PATHOGENICITY AND MANAGEMENT OF RECENTLY DETECTED *MELOIDOGYNE* SPP. ON VEGETABLE CROPS IN GEORGIA

by

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(Under the Direction of Intiaz Amin Chowdhury)

ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) can significantly impact vegetable production in Georgia. While *M. incognita* is the most prevalent species, recent surveys reported *M. enterolobii*, *M. floridensis*, and *M. haplanaria* for the first time in the state. This study evaluated the reproductive potential of *M. enterolobii*, *M. floridensis*, and *M. incognita* on major vegetable crops grown in Georgia, assessed *M. enterolobii* reproduction and potential yield suppression on different types of onion (Vidalia, red, and white), and investigated responses of these recently detected *Meloidogyne* species to non-fumigant nematicides. Our results showed that *M. enterolobii* had higher reproductive potential than *M. incognita* and *M. floridensis*, which had similar reproduction across most of the vegetable hosts. Moreover, *M. enterolobii* reproduced on onion and caused significant yield reduction in different onion types. In vitro assay showed *M. enterolobii* was less sensitive to nematicides, although all nematicides suppressed reproduction of *Meloidogyne* species tested under greenhouse conditions.

INDEX WORDS: root-knot nematodes, vegetables, reproduction, non-fumigant nematicides

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DEDICATION

This work is dedicated to my beloved parents, Mr. Pitamber Poudel and Mrs. Santu Maya Poudel. Their unwavering love, steadfast support, and constant encouragement have been the foundation of my journey. Every achievement in my life is a reflection of their sacrifices, values, and endless belief in me. I am forever grateful.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Vegetable production in Georgia

Georgia is one of the leading vegetable producing states in the country. The total value of the vegetables produced in Georgia in 2023 alone was approximately 1.3 billion dollars (Georgia farm gate value report 2023, University of Georgia 2025), this accounts for 7.67 % of total value of the agricultural commodities produced in the state. (Georgia farm gate value report 2023, University of Georgia 2025). Georgia produces almost 33 different kind of vegetables and the major vegetables in terms percentage of farm gate value are watermelon (*Citrullus lanatus*, 14.7 %), onions (*Allium cepa*, 14 %), sweet corn (*Zea mays* var. *saccharate*; 13.1 %), bell pepper (*Capsicum annuum*; 10.4 %), tomato (*Solanum lycopersicum*, 6.8 %), cucumbers (*Cucumis sativus*, 5.9 %), yellow squash (*Cucurbita pepo*; 3.4 %); cabbage (*Brassica oleracea* var. *capitata*; 2.8 %), zucchini (*Cucurbita pepo*; 2.7 %), carrot (*Daucus carota* subsp. *Sativus,* 2.2 %), cantaloupe (*Cucurbita melo*; 1.3 %), snapbeans (*Phaseolus vulgaris*; 1.2 %; Georgia farm gate value report 2023, University of Georgia 2025). Vegetable production in Georgia is constrained by various plant diseases caused by fungi, bacteria, viruses, and plant-parasitic nematodes (PPNs). Plant-parasitic nematodes are a significant pest in vegetable producing areas of Georgia, where relatively warm temperatures and sandy soil create a suitable environment for their infection and reproduction on numerous vegetable crops (Koenning 1996; Marquez et al. 2021). Several PPNs genera including root-knot nematode (*Meloidogyne* spp.), stubby-root

nematode (*Nanidorus* spp.), ring nematode (*Mesocriconema* spp.), spiral nematode (*Helicotylenchus* spp.), lesion nematode (*Pratylenchus* spp.), reniform nematode (*Rotylenchulus* spp.), lance nematode (*Hoplolaimus* spp.), cyst nematode (*Heterodera* spp.), stunt nematode (*Tylenchorhynchus* spp.), and dagger nematode (*Xiphinema* spp.) have been detected in multiple vegetable fields of Georgia infesting a range of vegetable crops (Marquez et al. 2021).

Root-knot nematodes (*Meloidogyne* spp.)

Root-knot nematodes are among the most economically important genera of PPNs, capable of causing substantial yield losses across a wide range of crops globally. These nematodes have an exceptionally broad host range, infecting nearly all major cultivated crops. Global economic losses attributed to RKNs infestations are estimated to exceed $100 billion annually (Elling, 2013). In vegetable crops, yield reductions of up to 85% have been reported, depending on the host species and severity of infestation by RKNs (Barker et al. 1976; Bélair 1992; Davis 2007; Ornat et al. 1997). Characteristic symptoms of RKNs infection include the formation of diagnostic root galls, chlorosis, stunting, and wilting (Duncan and Noling 1998). Additionally, RKNs often interact synergistically with other soilborne pathogens such as *Pythium* and *Fusarium*, leading to severe disease complexes and increased damage to the host plant (Morris et al. 2016; Seo and Kim 2017).

Root-knot nematodes eggs are encased in a gelatinous matrix typically deposited on the surface of galled roots. Following embryonic development, the first molt occurs within the egg, producing the infective second-stage juvenile (J2). Hatching of J2 is primarily regulated by temperature and can occur independently of plant root stimuli, although root exudates may enhance the process (Perry and Moens 2013). Upon emergence from the egg mass, J2s may

infect nearby galled regions or invade new root systems. Second-stage juvenile and the adult male are the only *Meloidogyne* life stages found freely in the soil. Second-stage juveniles can persist in the soil in a quiescent state for extended durations. They are guided toward host roots by detecting chemical gradients in root exudates (Perry and Moens 2013; Teillet et al. 2013). Penetration generally occurs just behind the elongation zone. After entering the root, J2s migrate intercellularly through the cortex toward the zone of cell differentiation. When confronted with the casparian strip at the endodermis, which their stylet cannot penetrate, they redirect their migration toward the root tip, passing into the apical meristematic region. From there, they turn and migrate upward within the vascular cylinder to reach the zone of differentiation (Perry and Moens 2013; Teillet et al. 2013).

Penetration and migration are achieved through a combination of mechanical force and enzymatic degradation. Second-stage juveniles secrete cell wall-degrading enzymes and use their stylet and anterior body to physically separate cells by weakening the middle lamella that binds them. After a short migration, the J2s become sedentary in the cortical tissue adjacent to the vascular region, embedding their head in the protoxylem or protophloem while the rest of the body remains aligned with the cortex. In susceptible hosts, feeding by J2s triggers significant physiological and morphological modifications, including the formation of giant cells. Typically ranging from 2 to 12 in number (commonly around 6), these specialized feeding sites originate in the phloem or adjacent parenchyma. Both the initiation and maintenance of giant cells are thought to be governed by secretions from the nematode's stylet, originating from the sub-ventral glands during early giant cell formation and the dorsal pharyngeal gland at later stages (Perry and Moens 2013).

Giant cells undergo mitotic divisions without cytokinesis, resulting in nuclear multiplication without cell wall formation-presumably through repeated endomitosis. Cell wall degradation is not observed within the first 72 hours of giant cell initiation. Early in their development, giant cells are primarily vacuolated, with peripheral localization of the nuclei. Synchronous mitosis of nuclei occurs, and as cytoplasmic volume increases, the cells expand laterally. Surrounding cortical cells divide and become hypertrophied, and the pericycle also proliferates (Berg et al. 2009), culminating in the formation of a characteristic gall, a pseudo-organ enclosing the giant cells.

Within this gall, J2s become sedentary as its somatic muscles atrophy-except for the head region-and it alternates feeding from different giant cells. The nematode undergoes three molts (to J3, J4, and the adult female). Only the J2 and the adult female are capable of feeding; J3 and J4 possess non-functional stylets and thus do not feed (Escobar et al. 2015).

Distribution of *Meloidogyne* species in vegetable fields of Georgia

In 2018, a comprehensive survey was conducted across 436 vegetable fields in southern Georgia, where the majority of the state's vegetables are grown-to assess the distribution of PPNs. These fields included both bare ground (n = 194) and plastic bed (n = 242) cropping systems, and were randomly selected from 29 counties in southern Georgia. The results indicated that *Meloidogyne* species were the most prevalent group of PPNs, detected in 67.3% of the surveyed fields (Marquez et al. 2021). This underscores the dominance and significance of *Meloidogyne* species in Georgia's vegetable production systems.

Among the *Meloidogyne*-infested fields, *M. incognita* was by far the most frequently detected species, present in 91.9% of cases. It was followed by *M. arenaria* (36.0%), *M. javanica*

(5.5%), *M. floridensis* (2.2%), and *M. haplanaria* (0.7%) (Marquez and Hajihassani 2023a). These findings highlight *M. incognita* as the most widespread and economically important *Meloidogyne* species in Georgia.

Notably, *M. floridensis* (peach RKN) and *M. haplanaria* (Texas RKN) were reported for the first time in Georgia during this survey (Marquez et al. 2021; Marquez and Hajihassani 2022; Marquez and Hajihassani 2023a). *Meloidogyne floridensis* was initially detected in a tomato field and has since been found infecting other crops such as watermelon, cucumber, collard, and cowpea in Georgia (Marquez and Hajihassani 2023b). Except for collard, *M. floridensis* was able to reproduce on all other associated vegetable crops under greenhouse conditions, confirming its pathogenic potential on multiple hosts. *Meloidogyne floridensis* was originally identified in Florida, where it was found infecting *M. incognita*-resistant peach cultivars 'Nemaguard' and 'Okinawa' (Sharpe 1968). Since its initial discovery, *M. floridensis* has been confirmed in 12 counties across Florida, where it infects a wide array of hosts, including weeds, ornamentals, vegetables, and row crops (Brito et al. 2015). Its presence has also been reported in almond orchards in two counties in California (Westphal et al. 2019) and on the peach rootstock cultivar 'Guardian' in a county in South Carolina (Reighard et al. 2019). Although historically associated with fruit trees, *M. floridensis* has demonstrated the ability to infect a broad spectrum of vegetable crops. Documented hosts include tomato, melon, sweet potato, cucumber, eggplant, and squash (Burelle and Nyczepir 2004; Marquez and Hajihassani 2023b; Ploeg and Edwards 2024). *Meloidogyne floridensis* can overcome resistance conferred to *M. incognita* in several vegetable cultivars. This includes resistant varieties of tomato, sweet potato, and pepper (Ploeg

and Edwards 2024; Ploeg and Stoddard 2024; Stanley et al. 2009), making it a growing concern for resistance-based nematode management programs.

In Georgia, *Meloidogyne haplanaria* was first detected in cowpea and watermelon fields; however, greenhouse trials confirmed that this nematode did not reproduce on either crop, suggesting that its reproduction likely occurred on a previous host crop grown in the rotation cycle. *Meloidogyne haplanaria* was first described on peanut (*Arachis hypogaea* L.) in Texas (Eisenback et al. 2003). Since then, it has been reported infecting a range of hosts, including vegetables, grasses, and perennial trees in Arkansas (Khanal et al. 2016), *M. incognita*-resistant tomato cultivars in Florida (Joseph et al. 2016), and the American pitcher plant in California (Subbotin 2021). *M. haplanaria* has also demonstrated the ability to reproduce on *M. incognita* resistant tomato cultivars (Joseph et al. 2016; Marquez and Hajihassani 2022), raising concerns over its potential to overcome resistance-based management strategies.

In addition to *M. floridensis* and *M. haplanaria*, another species-*M. enterolobii*, also known as the guava RKN-was detected for the first time in Georgia in sweet potato fields in Tattnall County in 2021 (Hajihassani et al. 2023). Beyond Georgia, *M. enterolobii* has been reported infecting a range of vegetable and row crops in Florida, Louisiana, North Carolina, and South Carolina (Gu et al. 2021; Overstreet et al. 2019; Riva et al. 2025; Rutter et al. 2019; Schwarz et al. 2020; Ye et al. 2013). This species is regarded as one of the most destructive RKNs due to its broad host range and its ability to infect and reproduce on several vegetable cultivars that possess resistance genes effective against other common *Meloidogyne* species such as *M. incognita* (Castagnone-Sereno 2012; Philbrick et al. 2020).

Root-knot nematodes management

There are several strategies available for the management of RKNs in vegetable crops. These strategies can be broadly categorized into biological, cultural, chemical, and host plant resistance-based methods. Among these, the use of chemical nematicides remains one of the most effective, practical, and reliable approaches for managing RKNs in vegetable production systems in Georgia. Chemical nematicides have historically been key tools in nematode management and are generally classified into two categories: fumigants and non-fumigants. However, the use of fumigants has declined due to growing concerns regarding environmental impact and worker safety. For instance, methyl bromide-once a widely used broad-spectrum fumigant effective against various soilborne pests, including nematodes-was globally phased out in 2015 because of its ozone-depleting properties and negative effects on non-target organisms (Desaeger et al. 2020; Oka 2020). Currently, fumigants such as 1,3-dichloropropene (1,3-D) and methyl isothiocyanate (MITC)-releasing compounds like metam sodium and metam potassium are still employed for RKNs management (Oka 2020). Although these fumigants can be effective during the early stages of crop development, they have significant limitations, including high application costs, stringent handling requirements, buffer zone mandates (minimum width of 7.6 meters), enforced pre-plant intervals, and occupational health risks (Desaeger et al. 2020; Oka 2020).

Non-fumigant nematicides, in contrast to fumigants, offer several advantages, including lower application rates, simpler delivery methods, and reduced risk of phytotoxicity, making them suitable for both pre- and post-plant applications (Desaeger et al. 2020; Oka 2020). Historically, the majority of non-fumigant nematicides used for managing RKNs belonged to the

organophosphate and carbamate chemical classes. These compounds exert their nematicidal effect by inhibiting acetylcholinesterase activity in the nematode's nervous system. However, due to their potential risks to human health and non-target organisms, many of these chemicals have been withdrawn from widespread use (Desaeger et al. 2020; Oka 2020). Despite this, a few compounds are still approved for use in specific contexts. For instance, aldicarb (a carbamate) is currently registered only for sweet potato production in Louisiana and Mississippi (Schloemer et al. 2024), and ethoprop (an organophosphate) is used on select vegetable crops such as sweet potatoes and potatoes (Hajihassani 2018). Oxamyl, another carbamate, continues to be widely used for RKN management in vegetable production systems.

