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The Effects of Seed-Applied Fluopyram on Root Penetration and Development of Meloidogyne incognita on Cotton and Soybean

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The Effects of Seed-Applied Fluopyram on Root Penetration and Development of *Meloidogyne incognita* on Cotton and Soybean

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ABSTRACT

Plant-parasitic nematodes are major pests of cotton and soybean in Arkansas, and across the Southern United States. These nematodes cause more than $3 billion worth of crop losses each year, in part due to lack of available control tactics, such as nematicides. Fluopyram has recently been registered as a seed-treatment nematicide in agronomic crops. The toxicity of fluopyram against *Meloidogyne incognita* infection has been reported, however, information on root protection provided by fluopyram against *Meloidogyne incognita* is lacking. The first objective of this research was to evaluate the effect seed-applied fluopyram had on nematode development, root galling, and reproduction on cotton and soybean roots. Fluopyram significantly reduced nematode penetration, gall development, and reproduction compared to the non-treated control in both cotton and soybean. This effect in root protection was similar to that of an industry standard nematicide, abamectin. Neither nematicide had an effect on nematode post-infection development in soybean and cotton. The second objective was to evaluate the distance in root protection provided by fluopyram-treated seed. Seed-applied fluopyram suppressed the root penetration of second-stage juveniles inoculated at a 2.5- and 5.0-cm depth in cotton, whereas only those inoculated at 2.5 cm were suppressed in soybean. These data indicate that the extent of root protection provided by seed-applied fluopyram has a greater effect on suppression of second-stage juvenile suppression than post-infection development and that protection is greatest in close proximity to the seed. Overall, the degree of nematode protection provided by seed-applied fluopyram was similar to root protection provided by abamectin.

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DEDICATION

This thesis is dedicated to my family Kelly, Bill, Erin, Will, and Cassidy. For always believing in me, inspiring me, encouraging me, and making fun of me.

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LITERATURE REVIEW

MELOIDOGYNE SPP.

Meloidogyne is derived from two Greek words that mean “apple-shaped” and “female” to describe the swollen female present in the nematode life cycle. Originally, all the root-knot nematodes were considered one species and there were many early conflicting reports on the resistance or susceptibility of plant hosts as well behavioral patterns in the literature (Berkeley, 1855; Chitwood, 1949; Perry, 2009). It wasn’t until 1949 that Chitwood differentiated and described four widespread and common *Meloidogyne* species: *M. incognita, M. javanica, M. arenaria,* and *M. hapla* (Chitwood, 1949).

Root-knot nematodes all have a similar life cycle. The nematodes overwinter in the soil as eggs in egg masses attached to roots from the previous growing season. Inside of the eggs, embryogenesis occurs resulting in the first-stage juvenile followed by a molt to the infective second-stage juvenile (J2) (Perry et al., 2009). When the soil temperature is between 25-30°C and soil air is near 100% humidity, the eggs hatch and the J2 migrate to the host root (Taylor and Sasser, 1978). Second-stage juveniles are vulnerable to environmental stresses in the soil and cannot survive long periods of time in the absence of a host root. The J2 invade the root behind the root tip and migrate through the root intercellularly until they initiate a permanent feeding site. Root-knot nematodes penetrate the host cell wall with a specialized feeding structure (stylet) and inject secretory proteins that induce giant cells. Giant cells form by repeated mitosis without cytokinesis and are multinucleate with each nucleus containing many sets of chromosomes (Jones and Goto, 2011). Each giant cell could have up to a 600-fold increase in copy number of plant genes, producing a large amount of protein for the nematode to ingest (Perry et al., 2009). Once a giant cell has been initiated and the J2 begins to develop within this permanent feeding

site, the nematode becomes sedentary and enlarges, undergoing three more molts to become an adult. If the juvenile differentiates into an adult male, the nematode will either exit the root and mate with a female, or, as is the case with *M. incognita,* the male will exit the root and die. If the juvenile differentiates into an adult female, she will continue to feed and enlarge, causing her posterior end to break through the epidermis of the root where she will deposit several hundred eggs in a gelatinous matrix outside her body (Perry et al., 2009). Because *M. incognita* reproduces via mitotic parthenogenesis, males are vestigial and are not often found in routine soil samples. Due to the short life cycle of root-knot nematodes (approximately 20-30 days), several life cycles will occur during one cropping season, exponentially increasing populations by the end of the season.

The primary symptom for identifying *Meloidogyne* spp. is the presence of galls on infected roots. Galls are formed by cellular hypertrophy and are the exterior appearance of giant cells on the roots. Galls are often coalesced and the number and size of galls per root system depends on host susceptibility and population density in the soil. Galling of roots below ground creates varying plant responses above ground. Galls interfere with normal root function, causing plants to display symptoms such as wilting, loss of vigor, nutritional deficiencies, stunting, and yield loss (Kirkpatrick et al., 2014). *Meloidogyne incognita* infection on cotton has been shown to inhibit water flow through roots up to 28%, mimicking the conditions observed in severe water stress environments (Kirkpatrick et al., 1991). The severity of yield suppression caused by *Meloidogyne* spp. is dependent on three main factors: i) the population density of the nematodes in the soil and on roots from the previous growing season ii) the severity of other stress factors in the field and iii) host susceptibility.

The southern root-knot nematode (*Meloidogyne incognita)* is the most prevalent nematode in Arkansas (Ye et al., 2019) and a major pathogen to cotton and soybean across the southern United States (Allen et al., 2017; Lawrence et al., 2017). According to the Cotton Disease Council, plant parasitic nematodes (*Meloidogyne incognita* and *Rotylenchulus reniformis*) were responsible for an average estimated loss in US cotton of about 4.6% in 2016 (Lawrence et al., 2017). In 2014, non-soybean cyst nematodes (including *Meloidogyne* spp.) were the second most destructive pathogens on soybean in the southern US, resulting in 510,399,487 kg of lost soybean production (Allen et al., 2017).

ROOT-KNOT NEMATODE MANAGEMENT PRACTICES

Nematode management strategies include the use of one or several of the following: resistant cultivars, crop rotation, chemical control, or biological control. Where resistant cultivars are available, host-plant resistance is an effective and economical strategy to manage plant-parasitic nematodes on soybean and cotton (Kirkpatrick et al., 2014). Although there are moderately-resistant group 4 and 5 soybean cultivars that yield significantly higher than susceptible cultivars in nematode infested fields (Emerson, et al., 2018), there are few highly resistant cultivars. There are also southern-root-knot-nematode-resistant cotton cultivars available, but these cultivars often yield less than susceptible cotton cultivars in the absence of nematodes (Starr et al., 2007). Because root-knot nematodes have a patchy and aggregated distribution in the field, some growers continue to select a high-yielding soybean or cotton cultivar that is susceptible to *M. incognita* with the assumption that they will perform well in the areas of the field where nematodes are not present. While this strategy may be economically feasible in certain fields, success requires a detailed understanding of the nematode distribution

and density fieldwide – information that is generally lacking. Additionally, weed control in the mid-south is a major issue, and some weeds that are nematode hosts are resistant to many herbicides. Unfortunately, because resistance to root-knot nematode in soybean is mainly found in the glyphosate-tolerant cultivars, farmers with glyphosate-resistant pigweed (*Amaranthus*) have even more limited options for cultivar selection. This is also the case in glufosinate-resistant cultivars or those tolerant to dicamba (Emerson et al., 2018). Thus, it is vital that growers have other options in nematode management, where a resistant cultivar may not be feasible.

Crop rotation is another option to manage nematode population densities. The inclusion of non-host plants in a crop rotation sequence is one of the most effective ways to maintain nematode populations below the action threshold. If *Meloidogyne incognita* is severe in a field, growing peanut (*Arachis hypogaea*), rice (*Oryza sativa*) or some grain sorghum (*Sorghum bicolor*) hybrids can effectively lower populations (Kirkpatrick et al., 2014; Hurd and Faske, 2017). While rice is considered a susceptible host for *Meloidogyne incognita*, it is an effective rotation crop because of the flooding that occurs during rice production, preventing nematode survival in soil. Peanut is considered a non-host to *M. incognita* and incorporating peanut into a cotton cropping sequence has been shown to effectively reduce the number of *M. incognita* in the soil as well as increase cotton yield (Johnson et al., 1998; Kirkpatrick and Sasser, 1984). Rotation to grain sorghum is effective as long as the hybrid selected is not susceptible to root-knot nematode reproduction. Some grain sorghum hybrids are resistant to *M. incognita*, but there is considerable variation between hybrids relative to host susceptibility, so nematode reproduction potential must be considered (Hurd and Faske, 2017; Xavier-Mis et al., 2014). Although crop rotation is a good strategy for managing nematode population densities, for

rotation crops to be practical, the rotational crop must provide an adequate income to the grower to justify rotating land from cotton or soybean production. For example, due to current commodity prices, grain sorghum is not as profitable as cotton or soybean. There is also not enough peanut acreage to rotate with cotton or soybean and some of the soils where cotton and soybean are grown are not suited for peanut or rice production.

Chemical control of nematodes involves the use of nematicides. In order for a nematicide to be effective, toxic concentrations of the chemical must come in contact with the nematode for sufficient time. Nematicides are classified as either fumigants or non-fumigants. Fumigants are generally broad-spectrum pesticides that are injected into the soil pre-plant and move through air spaces in the soil as a gas. They kill nematodes and other soil borne organisms that are present in the soil before planting occurs. They do not bind to soil particles, so residual pesticidal activity is short-lived. Fumigants can be categorized as either nematicidal, targeting only nematodes, or multi-purpose, targeting fungi, nematodes, weeds, and insects (Nyczepir and Thomas, 2009). While most fumigant nematicides are considered effective at controlling nematode populations, the Environmental Protection Agency (EPA) has suspended registration of many fumigants or they have been voluntarily withdrawn from use (Nyczepir and Thomas, 2009). A fumigant nematicide that is currently recommended for control of *M. incognita* and other nematodes in cotton and soybean production systems in Arkansas is 1,3-dichloropropene (Telone II®, Corteva Agriscience, Wilmington, DE). Telone II® is recommended when nematode pressure is severe (Faske et al., 2019).

Non-fumigant nematicides can be either liquid or granular formulations that have low volatility, are dispersed into the soil by water, and are more commonly used than fumigants. Historically, the two popular classes of non-fumigant nematicides included carbamates and

organophosphates. Both of these classes of chemistry inhibit the enzyme acetylcholinesterase that is responsible for degradation of the neurotransmitter acetylcholine. Over time, when this enzyme is inhibited, acetylcholine accumulates throughout the nervous system, causing malfunction of the muscular systems in the nematode. Disruptions of these systems can affect nematode movement, behavior, and infection process, leading to nematode paralysis. Aldicarb (AgLogic® 15GG, AgLogic Chemical, Chapel Hill, NC) is an example of a non-fumigant nematicide recommended for use in *M. incognita* infested cotton fields in Arkansas (Faske et al., 2019).

