# **RESEARCH/INVESTIGACIÓN**

# ASSESSMENT OF SEED-APPLIED FLUOPYRAM FOR MANAGEMENT OF *MELOIDOGYNE INCOGNITA* IN SOYBEAN

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# ABSTRACT

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The field efficacy of seed-applied fluopyram was evaluated in 2015, 2017, and 2019 to manage *Meloidogyne incognita* on a moderately resistant and susceptible soybean cultivar and in a greenhouse pot experiment at high, moderate, and low nematode population densities. Based on the percent of root system galled at 45 or 60 days after planting, the significance of *M. incognita* suppression by seed-applied nematicides was similar to that of abamectin, *Bacillus firmus* (strain I-1582) and fluopyram + *B. firmus* treated seed. Further, these nematicides provided similar grain yield protection that was most consistent at low nematode population densities on the susceptible cultivar. In contrast, grain yield was greater on the moderately resistant cultivars regardless of nematode population density. In the greenhouse pot experiment, fluopyram provided a numeric suppression of *M. incognita* infection compared to the nontreated control, but similar to that of other seed-applied nematicides. Overall, soybean root and grain yield protection by seed-applied fluopyram was similar to that of other seed-applied nematicides.

Key words: Abamectin, Bacillus firmus, Meloidogyne incognita, seed-applied nematicide, soybean

#### RESUMEN

Spinks, C. S., M. Emerson, y T. R. Faske. 2020. Evaluación de fluopiram aplicado a semillas para el manejo de *Meloidogyne incognita* en soja. Nematropica 50:118-126.

La eficacia de campo del fluopiram aplicado a las semillas se evaluó en 2015, 2017 y 2019 para gestionar *Meloidogyne incognita* en un cultivar de soja moderadamente resistente y susceptible y en un experimento de maceta de invernadero con densidades de población de nematodos altas, moderadas y bajas. Basado en el porcentaje de sistema radicular escogido a los 45 o 60 días después de la siembra, la importancia de la supresión de *M. incognita* por nematicidas aplicados a las semillas fue similar a la de abamectina, *Bacillus firmus* (cepa I-1582) y fluopiram + *B. firmus* semilla. Además, estos nematicidas proporcionaron una protección de rendimiento de grano similar que fue más consistente a bajas densidades de población de nematodos en el cultivar susceptible. En contraste, el rendimiento de grano fue mayor en los cultivares moderadamente resistentes, independientemente de la densidad de población de nematodos.

En el experimento de la maceta de invernadero, el fluopiram proporcionó una supresión numérica de la infección por *M. incognita* en comparación con el control no tratado, pero similar a la de otros nematicidas aplicados a las semillas. En general, la protección del rendimiento de la raíz y el grano de la soja mediante fluopiram aplicado a las semillas fue similar a la de otros nematicidas aplicados a las semillas fue similar a la de otros nematicidas aplicados a las semillas fue similar a la de otros nematicidas aplicados a las semillas fue similar a la de otros nematicidas aplicados a las semillas fue similar a la de otros nematicidas aplicados a las semillas fue similar a la de otros nematicidas aplicados a las semillas en la soja.

Palabras clave: Abamectina, Bacillus firmus, Meloidogyne incognita, nematicida aplicado a semillas, soja

### **INTRODUCTION**

*Meloidogyne incognita* (Kofoid and White), Chitwood, the southern root-knot nematode, is the most common root-knot nematode on soybean [*Glycine max* (L.) Merr.] in Arkansas (Kirkpatrick and Sullivan, 2018; Ye *et al.*, 2019). The total soybean grain yield loss in 2014 from *M. incognita* was estimated at 16 million bushels in Arkansas (Allen *et al.*, 2015). Root-knot nematodes were among the top five most destructive diseases of soybean from 2010-2014 in the Southern U.S., with a total estimated grain yield loss of 50 million bushels (Allen *et al.*, 2017).

