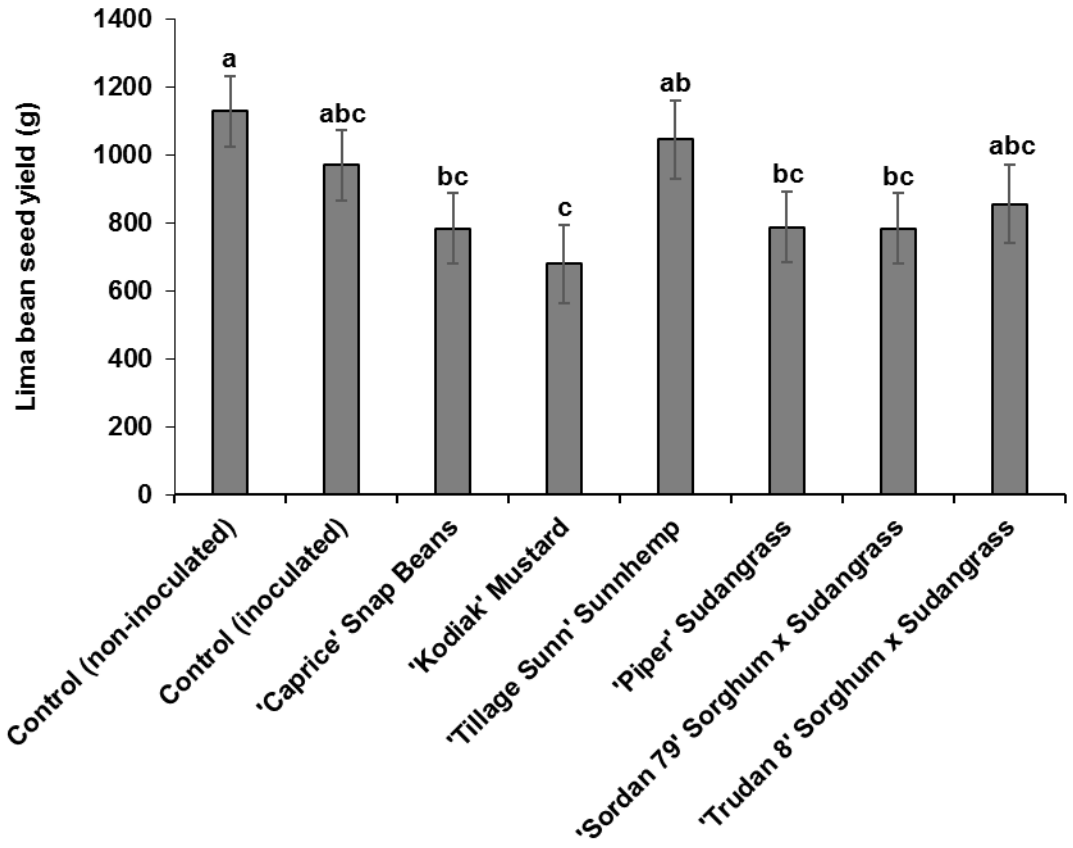

Figure 2.1: Biofumigant (BIO) 1 with lima bean in Georgetown DE, 2014. Mean lima bean seed yield (grams) harvested from 4.9 meters of row in each plot, following biofumigant cover crops. Bar values are means ± standard error of five replicates. Each error bar is constructed using 1 standard error from the mean. Treatments not connected by the same letter are considered significantly different according to Protected LSD (α=0.05). Control treatments were clean fallow during the biofumigant crop growth period.

Root Gall Ratings. In BIO 1, the biofumigant crops had a significant effect on the subsequent lima bean crop's root gall ratings (Chi-square = 25.76, p = 0.0006; Fig. 2.2). The non-inoculated control treatment had the fewest galls, a 77% reduction from the inoculated control. 'Kodiak' mustard increased lima bean galling compared to the inoculated control 238%, with results similar to snap beans and 'Sordan' 79. Lima

bean following sunnhemp and ‘Trudan 8’ had gall ratings similar to the inoculated control.

In BIO 2, the biofumigant crops again had significant effects on lima bean root gall ratings at harvest (Chi-square = 15.21, $p = 0.0853$; Figure 2.3). Although no treatments were statistically different from the control (weedy fallow), a large variation in galling in the lima beans occurred. As in BIO 1, the highest root gall rating was recorded following ‘Kodiak’ mustard, which was the only treatment used in both trials. Lima bean following ‘Dwarf Essex’ rapeseed had the lowest galling severity, a decrease of 74% compared with the ‘Kodiak’ mustard treatment.