SEED-TREATMENT NEMATICIDES

Seed treatments are the application of chemical or biological agents to the outside of seeds prior to planting to provide protection from pests and pathogens in early stages of growth and improve establishment of crops. Abamectin (Avicta® 500 FS, Syngenta, Greensboro, NC) is an example of a seed-treatment nematicide recommended for use against *M. incognita* and *R. reniformis* in cotton and soybean in Arkansas (Faske et al., 2019). The compound abamectin is a mixture of approximately 80% avermectin B1a and 20% avermectin B1b, both of which are natural fermentation byproducts of the actinomycete *Streptomyces avermitilis* (Campbell, 1989). Abamectin affects nematodes by blocking the transmission of electrical activity in nerve and muscle cells by enhancing the effects of the glutamate-gated chloride channels, causing an influx of chloride ions into cells, leading to muscular paralysis and eventually death (Campbell, 1989). In a study done by Faske and Starr (2006), a nematode mobility assay was conducted where 24-hr-old *M. incognita* J2 were exposed to varying concentrations of abamectin to observe nematode paralysis and mortality. When nematodes were exposed to the LD_{50} value 1.56µg

abamectin/ml for 2 hours, nematode mortality was observed (Faske and Starr, 2006) This work confirmed that abamectin is nematicidal, as its effects are not reversible. In greenhouse trials with abamectin-treated cotton seed inoculated with *M. incognita* eggs, it was observed that root galling was less severe and all abamectin seed treatments resulted in lower eggs per gram of root (Monfort et al, 2006). By contrast, in a similar study, it was shown that abamectin-treated cotton seed (150µg abamectin/seed) inoculated with *M. incognita* J2 had variable protection from penetration and there were no differences in nematode reproduction between abamectin-treated and non-treated control seed (Faske and Starr, 2007). It is likely that the difference between the two studies was related to the different concentrations of abamectin used, as it was found that in cotton seed treated by the manufacturer at 150µg abamectin/seed, more abamectin remains on the seed coat than the developing radicle and protection decreases as the cotton taproot length increases (Faske and Starr, 2007). Variability could have also been a result of different inoculation techniques. There has also been variability in the effect abamectin has on post-penetration development of nematodes. Stretton et al. (1987) reported that abamectin had no effect on *M. incognita* development on tomato roots, while Cayrol et al. (1993) reported that growth of *M. arenaria* after entering tomato roots was affected by abamectin.

Another example of a seed-treatment nematicide recommended for use against *M. incognita* in cotton in Arkansas is a mixture of a carbamate nematicide, thiodicarb and the insecticide imidacloprid (Aeris® 5 FS Bayer CropScience, Research Triangle Park, NC) (Faske et al., 2019). It has been reported that thiodicarb has poor mobility in soil and rapidly degrades to methomyl and field trials as a seed-treatment nematicide have been variable (Hurd et al., 2016; Jones et al. 1989; Lawrence and Lawrence, 2007).

Two other seed-treatment products that are marketed in cotton and soybean for nematode control in Arkansas include a combination of the insecticide clothianidin and the bacterium *Bacillus firmus* (Poncho/VOTiVO®, Bayer CropScience, Research Triangle Park, NC) and treatment with *Burkholderia rinojensis* (BioST® nematicide 100, Albaugh, Ankeny, IA). Both of these products are considered biological nematicides (Faske et al., 2019). Biologicals utilize living organisms that kill or damage nematodes or indirectly influence their establishment, function, and survival (Perry et al., 2009). Biological control nematicides are becoming more widely investigated because of the increasing interest in understanding effects of pest management practices on biodiversity in the soil. There are numerous reports of *Bacillus* spp. investigated as biological control organisms, and some studies show their ability to reduce root-knot nematode populations (Stirling, 1984; Xiang et al., 2017). However, soybean field trials with Poncho/VOTiVO® indicate little protection against *M. incognita* infection (Hurd et al., 2017; Land and Lawrence, 2014). Similarly, *Burkholderia rinojensis* A396 has shown no yield protection as a cotton seed treatment (Faske et al., 2019; Xiang et al., 2018)

FLUOPYRAM AS A FUNGICIDE

Fluopyram is a succinate dehydrogenase inhibitor (SDHI) fungicide with a fungicide resistance action committee (FRAC) code of seven. Succinate dehydrogenase inhibitor fungicides prevent energy production in fungal cells. They do this by binding to the succinate dehydrogenase (SDH) complex which then blocks the transport of electrons in the respiratory chain within the mitochondria, halting the production of adenosine triphosphate (ATP), a molecule essential to energy in living organisms.

Fluopyram as a fungicide was approved by the US EPA in 2012 and developed for the protection against a range of diseases caused by Ascomycetes and Deuteromycetes on various crops. Seed-applied fluopyram has been reported to suppress sudden death syndrome (SDS) of soybean, which is caused by the soilborne pathogen *Fusarium virguliforme*, and protect yield potential in field trials (Wang et al., 2014). Similarly, fluopyram seed-treatment and in-furrow application were effective at reducing SDS and increasing yield relative to the control in fields across the Midwestern U. S. (Kandel et al., 2016).

FLUOPYRAM AS A NEMATICIDE

Fluopyram is a succinate dehydrogenase inhibitor nematicide as well as a fungicide. Similarly to the mode of action against fungi, fluopyram targets the same enzymes in the mitochondrial respiration chain of nematodes as it does fungi. The mode of action against the nematodes was reported through the use of mutant *Caenorhabditis elegans* with a knockout gene, leading to reduced expression of succinate dehydrogenase (Heiken, 2017). The mutant *C. elegans* were more sensitive to exposure of fluopyram, indicating that succinate dehydrogenase is the target enzyme of fluopyram in nematodes (Heiken, 2017).

Fluopyram is translocated in plants upward through the xylem, so in order for fluopyram to be effective at reducing root infection, toxic concentrations must come in contact with the nematode. The 2-hr EC_{50} value of fluopyram for *M. incognita* was reported to be 5.18 μg/ml. Fluopyram is considered to be nematistatic, as 58% of nematodes recovered after 1-hr exposure to fluopyram EC_{50} (Faske and Hurd, 2015). *In planta* studies with different concentrations of fluopyram have had varying results. Faske and Hurd (2015) reported that the 2-hr EC_{50} value of fluopyram was effective at reducing *M. incognita* infection on tomato roots, while Wram and

Zasada (2019) reported that the same concentration of fluopyram had no effect on *M. incognita* reproduction on tomato.

Fluopyram is marketed as a non-fumigant nematicide as Velum® Prime and Velum® Total (Bayer CropScience, Research Triangle Park, NC) and as a soybean and cotton seed treatment as ILeVO® and COPeO® Prime (BASF Agricultural, Florham Park, NJ), respectively. Velum® Prime is labeled for use against root-knot and cyst nematode and several fungal diseases including white mold, early blight, powdery mildew, and Alternaria. Velum® Total is a mixture of fluopyram and the insecticide imidacloprid, to control insect and nematode populations on cotton and peanut. In cotton field trials, a 45% reduction in *M. incognita* reproduction was reported on treatments that received an in-furrow spray of fluopyram compared to a control (Till et al., 2017). However, other studies have shown no yield protection with fluopyram as an in-furrow spray on cotton (Faske et al., 2019; Hurd et al., 2016).

The movement of seed-applied fluopyram through sandy soil (100% sand) and its efficacy was evaluated against *M. incognita* on cotton and soybean and it was found that fluopyram had no effect on nematode mortality past a depth of 10-cm with the majority of mortality at 0-5 cm (Faske and Brown, 2019). These authors also reported that in the upper 5 cm of the soil, fluopyram concentrations accounted for 4.8 and 1.3% of the fluopyram applied to the cotton and soybean seed coat, respectively (Faske and Brown, 2019). In a root penetration experiment, fluopyram-treated soybean seeds were evaluated against *Heterodera glycines* at different inoculation depths (Beeman et al., 2019). There was an 87% reduction in the number of penetrating J2 at 2.5-cm compared to the non-treated control and there were no significant effects of fluopyram at the 5- or 7.5-cm inoculation depths (Beeman et. al, 2019). These studies indicate that fluopyram has moderate to poor mobility in soil.

In the greenhouse, fluopyram seed treatment was successful at reducing *M. incognita* infection on soybean and suppressing nematode reproduction at all levels evaluated, including the industry-recommended rate at both 30- and 60-days after inoculation (Hurd and Faske, 2016). In field trials with fluopyram-treated soybean seed in a *M. incognita* infested field in Arkansas, there were no statistical differences in yield between fluopyram-treated and untreated seed, however, fluopyram-treated seed had a numerically higher yield (Jackson et al., 2017). The same results were observed in field trials in a *H. glycines* infested field in Kentucky (Lawrence, 2017). In one of the field trials, seedling phytotoxicity ratings taken at 14 DAP revealed that fluopyram-treated seed had significantly higher phytotoxicity than non-nematicide treated seed (Jackson et al., 2017). Cotyledon phytotoxicity has been widely reported in association with fluopyram-soybean seed, often referred to as the halo-effect (Jackson et al., 2017; Lawrence, 2017; Kandel et al., 2018).

While it has been shown that fluopyram is effective at suppressing *M. incognita* infection in lab and greenhouse experiments, there is little information on the impact of seed-applied fluopyram on nematode development or characterization of root protection.

EFFECT OF SEED-APPLIED FLUOPYRAM ON *MELOIDOGYNE INCOGNITA*

INTRODUCTION

Upland cotton (*Gossypium hirsutum)* and soybean (*Glycine max)* are staple crops in the southern United States, and specifically in Arkansas. In 2018, over 1.2 million hectares of soybean and 154,000 hectares of cotton were harvested in Arkansas (USDA-NASS, 2018). In the southern U.S., there are many diseases and nematodes that affect cotton and soybean production (Thomas and Kirkpatrick, 2001; Hartman et al., 2016). According to the Cotton Disease Council, the total percent of cotton lost in the U.S. in 2016 was estimated at 12.5%, with plant-parasitic nematodes responsible for the greatest average percent loss, estimated at 4.3% (Lawrence et al., 2017). As for soybean, the top five diseases that contributed to the greatest soybean yield losses in the southern U.S. in 2014 were soybean cyst nematode (*Heterodera glycines*), non-soybean cyst nematodes (including *Meloidogyne* spp.), sudden death syndrome (*Fusarium virguliforme*), frogeye leaf spot (*Cercospora sojina*), and charcoal rot (*Macrophomina phaseolina*) (Allen et al., 2017). The impact of these pests and pathogens on soybean was estimated to cause an average annual loss of production of 350 million kg per year from 2010-2014, resulting in an average annual loss of US$158,244,000 in Arkansas (Allen et al., 2017).

Among plant-parasitic nematodes, root-knot nematodes are the most economically important with a worldwide distribution, resulting in devastating crop loss (Perry et al., 2009). Although there are four major species of *Meloidogyne* in the southern U.S., *M. incognita* is the most common species in cotton and soybean fields in Arkansas (Ye, et al., 2019). The host range is extremely broad that mostly consists of cultivated crops and ornamentals. The primary symptom for identifying *Meloidogyne* spp. is the presence of galls on infected roots. Galls are often coalesced and the number of galls per root system depends on host susceptibility and

nematode population density in the soil. The above-ground symptoms for both cotton and soybean can vary from wilting, loss of vigor, and nutrient deficiencies which can lead to yield suppression and increased susceptibility to other diseases (Thomas and Kirkpatrick, 2001). The severity of yield suppression caused by the root-knot nematode is dependent on three main factors: i) the population density of the nematodes in the soil and on roots from the previous growing season ii) the severity of other stress factors in the field and iii) host susceptibility.

Meloidogyne incognita overwinters as eggs in egg masses attached to roots from the previous growing season. First-stage juveniles (J1) develop while the nematode is inside the egg shell. After the first molt, the nematode hatches as a second-stage juvenile (J2), the only stage that can initiate infection. They attack root tips and enter roots intercellularly, migrating to an area of cell elongation where they begin to feed. Salivary secretions from the nematode cause physiological changes, transforming plant cells into multinucleate giant-cells. Three more molts occur *in situ* to give rise to an adult female or male. In a favorable host, several hundred eggs can be produced by each female (Karssen and Moens, 2006). Although males can be present in *M. incognita* populations, they are vestigial, with reproduction occurring exclusively via parthenogenesis. There is no sperm contribution from males (Calderon-Urrea et al., 2016). When present, vermiform males remain in the soil with no evidence of feeding (Perry et al., 2009).