Common management strategies for M. incognita include cultural practices, host plant resistance, and nematicides. A crop rotation sequence that includes a nonhost crop such as peanut (Arachis hypogea L.) can be beneficial; however, this option is limited in Arkansas due to total acreage of peanut compared to soybean. Some grain sorghum (Sorghum bicolor (L.) Moench) hybrids are less suitable hosts for M. incognita (Hurd and Faske, 2017; Xavier-Mis et al., 2017), but acreage has declined in recent years due to profitability of grain sorghum in the Mid-South. There are some soybean cultivars with a moderate level of resistance to M. incognita (Emerson et al., 2018; Emerson et al., 2019): however, host plant resistance is often underutilized in the Mid-South due to limited availability of a specific post-emergence herbicide technology or maturity group.

Over the past 15 years, there has been an increased use in seed-applied nematicides in soybean. This application method is convenient, reduces handler exposure to the pesticide, and delivers the nematicide in close proximity to the developing root system. *Bacillus firmus*, a grampositive, spore-forming bacterium, has been reported to affect *Meloidogyne* second-stage juvenile motility and suppress infection of cucumber (*Cucumis sativum* L.) and tomato (*Solanum lycopersicum* L.) (Giannakou *et al.*,

2004; Mendoza et al., 2008; Terefe et al., 2009). Furthermore, it was one of the first seed-applied bionematicides to be registered (2010) for use on soybean (US-EPA, 2010b). Abamectin was registered in 2010 as a seed-applied nematicide on soybean (US-EPA, 2010a) and has been reported to suppress M. incognita infection in tobacco (Nicotiana tabacum L.) and cotton (Gossypium hirsutum L.) (Sasser et al., 1982; Monfort et al., 2006). More recently, fluopyram, a succinate dehydrogenase inhibitor (SDHI) fungicide was registered in 2014 as a seed-applied nematicide on soybean (US-EPA, 2014). Fluopyram has been reported to affect M. incognita motility and infection of tomato (Faske and Hurd, 2015; Heiken, 2017); however, there is limited information as a seed treatment in field efficacy and at different nematode population densities. Therefore, the objectives of this study were to evaluate the field efficacy of seed-applied fluopyram on a M. incognita-susceptible and moderately resistant soybean cultivar and investigate the efficacy of seed-applied fluopyram at three *M. incognita* population densities.

# **MATERIALS AND METHODS**

#### Nematode culture and inoculum

*Meloidogyne incognita* was isolated from cotton and maintained in the greenhouse on tomato cv. Rutgers. Eggs collected from cultures with 0.5% NaOCl (Hussey and Barker, 1973) were used as inoculum.

#### Soybean cultivars and seed treatments

Two soybean cultivars were used: Delta Grow 'DG 4940 GLY' and 'DG 4970 GLY' (Delta Grow Seed Co. Inc., England, AR), which are moderately resistant and susceptible to *M. incognita*, respectively (Emerson *et al.*, 2019). Due to seed quality, 'DG 4880 GLY', a *M. incognita* usceptible cultivar, was used in 2019 instead of 'DG 4970 GLY'. All seed treatments were applied with a rotary seed treating system (UNICOAT 1200 CCS, Universal Coating Systems, Inc., Independence, OR). Four seed-applied nematicide treatments were used in this study with appropriate insecticide and without a fungicide treatment. Seed-applied treatments consisted of abamectin (Avicta® 500 FS) at 0.15 mg ai/seed + thiamethoxam (Cruiser<sup>®</sup> 5 FS, Syngenta Crop Protection, Greensboro, NC) at 0.12 mg ai/seed; fluopyram (ILEVO®, BASF Corporation, Florham Park, NJ) at 0.15 mg ai/seed + imidacloprid (Gaucho<sup>®</sup> 600 F) at 0.12 mg ai/seed; clothianidin + Bacillus firmus I-1582 (Poncho<sup>®</sup>/Votivo<sup>®</sup>, BASF Corporation, Florham Park, NJ) at 0.13 mg ai/seed; and a combination of fluopyram + clothianidin + Bacillus firmus I-1582. The nontreated control seed was coated with 0.15 mg imidacloprid/seed (Gaucho® 600 F, Bayer CropScience). Hereafter the nematicide treatments are referred to as abamectin, fluopyram, B. firmus, and fluopyram + B. firmus.