In recent years, a new class of fluorinated nematicides-distinguished by the presence of a trifluoromethyl (-CF_3) group-has emerged as a promising alternative for RKN management. This group includes fluazaindolizine, fluensulfone, and fluopyram, which offer safer and more targeted options compared to traditional nematicides (Oka 2020). Fluazaindolizine, an imidazopyridine compound, demonstrates high specificity against PPNs and does not interact with known target sites of existing commercial nematicides, suggesting a novel mode of action. Additionally, it is reported to have relatively low toxicity to non-target organisms (Lahm et al. 2017; Thoden and Wiles 2019). Fluensulfone, a member of the fluoroalkenyl thioether group, also appears to operate through a novel but currently unidentified mechanism (Kearn et al. 2014). Both fluazaindolizine and fluensulfone induce irreversible paralysis in RKN juveniles, ultimately resulting in nematode death (Kearn et al. 2014; Lahm et al. 2017). In contrast, fluopyram, a pyridinyl-ethyl-benzamide compound, functions as a succinate dehydrogenase (SDH) inhibitor,

disrupting mitochondrial respiration and ATP synthesis. Although fluopyram causes paralysis, its effects are reversible, making it nematostatic rather than nematicidal (Schleker et al. 2022).

Justification for the research and objectives

While the host status of various vegetable crops to *M. incognita* has been extensively studied, similar information for *M. enterolobii* and *M. floridensis* remains limited. In recent years, both *M. enterolobii* and *M. floridensis* have emerged as nematode species of increasing concern in the southern United States, owing to their broad host ranges and capacity to reproduce on vegetable cultivars previously considered resistant to *M. incognita*. Research on *M. enterolobii* has largely focused on evaluating host status and developing management strategies (Brito et al. 2007; Grabau et al. 2024; Poudel et al. 2025; Watson 2022; Wong et al. 2024), while studies involving *M. floridensis* have been fewer and mainly limited to a small number of crops for host suitability assessments (Burelle and Nyczepir 2004; Ploeg and Edwards 2024; Ploeg and Stoddard 2024). However, no comprehensive studies have been conducted comparing the host status of vegetable crops to both *M. enterolobii* and *M. floridensis*, nor evaluating the relative reproductive potential of *M. enterolobii*, *M. floridensis*, and *M. incognita* across key vegetable crops commonly grown in Georgia. Furthermore, the Georgia-specific isolates of *M. enterolobii* and *M. floridensis* have so far only been studied on the crops from which they were originally isolated (Hajihassani et al. 2023; Marquez and Hajihassani 2023b), leaving a gap in knowledge regarding their ability to parasitize other economically important vegetables in the region. Determining the host status and relative reproduction potential of these RKN species on specific crops is critical for assessing their economic threat and developing effective, species-specific management strategies. Therefore, one of the objectives of this study is to determine the host

status of major vegetable crops grown in Georgia-including beet, broccoli, cabbage, cantaloupe, pepper, snap bean, squash, and tomato-to *M. enterolobii* and *M. floridensis*, and to assess the relative reproductive potential of *M. enterolobii*, *M. floridensis*, and *M. incognita* on those crops identified as suitable hosts.

In Georgia, *M. enterolobii* was first detected in 2021 from a sweet potato field in Tattnall County (Hajihassani et al. 2023), a region that also produces approximately 40% of the state's onions (Stubbs 2024). Onion is the second most valuable vegetable crop in Georgia based on farm gate value. In 2023, Georgia produced approximately 115,000 tons of onions, with an estimated value of $159 million (USDA-NASS 2024). In Tattnall County, sweet potatoes fields are often rotated with onions. Given that sweet potatoes are highly susceptible hosts of *M. enterolobii*, this rotation system increases the risk of *M. enterolobii* persisting in the soil through galled root debris and second-stage juveniles, thereby posing a threat to the subsequent onion crop. Despite this risk, there is limited information regarding the interaction between *M. enterolobii* and onions, and to date no study has confirmed whether onions can serve as hosts for this nematode species. Therefore, the second objective of this study was to evaluate the reproductive potential of *Meloidogyne enterolobii* on commonly grown onion cultivars in Georgia and assess its potential impact on yield. The findings from this research will be critical for developing targeted management strategies to reduce potential yield losses and economic damage caused by *M. enterolobii*.

Because host resistance in commercial vegetable cultivars is largely ineffective against *M. enterolobii*, *M. floridensis*, and *M. haplanaria*, chemical nematicides remain the primary management strategy for these aggressive species. Among chemical options, non-fumigant

nematicides such as fluazaindolizine, fluensulfone, fluopyram, and oxamyl have emerged as promising alternatives to fumigants. However, recent studies have revealed that sensitivity to these non-fumigant nematicides can vary significantly among *Meloidogyne* species, indicating that species-specific responses must be considered when formulating management strategies. For instance, an in vitro assay by Thoden et al (2019) found that *M. javanica* was less sensitive to fluazaindolizine than *M. incognita*. Similarly, Wram and Zasada (2020) reported reduced sensitivity of *M. chitwoodi* to fluazaindolizine compared to *M. hapla* and *M. incognita* in an infectivity assay. In another study, *M. javanica* was less sensitive to fluensulfone but more sensitive to fluopyram than *M. incognita*, while *M. incognita* exhibited the opposite trend, greater sensitivity to fluensulfone but lower sensitivity to fluopyram (Oka and Soraya 2019). Field applications of non-fumigant nematicides have also yielded inconsistent results, ranging from complete suppression of nematode reproduction to negligible effects. These outcomes are influenced by host plant species, geographic location, and genetic variation within nematode populations (Watson 2022; Wram and Zasada 2020). Such variability highlights the complexity of nematicide efficacy and underscores the importance of tailoring nematode management strategies based on species-specific and environmental considerations. Despite this need, no studies to date have evaluated the sensitivity of the recently detected species *M. enterolobii*, *M. floridensis*, and *M. haplanaria* to non-fumigant nematicides. Therefore, the third objective of this study is to comprehensively assess the effects of sublethal doses of non-fumigant nematicides on the motility, egg hatching, and reproduction of *M. enterolobii*, *M. floridensis*, *M. haplanaria*, and *M. incognita*.

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CHAPTER 2

RELATIVE REPRODUCTION POTENTIAL OF RECENTLY DETECTED *MELOIDOGYNE* SPECIES IN GEORGIA ON MULTIPLE VEGETABLE CROPS[1]

[1] Poudel, N., Davis, R.F., Dutta, B., McAvoy, T., Timper, P., Chowdhury, I.C. To be submitted to Phytopathology

Abstract

Georgia ranks among the top vegetable-producing states in the U.S., with a farm-gate value of approximately $1.3 billion in 2023-accounting for 7.67% of the state's total agricultural commodity value. However, vegetable production in Georgia faces significant challenges from different diseases and pests and one of them is root-knot nematodes (*Meloidogyne* spp.). In a recent statewide survey in major vegetable-growing regions in Georgia, two *Meloidogyne* species-guava root-knot nematode (*M. enterolobii*) and peach root-knot nematode (*M. floridensis*)-were detected for the first time in addition to the commonly occurring southern root-knot nematode, *M. incognita*. Despite their emerging significance, comparative data on the reproductive capabilities of these recently detected species on key vegetable crops are lacking. This study evaluated the reproduction potential of *M. enterolobii*, *M. floridensis*, and *M. incognita* on eight economically important vegetable crops in Georgia: beet, broccoli, cabbage, cantaloupe, pepper, snap bean, squash, and tomato. Five independent greenhouse trials were conducted in Tifton, Georgia, during the summer and fall of 2023 and 2024 using a randomized complete block design with six replications per species. Inoculation was performed using either 8,000 eggs or 3,000 second-stage juveniles of each *Meloidogyne* species, and plants were harvested 10 weeks post-inoculation. Reproduction factor (final population/initial inoculum), galling index, and number of eggs per gram of root were quantified for each species in each vegetable crop. In most trials, *M. enterolobii* exhibited significantly higher reproduction factors (Rf), galling indices, and egg counts per gram of root compared to *M. incognita* and *M. floridensis* across the majority of tested vegetable crops, with the exception of squash and tomato. On these two crops, all three species showed comparable values for the measured

parameters. These findings highlight the high reproductive capacity and pathogenic potential of *M. enterolobii* on a broad range of vegetable hosts. Based on these findings, we hypothesize that in mixed-species populations within infested vegetable fields, *M. enterolobii* may become the predominant species due to its superior reproductive performance.

Keywords: galling index, reproduction, root-knot nematodes, vegetables

Introduction

Georgia is one of the leading vegetable-producing states in the United States, contributing significantly to the nation's agricultural economy. In 2023, the total farmgate value of vegetables produced in Georgia was estimated at $1.3 billion, representing approximately 7.67% of the total value of all agricultural commodities in the state (Georgia farm gate value report 2023, University of Georgia, 2025). However, vegetable production in Georgia faces considerable challenges from various soilborne pests and pathogens, including plant-parasitic nematodes (PPNs). One of the important PPNs that significantly reduce the yield of vegetable crops is root-knot nematodes (RKNs, *Meloidogyne* spp.). Root-knot nematodes induce the formation of specialized feeding structures known as giant cells, which arise through the redifferentiation and enlargement (hypertrophy) of vascular root cells, becoming multinucleate in the process. These giant cells serve as the exclusive nutrient source for the nematodes and are critical for their development and reproduction. The proliferation (hyperplasia) of neighboring root cells of the giant cell contributes to the formation of galls or root-knots-distinctive swellings that are characteristic symptoms of RKNs infection (Favery et al. 2016). Gall formation disrupts normal root function, impairing the plant’s ability to absorb water and nutrients from the soil. As a result, affected plants often display symptoms of nutrient and moisture deficiency, including wilting, stunted growth, chlorosis, and ultimately, a significant reduction in crop yield (Duncan and Noling 1998). In Georgia, RKNs has the greatest frequency and abundance over other PPNs, infesting 66.7% of vegetable fields (Marquez et al. 2021). The sandy soil and high temperatures for most part of the year in Georgia vegetable growing region foster conditions conducive to

RKNs infestation in multiple vegetable crops grown year-round (Koenning 1996; Marquez et al. 2021).

The southern RKN (*M. incognita*) is the most prevalent *Meloidogyne* species in vegetable fields across Georgia. In a recent survey of vegetable fields in southern Georgia, *M. incognita* was detected in 250 out of 292 RKN-infested fields, affecting a wide range of vegetable crops (Marquez and Hajihassani 2023a). While *M. incognita* remains the most significant RKN species in the region, emerging species such as *M. enterolobii* and *M. floridensis* have recently been detected in multiple agricultural fields in Georgia and neighboring states. These emerging species are particularly concerning because they are capable of reproducing on vegetable cultivars that are resistant to *M. incognita*, posing a new threat to existing RKNs management strategies.

Meloidogyne enterolobii (syn. *M. mayaguensis*), commonly known as the guava root-knot nematode (GRKN), was first detected in Georgia in 2021 in sweet potato fields. In addition to Georgia, *M. enterolobii* has been reported infecting various vegetable and row crops in Florida, Louisiana, North Carolina, and South Carolina (Gu et al. 2021; Overstreet et al. 2019; Riva et al. 2025; Rutter et al. 2019; Schwarz et al. 2020; Ye et al. 2013). This species is considered one of the most destructive RKNs due to its wide host range, and its ability to infect and reproduce on crops that carry resistance genes effective against more common species such as *M. incognita* (Castagnone-Sereno 2012; Philbrick et al. 2020).

Meloidogyne floridensis, commonly known as the peach root-knot nematode, was first detected in a tomato field in Georgia in 2018. Since then, it has also been reported on watermelon, cucumber, collard, and cowpea in the state (Marquez and Hajihassani 2023b). This

species was originally identified in Florida, where it was found infecting *M. incognita*-resistant peach cultivars ‘Nemaguard’ and ‘Okinawa’ (Sharpe 1968). Beyond Georgia, *M. floridensis* has been confirmed in 12 counties in Florida, infecting weeds, ornamentals, vegetables, and row crops (Brito et al. 2015); in almond orchards in two counties in California (Westphal et al. 2019); and on the peach rootstock cultivar ‘Guardian’ in one county in South Carolina (Reighard et al. 2019). Although historically associated with fruit trees, *M. floridensis* is capable of infecting a range of vegetable crops. Hosts include tomato, melon, sweet potato, cucumber, eggplant, and squash (Burelle and Nyczepir 2004; Marquez and Hajihassani 2023b; Ploeg and Edwards 2024). Similar to *M. enterolobii*, *M. floridensis* can also infect vegetable cultivars that possess resistance to *M. incognita*, such as those of tomato, sweet potato, and pepper (Ploeg and Edwards 2024; Ploeg and Stoddard 2024; Stanley et al. 2009).

Some studies have reported that *M. enterolobii* is more aggressive than *M. incognita* in terms of root galling and reproduction. For instance, Asmus et al. (2024) found that *M. enterolobii* exhibited a higher galling index and reproduction factor than *M. incognita* on both *M. incognita* resistant and susceptible cotton cultivars. In a microplot study, although no significant difference in reproduction was recorded, *M. enterolobii* produced coalesced and visually larger galls compared to *M. incognita* in tomato (Cetintas et al. 2007). Regarding *M. floridensis*, some studies have shown it to be more aggressive in terms of reproduction than *M. incognita* on certain vegetable crops, while in others, *M. incognita* was more aggressive. For example, in a study by Ploeg and Stoddard (2024), *M. floridensis* had a significantly higher reproduction factor than *M. incognita* on the tomato cultivar ‘Daniela’. However, in the same study, no significant difference was observed between the two species on sweet potato cultivar ‘Beauregard’. These

findings suggest that the reproductive potential of RKNs may vary depending on the host crop, and the nematode species.