Root-knot nematode management strategies in Arkansas include a combination of resistant cultivars, crop rotation, and chemical/biological control. In soybean, a few moderately-resistant maturity group IV and V cultivars are available that yield significantly greater than susceptible cultivars in nematode infested fields (Emerson, et al., 2018). There are southern-root-knot-nematode-resistant cotton cultivars available (Wheeler et al., 2018), but these cultivars often yield less than susceptible cotton cultivars in the absence of nematodes (Starr et al., 2007).

Crop rotation is another option to manage nematode population densities. Incorporating poor or non-host crops into a cropping sequence is one of the most economically effective ways to manage nematode populations. Crops suggested for rotation for management of *M. incognita* include peanut (*Arachis hypogaea*), rice (*Oryza sativa*) and some grain sorghum (*Sorghum bicolor*) hybrids (Kirkpatrick et al., 2014; Hurd and Faske, 2017). Unfortunately, these rotational crops are not always practical in Arkansas. For example, with 10,500 hectares of peanut grown in Arkansas in 2018 compared to 1,295,000 hectares of soybean and 196,000 hectares of cotton, rotation from cotton or soybean to peanut is limited (USDA-NASS, 2018). Rice may not be an appropriate rotation crop because soils that favor root-knot nematode are often coarse-textured compared to silt loam or loamy clay soil that are the most suitable for rice production (Hardke, 2018). Rotation to grain sorghum is effective as long as the hybrid selected is a poor host for root-knot nematode reproduction. Some grain sorghum hybrids are resistant to *M. incognita*, but there is considerable variation between hybrids, so nematode reproduction potential on the specific hybrid must be considered (Hurd and Faske, 2017; Xavier-Mis et al., 2017).

Nematicides are generally categorized as either chemical or biological agents. Biological control of nematodes utilizes the aid of living organisms that kill or damage nematodes or indirectly influence their infection of a host (Perry, 2009). Recently, biological agents have been widely investigated because of the increasing concern for the environment and the importance the effects pest management practices may have on biodiversity in the soil. While biological controls have promise for nematode management, the effectiveness of those that are currently suggested to reduce *M. incognita* have been quite variable and oftentimes ineffective (Joyce and Thiessen, 2018; Land and Lawrence, 2014; Xiang et al, 2017).

Chemical nematicides act directly to either kill the nematode or disrupt the infection process. In order for these pesticides to be effective, toxic concentrations must come in contact with the nematode for a sufficient time to adversely affect the pathogen. Nematicides can be classified as either fumigants or non-fumigants. Fumigants are broad-spectrum pesticides that are applied to the soil prior to planting, move through soil pore spaces as a gas, and are usually toxic to a broad range of organisms in addition to nematodes. Fumigants are not taken up by the plant or bound to soil particles. Non-fumigant nematicides can be either liquid or granular formulations that are dispersed into the soil by water or mechanical incorporation. These products may or may not be systemic in plants and have historically been more broadly used than fumigants. Currently, the list of available and effective nematicides is short, in part, due to the small market share of nematicides relative to herbicides or insecticides and due to public concerns over human health risk and environmental toxicity (Perry et al., 2009). Application of nematicides to seed is another method to deliver compounds in close proximity to developing root systems. Seed treatments can be chemical or biological and offer several advantages as a tactic for nematode management. For example, seed treatments place the product right on the seed, theoretically protecting seedling roots during the first critical stages of crop development (Munkvold, et al., 2014). Other advantages to seed treatments are ease of nematicide application for the grower, relatively low amount of product that is used per hectare, and reduced risk of exposure to grower and handler (Munkvold et al., 2014).

One of the first chemicals that was developed as a seed treatment for nematode management in cotton and soybean was a group of compounds called avermectins (Campbell, 1989). Abamectin, an avermectin originally produced as a natural fermentation product of the actinomycete *Streptomyces avermitilis*. Control of *M. incognita* by seed-applied abamectin has

been thoroughly examined and protection has been variable (Faske and Starr, 2007; Monfort et al., 2006). Another example of a seed-treatment nematicide is with the compound fluopyram. Fluopyram was originally marketed as an agricultural fungicide that was found to have an effect on nematodes. While fluopyram has been effective at suppressing *M. incognita* in cotton and soybean and protecting yield potential in some field trials (Hurd et al., 2017; Lawrence et al., 2016), little is known on the impact of seed-applied fluopyram on nematode development and further characterization of root protection is needed.

The objectives of this study were to evaluate the effects of seed-applied fluopyram on *M. incognita* infection, galling, and reproduction on cotton and soybean roots and to characterize the extent of seedling root protection provided by fluopyram as a seed treatment.

MATERIALS AND METHODS

Nematode inoculum and egg extraction

Cultures of *Meloidogyne incognita*, host race 3, originally collected from either cotton in Leachville, AR or soybean in Kerr, AR, were maintained on tomato (*Solanum lycopersicum* 'Rutgers'). Eggs were extracted from tomato roots with 0.05% NaOCl (Hussey and Barker, 1973). Collected eggs were placed in a hatching chamber (Vrain, 1977) and J2 were collected from hatching chamber every 24 hours. Only 24-48-hr old J2 were used as inoculum.

Seed treatments

Seeds of a *M. incognita*-susceptible cotton cultivar (cv. Stoneville ST 4848) were used throughout this study. Cotton seed treatments were commercially-applied and all cotton seed was treated with a base fungicide of 15 μg metalaxyl/seed + 5 μg penflufen/seed + 5 μg

prothioconazole/seed + 3 µg myclobutanil/seed (Allegiance® FL + EverGol® Prime + Proline® 480 SC, Bayer CropScience, Research Triangle Park, NC and Spera™ 240 FS, Nufarm Americas Inc., Alsip, IL). Treatment groups included a fluopyram-treated, abamectin-treated, and a non-treated control. Fluopyram-treated seed consisted of 250 µg fluopyram/seed (COPeO®, BASF Chemical, Florham, NJ) + 375 µg imidacloprid/seed (Gaucho® 600 FS Bayer CropScience, Research Triangle Park, NC). Abamectin-treated seed consisted of 150 µg abamectin/seed (Avicta® 500 FS, Syngenta Crop Protection, Greensboro, NC) + 340 µg thiamethoxam/seed (Cruiser® 5 FS, Syngenta Crop Protection, Greensboro, NC). The non-treated control seed consisted of 120 µg imidacloprid/seed (Gaucho® 600 FS Bayer CropScience, Research Triangle Park, NC).

A *M. incognita* susceptible soybean cultivar (cv. Delta Grow DG 4970) was used for soybean trials (Emerson et al., 2018). Soybean seed treatments were applied with a Unicoat 1200 CCS seed treater (Universal Coating Systems, Inc, Independence, OR). All soybean seed received a base fungicide treatment of 8 µg prothioconazole/seed + 4 µg penflufen/seed + 6.5 µg metalaxyl/seed (EverGol® Energy SB, Bayer CropScience, Research Triangle Park, NC). Fluopyram-treated seed included 150 µg fluopyram/seed (ILeVO®, BASF Chemical, Florham Park, NJ) + 120 µg imidacloprid/seed (Gaucho® 600 FS Bayer CropScience, Research Triangle Park, NC). Abamectin-treated seed received 150 µg abamectin/seed (Avicta® 500 FS, Syngenta Crop Protection, Greensboro, NC) + 150 µg thiamethoxam/seed (Cruiser® 5 FS, Syngenta Crop Protection, Greensboro, NC). The non-treated control seed consisted of 120 µg imidacloprid/seed (Gaucho® 600 FS Bayer CropScience, Research Triangle Park, NC).

Greenhouse experiments

A time course experiment was conducted to evaluate the effect of seed-applied fluopyram on suppression of *M. incognita* infection, development, and reproduction. Course (< 2.0-mm-diameter) pasteurized sand (90% sand, 6% silt, 4% clay; pH 7.3; CEC 3.6 cmol+/kg) was infested with nematode inoculum at a concentration of two J2/cm^3 soil and was packed into two different size Deepots (Stuewe and Sons, Inc, Corvallis, OR). One seed of either cotton or soybean was planted in each Deepot at a 1.75-cm depth. Treatments included abamectin, fluopyram, and non-treated control seeds, as indicated above. Treatments were arranged in a randomized complete block design with five replications per treatment. Roots were sampled at five collection dates (7, 14, 21, 28, and 35 days after planting (DAP)) and consisted of five replications. Plants that were collected on the first three sampling dates were planted in 164 cm^3 Deepots while plants collected on the last two sampling dates were planted in 656 cm^3 Deepots.

To promote germination and minimize evaporation, Deepots were covered with plastic wrap and incubated at 30°C until seedling emergence. Once seedlings began to emerge, usually after about 72 hr, the plastic wrap was removed and Deepots were moved to the greenhouse where ambient temperatures ranged from 26 to 33°C. Plants were overhead watered daily and fertilized with 1.85 ml of Osmocote 20-20-20 (Scotts Miracle-Gro, Marysville, OH) every 14 days. Each experiment was conducted twice.

Roots sampled at 7, 14, and 21 DAP were stained with acid fuchsin following methodologies derived from Byrd et al. (1983). Stained nematodes were enumerated with a stereomicroscope. In order to determine post-penetration development, four different life stages of *M. incognita* were recorded: J2, swollen J2, female, and female with eggs (Fig. 1). Roots collected at 21, 28, and 35 DAP were assessed for number of galls per root system. Eggs were

extracted from roots sampled at 28 and 35 DAP using 1% NaOCl and enumerated with a stereomicroscope.

Root penetration experiment

Four experiments were conducted to determine the depth of root protection provided by fluopyram-treated cotton and soybean seed. For this assay, 50-mlconical tubes (Thermo Fisher Scientific Inc., Waltham, MA) were filled with the soil mix described above to a bulk density of 1.4 g/cm^3. Nematicide-treated and non-treated soybean and cotton seeds were planted 2-cm deep and each tube received 5 mL of water. Tubes were incubated at 30°C in low light conditions and were over-head watered daily for 5 days. Developing cotton and soybean roots were inoculated at five DAP by depositing 250 24-hr-old *M. incognita* J2 in 200 µl of water. Inoculations were deposited into a small hole drilled at either 2.5-, 5-, or 7-cm deep in the side of the tube and inoculum was dispensed at the center of each tube. Forty-eight hours after inoculation, roots were removed from conical tubes, rinsed with water, and stained with acid fuchsin (Byrd et. al, 1983). The treatments were arranged in a randomized complete block design with each treatment and inoculation depth replicated four times. The experiment was conducted twice per host crop.

Statistical analysis

Data were log transformed [log(x+1)] to normalize for analysis. Untransformed values are reported. Data were analyzed using general linear mixed model analysis of variance (ANOVA) with experiment and treatment as fixed variables and replication modeled as a random variable using SPSS v 25 (SPSS Inc., Chicago IL). Mean separation were based on

Tukey's honest significant difference (HSD) test at $\alpha = 0.05$. Data from the post-penetration observations were subject to chi-square analysis using SPSS.

RESULTS

Greenhouse experiments

In the cotton greenhouse experiments, there was no ($P > 0.05$) experiment by treatment interaction for nematode penetration, infection or reproduction, so experiments were combined for final analysis. Although there was a general trend at 7 and 14 DAP with fewer total nematodes per root system from fluopyram and abamectin roots compared to the non-treated control, it was not until 21 DAP that the number of nematodes was statistically lower for both nematicide treatments compared to the control (Fig. 2). Fewer ($P \leq 0.05$) galls were observed on roots at 21 DAP from both fluopyram- and abamectin-treated seed compared to the non-treated group (Fig. 3), however, at 28 and 35 DAP, fewer *(*$P \leq 0.05$) galls were observed for fluopyram-treated seed compared to non-treated treated control (Fig. 3). Nematode reproduction was consistently lower ($P \leq 0.05$) for fluopyram at 28 and 35 DAP, and abamectin at 35 DAP compared to the non-treated control (Fig. 4). There were no differences among nematode developmental stages at 7 DAP among treatments, with similar percentages of vermiform nematodes and swollen J2 present among treatment group (Fig. 5). Though differences in the percent of nematode development stages differed between abamectin and fluopyram at 14 and 21 DAP, with the presence and absence of vermiform nematodes, they did not differ from the non-treated control (Figs. 6 and 7).