#### Field experiments

The field efficacy of seed-applied was evaluated in commercial nematicides production fields with a history of *M. incognita* in 2015 near Pine Bluff, AR, and in 2017 and 2019 near Kerr, AR. The 2015 field site was a Roxana silt loam (13% sand, 69% silt, and 18% clay, and <1% OM), while the 2017 and 2019 field site was a Rilla silt loam, but based on lab analysis, it was classified as a sandy loam (47% sand, 47% silt, 6% clay, and <1% OM).

Cultivars were planted on May 6, 2015; May 9, 2017; and May 28, 2019 at a seeding rate of 370,500 seed/ha. Weeds were controlled in plots based on recommendations by the University of Arkansas Cooperative Extension Service (Barber et al., 2019). The experimental design consisted of four, 7.62-m rows spaced 76 cm apart, separated by a 1-m fallow alley. Treatments were arranged in a randomized split-plot design with nematicide treatment as the main plot and soybean cultivar as sub plot. Each cultivar by treatment combination was replicated four times. Seedling population density (stand) was recorded at 14 days after planting (DAP) as total plants per meters of row. Seedling vigor was visually assessed at 14 DAP using a five-point scale with 1 = poor plant vigorand 5 = most vigorous plants. Phytotoxicity was

assessed at 14 DAP using a five-point scale with 1 = no damage and 5 = severe cotyledon necrosis. Soil samples were collected within each block at planting and at harvest. Soil samples were a composite of a minimum of 10 soil cores taken 15 to 20 cm deep with a 1.9-cm-diam. soil probe. Vermiform nematodes were collected with a Baermann funnel system and enumerated using a stereoscope. To determine nematode infection, 10 roots were arbitrarily sampled at 45 (2015 and 2017) or 60 (2019) DAP from non-harvest rows per plot. Gall rating was based on the percentage of root system galled. The center two rows of each plot were harvest on September 29, 2015; October 5, 2017; and November 5, 2019 with a K Gleaner combine (AGCO, Duluth, GA) equipped with a HarvestMaster<sup>TM</sup> Single BDS HiCap HM800 Weigh System (Harvest Master, Logan, UT). Grain yield was adjusted to 13% moisture.

#### Nematode population density experiments

A greenhouse pot assay was used to determine the efficacy of seed-applied fluopyram at different nematode population densities. Pasteurized sandy soil was filled into 10-cm-diam. (500 cm<sup>3</sup>) clay pots. Two seeds of the root-knot susceptible cultivar (DG 4970 GLY) were planted at 1.0 cm depth per pot. Plants were maintained in the greenhouse at 27±3°C. Seedlings were thinned at 7 DAP to one plant per pot. Seedlings were inoculated at the second-true leaf stage (10 DAP) with 50, 500, or 5,000 M. incognita eggs in 2 ml of water dispersed into 2 cm deep holes for an initial population density of 10, 100, and 1,000 M. incognita eggs/100 cm<sup>3</sup> soil. Roots were sampled at 30 DAP, blotted dry, and visually assessed for the percent of root system galled. Treatments, each nematicide by inoculum rate combination, were arranged in a randomized complete block design, replicated five times, and the experiment was conducted twice.

#### Statistical analysis

Data were analyzed using general linear mixed model analysis of variance with cultivars and nematicides modeled as fixed variables, and experiment repetitions and treatment replications modeled as random variables using IBM SPSS Statistics 25.0 (International Business Machines Corp., Armonk, NY). Data from greenhouse study were analyzed using a similar procedure with nematode rates as a fixed variable. Percent root system galled data were arsine transformed [arcsine (square root (x + 0.5)] to normalize for analysis and reverse transformed data are reported. Mean separation were based on Tukey's honest significant difference (HSD) test at  $\alpha = 0.05$ .

# RESULTS

There was an experiment by cultivar by nematicide interaction ( $P \leq 0.05$ ) for seedling population density, percent root system galled, and yield, thus data from each year are presented separately (Table 1). There was no effect of nematicide or cultivar on seedling population density in 2015 and 2019, but in 2017 there was a greater seedling population density with the moderately resistant cultivar 'DG 4940 GLY' than the susceptible cultivar 'DG 4970 GLY'. Treatments that contained fluopyram had an average phytotoxicity rating of 2.3 across experiments. The phytotoxic effect was a necrotic ring on 80-90% of the cotyledonary leaves in all experiments; however, this did not affect vigor or vield. There was no effect of nematicide or cultivar for seedling vigor. Overall seedling vigor ranged from 4.5-5.0 across experiments.