In recent years, both *M. enterolobii* and *M. floridensis* have gained increasing importance in the southern United States due to their broad host ranges and ability to reproduce on vegetable cultivars previously considered resistant to RKNs. Most research on *M. enterolobii* has focused on determining the host status of various crops and developing management strategies for this important species (Brito et al. 2007; Grabau et al. 2024; Poudel et al. 2025a; Watson 2022; Wong et al. 2024), while studies on *M. floridensis* have been limited to a few crops, primarily assessing host suitability (Burelle and Nyczepir 2004; Ploeg and Edwards 2024, Ploeg and Stoddard 2024). However, no comprehensive studies have been conducted to know the host status of vegetable crops to *M. enterolobii* and *M. floridensis* and relative reproductive potential of *M. enterolobii*, *M. floridensis*, and *M. incognita* across major vegetable crops grown in Georgia. Moreover, the Georgia-specific isolates of *M. enterolobii* and *M. floridensis* have only been studied on the crops from which they were originally recovered (Hajihassani et al. 2023; Marquez and Hajihassani 2023b), and there is currently no information regarding their parasitism on other economically important vegetable crops in the state. Understanding the host status and relative reproductive ability of these *Meloidogyne* species on specific vegetable crops is essential for assessing their potential to cause economic damage and for developing effective, species-specific management strategies. Therefore, the objective of this study is to determine the host status of major vegetable crops grown in Georgia-including beet, broccoli, cabbage, cantaloupe, pepper, snap bean, squash, and tomato-to *M. enterolobii* and *M. floridensis*, and to assess the

relative reproductive potential of *M. enterolobii*, *M. floridensis*, and *M. incognita* on those crops identified as suitable hosts.

Materials and Methods

Preparation of nematode culture: *Meloidogyne* species used in this study were maintained as pure cultures in separate compartments under controlled greenhouse conditions to avoid cross-contamination. These pure cultures of *M. enterolobii*, *M. floridensis*, and *M. incognita* were originally detected in vegetable fields in Georgia and maintained on tomato (*Solanum lycopersicum* cv. 'Rutgers') under greenhouse conditions at the University of Georgia, Tifton campus, for at least three months prior to their use in the experiment. To prepare juvenile inoculum, the three-month-old inoculated plants were uprooted, and the infected roots were rinsed free of soil, chopped into small fragments, and placed on a screen that rested on top of stainless-steel collection pots. The pots were then placed in a mist chamber for 7 days to encourage egg hatching, after which the collected water was passed through nested 149 and 25 µm pore sieves. Fresh second-stage juveniles (J2s) remaining on the 25 µm pore sieve were then collected and enumerated under an inverted compound microscope (ZEISS Axio Vert.A1, Jena, Germany) at 40× magnification. For egg inoculum preparation, eggs were extracted by soaking galled roots in 0.5 % sodium hypochlorite solution while rotating at 250 rpm for 4 minutes. The mixture was poured through a series of mesh sieves (150 µm, 75 µm, and 25 µm), rinsed thoroughly with tap water, and the contents retained on the sieves were collected in 50 ml falcon tubes. The egg suspension was then purified using a centrifugal sugar flotation method. The rinsate was centrifuged at 3000 rpm for 5 minutes, and the supernatant discarded. The resulting pellet was resuspended in a sucrose solution (454 g/L) and centrifuged again at 3000 rpm for 1

minute. The egg-containing supernatant was poured over a 25 µm sieve and rinsed thoroughly with tap water to remove residual sugar (Jenkins 1964). Eggs were collected from the sieve in 50 ml falcon tubes and enumerated under an inverted compound microscope (ZEISS Axio Vert.A1, Jena, Germany) at 40× magnification. Eggs were stored in tap water at 4°C for 24 hours before inoculation. After extracting the eggs, the hatching percentage of each *Meloidogyne* species was calculated. For the hatching experiment, a kimwipe paper was placed over a metal basket, which was positioned above a bowl filled with water until the paper just touched the water's surface. The egg suspension containing 8,000 eggs was then poured onto the kimwipe paper, and the entire hatching setup was placed in an incubator at a temperature of 25 ± 1°C. Emerging J2s were collected daily over a 25 µm mesh sieve for seven days. The hatching percentage was calculated as the total number of J2s recovered divided by the initial number of eggs poured over the hatching setup (Poudel et al. 2025b).

Vegetable crops: Eight different vegetable crops including beetroot cv. Detroit Dark Red (*Beta vulgaris*), broccoli cv. Emerald Crown (*Brassica oleracea* var. *italica*), cabbage cv. Bravo (*Brassica oleracea* var. *capitata*), cantaloupe cv. Athena (*Cucumis melo* var. *cantalupensis*), pepper cv. Regulator (*Capsicum annuum*), snap bean cv. Blue Lake (*Phaseolus vulgaris*), squash cv. Lioness (*Cucurbita pepo*), and tomato cv. Rutger (*Solanum lycopersicum*) were used in this study. All the vegetables cultivar selected in this study are commercially grown in Georgia and susceptible to *M. incognita*.

Vegetable seedlings preparation, transplantation and timing of inoculation: Seed of cabbage, broccoli, cantaloupe, pepper and tomato were planted in Miracle-Gro Moisture Control potting mix (The Scotts Miracle-Gro Company, Marysville, OH, U.S.A.) in seed trays

(Speedling Incorporated, Ruskin, FL, U.S.A.). When seedlings were four weeks old, they were transplanted individually into 10 cm diameter pots filled with sterilized sandy loam soil. These vegetable crops were inoculated with particular *Meloidogyne* species 1 week after transplantation. In contrast, beetroot, snap bean, and squash were directly sown into pots filled with sterilized sandy loam soil and thinned to one plant per pot ten days after emergence. Beetroot plants were inoculated 20-25 days after thinning, while snap bean and squash were inoculated 12 days after sowing. These staggered inoculation times were chosen to ensure that all plants had developed a consistent root biomass suitable for nematode infection.

Experimental setup for egg inoculation experiments: For each vegetable crop, two independent experiments were conducted in fall 2023 in twenty-five days differences. For each crop, treatments included egg inoculation with one of three *Meloidogyne* species. Each pot was inoculated with 8,000 eggs of the particular *Meloidogyne* species in two 4 cm deep holes near the root region. Pots were arranged in a randomized complete block design (RCBD) with six replications for each crop in each experiment. The timing of inoculum preparation varied among crop groups. Broccoli and cabbage experiments were established concurrently, using the same batch of egg inoculum. Similarly, cantaloupe, beetroot, squash, tomato, and snap bean experiments were established together and shared the same egg inoculum. Pepper experiments were conducted separately from the others in both experiments and used different batches of egg inoculum.

Because egg batches differed in their hatching percentages, the number of viable J2s applied to each pot varied among the nematode species and across experiments. The estimated number of hatched J2s and corresponding hatch percentages for each crop and nematode species

for two experiments are presented in Tables 2.1 and 2.2. Plants were watered as necessary throughout the experiment to maintain adequate soil moisture. Plants were once fertilized with 2 g of Osmocote smart-release fertilizer (15-9-12 N-P-K; The Scotts Miracle-Gro Company, Marysville, OH, USA) at the time of planting. Knack® (a.i. Pyriproxyfen 11.23%, Valent®, San Ramon, CA, USA) and PQZ® insecticide (a.i. Pyrifluquinazon 20.0%, Nichino America, Wilmington, DE, USA) were applied in rotation at the rate of 65.6 mL a.i./ha and 41.0 mL a.i./ha, respectively, targeting whiteflies. Coragen® (a.i. Chlorantraniliprole 18.4%, FMC Corporation, Philadelphia, PA, USA) was used at a rate of 67.1 mL a.i./ha to control armyworms. All these products were applied as foliar sprays using a backpack sprayer. Based on label information and the available literature, the active ingredients of these insecticides lack nematicidal activity and are, therefore, not capable of directly influencing RKNs reproduction.

Experimental setup for J2s inoculation experiments: For each vegetable crop, three independent experiments were conducted in summer and fall of 2024. For each crop, treatments included inoculation with one of three *Meloidogyne* species. Each pot was inoculated with 3000 J2s of the particular *Meloidogyne* species in two 4 cm deep holes near the root region. Pots were arranged in a randomized complete block design (RCBD) with six replications for each crop in each experiment. Watering, fertilization and insect management practices were conducted as described above in egg inoculation experiments.

Data collection: Each experiment for each crop was terminated ten weeks after the nematode inoculation. At harvest, plant materials such as shoots and roots were gently separated from the soil and wrapped in a paper towel. Roots were then examined to determine root gall index. Galling indices were based on a combination of rating scales, where 0 = no galls and 1 = 1

to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100 galls. Plants with more than 100 root galls were rated as 5 = 25%, 6 = 50%, and 7 = 75% of roots are galled; 8 = roots are completely galled; 9 = roots are completely galled and rotting; and 10 = dead plant (Marquez and Hajihassani 2023b). Vegetative/growth parameters, like fresh root weight and fresh shoot weight, were measured using the weighing scale (Mettler) after uprooting the plants. Nematode eggs were extracted from the plant roots by soaking them in a 1% sodium hypochlorite solution and agitating the roots on an orbital shaker (Thermo MaxQ 2000, Thermo Fisher Scientific, Waltham, MA, USA) at 250 RPM for 4 min to facilitate egg release. Subsequently, the root material and hypochlorite solution were passed through a series of sieves (150 µm, 75 µm, and 25 µm) to separate and retain the nematode eggs, followed by rinsing with tap water to remove any remaining debris. The resulting suspension was then collected in a 50 mL tube, and eggs were counted under an inverted microscope. Eggs were enumerated with a nematode counting slide (Chalex LLC, Park City, UT, USA) under an inverted compound microscope (ZEISS Axio Vert.A1, Jena, Germany) at 40× magnification. For the experiments where eggs were used as the inoculum source, the reproduction factor (Rf) was calculated as the number of eggs extracted at harvest divided by the initial inoculum level adjusted for the percentage hatch (shown in Table 2.1 and Table 2.2). For the experiments, where juveniles were used as the inoculum source, Rf was calculated as the number of eggs extracted at harvest divided by the initial inoculum level (3000 J2s). In trials where J2s were used as the inoculum, the final egg count was divided by the fresh root weight to calculate the number of eggs per gram of root. However, this parameter was not calculated in trials where eggs were used as the inoculum, as the hatching percentages varied among

Meloidogyne species, resulting in different effective initial inoculum levels and making direct comparisons unreliable.

Data analysis: Data were analyzed using R Studio version 2021.09.0 Build 351 (R Core Team, 2020). In experiments where eggs were used as the inoculum, nematode species and experiments were considered as fixed effects and replications as a random effect in a linear mixed model, and data from two experiments (where eggs were used as inoculum) were pooled where no nematode species × experiments effect existed at $P = 0.05$ for each of the vegetable crop. In experiments where J2s were used as the inoculum, the nematode species × experiments effect existed for most of the vegetable crops, hence, data for each experiment were analyzed separately. Data for the reproduction factor and galling index which were not normally distributed were $\log_{10}(x)$ transformed to meet the assumptions of normality. However, non-transformed means of replicates from each experiment are presented in the table. Mean comparisons were performed using Tukey's Honestly Significant Difference (HSD) at $\alpha = 0.05$.

Results

Egg inoculation experiments: All vegetable crops evaluated supported reproduction of each *Meloidogyne* species, with Rf values greater than 1, indicating host suitability, except for pepper, which exhibited an Rf less than 1 for *M. floridensis*, suggesting it is a poor or non-host for this species (Table 2.3 and Table 2.4). In beetroot, *M. enterolobii* exhibited a greater Rf ($p=0.04$) than *M. incognita*, while *M. floridensis* had an Rf statistically similar to both *M. enterolobii* and *M. incognita*. A comparable trend was observed for the galling index, with *M. enterolobii* recording greater($p<0.0001$) galling index, followed by *M. floridensis* and *M. incognita* (Table 2.3). In broccoli and cabbage, *M. enterolobii* recorded greater ($p < 0.0001$) Rf

values than both *M. floridensis* and *M. incognita*, which were statistically similar to each other. This pattern was consistent in galling index, where *M. enterolobii* recorded greater galling ($p<0.0001$) compared to the other two species (Table 2.3). For cantaloupe, no significant differences were observed between *M. floridensis* and *M. incognita* in terms of Rf and galling index. However, *M. enterolobii* had greater ($p = 0.03$) Rf and greater ($p=0.003$) galling index compared to the other two species (Table 2.3). Pepper was confirmed to be a non-host for *M. floridensis* ($Rf < 1$). In contrast, *M. enterolobii* had greater ($p<0.0001$) reproduction and galling than *M. incognita* (Table 2.4). In snapbean, both *M. enterolobii* and *M. incognita* had greater ($p=0.03$) Rf values than *M. floridensis*, while no significant differences in galling index were observed among the three species (Table 2.4). For both squash and tomato, no significant differences were detected among *Meloidogyne* species for either reproduction factor or galling index (Table 2.4).

Second stage juveniles' inoculation experiments

Beetroot: Beetroot supported the reproduction of all three *Meloidogyne* species, with reproduction factors greater than one across all three experiments (Table 2.5). In first experiment, there was no differences for Rf, galling index and number of eggs/gram root among three *Meloidogyne* species. In second and third experiments, *M. enterolobii* recorded significantly greater reproduction factor compared to *M. floridensis* and *M. incognita*, while the reproduction factors of *M. floridensis* and *M. incognita* were statistically similar. A similar trend was observed for galling index and number of eggs/gram root, where *M. enterolobii* exhibited a significantly greater galling index and number of eggs/gram root than *M. floridensis* and *M. incognita* (Table 2.5).

Broccoli: Broccoli supported reproduction of *M. enterolobii*, with reproduction factor (Rf) values exceeding 1 in all three experiments (Table 2.6). In contrast, *M. floridensis* and *M. incognita* exhibited Rf values less than 1 in two of the three experiments, indicating that broccoli is a poor host for these two species. In the first and second experiments, *M. enterolobii* recorded significantly higher Rf, galling index, and number of eggs per gram of root compared to *M. floridensis* and *M. incognita*. No significant differences were observed between *M. floridensis* and *M. incognita* for any of these parameters. In the third experiment, *M. enterolobii* exhibited a significantly greater Rf and number of eggs per gram of root than *M. incognita*, but these values were statistically similar to those of *M. floridensis*. No significant differences among the *Meloidogyne* species were observed for galling index in the third experiment (Table 2.6).