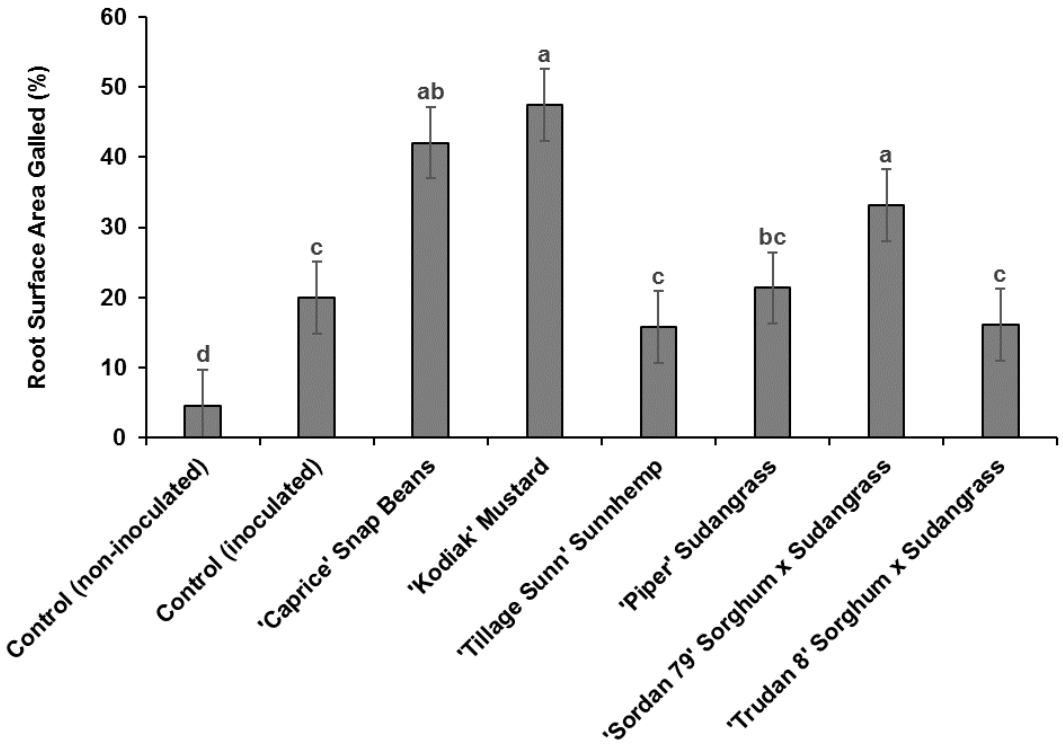

Figure 2.2: Biofumigant (BIO) 1 with lima bean in Georgetown, DE, 2014: Root gall ratings from *Meloidogyne incognita* on lima bean at harvest, following biofumigant cover crops. Bar values represent means ± standard error of five replicates. Each error bar is constructed using 1 standard error from the mean. Treatments connected by the same letter are not significantly different according Wilcoxon Rank Sums Test (Kruskal-Wallis) ($\alpha = 0.10$). Root galls were rated on a 0-10 scale, comparing the root surface area with galls to the total root surface area: 0 = 0-5%, 1 = 6-15%, 2 = 16-25%, 3 = 26-35%, 4 = 36-45%, 5= 46-55%, 6 = 56-65%, 7 = 66-75%, 8 = 76-85%, 9 = 86-95% and 10 = 96-100% of the root surface area galled. Control treatments were clean fallow during the biofumigant crop growth period.