The percent root system galled in 2015 was very low at 45 DAP with an average of 0.3% across nematicide treatments. In contrast, in 2017 and 2019, there was a cultivar by nematicide interaction (P < 0.01) for percent of root system galled. In 2017, the percent root system galled was lowest (P $\leq$  0.05) on the moderately resistant cultivar regardless of nematicide used. While on the susceptible cultivar, fluopyram + B. firmus contributed to a lower ( $P \leq 0.05$ ) percent root galled compared to the nontreated control. Root galling, across cultivars, was lower ( $P \le 0.05$ ) with B. firmus compared to fluopyram, fluopyram + B. firmus and the nontreated control. In contrast, in 2019, abamectin and fluopyram contributed to a lower ( $P \leq 0.05$ ) percent roots galled on the moderately resistant cultivar than B. firmus and the nontreated control. While on the susceptible cultivar, fluopyram + B. firmus contributed to lower ( $P \le 0.05$ ) galling than the nontreated control. Root galling, across cultivars, was lower  $(P \leq 0.05)$  with abamectin, fluopyram, and fluopyram + B. firmus than B. firmus and the nontreated control. In both 2017 and 2019, the

moderately resistant cultivar, 'DG 4940 GLY', had fewer ( $P \le 0.05$ ) galled roots than the susceptible cultivar, 'DG 4970 GLY' and 'DG 4880 GLY'.

No effect of cultivar or nematicide was observed in 2015 for grain yield with an average yield of 3,652 kg/ha. In 2017, there was no interaction between cultivar and nematicide for grain yield, but the moderately resistant cultivar had a greater ( $P \le 0.05$ ) grain yield than the susceptible cultivar. Alternately, in 2019, there was a cultivar by nematicide interaction (P < 0.01) for grain yield, as fluopyram and fluopyram + B. firmus had a greater ( $P \le 0.05$ ) yield on the moderately resistant cultivar and no significant difference among nematicides on the susceptible cultivar. Further, across cultivars, fluopyram + B. *firmus* had the greatest ( $P \le 0.05$ ) yield compared to *B. firmus* and the nontreated control. There was a negative correlation in 2015 (r = -0.42, P =(0.007), 2017 (r = -0.95, P = 0.0001), and 2019 (r =-0.40, P = 0.001) between percent root system galled and yield. Based on soil samples collected at harvest the damage threshold for *M. incognita* on soybean in Arkansas was low in 2015 at 50 J2/100 cm<sup>3</sup> soil, but severe in 2017 and 2019 at 913 and 1,628 J2/100 cm<sup>3</sup> soil, respectively (Kirkpatrick et al., 2014).

There was a significant interaction between seed-applied nematicides and inoculum density in the greenhouse study. However, the effect of nematicide treatment was similar within low, moderate, and severe inoculum densities (Fig. 1). Fluopyram (0.3%), abamectin (0.5%) and fluopyram + *B. firmus* (0.6%) had a lower ( $P \le 0.05$ ) percent root system galled across inoculum densities compared to the nontreated control (1.2%).

#### DISCUSSION

The field efficacy of seed-applied fluopyram was similar to abamectin, *B. firmus* (strain I-1582), and fluopyram + *B. firmus* treated seed to suppress *M. incognita* infection and protect grain yield potential in soybean. There was a lower percent root system galled trend with a seed-applied nematicide, which in most cases (79%) contributed to a greater numeric grain yield. The most consistent protection of grain yield occurred when nematode population densities were low, which supports the use of seed-applied nematicides