In the soybean greenhouse experiments, there was no ($P > 0.05$) experiment by treatment interaction for nematode penetration, root galling, or reproduction so experiments were

combined for final analysis. Fewer ($P \leq 0.05$) total nematodes were observed inside the root systems of fluopyram-treated seed than roots of the non-treated control at 7, 14, and 21 DAP (Fig. 8). The number of galls observed on roots from the fluopyram treatment group were similar to that on the non-treated control at 21 DAP but statistically different at 28 and 35 DAP (Fig. 9). Whereas, the number of galls observed from the abamectin treatment group was similar to that of the non-treated at all sampling dates except 28 DAP (Fig. 9). Nematode reproduction observed from fluopyram-treated seed was lower ($P \leq 0.05$) compared to the non-treated at 28 DAP and lower ($P \leq 0.05$) than the non-treated and abamectin-treated at 35 DAP (Fig. 10). The suppression of nematode development by either nematicide was inconsistent at the three sample times in soybean (Figs. 11-13). Nematode development in nematicide treatments differed from the non-treated control at 7 DAP with more vermiform nematodes present (Fig. 11). Differences in nematode development at 14 DAP were observed between abamectin and fluopyram, with vermiform nematodes present in abamectin and absent in fluopyram, however, the percent of nematode development stages present did not differ from the non-treated control (Fig. 12). Differences in nematode development at 21 DAP were observed between fluopyram and the non-treated control, with a lower percentage of females maturing in the root systems from fluopyram-treated seed (Fig. 13).

Root Penetration Experiments

There was no experiment by treatment by inoculation depth interaction for the cotton experiments, however, there was an interaction ($P \leq 0.05$) between treatment and inoculation depth. Fewer ($P \leq 0.05$) J2 were observed in roots from fluopyram- and abamectin-treated cotton seed at the 2.5-cm inoculation depth compared to that of the non-treated control (Fig. 14). This

suppression conferred to 99 and 98% fewer J2 in roots from seed treated with fluopyram and abamectin, respectively, compared to the roots from the non-treated control. Only seed treated with fluopyram contributed to a suppression of J2 infection at the 5.0-cm inoculation depth compared to the non-treated control, whereas no suppression of nematode infection was observed at 7.0 cm for either nematicide (Fig. 14). Overall, the average number of J2 per cotton root system across sample depths was lower ($P \leq 0.05$) in roots from abamectin and fluopyram treated seed than roots from the non-treated control (Fig. 15).

There was no experiment by treatment by inoculation depth interaction for the soybean experiments, however there was a ($P \leq 0.05$) treatment by inoculation depth interaction. Roots from fluopyram- and abamectin-treated seed had 89 and 80% less J2 penetrating compared to roots from the non-treated control at 2.5 cm (Fig. 16). There were no differences in the number of nematodes that had penetrated the roots at 5 and 7 cm among treatments (Fig. 16). The accumulative average number of nematodes in fluopyram and abamectin-treated root systems was significantly less than the accumulative average from non-treated root systems (Fig. 17).

DISCUSSION

Seed-treatment with fluopyram provided a similar degree in seedling protection against *M. incognita* root penetration, gall formation, and reproduction to that of abamectin in cotton and soybean. On average, across sampling dates, seed-applied fluopyram and abamectin provided a 73 and 72% reduction in nematode penetration on cotton and a 74 and 45% reduction on soybean seed. Similar reductions were observed in root galling. Fluopyram and abamectin treatment provided a 78 and 62% reduction in root galling for cotton and a 66 and 49% for soybean, respectively. Nematode reproduction was suppressed by fluopyram and abamectin treatment by

95 and 83% on cotton and 84 and 51% on soybean. These results show the effectiveness of fluopyram and abamectin at reducing nematode infection in the first month after planting. However, similar greenhouse experiments with fluopyram and abamectin-seed treatments have been variable in reducing *M. incognita* infection (Hurd and Faske, 2016; Faske and Starr, 2007; Monfort et al., 2006).

These data indicate that seedling root protection by these seed-applied nematicides had the greatest impact on nematode penetration with little impact on post-penetration development. Nematode development was neither delayed nor accelerated by abamectin or fluopyram and consequently, the greatest impact from seed-applied fluopyram in our experiments is likely prior to or at the time of nematode penetration. Similarly, exposure of *M. incognita* to 1.0 μg abamectin/ml for 72hr did not affect post-invasion development on tomato (Stretton et al., 1987). In contrast, soil-applied abamectin at concentrations of 600 μg/100 cm^3 of soil did impact *M. arenaria* development in tomato roots (Cayrol et al., 1993). Furthermore, in an evaluation of fluopyram seed treatment at three different rates, only the highest rate of 0.25 mg ai/seed reduced *M. incognita* reproduction on soybean (Hurd and Faske, 2016). While there was no effect on nematode development by the labeled rates of fluopyram and abamectin as a soybean and cotton seed treatment in our experiments, a higher rate of contact with the nematode in the soil may have effect; however, that was not part of our study.

Protection of the root from *M. incognita* penetration was greatest within a few centimeters from the seed for both nematicides. Fluopyram as a seed treatment provided protection to cotton roots from *M. incognita* penetration at the 2.5- and 5-cm soil depth, which was approximately 0.5 and 3.0 cm below the seed. Protection of soybean roots occurred at the 2.5-cm depth, 0.5 cm below the seed. These results indicate that the protection provided by

fluopyram to suppress nematode penetration is greatest in close proximity to the seed. Similar results were reported for roots of fluopyram-treated soybean seed, where suppression of *H. glycines* penetration occurred at 2.5-cm and no protection was seen at greater depths (Beeman et al., 2019). In our studies, abamectin treatment conferred protection to cotton and soybean roots only at the 2.5-cm depth, 0.5 cm below the seed. Similarly, Faske and Starr (2007) reported that suppression of penetration on cotton taproots from abamectin-treated seed was greatest at a taproot length of 5 cm and decreased as taproots elongated. These studies were conducted in a sandy soil and given the limited movement by the nematicides, revealed by the inability to provide protection at greater depths, movement may be even more limited in finer textured soils.

These data indicate that fluopyram provides suppression of nematode penetration that subsequently affects galling and reproduction. The impact on nematode penetration is greatest shortly after planting and in close proximity to the seed. Protection of seedling is often considered to be the most important stage of root protection from nematode infection and fluopyram provides a similar degree of root protection to that of abamectin.

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FIGURES

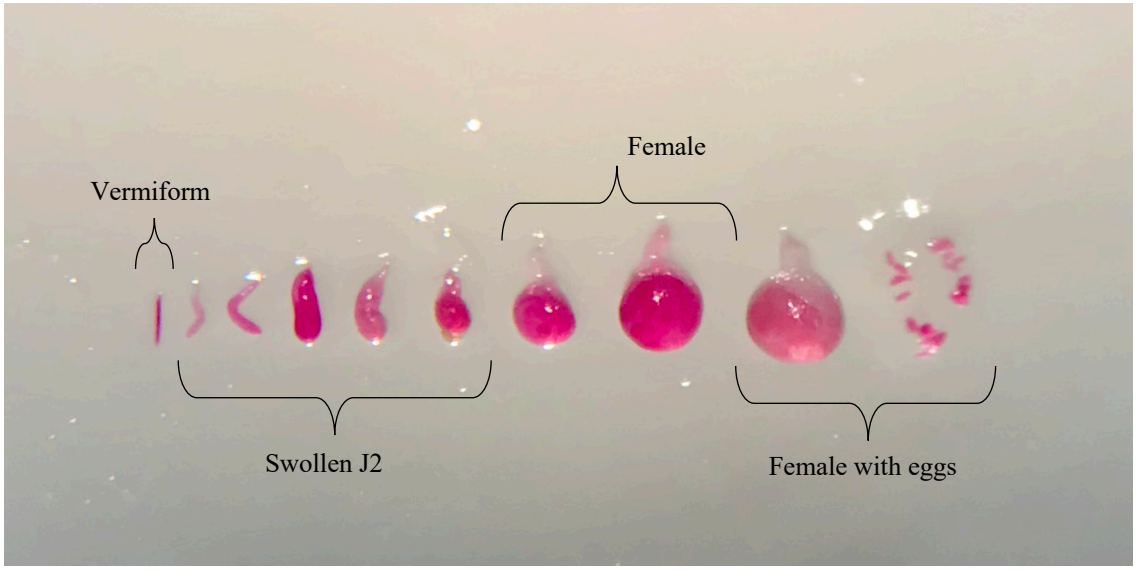

Figure 1. Various life stages of *Meloidogyne incognita* stained with acid fuchsin. Life stages include vermiform nematode, swollen second stage juvenile (J2), female, and female with eggs.

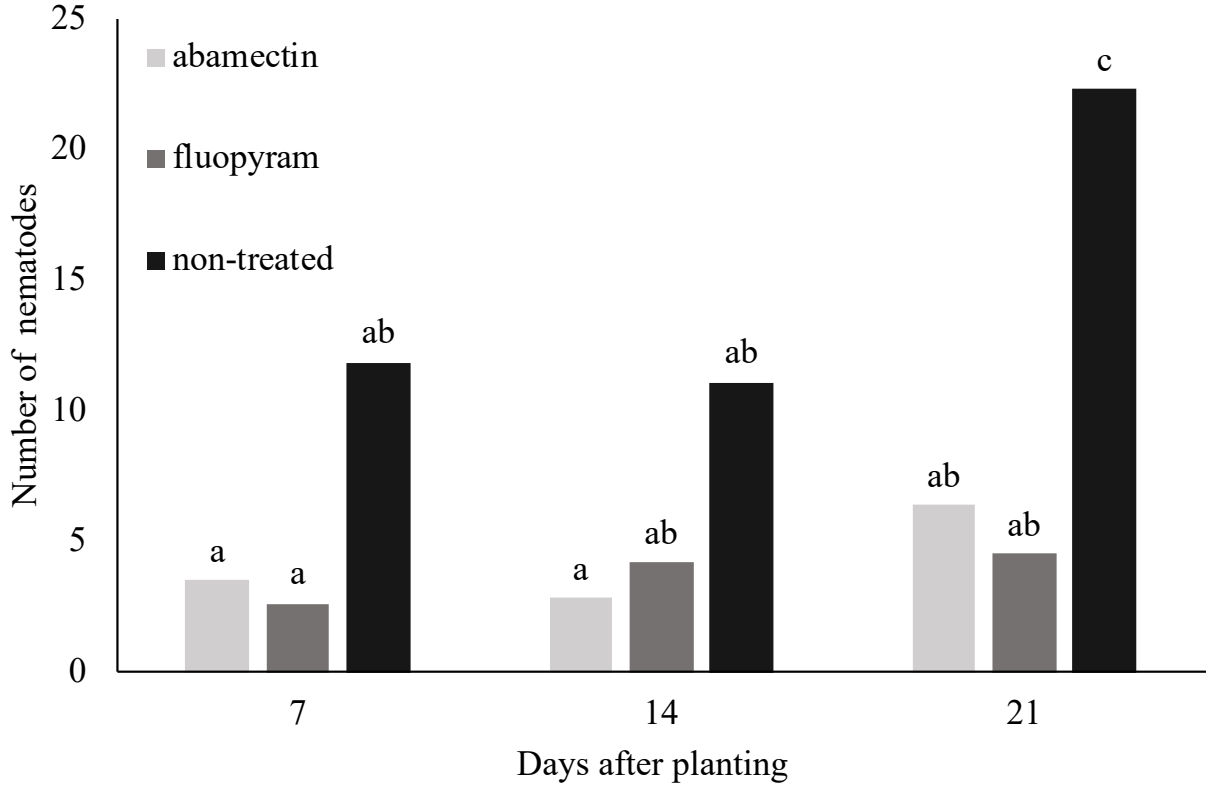

Figure 2. Number of *Meloidogyne incognita* per cotton root system at three sample times in a seed-applied nematicide pot experiment. Initial population density of *M. incognita* was 200 J2/100 cm^3 soil. Different letters above bars indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