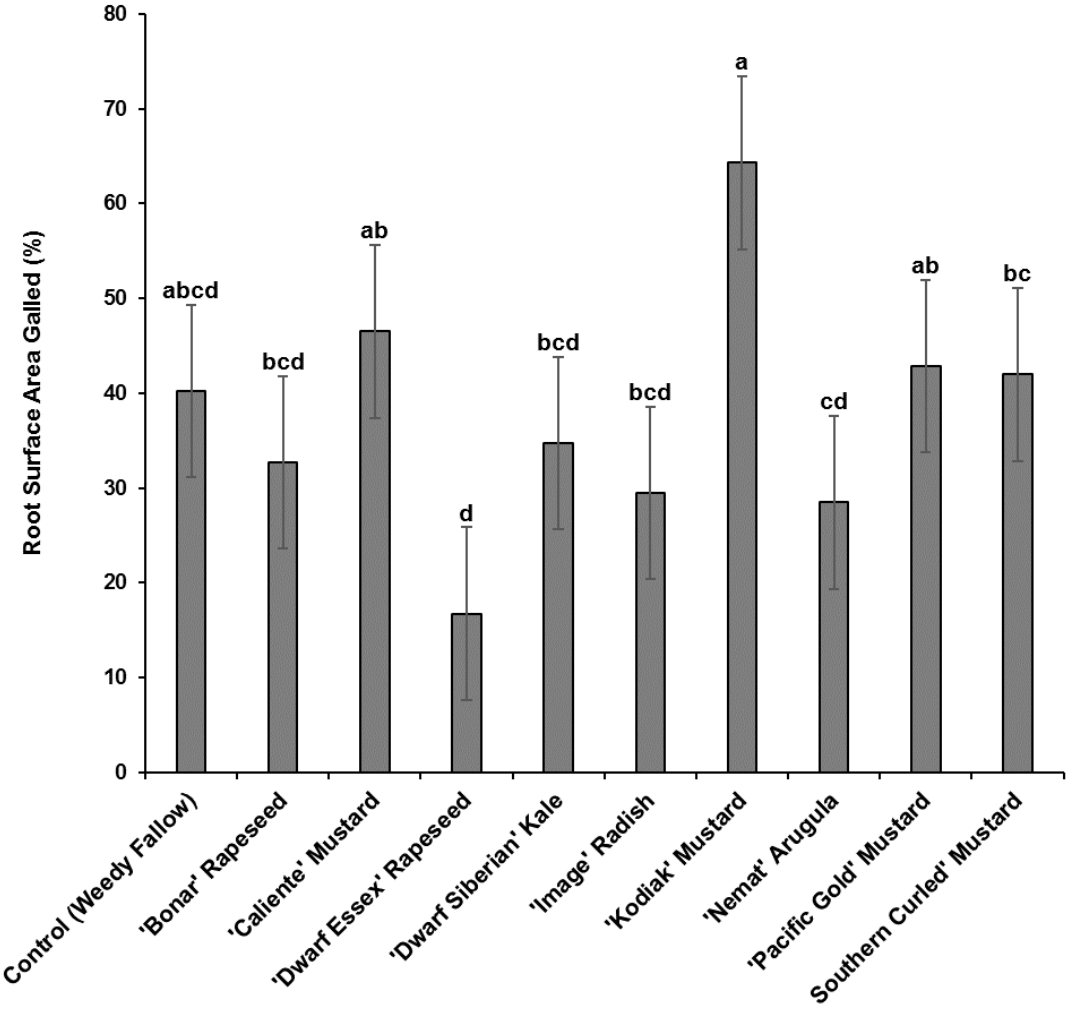

Figure 2.3: Biofumigant (BIO) 2 with lima bean in Harbeson, DE, 2015. Root gall ratings from *Meloidogyne incognita* on lima bean, midseason and harvest, following biofumigant cover crops. Bar values represent means ± standard error of five replicates. Each error bar is constructed using 1 standard error from the mean. Treatments connected by the same letter are not significantly different according Wilcoxon Rank Sums Test (Kruskal-Wallis) ($\alpha = 0.10$). Root galls were rated on a 0-10 scale, comparing the root surface area with galls to the total root surface area: 0 = 0-5%, 1 = 6-15%, 2 = 16-25%, 3 = 26-35%, 4 = 36-45%, 5= 46-55%, 6 = 56-65%, 7 = 66-75%, 8 = 76-85%, 9 = 86-95% and 10 = 96-100% of the root surface area galled.

Soil Amendment Trials with Lima Bean and Cucumber

Root Galling. In SAT 1, the soil amendments had a significant effect on lima bean root gall ratings (Chi-square = 10.96, $p = 0.0897$; Fig. 2.4). The non-amended

control had the highest percentage of root surface area covered in galls (75.1%), indicative of the high pressure of RKN in this trial, while Jones Poultry Litter (6.7 t ha^{-1}) had the lowest amount of galling, a reduction of 18% compared to the non-amended control. In SAT 2, the soil amendments did not have a significant effect on cucumber root gall counts ($F_{6,28}$= 0.43, p = 0.8512; data not shown).