Cabbage: Cabbage supported reproduction of *M. enterolobii*, with reproduction factor (Rf) values exceeding 1 in all three experiments (Table 2.7). In contrast, *M. floridensis* exhibited Rf values less than 1 in two of the three experiments, indicating that cabbage is a poor host for this species. *Meloidogyne incognita* had reproduction factor greater than 1 in two out of three experiments indicating that cabbage supports the reproduction of *M. incognita*. In first and second experiments, *M. enterolobii* recorded significantly higher Rf, galling index, and number of eggs per gram of root compared to *M. floridensis* and *M. incognita*. In the third experiment, no significant differences were observed among three *Meloidogyne* species for reproduction factor, galling index and number of eggs/gram root (Table 2.7).

Cantaloupe: Cantaloupe supported the reproduction of all three *Meloidogyne* species, with reproduction factors greater than one across all three experiments (Table 2.8). No statistical differences were observed among the *Meloidogyne* species for Rf, galling index and number of

eggs/gram root in the first experiment. In the second experiment, *M. enterolobii* recorded a significantly greater reproduction factor compared to *M. floridensis* and *M. incognita*, while the reproduction factors of *M. floridensis* and *M. incognita* remained statistically similar (Table 2.8). However, there was no significant differences among the *Meloidogyne* species for galling index in the second experiment. In second experiment, *M. enterolobii* recorded singificantly higher number of eggs/gram root compared to that of the *M. incognita* but similar to that of *M. floridensis*. *Meloidogyne floridensis* and *M. incognita* recorded statstically similar number of eggs/gram root. In third experiment, *M. enterolobii* recorded significantly greater reproduction factor, galling index and number of eggs/gram root compared to that of the *M. floridensis* and *M. incognita* (Table 2.8).

Snap bean: Snap bean was a good host for all three *Meloidogyne* species, with the reproduction factor being greater than one in all three experiments (Table 2.9). In the first experiment, *M. enterolobii* had greater reproduction factor (p=0.02) and greater (p=0.03) galling index than *M. floridensis* and *M. incognita*. Number of eggs/gram root was greater (0.01) in *M. enterolobii* infested snapbean compared to that of *M. floridensis* infested snap beans but statistically similar to that of *M. incognita*. In the second experiment, *M. enterolobii* recorded significantly higher Rf, galling index and number of eggs/gram root compared to that of *M. floridensis* but statistically similar to that of *M. incognita*. In the third experiment, there was no significant difference among the *Meloidogyne* species for the reproduction factor, galling index and number of eggs/gram root (Table 2.9).

Squash: Squash supported the reproduction of all three *Meloidogyne* species, with reproduction factors greater than one across all experiments (Table 2.10). There were no

significant differences in reproduction factor among the species in any of the three experiments. Similarly, no significant differences were observed among the species for the galling index and number of eggs/gram root across all three experiments (Table 2.10)

Tomato: Tomato supported the reproduction of all three *Meloidogyne* species, with reproduction factors greater than one across all experiments (Table 2.11). There were no significant differences in reproduction factor among the species in any of the three experiments. Similarly, no significant differences were observed among the species for the galling index and number of eggs/gram root across all three experiments (Table 2.11).

Discussion

Host status: This study is the first to evaluate the pathogenicity and relative reproductive potential of *M. enterolobii*, *M. floridensis*, and *M. incognita* on several vegetable crops commonly grown in Georgia. Among these, beetroot was identified as a good host for all three *Meloidogyne* species, with Rf exceeding 1 in all trials. To the best of our knowledge, this is the first report documenting the host status of beetroot to *M. floridensis*. Similar to our study, previous studies have reported the reproductive potential of *M. enterolobii* and *M. incognita* on the beetroot (Mashela 2017; Rodriguez et al. 2003). Cabbage variety used in this study 'Bravo' was a suitable host to *M. enterolobii*, similar to cabbage 'Esculenta' reported by by Brito et al. (2007) and 'Stonehead' reported by Wong et al. (2024). However, cabbage variety 'Premium' was considered poor host for *M. enterolobii* population from Cuba, suggesting differential response of cultivar or pathogen isolate (Rodriquez et al. 2003). In our study, cabbage was a poor host to *M. floridensis* and *M. incognita* in two of the three trials. This study is the first to report the host status of cabbage to *M. floridensis*. Similar to cabbage, broccoli supported the

reproduction of *M. enterolobii* but not that of *M. floridensis* and *M. incognita*. The good host status of broccoli to *M. enterolobii* has been previously reported by Brito et al. (2007). This is the first study to document the host status of broccoli to *M. floridensis*. Additionally, the poor host suitability of broccoli to *M. incognita* is consistent with earlier findings by Edwards and Ploeg (2014). Both cucurbit crops tested in this study-cantaloupe and squash-were highly susceptible to all three *Meloidogyne* species, with reproduction factors (Rf) exceeding 7 in all experiments for each species. The good host status of cantaloupe and squash to *M. enterolobii*, *M. floridensis*, and *M. incognita* has been previously reported (Brito et al. 2007; Bui and Desaeger 2022; Burelle and Nyczepir 2004). Similarly, tomato was a good host for all three *Meloidogyne* species evaluated, consistent with numerous earlier studies documenting its high susceptibility. Snap bean also supported the reproduction of *M. enterolobii*, *M. floridensis*, and *M. incognita*. In agreement with our findings, Rodriguez et al. (2003) reported that common bean supports reproduction of *M. enterolobii*, with Rf values greater than 1. Brito et al. (2008) detected *Meloidogyne floridensis* in snap bean during a survey of *Meloidogyne* species affecting horticultural crops in Florida. Although the detection confirmed that snap bean can serve as a host for *M. floridensis*, the study did not assess whether it was a good or poor host. In contrast, our study clearly demonstrates that snap bean is a good host to *M. floridensis*, with Rf values exceeding 1. As for *M. incognita*, its ability to reproduce on snap bean has been well documented in studies, consistently classifying snap bean as a good host. Pepper yielded particularly interesting results in our study. It was a good host for both *M. enterolobii* and *M. incognita*, but did not support the reproduction of *M. floridensis*. *Meloidogyne enterolobii* has previously been shown to reproduce on both *M. incognita*-susceptible and resistant pepper

cultivars carrying the *N* gene (Kiewnick et al. 2009; Maquilan et al. 2020). In contrast, reports on the host status of pepper to *M. floridensis* have been inconsistent. Some studies have identified *M. incognita*-susceptible pepper as a non-host (Burelle and Nyczepir 2004; Handoo et al. 2004), whereas others have reported that both *M. incognita*-susceptible and -resistant pepper cultivars can serve as good hosts to *M. floridensis* (Ploeg and Edwards 2024; Stanley et al. 2009). These discrepancies may be attributed to differences in *M. floridensis* isolates, environmental or experimental conditions, or variation among pepper cultivars used. It is also possible that the *M. floridensis* population present in Georgia represents a distinct race incapable of reproducing on pepper.

Relative reproduction potential: The relative reproduction ability of *Meloidogyne enterolobii*, *M. floridensis*, and *M. incognita* was first assessed by Cetintas et al (2007) in tomato. Our study is the first to evaluate and compare the reproductive potential of these three *Meloidogyne* species across a broader range of vegetable crops. Results indicated that, except for squash and tomato, *M. enterolobii* consistently exhibited significantly higher Rf compared to *M. incognita* and *M. floridensis* in most crops tested. This finding suggests that even among susceptible crops, the degree of susceptibility can vary depending on the *Meloidogyne* species involved. For instance, although beetroot, snap bean, and cantaloupe supported reproduction of all three species, *M. enterolobii* consistently achieved higher Rf values. In contrast, no significant differences in Rf or galling index were observed among the three species on squash and tomato, despite both being good hosts. These results highlight species-specific interactions that may influence disease severity and management strategies. Contrary to our findings, Cetintas et al. (2007) observed significant differences in galling indices among *M. enterolobii*,

M. floridensis, and *M. incognita* in a microplot study conducted on tomato. In their study, *M. floridensis* consistently induced significantly lower galling compared to *M. enterolobii* and *M. incognita* across all three trials. Additionally, in two of the three trials, both *M. enterolobii* and *M. incognita* recorded significantly higher numbers of second-stage juveniles (per 100 cm^3 of soil) and eggs per gram of root than *M. floridensis*. Interestingly, *M. incognita* had either similar or higher J2s counts and egg production compared to *M. enterolobii* in those trials. The differences between their findings and ours may be attributed to several factors, including variation in tomato cultivars used, environmental conditions, differences in *Meloidogyne* isolates, and experimental conditions. While Cetintas et al. (2007) conducted their experiments in microplots under field-like conditions, our study was conducted in a controlled greenhouse environment, which may have influenced the host-nematode interactions observed. In our study, most of the crops where *M. enterolobii* exhibited significantly higher Rf also recorded higher numbers of eggs per gram of root. This parameter is a critical indicator of the intensity of root infection and reproductive success, as a higher number of eggs per gram root generally correlates with greater damage potential. Based on our findings, *M. enterolobii* appears to pose a greater threat to crop productivity compared to *M. floridensis* and *M. incognita*. However, to accurately assess the yield reduction potential of these species, further studies under microplot or field conditions are necessary. Interestingly, in our study, there were no significant differences between *M. floridensis* and *M. incognita* in terms of Rf and galling index across almost all tested crops. This contrasts with the findings of Ploeg and Edward (2024), who reported consistently higher reproduction of *M. incognita* compared to *M. floridensis* in most vegetable crops, including tomato, which was also evaluated in our study. A key methodological difference lies in

the source of inoculum: Ploeg and Edwards (2024) used eggs as inoculum, without adjusting for hatch rates. Since hatching percentages can vary among *Meloidogyne* species, equal numbers of eggs may not translate to equal numbers of infective juveniles, potentially skewing the results. In our study, we either used J2s directly as inoculum or, when eggs were used, we accounted for species-specific hatch rates to standardize the number of infective juveniles applied. This approach not only improves the accuracy of reproduction factor calculations but also better simulates field conditions, where infection typically occurs through hatched juveniles rather than unhatched eggs. Since *M. enterolobii* exhibited either higher or comparable reproduction compared to *M. floridensis* and *M. incognita* in most of the vegetable crops tested, this suggests that, under temperature conditions similar to those in our experiments, a mixed field population of these species on a susceptible crop may gradually shift over time toward a dominance of *M. enterolobii* (Ploeg and Edwards 2024).

Our findings demonstrate that several important vegetable crops grown in Georgia are good hosts to the recently detected *M. enterolobii* and *M. floridensis*. *Meloidogyne enterolobii* exhibited consistently higher reproductive potential compared to the widely prevalent *M. incognita*, while *M. floridensis* showed a reproductive ability similar to that of *M. incognita*. The presence and reproductive success of *M. enterolobii* and *M. floridensis* in key vegetable crops are of particular concern, as both species can compromise resistance-based management strategies traditionally effective against *M. incognita*. This underscores the need for integrated nematode management approaches-incorporating cultural practices, biological control, and chemical nematicides-to limit the spread and establishment of these aggressive *Meloidogyne* species in Georgia's vegetable production systems. Furthermore, the development and deployment of crop

cultivars with resistance to *M. enterolobii* and *M. floridensis* should be prioritized to ensure sustainable nematode management and protect crop yields in the region.

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Table 2.1. Hatch of second-stage juveniles from 8,000 eggs of three *Meloidogyne* species on eight vegetable crops in first experiment.

Vegetable crops	*Meloidogyne* species		
	M. enterolobii	*M. floridensis*	*M. incognita*
Beetroot	3,520[y] (44)[z]	2,560 (32)	2,080 (26)
Broccoli	1,520 (19)	1,920 (24)	1,600 (20)
Cabbage	1,520 (19)	1,920 (24)	1,600 (20)
Cantaloupe	3,520 (44)	2,560 (32)	2,080 (26)
Pepper	3,440 (43)	1,680 (21)	2,400 (30)
Snap bean	3,520 (44)	2,560 (32)	2,080 (26)
Squash	3,520 (44)	2,560 (32)	2,080 (26)
Tomato	3,520 (44)	2,560 (32)	2,080 (26)

[y]Values are number of second-stage juveniles hatched from 8,000 eggs.

[z]Values indicate the percentage hatch from 8,000 eggs.

Table 2.2. Hatch of second-stage juveniles from 8,000 eggs of three *Meloidogyne* species on eight vegetable crops in second experiment.

Vegetable crops	*Meloidogyne species*		
	M. enterolobii	*M. floridensis*	*M. incognita*
Beetroot	3,760[y] (47)[z]	2,880 (36)	2,800 (35)
Broccoli	1,680 (21)	2,080 (26)	2,160 (27)
Cabbage	1,680 (21)	2,080 (26)	2,160 (27)
Cantaloupe	3,760 (47)	2,880 (36)	2,800 (35)
Pepper	2,800 (35)	2,000 (25)	2,320 (29)
Snap bean	3,760 (47)	2,880 (36)	2,800 (35)
Squash	3,760 (47)	2,880 (36)	2,800 (35)
Tomato	3,760 (47)	2,880 (36)	2,800 (35)

[y]Values are number of second stage juveniles hatched from 8,000 eggs.

[z]Values indicate the percentage hatch from 8,000 eggs.

Table 2.3. Reproductive potential of *Meloidogyne enterolobii*, *M. floridensis*, and *M. incognita* on beetroot, broccoli, cabbage, and cantaloupe following inoculation with 8,000 eggs.

Meloidogyne species	Beetroot		Broccoli		Cabbage		Cantaloupe	
	Rf[w]	GI[x]	Rf	GI	Rf	GI	Rf	GI
M. enterolobii	10.5[y] b[z]	7.0 c	2.3 b	2.2 b	4.9 b	2.9 b	11.1 b	6.0 b
M. floridensis	8.0 ab	6.1 b	1.5 a	1.3 a	2.3 a	1.4 ab	7.3 a	4.7 a
M. incognita	5.9 a	4.9 a	1.3 a	1.1 a	2.1 a	2.0 a	8.0 a	5.4 a
P value								
Nematode	0.04	<0.001	<0.001	0.001	<0.001	0.003	0.03	0.003
Experiment	0.36	0.70	0.30	0.32	0.15	0.50	0.77	0.04
Nematode×Experiment	0.51	0.60	0.17	0.80	0.30	0.89	0.65	0.09

[w]Reproduction factor, Rf = final total egg count/initial egg count adjusted for the percentage hatch

[x]Galling index, GI = Root gall indices for vegetables following *Meloidogyne* species inoculation (0 to 10 scale).