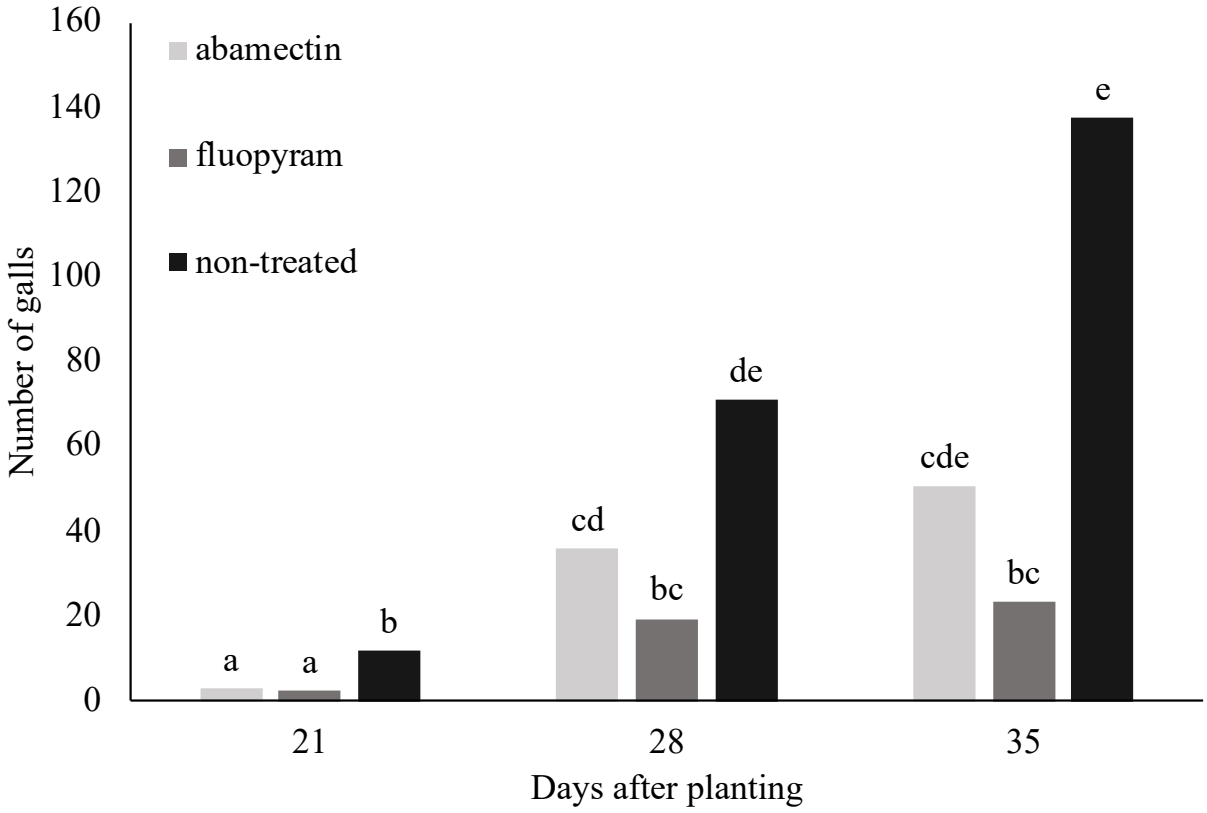

Figure 3. Number of galls per cotton root system at three sample times in a seed-applied nematicide pot experiment. Initial population density of *Meloidogyne incognita* was 2 J2 cm^{-3} soil. Different letters above bars indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

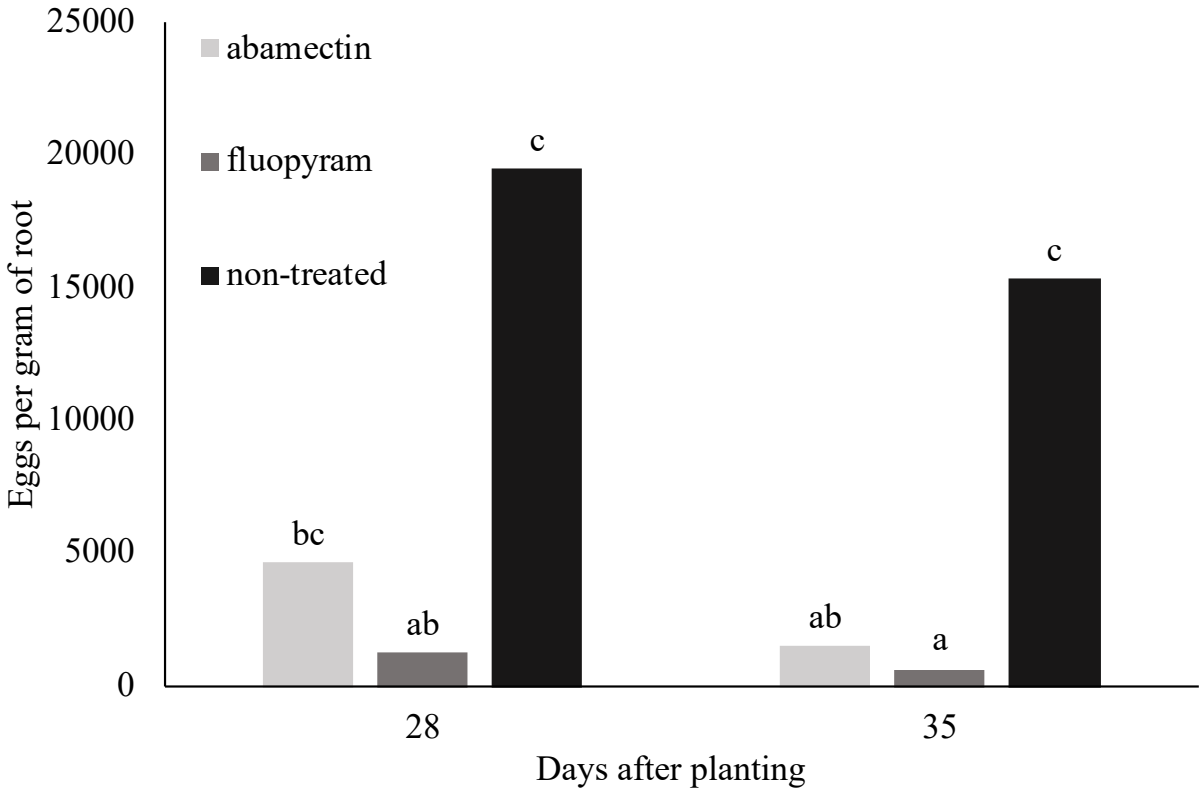

Figure 4. Number of eggs per gram of root per cotton root system at two sample times in a seed-applied nematicide pot experiment. Initial population density of *Meloidogyne incognita* was 200 J2/100 cm^3 soil. Different letters above bars indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

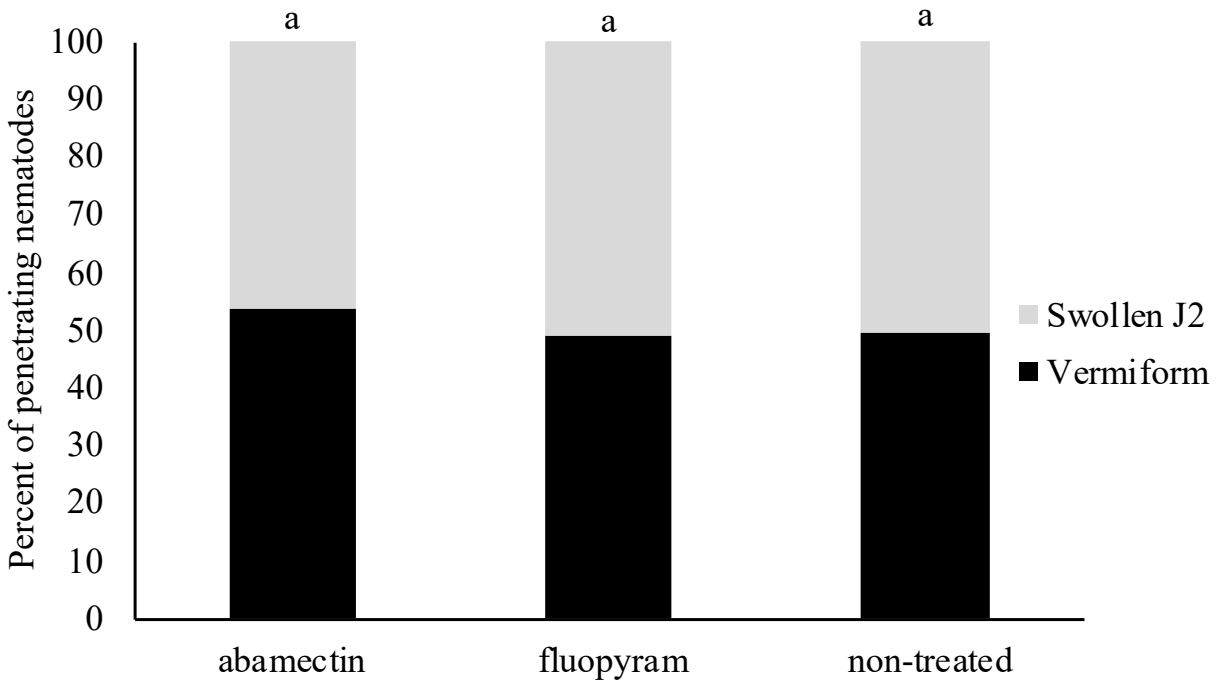

Figure 5. Post-infection development of *Meloidogyne incognita* on cotton roots at 7 days after planting. Initial population density of *M. incognita* was 2 J2 cm^{-3} soil. Different letters above bars indicate significant differences at $\alpha = 0.05$ according to chi-square analysis.

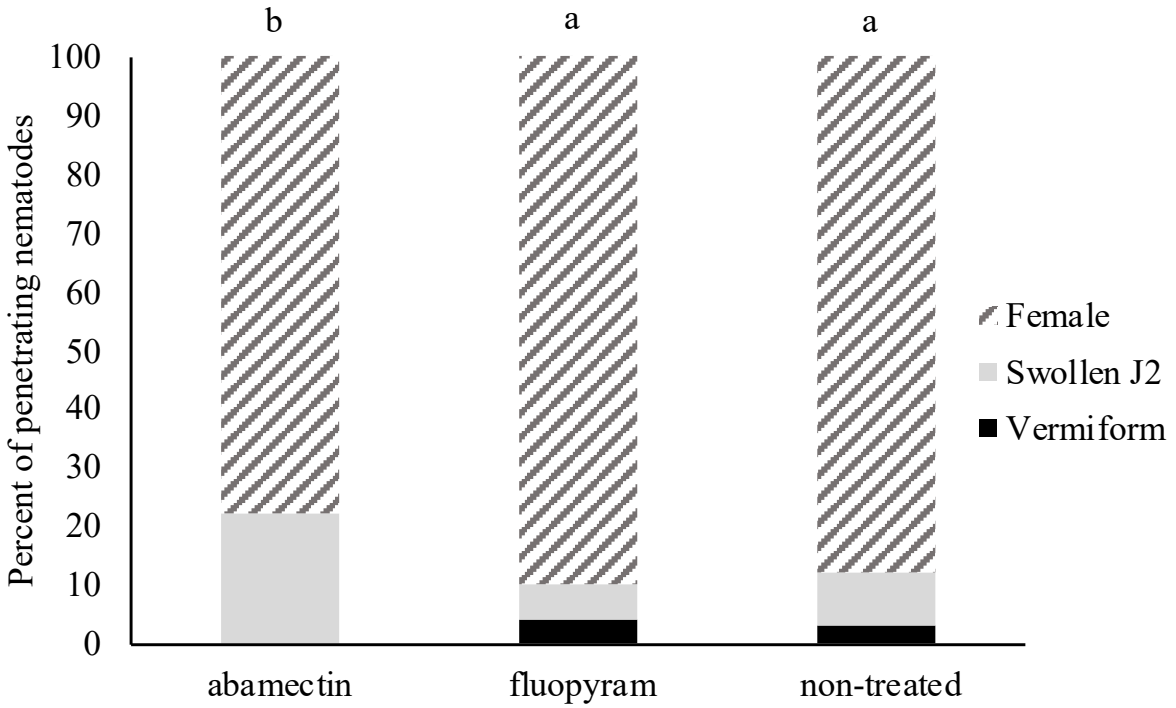

Figure 6. Post-infection development of *Meloidogyne incognita* on cotton roots at 14 days after planting. Initial population density of *M. incognita* was 2 J2 cm^{-3} soil. Different letters above bars indicate significant differences at $\alpha = 0.05$ according to chi-square analysis.