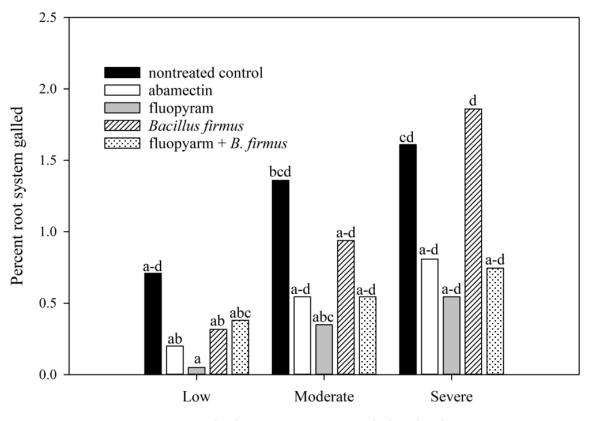
Table 1. Effect of seed-applied nematicides and soybean cultivars to manage <i>Meloidogyne incognita</i> in three field trials.	iematicides ar	id soybean cultivars	to manage <i>M</i>	<u> 1eloidogyne</u>	<i>incognita</i> in three	field trials.		0100	
		C107	Vialdy		7017	Viald		5013	Viald
Cultivar	$Stand^w$	% Root Galled <sup>x</sup>	(kg/ ha)	Stand	% Root Galled	t tetu (kg/ha)	Stand	% Root Galled	t tetu (kg/ ha)
Delta Grow 'DG 4940 GLY'	15.0	0.22	3,734	20.3 b	0.98 a	5,034 b	22.0	0.48 a	4,027 b
Delta Grow 'DG 4970 GLY'	15.9	0.37	3,570	17.2 a	3.21 b	2,260 a	20.8	3.15 b	3,203 a
Nematicide									
nontreated control	16.4	0.48	3,362	19.5	$2.28 b^z$	3,706	20.8	2.76 b	3,471 a
abamectin	15.7	0.04	3,616	18.6	1.98 ab	3,607	21.9	1.65 a	3,712 ab
fluopyram	14.0	0.42	3,923	18.1	2.16 b	3,355	20.9	0.80 a	3,601 ab
Bacilius firmus	15.2	0.31	3,588	18.9	1.67 a	3,822	21.6	2.56 b	3,407 a
fluopyram $+ B$ . firmus	16.1	0.32	3,740	18.1	2.23 b	3,729	21.7	1.26 a	3,885 b
Cultivar x Nematicide									
DG 4940, nontreated	15.3	0.27	3,591	21.1	1.00 a	4,877	20.8	1.51 bc	4,071 b
DG 4940, abamectin	15.7	0.26	3,577	20.7	1.37 a	4,984	23.0	0.05 a	4,004 ab
DG 4940, fluopyram	14.4	0.06	4,055	19.5	0.75 a	4,625	20.9	0.08 a	4,119 b
DG 4940, B. firmus	14.0	0.22	3,638	20.3	0.65 a	5,185	21.5	1.71 bc	3,695 ab
DG 4940, fluopyram +									
B. firmus	15.9	0.43	3,808	19.6	0.97 a	5,591	22.9	0.24 ab	4,242 b
DG 4970, nontreated	17.4	0.70	3,191	17.5	3.58 c	2,146	19.8	4.00 e	2,871 a
DG 4970, abamectin	15.7	0.05	3,656	17.1	3.21 bc	2,229	20.9	3.36 de	3,419 ab
DG 4970, fluopyram	13.7	0.57	3,792	17.1	2.94 bc	2,085	20.8	2.58 cde	3,083 ab
DG 4970, B. firmus	16.4	0.40	3,538	17.1	2.68 b	2,458	21.8	3.41 de	3,119 ab
DG 4970, fluopyram +									
B. firmus	16.3	0.22	3,672	16.7	3.64 c	2,332	20.6	2.32 cd	3,526 ab
Statistics: $P > F$									
Cultivar	0.31	0.18	0.38	0.02	<0.01	<0.01	0.15	0.05	0.01
Nematicide	0.18	0.10	0.16	0.78	<0.01	0.22	0.28	<0.01	0.01
Cultivar x Nematicide	0.45	0.55	0.82	0.94	<0.01	0.34	0.65	<0.01	0.04
<sup>v</sup> Delta Grow 'DG 4940 GLY' is moderately resistant while 'DG 4970 GLY' (2015 and 2017) and 'DG 4880 GLY' (2019) are susceptible to Meloidogyne	moderately re	sistant while 'DG 4	970 GLY <sup>,</sup> (2	2015 and 20	17) and 'DG 4880 (	GLY <sup>,</sup> (2019)	are suscep	otible to <i>Meloidogy</i>	ie
incognita.									
<sup>w</sup> Plants per row meter.									