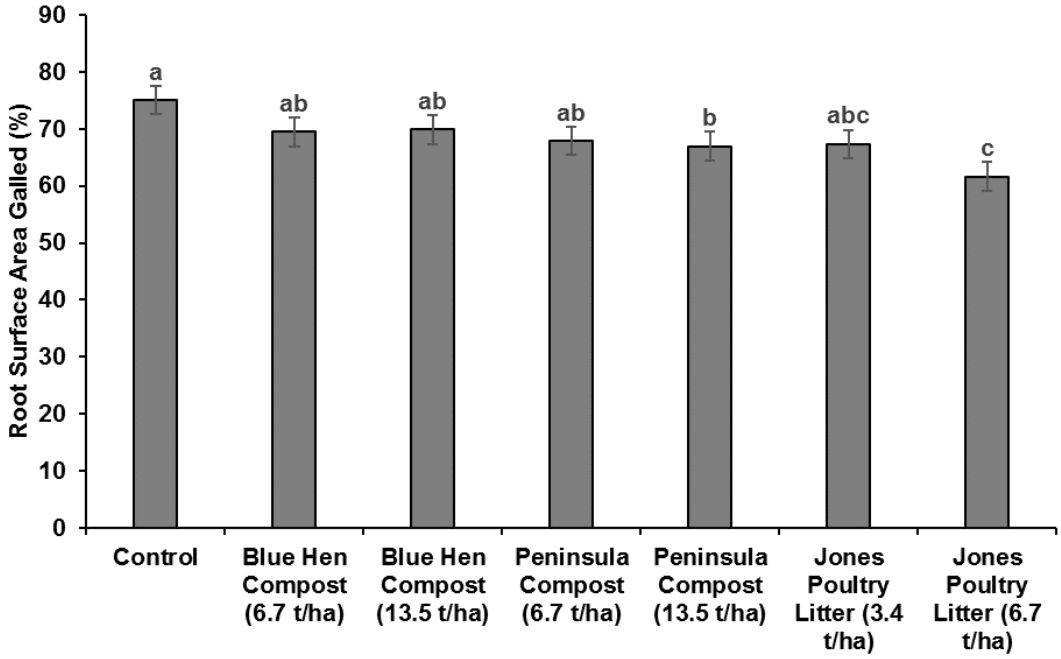

Figure 2.4: Soil Amendment (SAT) 1 with lima bean in Milford, DE, 2014. Root gall ratings from *Meloidogyne incognita* on lima bean as affected by compost and litter treatments at harvest. Bar values represent Means ± standard error of five replicates. Treatments within the same experiment, connected by the same letter are not significantly different according to separate Wilcoxon Rank Sums Tests (α = 0.10). Root galls were rated on a 0-10 scale, comparing the root surface area with galls to the total root surface area: 0 = 0-5%, 1 = 6-15%, 2 = 16-25%, 3 = 26-35%, 4 = 36-45%, 5= 46-55%, 6 = 56-65%, 7 = 66-75%, 8 = 76-85%, 9 = 86-95% and 10 = 96-100% of the root surface area galled.

RKN Egg Production. In SAT 1, RKN eggs were not collected from the lima bean roots, but eggs were extracted and enumerated from cucumber in SAT 2. The soil

amendments did not have significant effects on number of eggs per (g) cucumber root fresh weight ($F_{6,28}$= 0.69, p = 0.6614; data not shown). A large amount of variability in egg density on roots occurred within treatments. The greatest variability was in Perdue Poultry Litter (3.4 Mg ha^{-1}), with a minimum of 100 eggs and a maximum of 5,350 eggs per eight cucumber roots.

Discussion

Survival and reproduction of RKN on small grains used as winter cover crop varies with cultivar (Wang et al. 2004, Johnson and Motsinger 1989). A common practice among growers is to plant cover crops of unknown varieties and thus it is difficult to assess the host status without testing individual lots. Rye cultivars can range from moderate RKN hosts, similar to wheat and barley, to poor/non-hosts, depending on cultivar (Zasada et al. 2005, Timper et al. 2006, Johnson and Motsinger 1989). In the two cover crop trials, all small grains obtained locally were shown to be RKN hosts and had high soil J2 levels in CC 2. 'Nemat' arugula ranked among the poorest hosts for *M. incognita* in a 2014 study by Edwards and Ploeg (2014), while rapeseed was an intermediate host. However, in our CC 2trial, both the arugula and rapeseed served as hosts under high RKN pressure.

To manage RKN reproduction in winter cover crops, it has been recommended to delay planting until soil temperatures drop below 18°C and RKN can no longer infect roots (Johnson and Motsinger 1989, Johnson and Motsinger 1990, Roberts and Van Gundy 1981, Roberts et al. 1981). This corresponds to a mid-October planting date in the mid-Atlantic region. However, in CC 2, with a planting date of October 27, all cover crop species supported high populations of RKN in a heavily infested field. Unfortunately, as we observed in our research, the effects of any winter cover

crop are likely not going to be seen, perhaps even in the immediately subsequent crop (Johnson and Motsinger 1990, Wang et al. 2004).