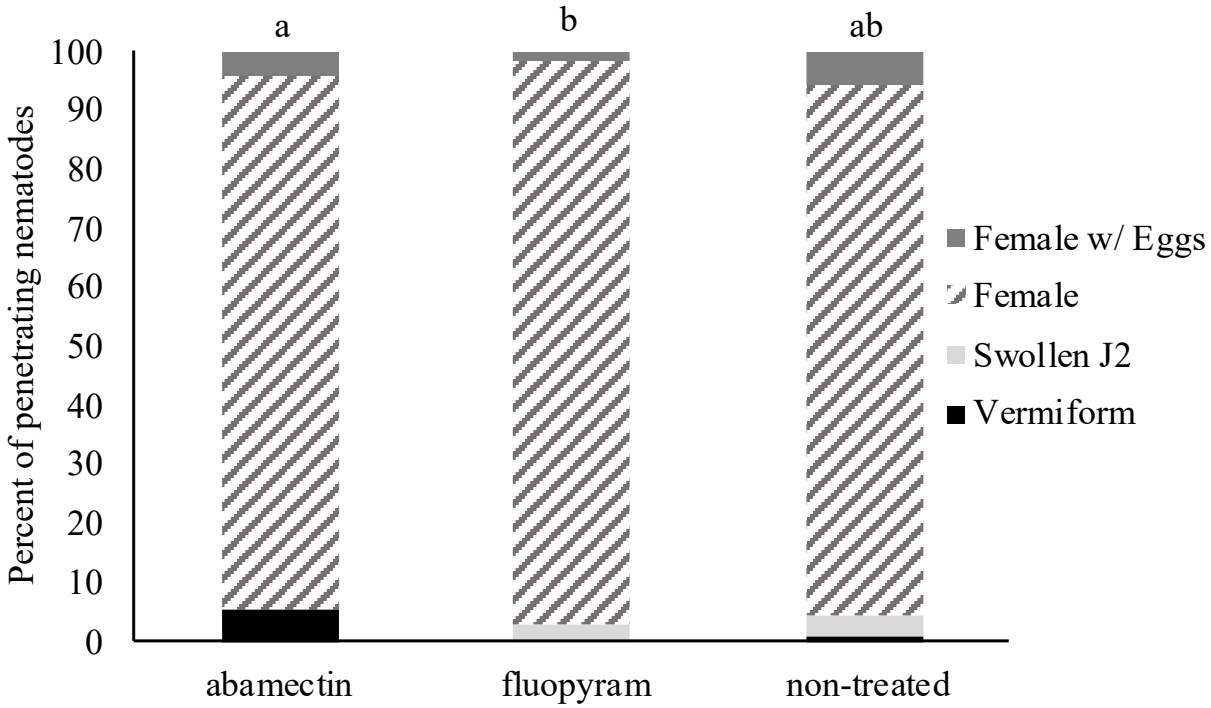

Figure 7. Post-infection development of *Meloidogyne incognita* on cotton roots at 21 days after planting. Initial population density of *M. incognita* was 2 J2 cm^{-3} soil. Different letters above bars indicate significant differences at $\alpha = 0.05$ according to chi-square analysis.

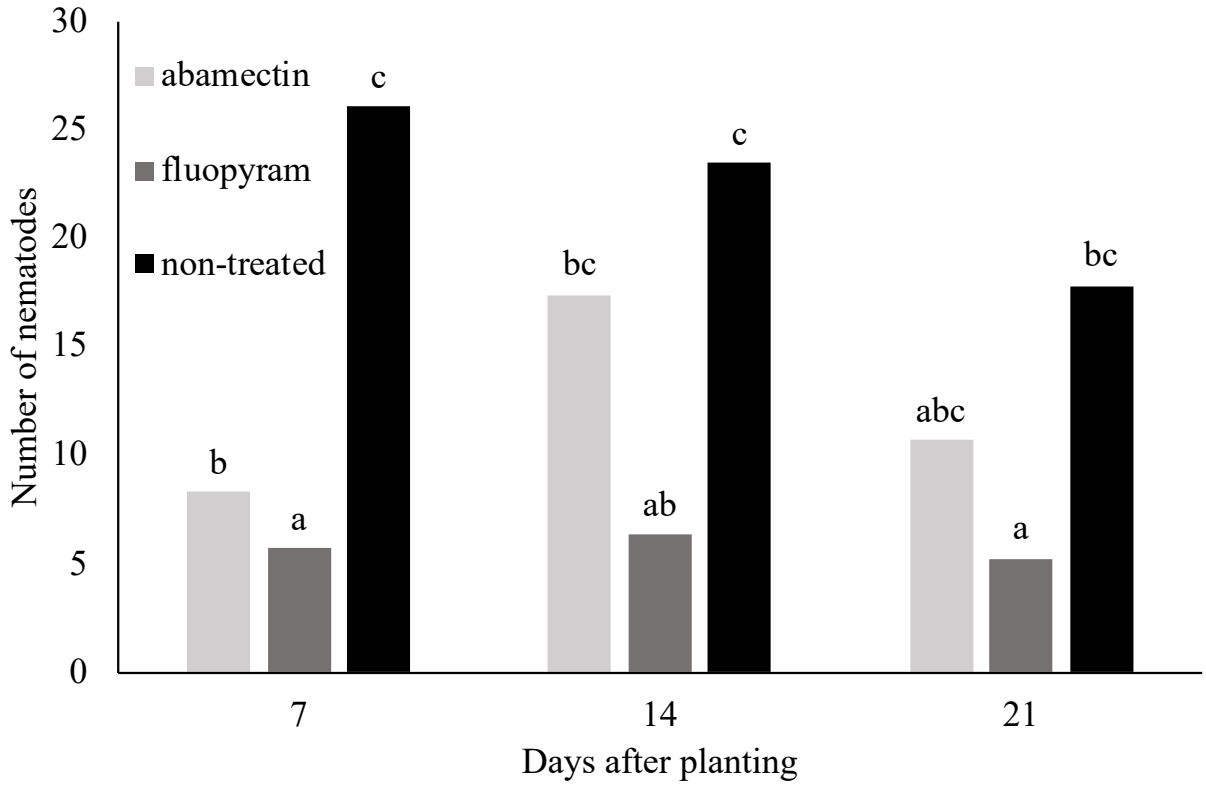

Figure 8. Number of *Meloidogyne incognita* per soybean root system at three sample times in a seed-applied nematicide pot experiment. Initial population density of *M. incognita* was 2 J2 cm^{-3} soil. Different letters above bars indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

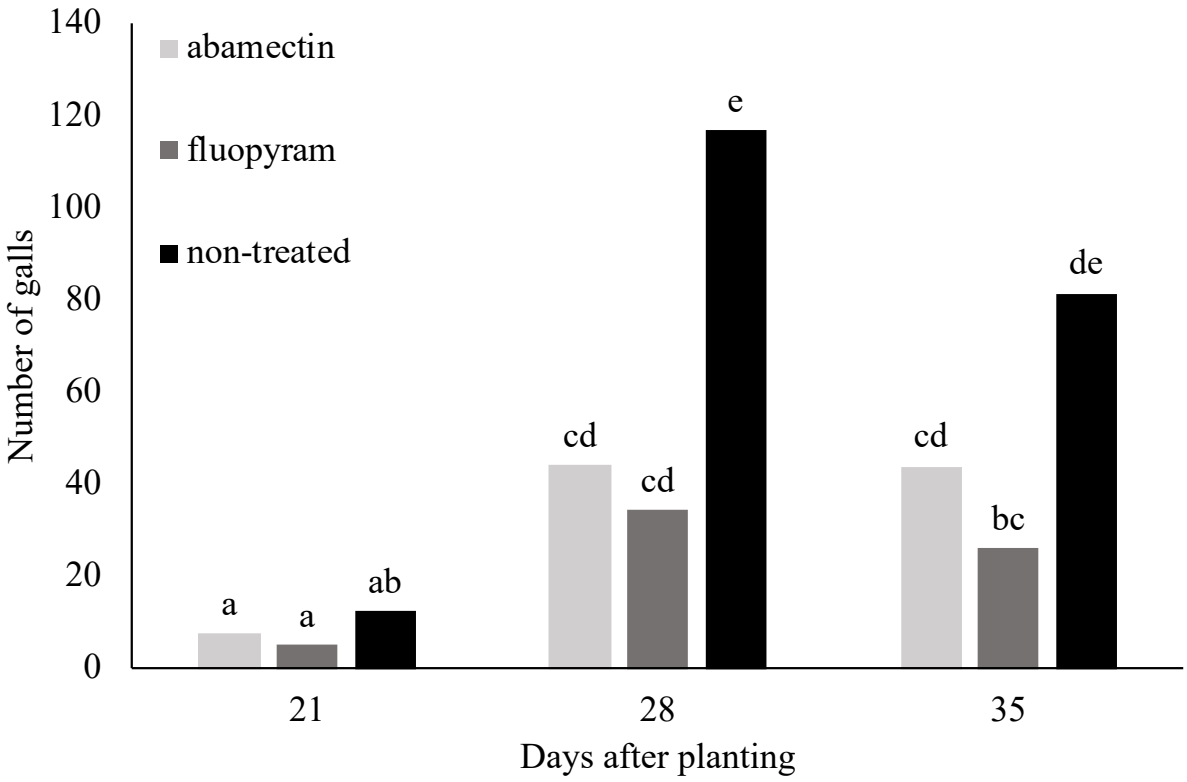

Figure 9. Number of galls per soybean root system at three sample times in a seed-applied nematicide pot experiment. Initial population density of *Meloidogyne incognita* was 2 J2 cm^{-3} soil. Different letters above bars indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

