EVALUATION OF NEMATICIDES FOR SOUTHERN ROOT-KNOT NEMATODE MANAGEMENT IN LIMA BEAN

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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-infested tomato root tissue and soil grown in the greenhouse. Root galling, RKN egg

and juvenile densities, and aboveground plant dry masses were determined and analyzed using Mixed Model ANOVA. In the GH trials, 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 trial, all treatments except spirotetramat significantly reduced RKN populations and had significantly greater yields relative to the untreated control. Oxamyl, ethoprophos, and fluensulfone (1.64 L ai/ha and 2.34 L ai/ha) 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.

1. 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 and 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 (NASS and DDA 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 their 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 humans and the environment, with the majority lacking specificity in their toxicity, leading to heavy regulation and scrutiny (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).

As a consequence of the deregistration of synthetic 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 infested fields and biofumigation with rapeseed. The resulting RKN reduction from crop rotation and rapeseed is often transient and inconsistent (Everts et al. 2006). However, several non-carbamate/organophosphate active ingredients have recently received EPA registration as nematicides. These include spirotetramat (2010), fluensulfone (2014) and fluopyram (2015) (Table 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 for the effectiveness. 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 on 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 and RKN are unknown (Kandel et al. 2016).

The objective of this research was to compare the efficacy of these new nematicides to each other and to other select nematicides for suppression of RKN in lima bean. To accomplish this, we evaluated the nematicides for RKN control in greenhouse and microplot experiments.

2. Materials and Methods

2.1. 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). Each experimental combination was replicated six times and the entire experiment was replicated twice (GH 1, GH 2), for a total of 288 units in the combined trials (144 experimental units per trial). 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 GH 1 was 22.8°C, with a low of 18.8°C and a high of 27.4°C, while the average temperature in GH 2 was 23.2°C, with a low of 18.8°C and high of 32.3°C. 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. 2016).

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 growth of foliage was sufficient to apply the foliar spray on 19 (GH 1) and 16 DAP (GH 2). See Table 1 for a complete list of chemical treatments, modes-of-action, rates, and application methods.

On 32 (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 dry mass at 60°C. Roots were rinsed in running tap water to remove soil and debris, and then stored in sealed plastic bags at 4.5°C until egg masses were counted. RKN egg masses 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 egg masses 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 analyzed separately to test the effects of chemical treatments on overall plant growth and productivity in the absence of RKN. Means were separated following significant ANOVA using Student's Protected LSD ($\alpha=0.05$). 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.).

2.2. 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 (diameter x height) 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). 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 *Lycopersicon 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 into the upper 30 cm of soil.

Nematicide treatments included: 1) fluopyram (0.22 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 (0.22 L ai/ha and 0.37 L ai/ha, respectively [MP 2]), and 8) an untreated control [MP 1, MP 2] (Table 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 swath 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).

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 were collected as described above 62 DAP in MP 1 (3 root systems), 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 to 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 and sieving and sucrose centrifugation (Byrd et al. 1976, Jenkins 1964). RKN were identified morphologically and enumerated under a light microscope. Initial RKN J2 populations were determined to be uniformly absent via soil sampling in untreated control plots, before the addition of inoculum. 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 from the remaining plants in each treatment plot, as a plot average of numbers and weights (g) of full, flat, and dry lima bean pods, along with average shelled bean weight for each treatment plot.

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 were 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 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.

221 **Table 1:** Microplot (MP) and Greenhouse (GH) Trials: Chemical treatments, active ingredients,
 222 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 ^x	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

223 ^xEthoprophos band treatment rate was applied to the entire 10.2 cm greenhouse pot width.

224 ^ySpirotetramat only applied once in GH 1 and 2.

225 ^zPutative pathway (Holden-Dye et al. 2015)

226

3. Results

3.1. 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.

3.1.1. 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 trial (data not shown). No evidence of phytotoxicity (i.e., chlorosis, growth deformities, etc.) was observed in either trial.

3.1.2. Effects of Nematicides on Root Gallings

Main effects of nematicide treatments and egg level, but not their interactions, were significant for root gallings in both GH trials (Table 2). Root gallings 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 the GH 1 and GH 2 trials, respectively (data not shown).

Table 2: Results of ANOVA on LN transformed *Meloidogyne incognita* (RKN) egg masses per gram lima bean root dry weight in Greenhouse (GH) trials.

Number of RKN egg masses per (g) dry root weight						
Factor	GH 1			GH 2		
	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		

^xDegrees of freedom.

^yF-statistic.

^zP-value.

In GH 1, all nematicide treatments except spirotetramat significantly reduced root galling relative to the untreated control (Table 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 3). In GH 2, spirotetramat and ethoprophos did not reduce root galling compared to the untreated control (Table 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 galling and plant biomass measurements were detected.

Table 3: *Meloidogyne incognita* (RKN)egg masses per gram lima bean root dry weight as affected by nematicide treatment in Greenhouse (GH) trials.

Nematicide Treatment	RKN egg masses per (gram) root dry weight	
	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

^zLS means ± the 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 LN transformed data. Back transformed data depicted.

3.2. Microplot Experiments

3.2.1. 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 4). The greatest reduction of emergence occurred in ethoprophos and fluensulfone treatments (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 4).

Table 4: Mean emergence (%) of lima bean seedlings as affected by nematicide treatment in Microplot (MP) experiments.