[y]Values are the mean of twelve replicates.

[z]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 2.4. Reproductive potential of *Meloidogyne enterolobii*, *M. floridensis*, and *M. incognita* on pepper, snapbean, squash, and tomato following inoculation with 8,000 eggs.

Meloidogyne species	Pepper		Snapbean		Squash		Tomato	
	Rf[w]	GI[x]	Rf	GI	Rf	GI	Rf	GI
M. enterolobii	14.4[y] c[z]	3.9 c	6.9 b	4.6 a	16.0 b	5.9 a	17.9 a	5.9 a
M. floridensis	0.5 a	0.8 a	4.4 a	3.9 a	9.1 a	5.4 a	15.9 a	5.6 a
M. incognita	10.9 b	2.3 b	6.4 b	4.5 a	13.5 ab	5.2 a	16.3 a	6.3 a
P value								
Nematode	<0.001	<0.001	0.03	0.22	0.002	0.38	0.85	0.37
Experiment	0.12	0.38	0.31	0.63	0.41	0.24	0.16	0.81
Nematode×Experiment	0.52	0.71	0.82	0.87	0.75	0.39	0.80	0.55

[w]Reproduction factor, Rf = final total egg count/initial egg count adjusted for the percentage hatch

[x]Galling index, GI = Root gall indices for vegetables following *Meloidogyne* species inoculation (0 to 10 scale).

[y]Values are the mean of twelve replicates.

[z]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 2.5. Reproductive potential of *Meloidogyne enterolobii*, *M. floridensis*, and *M. incognita* in beetroot cv. Detroit Dark Red following inoculation with 3,000 second-stage juveniles.

Meloidogyne species	Experiment 1			Experiment 2			Experiment 3		
	Rf[w]	GI[x]	Egg/g root	Rf	GI	Egg/g root	Rf	GI	Egg/g root
M. enterolobii	14. 5[y] a[z]	6.0 a	3,847 a	10.4 b	6.6 b	1,530 b	14.7 b	7.1 b	2,322 b
M. floridensis	11.4 a	5.1 a	1,676 a	6.6 a	5.8 a	1,044 ab	6 a	5.1 a	794 a
M. incognita	14.2 a	5.0 a	3,105 a	5.7 a	5 a	611 a	7 a	5.3 a	1,010 a
P value	0.09	0.28	0.15	0.007	0.002	<0.001	<0.001	<0.001	0.002

[w]Reproduction factor, Rf = final total egg count/initial inoculum egg count.

[x]Galling index, GI = Root gall indices for vegetables following *Meloidogyne* species inoculation (0 to 10 scale).

[y]Values are the mean of six replicates per trial.

[z]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 2.6. Reproductive potential of *Meloidogyne enterolobii*, *M. floridensis*, and *M. incognita* in broccoli cv. Emerald Crown following inoculation with 3,000 second-stage juveniles.

Meloidogyne species	Experiment 1			Experiment 2			Experiment 3		
	Rf[w]	GI[x]	Egg/g root	Rf	GI	Egg/g root	Rf	GI	Egg/g root
M. enterolobii	2.3[y] b[z]	2.5 b	545 b	1.6 b	1.6 b	330 b	1.3 b	0.8 a	400 b
M. floridensis	0.7 a	0.5 a	150 a	0.7 a	0. 5 a	138 a	1.1 ab	0 .6 a	358 ab
M. incognita	0.5 a	0.3 a	118 a	1.0 a	1.0 a	194 a	0.7 a	0.3 a	194 a
P value	<0.001	<0.001	<0.001	0.02	0.03	0.02	0.01	0.3	0.002

[w]Reproduction factor, Rf = final total egg count/initial inoculum egg count.

[x]Galling index, GI = Root gall indices for vegetables following *Meloidogyne* species inoculation (0 to 10 scale).

[y]Values are the mean of six replicates per trial.

[z]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 2.7. Reproductive potential of *Meloidogyne enterolobii*, *M. floridensis*, and *M. incognita* in cabbage cv. Bravo. following inoculation with 3,000 second-stage juveniles.

Meloidogyne species	Experiment 1			Experiment 2			Experiment 3		
	Rf[w]	GI[x]	Egg/g root	Rf	GI	Egg/g root	Rf	GI	Egg/g root
M. enterolobii	3.5[y] b[z]	2.3 b	943 b	2.6 b	2.3 b	737 b	1.2 a	1 a	383 a
M. floridensis	0.8 a	0.5 a	222 a	1.2 a	1.0 a	349 a	0.7 a	0.5 a	231 a
M. incognita	1.5 a	1.5 a	431 a	1.5 a	1.7 a	489 a	0.9 a	0.6 a	249 a
P value	<0.001	<0.001	<0.001	0.006	0.02	0.04	0.29	0.50	0.18

[w]Reproduction factor, Rf = final total egg count/initial inoculum egg count.

[x]Galling index, GI = Root gall indices for vegetables following *Meloidogyne* species inoculation (0 to 10 scale).

[y]Values are the mean of six replicates per trial.

[z]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 2.8. Reproductive potential of *Meloidogyne enterolobii*, *M. floridensis*, and *M. incognita* in cantaloupe cv. Athena following inoculation with 3,000 second-stage juveniles.

Meloidogyne species	Experiment 1			Experiment 2			Experiment 3		
	Rf[w]	GI[x]	Egg/g root	Rf	GI	Egg/g root	Rf	GI	Egg/g root
M. enterolobii	14.8 [y]a[z]	6.6 a	3,823 a	20.9 b	6.3 a	5,690 b	22.1 b	7.1 b	5,965 b
M. floridensis	15.9 a	6.5 a	4,382 a	15.5 a	5.8 a	4,614 ab	15.2 a	6 a	4,399 a
M. incognita	16.4 a	6.0 a	4,536 a	14.7 a	5.3 a	3,766 a	16.6 a	5.6 a	4,283 a
P value	0.75	0.5	0.43	0.001	0.14	0.01	0.002	<0.001	0.002

[w]Reproduction factor, Rf = final total egg count/initial inoculum egg count.

[x]Galling index, GI = Root gall indices for vegetables following *Meloidogyne* species inoculation (0 to 10 scale).

[y]Values are the mean of six replicates per trial.

[z]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 2.9. Reproductive potential of *Meloidogyne enterolobii*, *M. floridensis*, and *M. incognita* in snap bean cv. Blue lake following inoculation with 3,000 second-stage juveniles.

Meloidogyne species	Experiment 1			Experiment 2			Experiment 3		
	Rf[w]	GI[x]	Egg/g root	Rf	GI	Egg/g root	Rf	GI	Egg/g root
M. enterolobii	5.8[y] b[z]	5.7 b	1,921 b	6.0 b	5.5 b	2,097 b	5.8 a	5.1 a	2,488 a
M. floridensis	2.6 a	3.3 a	8,55 a	3.8 a	3 a	1,141 a	5.1 a	5.5 a	1,875 a
M. incognita	3.7 a	3.6 a	1,214 ab	4.6 ab	4.3 ab	1,383 ab	5.9 a	4.6 a	2,252 a
P value	0.02	0.03	0.01	0.01	<0.001	0.001	0.54	0.7	0.37

[w]Reproduction factor, Rf = final total egg count/initial inoculum egg count.

[x]Galling index, GI = Root gall indices for vegetables following *Meloidogyne* species inoculation (0 to 10 scale).

[y]Values are the mean of six replicates per trial.

[z]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 2.10. Reproductive potential of *Meloidogyne enterolobii*, *M. floridensis*, and *M. incognita* in squash cv. Lioness following inoculation with 3,000 second-stage juveniles.

Meloidogyne species	Experiment 1			Experiment 2			Experiment 3		
	Rf[w]	GI[x]	Egg/g root	Rf	GI	Egg/g root	Rf	GI	Egg/g root
M. enterolobii	17.8[y] a[z]	6.6 b	4,744 a	19.8 a	6.3 a	5,965 a	12.8 a	5.0 a	3,424 a
M. floridensis	19.1 a	6.0 a	5,402 a	17.7 a	5.3 a	4,712 a	11.3 a	4.1 a	3,211 a
M. incognita	17.9 a	5.8 a	4,964 a	17.8 a	5.6 a	4,542 a	12.7 a	5.1 a	3,080 a
P value	0.54	0.41	0.71	0.81	0.19	0.42	0.73	0.30	0.89

[w]Reproduction factor, Rf = final total egg count/initial inoculum egg count.

[x]Galling index, GI = Root gall indices for vegetables following *Meloidogyne* species inoculation (0 to 10 scale).

[y]Values are the mean of six replicates per trial.

[z]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 2.11. Reproductive potential of *Meloidogyne enterolobii*, *M. floridensis*, and *M. incognita* in tomato cv. Rutger following inoculation with 3,000 second-stage juveniles.

Meloidogyne species	Experiment 1			Experiment 2			Experiment 3		
	Rf[w]	GI[x]	Egg/g root	Rf	GI	Egg/g root	Rf	GI	Egg/g root
M. enterolobii	17.8[y] a[z]	5.8 a	6,685 a	22.9 a	5.6 a	6,864 a	15.3 a	5.3 a	4,549 a
M. floridensis	21.4 a	6.3 a	7,137 a	20.6 a	5.3 a	7,361 a	13.7 a	5.1 a	3,903 a
M. incognita	20.5 a	6.0 a	5,992 a	19.9 a	5.5 a	5,879 a	13.0 a	5.5 a	3,861 a
P value	0.61	0.80	0.70	0.65	0.87	0.43	0.21	0.81	0.48

[w]Reproduction factor, Rf = final total egg count/initial inoculum egg count.

[x]Galling index, GI = Root gall indices for vegetables following *Meloidogyne* species inoculation (0 to 10 scale).

[y]Values are the mean of six replicates per trial.

[z]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

CHAPTER 3

REPRODUCTION OF *MELOIDOGYNE ENTEROLOBII* ON ONION AND POTENTIAL YIELD SUPPRESSION[2]

[2] Poudel, N., Davis, R.F., McAvoy, T., Dutta, B., and Chowdhury, I.A. 2025. Published in Journal of Nematology, (https://doi.org/10.2478/jofnem-2025-0005). Reprinted here with permission of publisher.

Abstract

Meloidogyne enterolobii, is an emerging root-knot nematode species in the southern United States. To date, no studies have evaluated the host status of onions to *M. enterolobii*. This study aimed to assess the reproduction and pathogenicity of *M. enterolobii* on onion cultivars commonly grown in Georgia. Six Vidalia onion cultivars (‘Rio del Sol’, ‘Sapelo’, ‘Sweet Magnolia’, ‘Tania’, ‘Vidora’, and ‘NUN 1011’), three red onion cultivars (‘Red Duke’, ‘Red Halen’, and ‘Red Maiden’) and a white onion cultivar (‘Monjablanca’) were evaluated. Each cultivar was inoculated with 8,000 eggs of *M. enterolobii* in a repeated greenhouse trial with six replications each. Twelve weeks post-inoculation, plants were harvested to determine reproduction and pathogenicity based on the reproduction factor (Rf = final nematode population/initial nematode inoculum) and reductions in bulb and shoot weights, respectively. All tested cultivars were susceptible to *M. enterolobii*, with Rf values greater than 1, though significant differences were observed. ‘Vidora’, and ‘Tania’, exhibited the highest galling index and Rf values, while ‘Sweet Magnolia’ and ‘Sapelo’ had the lowest. All red onion cultivars showed significant reductions in weight for both bulbs and shoots, whereas among the Vidalia cultivars, only ‘NUN 1011’ exhibited notable reductions in bulb and shoot weights. These findings suggest that onions are suitable hosts for *M. enterolobii*, and that the nematode's reproduction and pathogenicity vary with onion type and cultivar.

Keywords**:** *Allium cepa*, guava root-knot nematodes, nematode reproduction, pathogenicity, red onion, Vidalia onion

Introduction

Onion (*Allium cepa*) is one of the economically important vegetable crops grown in the United States. According to the United States Department of Agriculture (USDA) National Agriculture Statistics Service (NASS), the total production of onions in 2023 was approximately 3.32 million tons with an estimated value of $1.5 billion (USDA-NASS, 2024). Georgia is one of the leading onion-producing states in the United States. In 2023, total onion production in Georgia was approximately 115 thousand tons, with an estimated value of $159 million (USDA-NASS, 2024). Georgia is known for the production of the Vidalia onion, a short-day type Yellow Granex onion. Vidalia onions are exclusively grown in the southeastern portion of Georgia, where mild winters, low sulfur soils, and sufficient water supply enhance their sweetness (Boyhan et al., 2007). In addition to Vidalia onions, red onions, and white onions are also grown in Georgia.

Onion production in Georgia is constrained by various plant diseases caused by fungi, bacteria, viruses, and plant-parasitic nematodes (Hajihassani et al., 2019; Dutta, 2024). Plant-parasitic nematodes (PPN) are a significant pest in southern Georgia, where relatively warm temperatures create a suitable environment for infection and reproduction on numerous vegetable crops (Marquez et al., 2021). Several PPN genera including cyst nematode (*Heterodora* spp.), lance nematode (*Hoplolaimus* spp.), ring nematode (*Mesocriconema* spp.), root-knot nematodes (*Meloidogyne* spp.), spiral nematode (*Helicotylenchus* spp.), and the stubby-root nematode (*Nanidorus* spp.) have been detected in onion fields of Georgia (Hajihassani et al., 2019; Marquez et al., 2021). Among these, root-knot nematodes (RKN) are one of the most prevalent nematode genera infesting onion fields of the state (Hajihassani et al., 2019). Infestation by RKN

can cause the formation of small galls in onion roots leading to poor uptake of water and nutrients from the soil. This can lead to stunting, chlorosis, and ultimately a reduction in crop yield (Gregon et al., 2002; Mitkowski et al., 2002).