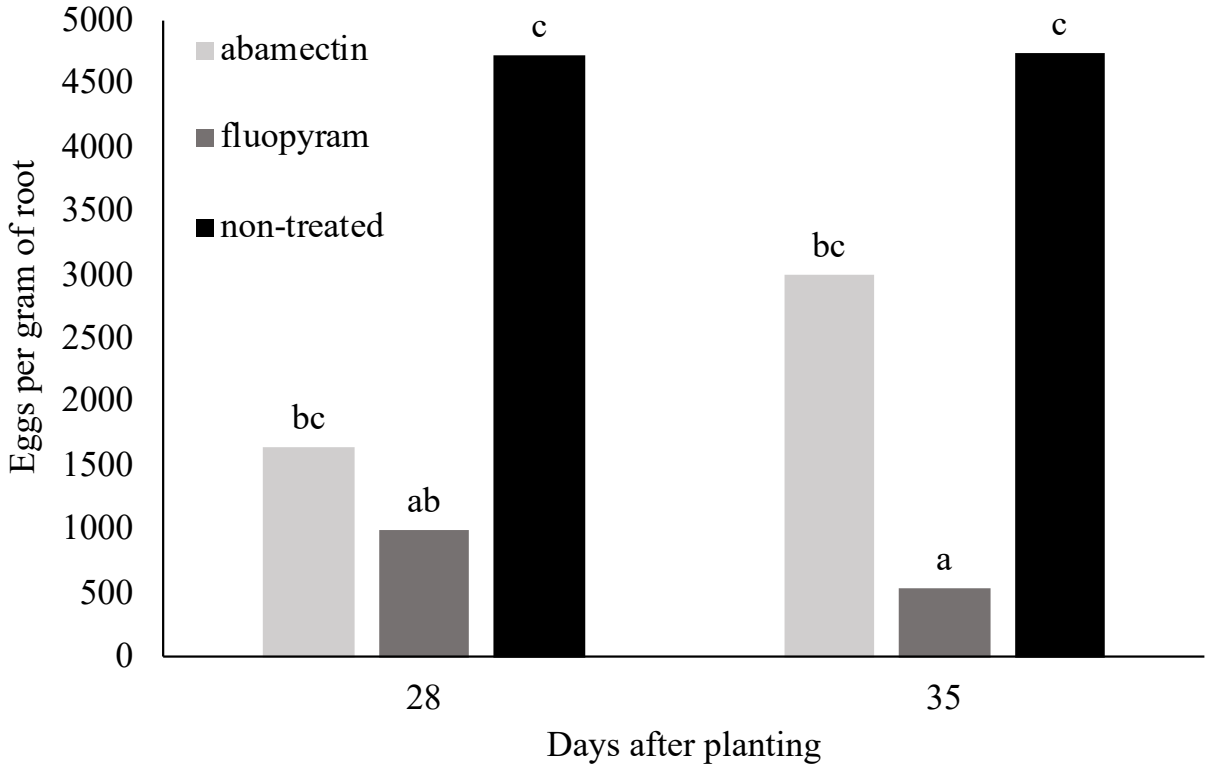

Figure 10. Number of eggs per gram of root per soybean root system at two sample times in a seed-applied nematicide pot experiment. Initial population density of *Meloidogyne incognita* was 2 J2 cm^{-3} soil. Different letters above bars indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

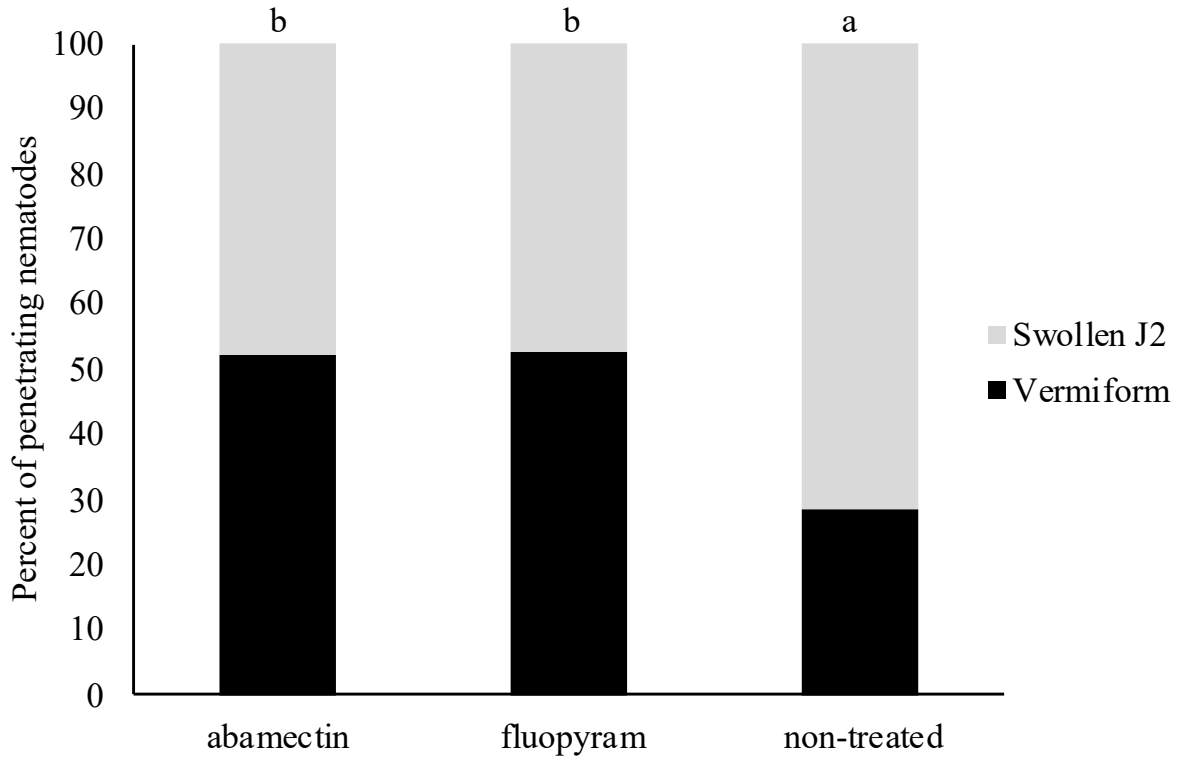

Figure 11. Post-infection development of *Meloidogyne incognita* on soybean roots at 7 days after planting. Initial population density of *M. incognita* was 2 J2 cm^{-3} soil. Different letters above bars indicate significant differences at $\alpha = 0.05$ according to chi-square analysis.

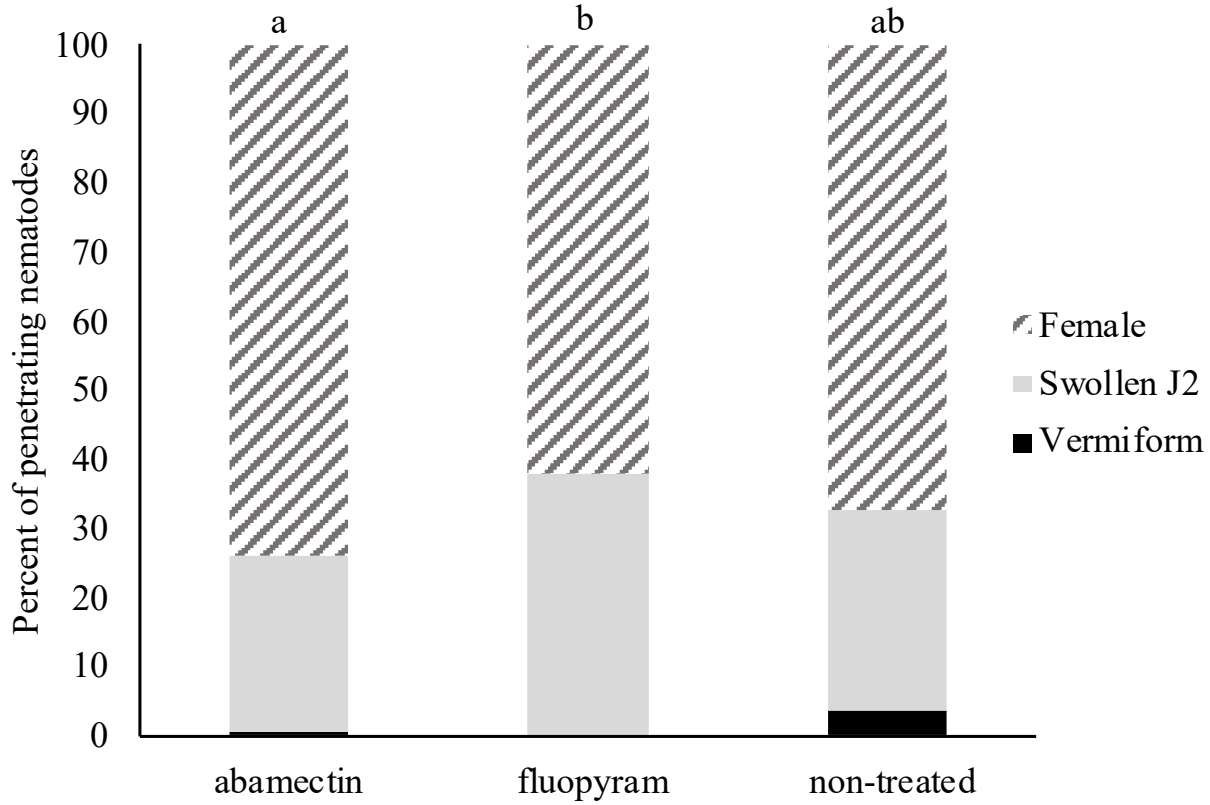

Figure 12. Post-infection development of *Meloidogyne incognita* on soybean roots at 14 days after planting. Initial population density of *M. incognita* was 2 J2 cm^{-3} soil. Different letters above bars indicate significant differences at $\alpha = 0.05$ according to chi-square analysis.

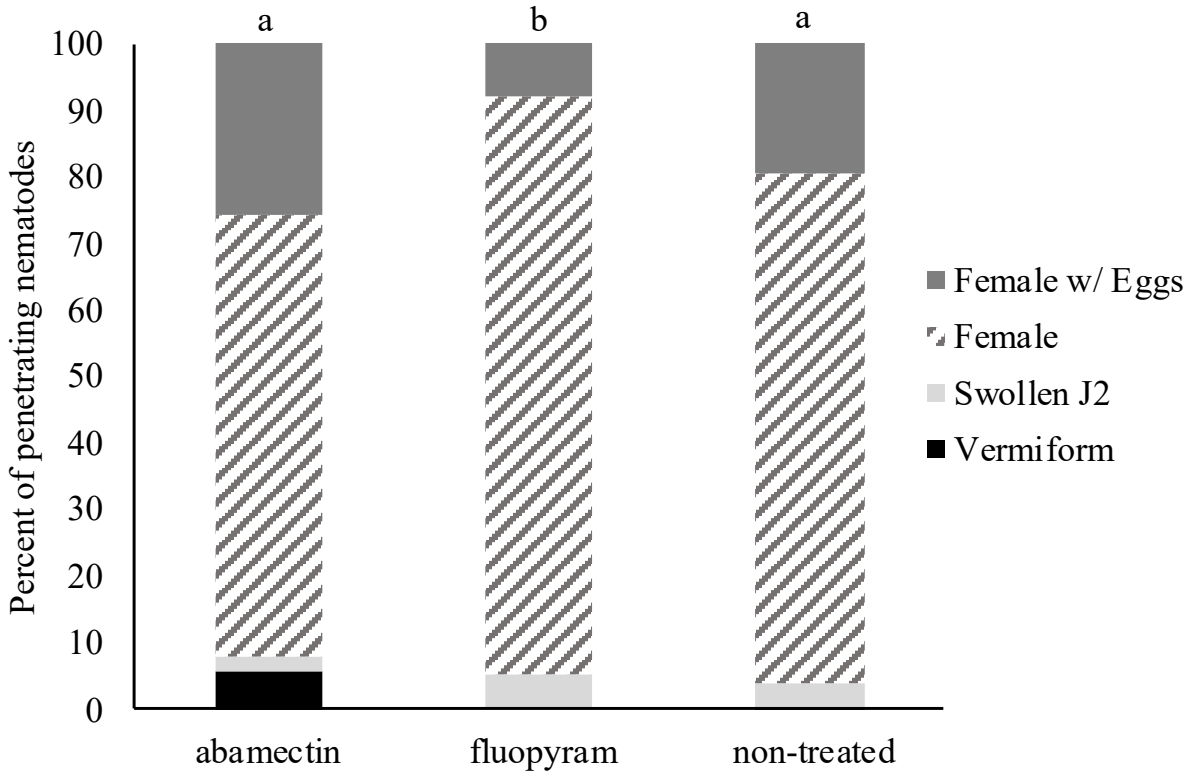

Figure 13. Post-infection development of *Meloidogyne incognita* on soybean roots at 21 days after planting. Initial population density of *M. incognita* was 2 J2 cm^{-3} soil. Different letters above bars indicate significant differences at $\alpha = 0.05$ according to chi-square analysis.