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<sup>x</sup> Percent of root system galled at 45 to 60 days after planting.

<sup>y</sup> Grain yield was adjusted to 13.0% moisture. <sup>z</sup> Numbers in a column followed by a different letter are significantly different at  $\alpha = 0.05$  according to Tukey's honest significant difference test.



Meloidogyne incognita population density

Figure 1. Suppression of *Meloidogyne incognita* infection of soybean roots 30 days after inoculate at three nematode population densities in a greenhouse pot study. Population densities of low, moderate, and severe were 10, 100, and 1,000 *M. incognita* eggs/100 cm<sup>3</sup> soil, respectively. Different letters over bars indicate a significant difference at P = 0.05 according to Tukey's HSD test.

(2015) compared to when severe (2017 and 2019). Only one seed applied nematicide, fluopyram + B. firmus (I-1582), contributed to a greater numeric grain yield when nematode damage threshold were severe. Fluopyram is a fungicide and its benefits to suppress soilborne and foliar diseases has been reported (Kandel et al., 2016a, 2016b). Though no fungal diseases were observed in this study, it is unknown if the suppression of fungal pathogens played a role in yield protection. Other studies have reported a similar variability among seedapplied nematicides to suppress root infection and protect grain yield potential against *M. incognita*, Rotylenchulus reniformis Linford and Oliveira, and Heterodera glycines Ichinohe (Kandel et al., 2017; Hurd et al., 2018a, 2018b; Rondon et al., 2019a, 2019b; Roth et al., 2020). Limited movement of fluopyram from the seed coat and within sandy loam soils could account for some of the variability (Beeman et al., 2019; Faske and Brown, 2019).

These data support the use of host plant

resistance to provide season-long protection against *M. incognita*. The moderately resistant soybean cultivar contributed to a significant reduction in root galling and increase in grain yield when *M. incognita* population densities were severe. In the 2017 Arkansas soybean performance test, the average yield for the susceptible cultivar 'DG 4880 GLY' was 4,519 kg/ha and 'DG 4970 GLY' was 4,324 kg/ha, which is 8.9% and 4.2% greater, respectively, than the 4,149 kg/ha by the moderately resistant cultivar 'DG 4940 GLY' (Bond et al., 2017). In contrast, in this study, the reverse was true as 'DG 4940 GYL' had a 25.7% and 50.4% greater yield compared to 'DG 4880 GLY' and 'DG 4970 GLY', respectively, which supports the use of host plant resistance in soybean fields infested with M. incognita in Arkansas and the Mid-South.

Seed-applied nematicides provided a numeric suppression of *M. incognita* infection across low, moderate, and severe population densities. These

trends were similar to that observed in the field; however, in the greenhouse, seed treated with abamectin and fluopyram had fewer galls than seed treated with *B. firmus*. Though the water solubility of abamectin and fluopyram are very low and moderate, respectively (Wislocki et al., 1989; ESFA, 2013), overhead irrigation in the greenhouse may have distribute a greater proportion of the nematicide from the seedcoat downward into contact with second-stage juveniles in sandy soil. In a similar study, seed-applied fluopyram provided better suppression of H. glycines than B. firmus on soybean (Beeman and 2018). Furthermore, seed-applied Tylka, fluopyram was reported to provide a numeric suppression of M. incognita reproduction compared to B. firmus (Hurd et al., 2015). Bacillus firmus has been reported to suppress galling of M. incognita on cucumber and tomato; however, it was incorporated into the soil, which potentially provided better opportunity for contact between the bionematicide and infective second-stage juveniles (Giannakou et al., 2004; Terefe et al., 2009).

Suppression of *M. incognita* infection and yield protection by seed-applied fluopyram was similar to that of other seed-applied nematicides in soybean. Given that fluopyram is a fungicide, it may be more beneficial in fields with a history of fungal diseases and plant-parasitic nematodes of soybean.

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