Multiple species of RKN have been documented to parasitize onion including *M.arenaria*, *M. chitwoodi*, *M. incognita*, *M. hapla*, *M. javanica*, and *M. graminicola* (Gergon et al., 2002; Mitkowski et al., 2002; Davis and Langston, 2003; Pang et al., 2009a). Moreover, most of these species are capable of causing significant yield loss. For instance, Corgan et al. (1985) observed up to a 76% reduction in onion bulb yield due to *M. incognita* infestation. Gergon et al. (2002) reported yield reductions between 7% and 82% in onion bulb weight under greenhouse conditions when inoculated with 50-10,000 second-stage juveniles (J2) of *M. graminicola* and documented up to a 35% yield loss in field trials. Similarly, total onion weight was reduced by 40.6% to 59.6% in greenhouse conditions when onions were inoculated with *M. hapla* at levels of 3,000 to 12,000 J2/1,500 cm^3. In microplot trials inoculated with 160,000 J2 of *M. hapla* per 20,000 cm^3 soil, there was a yield reduction of up to 41.3% in onion bulbs (Pang et al., 2009a). These studies clearly show that RKN species can be a major threat in onion production.

In recent years, an aggressive species of RKN (*M. enterolobii* [syn. *M. mayaguensis*]) has been detected in multiple states of the U.S. (Philbrick et al., 2020; Hajihassani et al., 2023). This species of RKN is considered one of the most damaging species due to its wide host range, pathogenicity, and ability to develop and reproduce on several crops carrying resistance genes effective against other common RKN species including *M. incognita*, *M. javanica* and *M. arenaria* (Castagnone-Sereno, 2012; Philbrick et al., 2020). In Georgia, *M. enterolobii* was first

detected in 2021 from a sweet potato field in Tattnall County (Hajihassani et al., 2023), where approximately 40% of the onions in the state are produced (Stubbs, 2024). Sweet potatoes, which are often rotated with onions in this region of Georgia, are excellent hosts for *M. enterolobii*. This rotation enables the *M. enterolobii* galled root debris and second-stage juveniles to remain in the soil after sweet potato cultivation, increasing the likelihood of onions encountering the nematode. There is limited information about the interactions of *M. enterolobii* with onion. To our knowledge, there has been no study of whether *M. enterolobii* can parasitize onion. Hence the objectives of this study were to assess the reproductive ability and pathogenicity of *M. enterolobii* on different onion cultivars grown in Georgia. The findings from this study are critical for developing effective management strategies to mitigate the economic losses caused by this nematode.

Materials And Methods

Plant materials: Six Vidalia onion cultivars (‘Sapelo’, ‘Sweet Magnolia’, ‘Tania’, ‘Vidora’, ‘Rio del Sol’, and ‘NUN 1011’), three red onion cultivars (‘Red Halen’, ‘Red Duke’, and ‘Red Maiden’), and one white onion cultivar (‘Monjablanca’) were evaluated in this study. All the selected cultivars are commercially grown in Georgia. Nine-week-old transplants of all onion cultivars were sourced from the University of Georgia, Vidalia Onion and Vegetable Research Center in Lyons, GA.

Preparation of nematode inoculum: The *M. enterolobii* isolate used in this trial was originally isolated from sweet potatoes in South Georgia (Hajihassani et al., 2023) and was used as the inoculum source. A pure culture of *M. enterolobii* was maintained on eggplant cv. ‘Black Beauty’ and tomato cv. ‘Rutger’ for three months before use. To prepare the inoculum, eggs, and

second-stage juveniles were extracted by soaking galled roots in a 0.5% sodium hypochlorite solution while rotating at 250 rpm for two minutes. After shaking, roots and sodium hypochlorite solution were poured through mesh sieves (150 µm, 75 µm, and 25 µm), rinsed with tap water, and collected in a 50 ml tube. The rinsate collected from a sieve was then subjected to the centrifugal sugar flotation technique to separate eggs from other debris. Specifically, the rinsate was centrifuged at 3000 rpm for 5 minutes. The supernatant was discarded, and the pellet was resuspended in a sucrose solution (454 g/L). This suspension was then centrifuged at 3000 rpm for 1 minute. The supernatant containing the eggs was poured over a 25 µm sieve and rinsed thoroughly with tap water to remove the sugar (Jenkins, 1964). Eggs were collected from the sieve and stored in tap water at 4^0C for 24 hours before inoculation. After extracting the eggs, the hatching percentage of nematode eggs were calculated. For the hatching experiment, a Kimwipe paper was placed over a metal basket, which was positioned above a bowl filled with water until the paper just touched the water's surface. The egg suspension containing 8,000 eggs was then poured onto the Kimwipe paper, and the entire hatching setup was placed in an incubator at a temperature of 25 ± 1°C. Emerging J2s were collected daily over a 25 µm mesh sieve for seven days. The hatching percentage was calculated as the total number of J2s recovered divided by the initial number of eggs poured over the hatching setup.

Establishment of trials: Trials were conducted in greenhouse conditions at the University of Georgia, Tifton Campus. The first trial was conducted during spring 2023 (January–March), and the second trial was conducted during spring 2024 (January–March). Plastic pots with 20 cm diameter were filled with sterilized sandy loam soil. Each pot was planted with a single 9-week-old onion seedling with two to four true leaves. A week after

planting the onion seedlings, each pot was inoculated with approximately 8,000 eggs of *M. enterolobii*. Eggs were sourced exclusively from eggplant in first experiment and tomato in the second experiment, to ensure that the inoculum remained consistent within each trial. The suspension of eggs was inoculated into three 1.5-cm-deep holes made 1 cm from the base of the onion seedlings, and then the holes were covered with a light layer of soil and moistened with water. Treatments included *M. enterolobii*-inoculated and non-inoculated control pots for each onion cultivar, and the pots were arranged in a randomized complete block design with six replications. Inoculated pots allowed evaluation of nematode reproduction, and the comparison of inoculated and non-inoculated pots allowed the evaluation of the nematode's effect on plant growth. Plants were watered daily, and each pot was fertilized with 3 g of Osmocote 15-9-12 N-P-K controlled-release fertilizer (Scotts-Sierra Horticultural Products Co, Marysville, OH, U.S.A.) at the time of planting and 2 g after one and a half month of planting.

Data collection: The trials were terminated 12 weeks after inoculation. At harvest, plant materials including shoot, root, and bulb were gently separated from the soil and wrapped in a paper towel. The fresh shoot, root, and bulb weight of each plant were then recorded on a weighing scale. Roots were then examined to determine the root gall index. Galling indices were based on a rank scale described by Marquez and Hajihassani (2023), which was adapted from Taylor and Sasser (1978) and Bridge and Page (1980), where 0 = no galls and 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100 galls. Plants with more than 100 root galls were rated as 5 = 25%, 6 = 50%, and 7 = 75% of roots are galled; 8 = roots are completely galled; 9 = roots are completely galled and rotting; and 10 = dead plant. After rating the galling indices, eggs were extracted using the sodium hypochlorite-based extraction method described previously.

However, 1% sodium hypochlorite solution was used for at-harvest egg extraction for increased extraction efficiency, unlike inoculum preparation where 0.5% sodium hypochlorite was used to ensure the viability of eggs (Hussey and Barker, 1973; Marquez and Hajihassani, 2023). Eggs were enumerated with a nematode counting slide (Chalex LLC, Park City, UT, U.S.A) under an inverted compound microscope (ZEISS Axio Vert.A1, Jena, Germany) at 40X magnification. The reproduction factor (Rf) was calculated as the number of eggs extracted at harvest divided by the initial inoculum level adjusted for the percentage hatch. Initial inoculum was 3,600 for the first trial (45 % hatch of 8,000 eggs) and 3,120 for the second trial (39 % hatch of 8,000 eggs). These inoculum levels represent typical RKN density (1-2 J2/cm^3 soil) observed in vegetable fields of Georgia. Roots and shoots of each onion seedlings were dried at 80 °C for 120 hours to measure dry weights.

Data analysis: Data were analyzed using R studio version 2021.09.0 Build 351 (R Core Team, 2020). Cultivars and trials were considered as a fixed effect and replications as a random effect in a linear mixed model, and data from two trials were pooled where no cultivars × trials effect existed at $P = 0.05$. In a scenario where the cultivars × trials effect existed, data for each trial were analyzed separately. Data for the number of eggs per root system, number of eggs per gram of root, Rf, and galling index were $\log_{10}$ (x) transformed to meet the assumptions of normality, however, non-transformed means of replicates from each trial are presented in the table. Mean comparisons were performed using Tukey's Honestly Significant Difference (HSD) at $\alpha = 0.05$. To compare the mean difference in growth and yield between inoculated and non-inoculated onion plants of each variety, two sample independent t-test was performed at $\alpha =$

0.05. Before performing the t-test, assumptions for t-test including normality of the dependent variable and homogeneity of variances among the independent samples were tested

Results

Nematode population, root gall index, and reproduction factor: There was a significant trial x cultivar interaction for the number of eggs per root system, number of eggs per gram of root, and the Rf. Therefore, data were analyzed separately for each trial. However, there was no trial x cultivar interaction for root gall index, so data from both trials were pooled for this parameter.

Significant differences were observed among the tested onion cultivars for the number of eggs per root system in both trials (Table 3.1). In the first trial, 'Vidora' supported the most eggs per root system, which was significantly greater than the counts for 'Sapelo' and 'Sweet Magnolia' but statistically similar to the other onion cultivars tested. The number of eggs per root system for 'Tania', 'NUN 1011', 'Monjablanca', 'Rio del Sol', 'Red Duke', 'Red Halen', and 'Red Maiden' were intermediate and did not differ from any cultivar tested. In the second trial, 'Tania' had the most eggs per root system, which was significantly greater than those recorded for 'Sapelo', 'Sweet Magnolia', 'Monjablanca', and 'Rio del Sol' (Table 3.1). The number of eggs per root system for 'Vidora', 'NUN 1011', 'Red Duke', 'Red Halen', and 'Red Maiden' were intermediate and did not differ from any cultivar tested.

Significant differences were observed among onion cultivars for the number of eggs per gram of root in both trials (Table 3.1). In the first trial, 'Vidora' had the most eggs per gram of root and was significantly greater than 'Sapelo' and 'Sweet Magnolia', but not significantly different from the other cultivars tested. The number of eggs per gram of root for 'Monjablanca',

‘Tania’, ‘NUN 1011’, ‘Red Duke’, ‘Red Halen’, and ‘Red Maiden’ were intermediate and statistically similar to all other cultivars tested. In the second trial, ‘Tania’ had the most eggs per gram of root, which was significantly greater than ‘Sweet Magnolia’, ‘Monjablanca’, ‘Sapelo’, ‘Rio del Sol’, and ‘Red Maiden’. The number of eggs per gram of root in ‘Vidora’, ‘NUN 1011’, ‘Red Duke’, and ‘Red Halen’ were intermediate and did not differ from any cultivar tested.

The Rf varied significantly among onion cultivars in both trials. In the first trial, ‘Vidora’ had the highest Rf, which was significantly greater than those observed for ‘Sapelo’ and ‘Sweet Magnolia’, but not significantly different from the other cultivars tested (Table 3.1). The Rf values for ‘Tania’, ‘NUN 1011’, ‘Red Duke’, ‘Red Halen’, ‘Monjablanca’, ‘Rio del Sol’, and ‘Red Maiden’ were intermediate and did not differ from any cultivar tested. In the second trial, ‘Tania’ had the highest Rf, which was significantly higher than those of ‘Monjablanca’, ‘Rio del Sol’, ‘Sapelo’, and ‘Sweet Magnolia’ (Table 3.1). The Rf values for ‘Vidora’, ‘NUN 1011’, ‘Red Duke’, ‘Red Halen’, and ‘Red Maiden’ were intermediate and did not differ from any cultivar tested.

There were significant differences among the onion cultivars for galling index (Fig. 3.1). ‘Tania’ and ‘Vidora’ had the highest galling index, which was significantly greater than ‘Sapelo’, ‘Sweet Magnolia’, and ‘Monjablanca’, but similar to other cultivars tested.

Pathogenicity of *M. enterolobii* on onion: *Meloidogyne enterolobii* caused a significant reduction in bulb weights of all three red onion cultivars tested (Fig. 3.2). For ‘Red Duke’, ‘Red Halen’, and ‘Red Maiden’, the average fresh bulb weight without *M. enterolobii* inoculation was statistically greater than the bulb weight obtained from the inoculated plants. Nematode parasitism reduced the bulb weight of ‘Red Duke’ by 31.1%, ‘Red Halen’ by 26.6%, and ‘Red

Maiden' by 24.6%. Among the Vidalia onion cultivars, only 'NUN 1011' showed a significant reduction in bulb weight (17.4%). The other Vidalia onion cultivars tested had numerically lower fresh bulb weights following *M. enterolobii* inoculation, but the differences were not statistically different. Similar result was obtained for Monjablanca, where the differences in bulb weight between inoculated and non-inoculated control treatments were not statistically different.

There were significant differences in the fresh shoot weights of all three red onion cultivars between inoculated and non-inoculated control treatments (Fig. 3.3). Nematode parasitism reduced the fresh shoot weight of 'Red Duke' by 23.2%, 'Red Halen' by 27.4%, and 'Red Maiden' by 22.2%. A similar trend was observed for dry shoot weight; all three red onion cultivars displayed significant reductions in dry shoot weight when inoculated with *M. enterolobii* (Fig 3.4). Among Vidalia onion cultivars, only 'NUN 1011' showed a significant reduction in fresh shoot weight, with a reduction of 22.2%; the dry shoot weight of 'NUN 1011' was not significantly affected by nematode parasitism. There was no statistical difference in fresh and dry shoot weight of Monjablanca between inoculated and non-inoculated treatments. There was a clear trend in which all cultivars tested had numerically lower fresh and dry shoot weights following *M. enterolobii* inoculation, but the only statistically significant differences were those noted above (Fig. 3.3, 3.4). There were also no statistical differences among the fresh or dry root weights of the onion cultivars tested (data not shown).