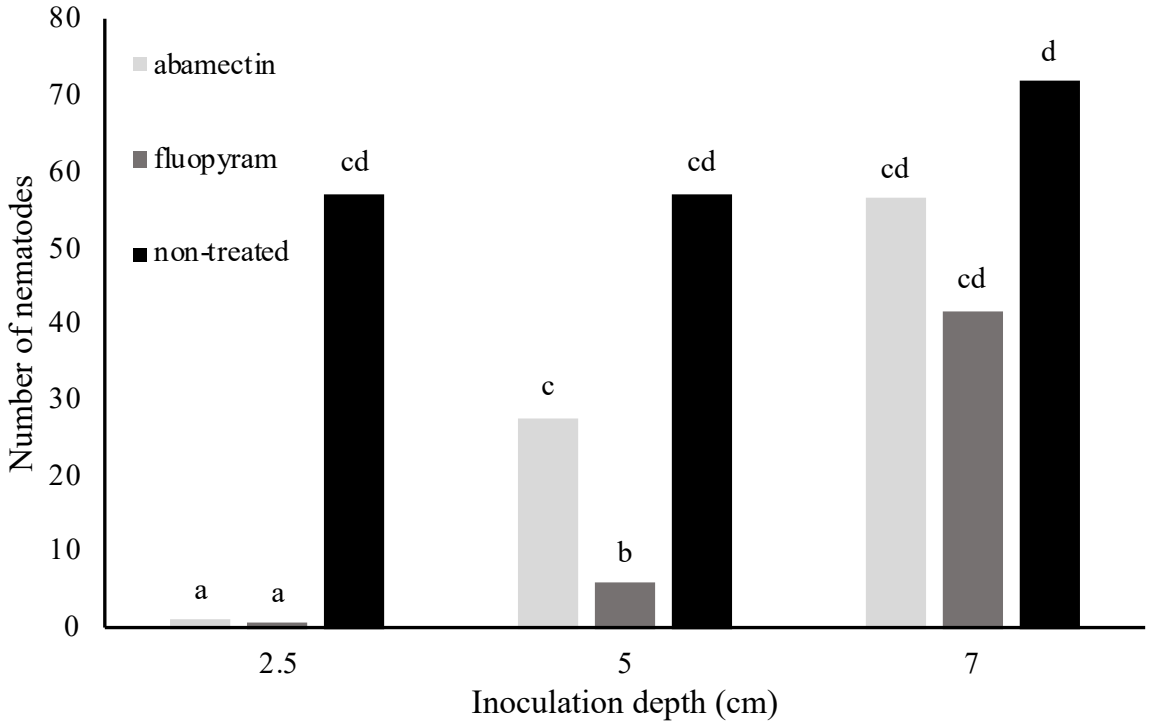

Figure 14. Number of *Meloidogyne incognita* J2 observed in cotton roots at three different inoculation depths. Inoculation rate was 250 J2 in 200 µl of water. Different letters above bars indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

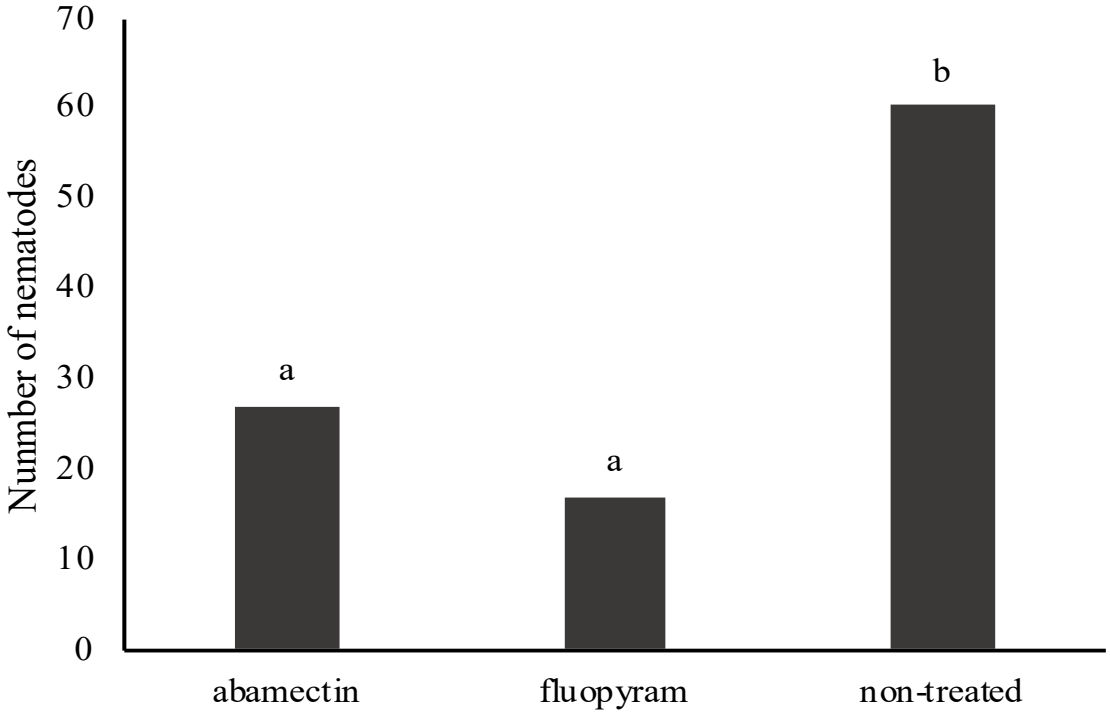

Figure 15. Main effect of seed-applied nematicides across three inoculation depths in cotton. Different letters above bars indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

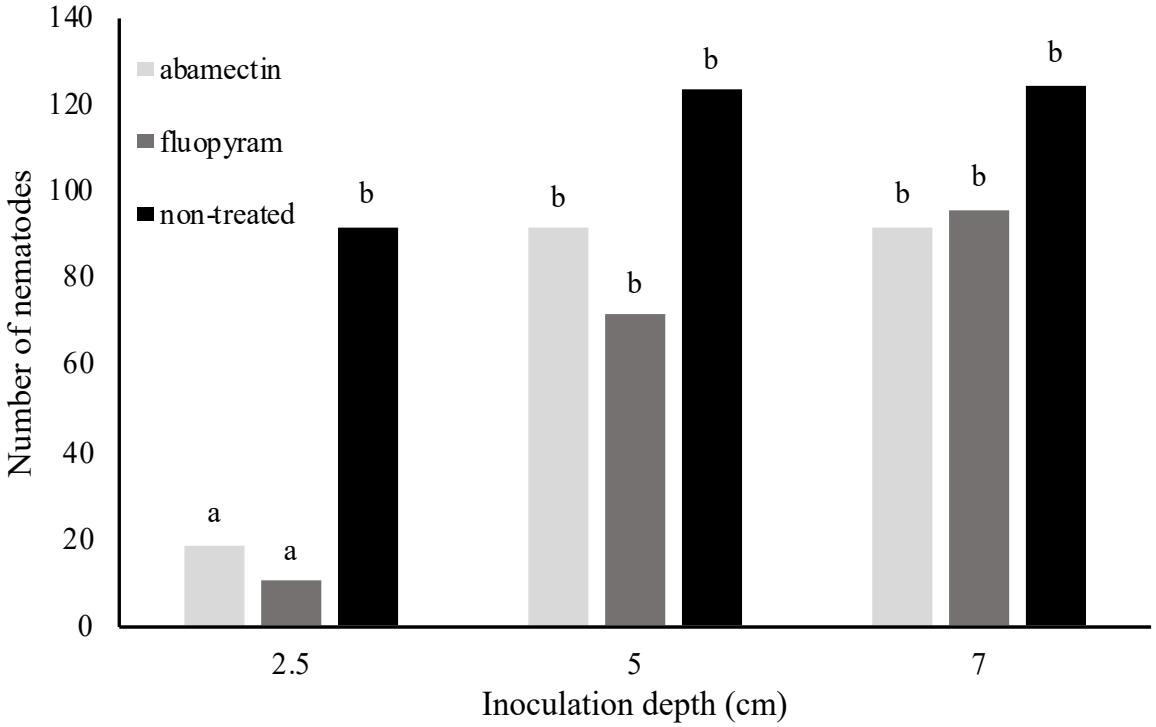

Figure 16. Number of *Meloidogyne incognita* J2 observed in soybean at three different inoculation depths. Inoculation rate was 250 J2 in 200 µl of water. Different letters above bars indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

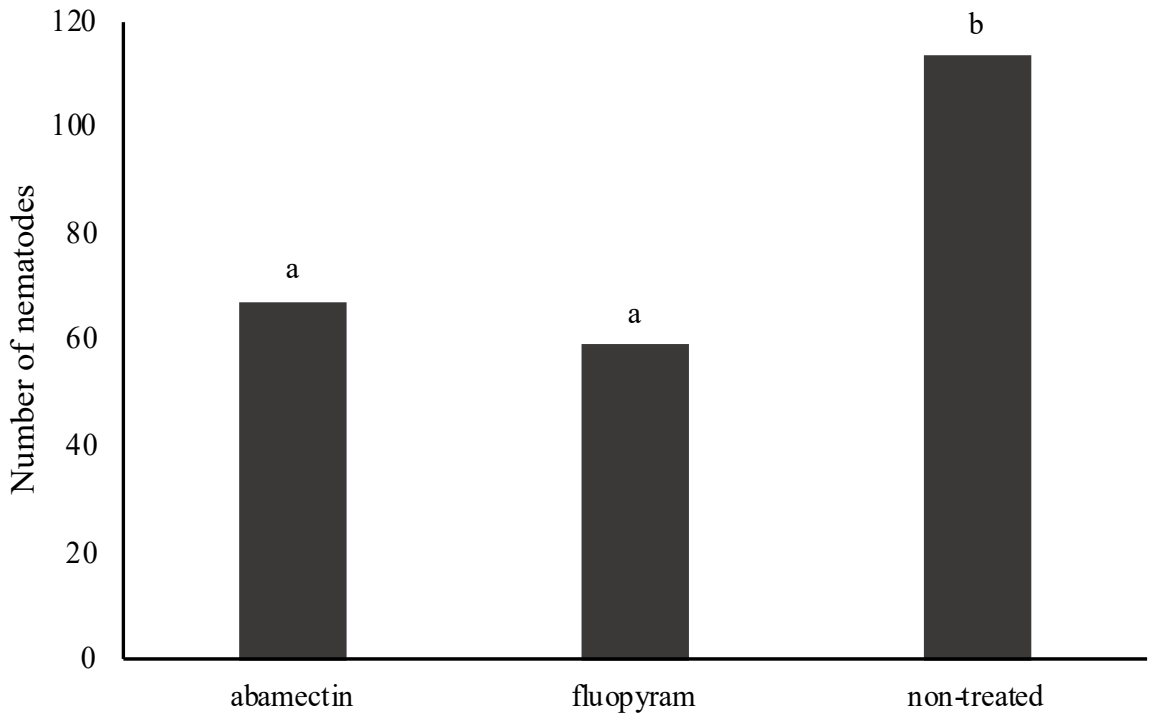

Figure 17. Main effect of seed-applied nematicides across three inoculation depths in soybean. Different letters above bars indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).