With biofumigant cover crops, if reduction of RKN populations occurs, it is often temporary and may not result in lasting benefits in the subsequent crop (Nyczepir and Thomas 2009). Kirkegaard et al. (1999) suggested the ideal biofumigant has high glucosinolate content and produces a large amount of biomass for incorporation. Although, phytotoxicity can occur in the following crop if the biofumigant is not allowed sufficient time to decompose completely and this cannot be ruled out as a confounding factor for yield reductions in BIO 1 and BIO 2 (Wang et al. 2001).

The use of biofumigants is an important part of a RKN management plan, and knowledge of biofumigant crops as RKN hosts is a precursor to successful plan (Monfort et al. 2007). Care must be taken to prevent the build-up of RKN during the biofumigant crop growth period, since these crops are often hosts of RKN and don't reduce the nematode population until chopping and incorporation is performed. Our BIO 1 and BIO 2 trials support the importance placed on host status when considering which biofumigant crop to plant (Zasada et al. 2007). The biofumigants we tested did not improve yields or reduce RKN infection in when compared to fallow control treatments, showing the value of fallowing as an RKN management strategy, due to the lack of host roots for RKN reproduction. Ornat et al. (1999) found reductions of RKN populations on average of 54% following summer fallow periods. All biofumigant crops tested were shown to be hosts for RKN in BIO 2. 'Image' radish in BIO 2, had significantly lower levels of infection, although this did not translate into a yield benefit. In BIO 1, lima bean root galling was higher than the inoculated fallow

control in the ‘Kodiak’ mustard and ‘Sordan 79’ *sorghum x sudangrass* treatments. ‘Kodiak’ mustard also performed poorly in BIO 2, with the highest percentage of root galling, likely due to due to its RKN host status. Rudolph et al. (2015) warn against using ‘Pacific Gold’ and ‘Caliente’ mustards in a field with RKN populations because they are RKN hosts, and they would likely increase the nematode populations when planted as overwintering cover crops. In our BIO 2 trial, they had among the highest root gall ratings in the subsequent crop. ‘Dwarf Essex’ rapeseed is used for nematode control in the region and lowered root galling substantially in BIO 2, suggesting merit behind the practice. ‘Nemat’ arugula, reduced root galling on the following lima bean crop in BIO 2 and merits further research. In BIO 1, ‘Tillage Sunn’ sunnhemp and ‘Trudan 8’ *sorghum x sudangrass* lowered galling in the subsequent lima bean crop compared to the susceptible snap bean crop. Biofumigation using early spring planted biofumigant crops such as ‘Image’ radish, ‘Dwarf Essex’ rapeseed, or ‘Nemat’ arugula showed potential for managing RKN populations in fields to be planted to lima beans; however, more research is needed. The late spring planted warm-season biofumigant sorghum and sunnhemp species warrant further research; however, their use may be limited by the short window of time they can be grown prior to incorporation, a waiting period, and late lima bean planting.

In the studies with organic soil amendments, the higher rate of yard-waste compost (Peninsula Compost) and the higher rate of crust-out poultry litter marginally lowered RKN population densities in lima beans compared to the untreated control treatment in SAT 1. Although minor, the RKN reduction benefits provided by the yard-waste compost and poultry litter at high rates, indicates some secondary benefits to correctly applying poultry litter and composts to agricultural fields (Bolan et al.

2010). In SAT 2, there were not effects of any organic soil amendment treatment. The shorter growing season for cucumbers and lower RKN pressure than expected may have contributed to this result. Consecutive applications of organic amendments can lead to more substantial reduction in RKN and should be examined in future research in RKN management in lima bean (Lamberti et al. 2007).

In a study by Everts et al. (2006) poultry litter was studied in combination with cover crops, crop rotation, and resistant crop varieties for nematode management, allowing for examination of a more cumulative RKN management plan, they found fallowing followed by poultry litter and tillage provided the greatest RKN reduction for two out of the three study years. Future research on RKN management in lima bean and cucumbers should use a similar approach with combinations of cultural practices including winter cover crops, biofumigation and organic soil amendment additions. The direct and indirect costs of these practices should also be determined in order to determine if they will be profitable for the growers to implement. With limited chemical control options, lima bean and cucumber growers must continue to adopt new RKN management practices and develop comprehensive management plans in order to limit immediate yield losses and reduce the spread and proliferation of RKN in the future.

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