Discussion

Meloidogyne enterolobii is emerging as a significant threat to the vegetable industry in the United States. This species of RKN has been documented in multiple states, including Florida, Georgia, Louisiana, North Carolina, and South Carolina. *Meloidogyne enterolobii*

affects a variety of weeds and crops including vegetables, fruit trees, and ornamental plants (Brito et al., 2007; Philbrick et al., 2020; Hajihassani et al., 2023; Schwarz et al., 2024). In Georgia, *M. enterolobii* was first detected in 2021 from a sweet potato field in Tattnall County (Hajihassani et al., 2023), located at the heart of Georgia's world-famous Vidalia onion-producing region. Importantly, most of the sweet potato cultivars that have intermediate to high levels of resistance to the southern RKN, *M. incognita,* are susceptible to *M. enterolobii* (Galo et al., 2024), which increases the risk of damage to onion when onion is rotated with sweet potato in fields infested by *M. enterolobii*. Moreover, the sandy soils and warm temperatures of Georgia's onion-growing region foster conditions highly conducive to *Meloidogyne enterolobii* infestation in onions (Koenning et al., 1996; Marquez et al., 2021). Compared to *M. incognita*, *M. enterolobii* has a relatively shorter life cycle, and thus can have rapid population build up and increased host damage (Collett et al., 2024). Yet there is no information in literature regarding onion's hosting ability to *M. enterolobii.* To more accurately assess the potential for damage and the need for management of *M. enterolobii* in onion, it is necessary to evaluate the host status of onion to *M. enterolobii.*

The results obtained from our study showed that Vidalia, red, and white onions are good hosts to *M. enterolobii* with Rf values ranging from 3.2 to 9.0. Though all the cultivars tested showed good hosting ability to *M. enterolobii*, significant differences were observed among the cultivars. 'Vidora', and 'Tania' had significantly higher Rf values compared to other cultivars, which documents that there can be significant differences among onion cultivars in *M. enterolobii* reproduction. This result is reminiscent of the results obtained by Kotcon et al. (1985), where the authors reported significant differences among onion cultivars for the

reproduction of *M. hapla*. Likewise, in a screening assay done by Pang et al. (2009b), there was a significant difference among onion cultivars to the reproduction of *M. hapla*, with some cultivars showing resistance. However, in our study, none of the tested cultivars of either Vidalia or red onions showed resistance to *M. enterolobii*. 'Sapelo' and 'Sweet Magnolia' recorded lowest number of eggs per root system and eggs per gram root, in both experiments and statistically lower than 'Tania' and 'Vidora' in one of the two experiments, showing that there can be differences in onion cultivars for susceptibility to *M. enterolobii.*

Our study showed differences in plant growth between the non-inoculated control and the inoculated treatment for some cultivars by the end of the trial. All three red onion cultivars showed a significant reduction in the growth and yield parameters like fresh shoot weight, fresh bulb weight, and dry shoot weight, whereas only one Vidalia onion cultivar ('NUN 1011') showed reduction in these parameters. The percentage reduction of these growth parameters was significantly higher in red onion cultivars compared to that of the Vidalia onion cultivars and a white onion cultivar, which suggests that the pathogenicity of *M. enterolobii* can be different among onion types. Although the eggs per gram of fresh root weight in red onion cultivars were either statistically lower or comparable to those in Vidalia onion cultivars and a white onion cultivar, the percentage reduction in bulb weight and shoot weight was still significantly higher in the red onion cultivars.

In our study, *M. enterolobii* had no significant effect on the growth parameters of a white onion cultivar and most Vidalia onion cultivars, except for 'NUN 1011', though a general trend of numerical reduction in plant weight was observed in inoculated white and Vidalia onion cultivars, affirming the potential of *M. enterolobii* to damage onion crop (Fig. 2, 3, and 4).

Meloidogyne enterolobii might need higher inoculum densities than we used in our trial to reduce the yield of Vidalia onion cultivars. The hatching rate of *M. enterolobii* in our trials was 45% in the first trial and 39% in the second, resulting in an inoculation density of 3,600 J2/1,500 cm^3 soil in the first trial and 3,120 J2/1,500 cm^3 soil in the second. A study by Pang et al. (2009a) found that while *M. hapla* began to reduce onion growth and yield at an inoculation density of 3,000 J2/1,500 cm^3 soil, maximum yield reduction occurred at 10,000 J2/1,500 cm^3 soil. Similarly, Gergon et al. (2002) observed a yield reduction ranging from 7% to 82% in onions inoculated with 50 to 10,000 second-stage juveniles (J2) of *M. graminicola*. Further investigation into the correlation between *M. enterolobii* inoculum density and yield reduction in Vidalia onion cultivars is necessary to determine whether higher densities could lead to more significant yield losses. It is also important to note that the response of onions to RKN may differ between greenhouse and field conditions. For example, onions inoculated with *M. hapla* showed significant yield reductions in both environments, but there was a greater reduction in total onion weight in greenhouse conditions compared to microplot trials, despite using the same inoculum density (Pang et al., 2009a). Gergon (2002) also observed variations in the pathogenicity of *M. graminicola* between greenhouse and field trials, with yield reductions being greater in greenhouse conditions. Therefore, conducting trials in *M. enterolobii*-infested fields would provide valuable insights into the pathogenicity of *M. enterolobii* on large scale onion production.

Previous studies have shown different species of RKN including *M. incognita*, *M. chitwoodi*, *M. graminicola*, and *M. hapla* significantly reduce the yield of onion. However, prior to this work, the response of onion to *M. enterolobii* was not known. To the best of our

knowledge, this is the first data to begin evaluating the reaction of onion cultivars to *M. enterolobii*. In summary, this study identified Vidalia onion, white onion, and red onion as a host to *M. enterolobii* and suggested differences in response of onion types and cultivars to *M. enterolobii* reproduction and pathogenicity.

Acknowledgments

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Table 3.1. Susceptibility of ten onion cultivars to *Meloidogyne enterolobii*.

Onion cultivars	Onion type	Number of eggs/roots system		Number of eggs/grams of root		Reproduction factor (Rf)[x]	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Monjablanca	White	17190[y]ab[z]	10,560 b	2,223 ab	1,447 b	4.78 ab	3.38 b
Rio del Sol	Vidalia	16,280 ab	12,978 b	1,982 ab	1,596 b	4.52 ab	4.16 b
Sapelo	Vidalia	11,546 b	13,667 b	1,407 b	1,709 b	3.21 b	4.58 b
Sweet Magnolia	Vidalia	11,997 b	11,574 b	1,474 b	1,441 b	3.33 b	3.71 b
Tania	Vidalia	22,999 ab	28,160 a	2,806 ab	3,657 a	6.39 ab	9.03 a
Vidora	Vidalia	27,930 a	20,915 ab	3,406 a	2,477 ab	7.76 a	6.70 ab
NUN 1011	Vidalia	21,137 ab	20,519 ab	2,632 ab	2,374 ab	5.87 ab	6.58 ab
Red Duke	Red	20,322 ab	20,415 ab	2,257 ab	2,258 ab	5.64 ab	6.54 ab
Red Halen	Red	21,373 ab	17,883 ab	2,466 ab	2,042 ab	5.94 ab	5.73 ab
Red Maiden	Red	17,947 ab	16,090 ab	2,145 ab	1,886 b	4.99 ab	5.16 ab
***P*-value**		0.01	0.0012	0.0251	0.0042	0.01	0.0012

[x]Reproduction factor, Rf = final total egg count/initial inoculum egg count.

[y]Values are mean of six replicates per trial.

[z]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).

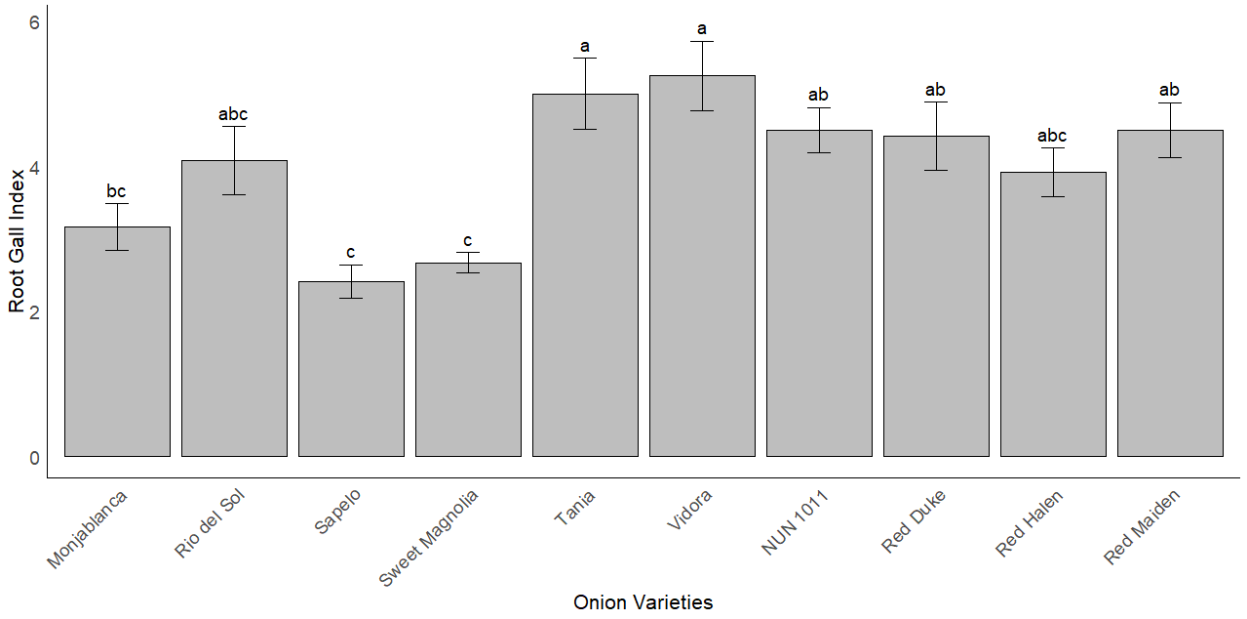

Figure 3.1. Root gall indices for cultivars of onion following *M. enterolobii* inoculation (0 to 10 scale). Bars represent the Root Gall Index (mean ± standard error). Values are pooled data from two trials. Bars with the same letter are not significantly different according to Tukey's HSD test (α=0.05). Monjablanca is a white onion, Red Duke, Red Halen and Red Maiden are red onions and all other onion cultivars are Vidalia onions.

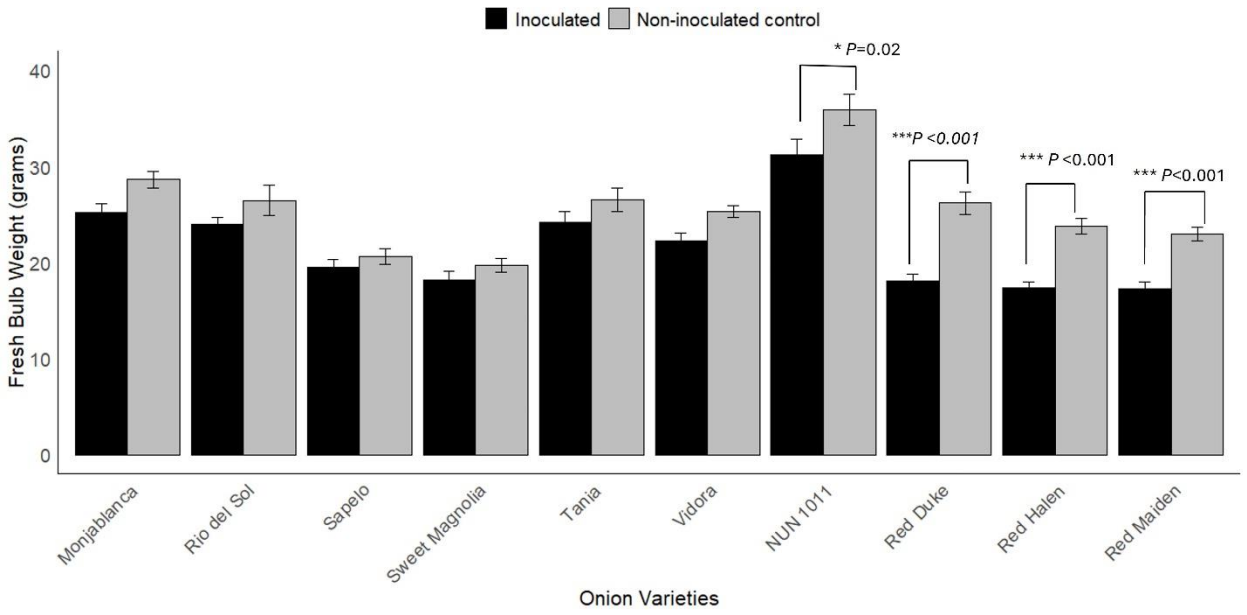

Figure 3.2. Effect of *Meloidogyne enterolobii* on fresh bulb weights of onion cultivars. Bars represent the fresh bulb weight (mean ± standard error). Values are pooled data from two trials. Two sample independent t-tests were done to identify differences between inoculated and non-inoculated control treatments. * and *** represents the 5% and 0.1% level of significance respectively. Monjablanca is a white onion, Red Duke, Red Halen and Red Maiden are red onions and all other onion cultivars are Vidalia onions.