Nematicide Treatment	MP 1	MP 2
	Emergence (%)	Emergence (%)
Control	94 ± 5.05 a ^y	95 ± 4.68 a
Fluopyram	73 ± 5.05 b	84 ± 4.68 abc
Ethoprophos	48 ± 5.05 c	67 ± 4.68 c
Fluensulfone (2.34L a.i. ha ⁻¹)	61 ± 5.05 bc	82 ± 4.68 bc
Fluensulfone (1.64L a.i. ha ⁻¹)	- ^z	83 ± 4.68 ab
Fluopyram + spirotetramat	-	85 ± 4.68 ab
Spirotetramat (2x)	-	79 ± 4.68 bc
Oxamyl	-	91 ± 4.68 ab

^yLS 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.

^zNematicides were not treatments in MP 1, only MP 2.

3.2.2. 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 5). Ethoprophos reduced J2 abundance by over 99% compared to the untreated control and nearly 94% compared to the closest treatment, fluopyram (Table 5). J2 abundance was negatively correlated with lima bean pod yield (Spearman's $\rho(38) = -0.47, p = 0.0026$).

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 the untreated control by nearly 293% (Table 5). Egg abundance was negatively associated with J2 abundance [Spearman's $\rho(38) = -0.41, p = 0.0083$].

Table 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 (#per 100cc soil)	RKN Eggs (# per gram root weight)
Control	16,669 ± 1,542 a ^z	39.9 ± 17.2 cde
Fluopyram	1,943 ± 1,542 d	81.4 ± 17.2 ab
Ethoprophos	121 ± 1,542 e	116.9 ± 17.2 a
Fluensulfone (2.34L a.i. ha ⁻¹)	2,603 ± 1,542 d	44.7 ± 17.2 bc
Fluensulfone (1.64L a.i. ha ⁻¹)	5,995 ± 1,542 bc	38.1 ± 17.2 cd
Fluopyram + spirotetramat	2,165 ± 1,542 d	37.0 ± 17.2 cd
Spirotetramat (2x)	11,780 ± 1,542 ab	20.9 ± 17.2 e
Oxamyl	3,522 ± 1,542 cd	23.1 ± 17.2 de

^zLS 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 Log₁₀ transformed data. Back transformed data depicted.

3.2.3. Effects of Nematicides on Root Gallings

In both MP experiments, nematicide treatments had significant effects on root gallings at harvest according to Kruskal-Wallis tests (Chi-square = 16.39, $p = 0.0009$ in MP 1; Chi-square = 34.33, $p = <0.0001$ in MP 2). In MP 1, all nematicide treatments except fluopyram reduced root gallings relative to the untreated control (Fig. 1). However, fluensulfone (2.34L a.i. ha⁻¹) reduced root gallings to the greatest degree, 74% compared to the untreated control (Figure 1). In MP 2, all nematicide treatments except spirotetramat significantly reduced root gallings compared to the untreated control, with ethoprophos reducing root gallings the most with an 81% reduction (Figure 1). In both experiments, root gallings at harvest was negatively associated with lima bean pod yield, [Spearman's $\rho(18) = -0.68$, $p = 0.001$ (MP 1); Spearman's $\rho(37) = -0.41$, $p = 0.0092$ (MP 2)].

Fig. 1. Root gall ratings from *Meloidogyne incognita* on lima bean as affected by nematocidal treatments at harvest for Microplot (MP) experiments. Bar values represent Means $\pm$ 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.

3.2.4. 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 the untreated control, respectively (Table 6). Fluopyram did not significantly increase yields. In MP 2, all treatments except spirotetramat increased yields compared to the untreated control (Table 6). Both fluensulfone rates (1.64L and 2.34L a.i. ha⁻¹), oxamyl, and ethoprophos treatments resulted in the greatest yields.

Table 6: Mean lima bean pod weights(grams) per plot as affected by nematicide treatments in Microplot (MP) experiments.

Nematicide Treatment	MP 1	MP 2
	Pod Weight (g)	Pod Weight (g)
Control	256 ± 60.7 c ^y	30 ± 20.9 d
Fluopyram	342 ± 60.7 bc	99 ± 20.9 bc
Ethoprophos	488 ± 60.7 ab	128 ± 20.9 ab
Fluensulfone (2.34L a.i. ha ⁻¹)	562 ± 60.7 a	127 ± 20.9 ab
Fluensulfone (1.64L a.i. ha ⁻¹)	- ^z	130 ± 23.7 ab
Fluopyram + spirotetramat	-	94 ± 20.9 bc
Spirotetramat (2x)	-	50 ± 20.9 cd
Oxamyl	-	196 ± 20.9 a

^yLS 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 ($\alpha = 0.05$). Analysis performed on square root transformed data. Back transformed data shown.

^zNematicides were not treatments in MP 1, only MP 2.

4. 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 bean. Data generated here may be useful in developing 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, 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 controls 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 (GH 1), 16 (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 egg mass production 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 the number of egg masses or extent of galling for determining 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). In a study by McKenry et al. in 2011, nematode control by spirotetramat was enhanced by waiting nine days before resumption of irrigation. No break in irrigation was performed in our studies, perhaps contributing to the lack of efficacy of spirotetramat.

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 (ILeVO®) and as an in-furrow application, or chemigated through drip, trickle or subsurface irrigation systems in cotton and peanuts (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, CA). 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 be phytotoxic to carrot seedlings.

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. However, the results suggest that 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 additional research on phytotoxicity 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.

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