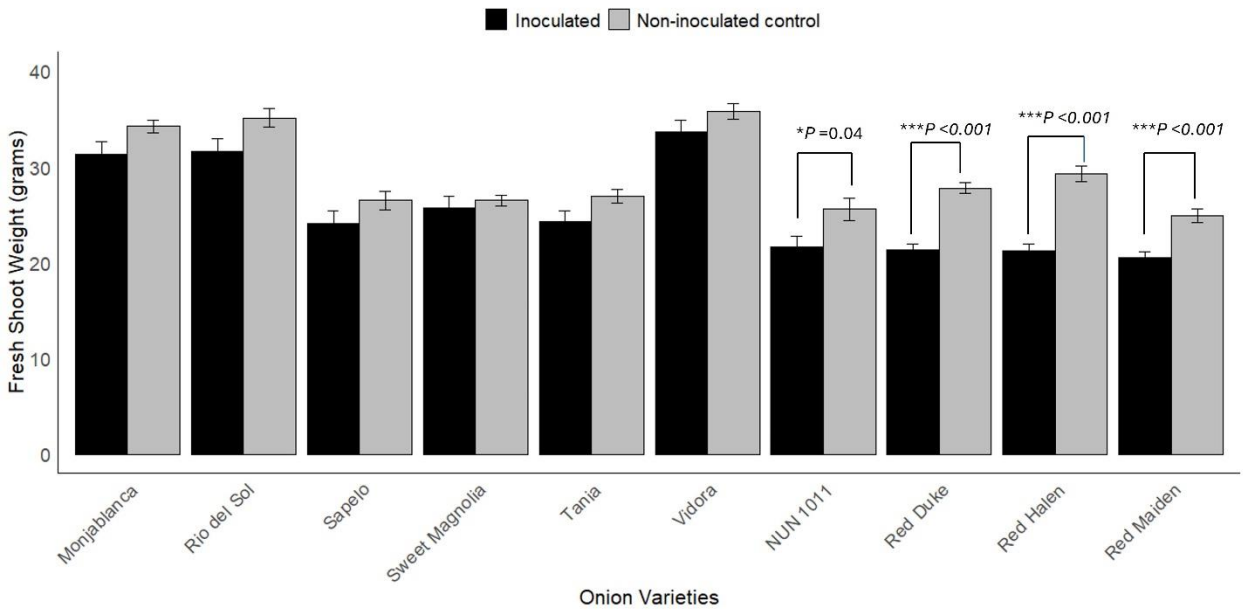

Figure 3.3. Effect of *Meloidogyne enterolobii* on fresh shoot weights of onion cultivars. Bars represent the fresh shoot weight weight (mean ± standard error). Values are pooled data from two trials. Two sample independent t-tests were done to identify differences between inoculated and non-inoculated control treatments. * and *** represents the 5% and 0.1% level of significance respectively. Monjablanca is a white onion, Red Duke, Red Halen and Red Maiden are red onions and all other onion cultivars are Vidalia onions.

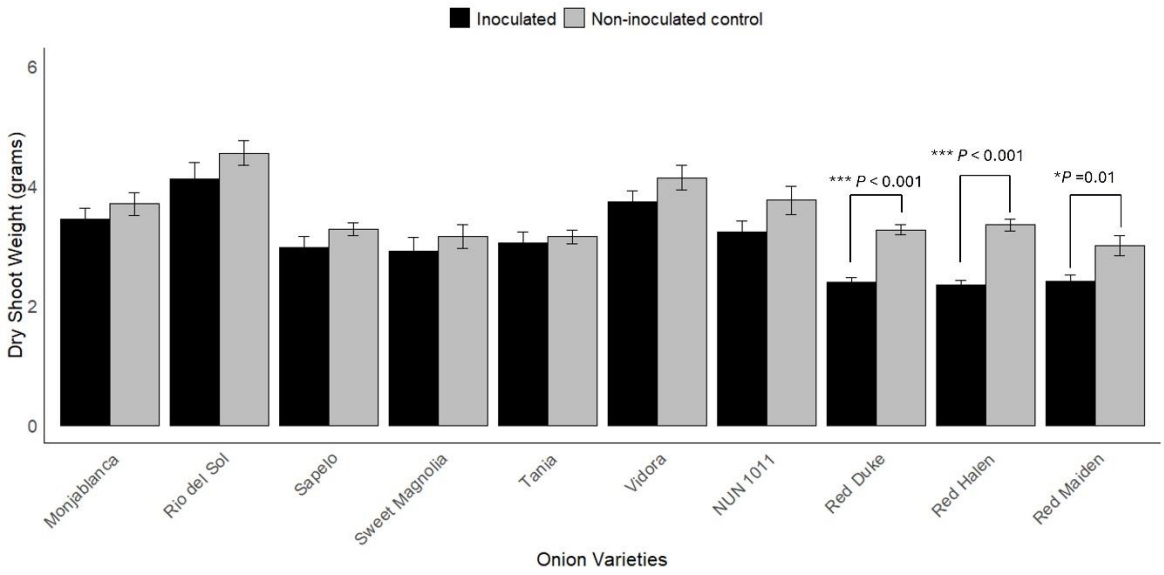

Figure 3.4. Effect of *Meloidogyne enterolobii* on dry shoot weights of onion. Bars represent the fresh bulb weight (mean ± standard error). Values are pooled data from two trials. Two sample independent t-tests were done to identify differences between inoculated and non-inoculated control treatments. * and *** represents the 5% and 0.1% level of significance respectively. Monjablanca is a white onion, Red Duke, Red Halen and Red Maiden are red onions and all other onion cultivars are Vidalia onions.

CHAPTER 4

DIFFERENTIAL RESPONSE OF *MELOIDOGYNE ENTEROLOBII*, *M. FLORIDENSIS*, *M. HAPLANARIA*, AND *M. INCOGNITA* TO SUB-LETHAL DOSES OF NON-FUMIGANT NEMATICIDES[3]

[3] Poudel, N., Davis, R. F., Severns, P. M., McAvoy, T., Jagdale, G. B., Brenneman, T. B., and Chowdhury, I. A. 2025. Accepted by Phytopathology, (https://doi.org/10.1094/PHYTO-01-25-0028-R), Reprinted here with permission of publisher.

Abstract

Root-knot nematodes (RKN; *Meloidogyne* spp.) are among the most widespread and damaging plant-parasitic nematodes known. While *M. incognita* is the most prevalent RKN species, other species, including *M. enterolobii*, *M. floridensis*, and *M. haplanaria* have recently been detected in multiple Georgia, USA, vegetable fields. Producers use chemical nematicides to manage *M. incognita* populations because most vegetable crops do not yet have effective RKN host resistance. We evaluated the effects of sublethal doses of non-fumigant nematicides-fluazaindolizine, fluensulfone, fluopyram, and oxamyl-on motility, egg hatching, and reproduction of these four RKN species. In vitro behavioral response assays revealed significant variation in motility between species, with *M. enterolobii* being the less sensitive to the evaluated nematicides. Root-knot nematode population response in greenhouse conditions indicated that all the nematicides impaired the reproduction of the evaluated *Meloidogyne* species. While most nematicides displayed a strong effect on reducing the egg-hatching, fluazaindolizine appeared to have a poor effect on suppressing the egg-hatching across all RKN species. No differences were observed among *Meloidogyne* species in their egg-hatching responses to nematicides. In this study, we conducted the first comprehensive evaluation of these nematicides on motility, reproduction, and egg hatching in these RKN species. This study enhances our understanding of the species-specific responses of different *Meloidogyne* spp. to non-fumigant nematicides.
Keywords: motility, *Meloidogyne*, nematodes, nematicides, non-fumigant, reproduction, sensitivity

Introduction

Plant-parasitic nematodes (PPNs) are serious agricultural pests that can cause significant reductions in the growth and yield of crops. Root-knot nematodes (RKNs, *Meloidogyne* spp.) are considered the most damaging genera of PPNs (Wram and Zasada 2020). They establish and maintain nutritional structures composed of multinucleate and hypertrophied giant cells, which result from the redifferentiation of vascular root cells. Hyperplasia and hypertrophy of the surrounding cells lead to the formation of the typical root gall, the primary visible symptom of infection (Favery et al. 2016). The formation of root galls reduces the absorption and translocation of water and dissolved nutrients, which leads to the typical RKN-infected plant symptoms like chlorosis, temporary plant wilting, and reduced shoot growth/stunting, which ultimately reduces the yield of the crop (Perry and Moens 2013). *Meloidogyne incognita* is the most prevalent RKN species in the United States, distributed across 29 states (Wram and Zasada 2019). Consequently, most management practices are tailored to this species. However, other RKN species, including *M. enterolobii*, *M. floridensis*, and *M. haplanaria*, have recently been detected in multiple vegetable fields in the southern United States (Bui et al. 2022; Church 2005; Espinoza-Lozano et al. 2022; Gu et al. 2021; Hajihassani et al. 2023; Joseph et al. 2016; Marquez et al. 2021; Marquez and Hajihassani 2022; Ye et al. 2013).

Although host resistance to *Meloidogyne incognita* and other root-knot nematodes exists in very few crops, its practical use often faces challenges, such as reduced yield potential or limited profitability (Espinoza-Lozano et al. 2022; Joseph et al. 2016; Philbrick et al. 2020). Chemical nematicides have been among the most effective tools for managing RKNs for decades. These nematicides are generally classified as fumigants and non-fumigants. However,

concerns about the environmental impact and worker safety associated with fumigants have been increasing. Methyl bromide, a widely used fumigant with a broad spectrum of efficacy against various pests, including nematodes, was phased out globally in 2015 because it is a major ozone-depleting substance and harmful to a diversity of non-target organisms (Desaeger et al. 2020; Oka 2020). Other fumigants such as 1,3-dichloropropene (1,3-D) and methyl isothiocyanate-generating compounds like metam sodium and metam potassium, are now employed for RKNs management (Oka 2020). While effective in controlling nematodes during the early stages of crop growth, these fumigants come with significant limitations, including high costs, complex application procedures, buffer zone requirements (minimum width of 7.6 m), long intervals between treatment and planting, and exposure safety concerns for workers (Desaeger et al. 2020; Oka 2020). Non-fumigant nematicides offer several advantages, including lower application rates, straightforward application methods, and reduced phytotoxicity, making them suitable for both pre-and post-planting applications (Desaeger et al. 2020; Oka 2020). Historically, most non-fumigant nematicides used for managing RKNs were organophosphates and carbamates. Nematicides from both the organophosphate and carbamate groups impact the central nervous system of nematodes by inhibiting acetylcholinesterase activity. These pesticides also pose threats to humans and non-target organisms and most of them have been withdrawn from use (Desaeger et al. 2020; Oka 2020). However, some non-fumigant nematicides belonging to carbamates and organophosphates are still employed for managing RKNs in vegetables. For instance, aldicarb (carbamate) is currently restricted to use on sweet potatoes and is only authorized in Louisiana and Mississippi (Schloemer et al. 2024). Ethoprop, an organophosphate, is used in certain vegetable crops, such as sweet potatoes and potatoes. (Hajihassani 2018).

Oxamyl, another carbamate, continues to be used in vegetable production for RKNs management. Several fluorinated nematicides, characterized by the presence of trifluoromethyl (-CF3) group, such as fluazaindolizine, fluensulfone, and fluopyram (Oka 2020), have emerged as newer alternatives to traditional carbamates and organophosphates, offering growers additional options for RKNs management.

Fluazaindolizine, an imidazopyridine compound, demonstrates specificity against PPN and lacks activity against the target sites of existing commercial nematicides, suggesting a novel mode of action. Fluazaindolizine is reported to have relatively low toxicity to non-target organisms (Lahm et al. 2017; Thoden and Wiles 2019). Fluensulfone, a member of the fluoroalkenyl thioether group, has a novel but currently unknown mode of action (Kearn et al. 2014). When RKN juveniles are exposed to both fluazaindolizine and fluensulfone, they display irreversible paralysis, ultimately leading to juvenile RKN death (Kearn et al. 2014; Lahm et al. 2017). Fluopyram, a member of the pyridinyl-ethyl-benzamide group, inhibits succinate dehydrogenase (SDH), disrupting ATP generation and it also leads to nematode paralysis. However, the paralysis caused by fluopyram is reversible, making it nematostatic rather than nematicidal (Schleker et al. 2022).

Multiple studies have assessed the sensitivity of various *Meloidogyne* species to non-fumigant nematicides, highlighting species-specific responses to these treatments. For example, an *in vitro* assay by Thoden et al. (2019) revealed that *M. javanica* is less sensitive to fluazaindolizine compared to *M. incognita*. In an infectivity assay by Wram and Zasada (2020), *M. chitwoodi* showed reduced sensitivity to fluazaindolizine compared to *M. hapla* and *M. incognita*. In a different study, *M. javanica* was less sensitive to fluensulfone but more sensitive

to fluopyram than *M. incognita*, whereas *M. incognita* exhibited greater sensitivity to fluensulfone but lower sensitivity to fluopyram than *M. javanica* (Oka and Soraya 2019). Field applications of non-fumigant nematicides have demonstrated variable outcomes, ranging from complete suppression of nematode reproduction to no significant effect. These variations are influenced by factors such as host plant, geographic location, and the genetic diversity of nematode populations (Watson 2022; Wram and Zasada 2020). These findings underscore the complexity of nematicide efficacy and the critical need to consider species-specific and environmental factors when developing nematode management strategies.

The efficacy of the nematicides depends on achieving adequate concentrations in the soil solution, a factor influenced by soil texture, moisture content, organic matter, and microbial degradation (Morris et al. 2018; Wram and Zasada 2019). These variables contribute to the likelihood of nematodes being exposed to a range of sublethal concentrations in the soil. The critical question is whether these sublethal concentrations are sufficient to reduce the infectivity of *Meloidogyne* spp. or if there are differences in sensitivity among the *Meloidogyne* spp. There have been some studies on the effects of sublethal concentrations of fluorinated nematicides against commonly prevalent *M. incognita* (Faske and Hurd 2015; Watson 2022; Wram and Zasada 2019). To date, only one study has evaluated *M. enterolobii* (Watson 2022), and no studies have assessed the short-term effects of varying nematicide concentrations on *M. floridensis* and *M. haplanaria*. Given the increasing threat these nematode species pose to agricultural productivity in the southeastern United States, our study aimed to comprehensively evaluate the effects of sublethal doses of non-fumigant nematicides on the motility, egg hatching, and reproduction of *M. enterolobii, M. floridensis, M. haplanaria,* and *M. incognita.*

Materials and methods

Preparation of nematode inoculum: Pure cultures of *M. enterolobii*, *M. floridensis*, *M. haplanaria*, and *M. incognita* originally collected from vegetable fields of Georgia were maintained on tomato (*Solanum lycopersicum* L., cv. 'Rutgers') under greenhouse conditions at the University of Georgia, Tifton, Georgia, USA, for three months before use. Eggs were extracted by soaking galled roots in a 0.5 % sodium hypochlorite solution while rotating at 250 rpm for four minutes. After shaking, roots and the hypochlorite solution were poured through mesh sieves (150 µm, 75 µm, and 25 µm), rinsed with tap water, and collected in a centrifuge tube. The rinsate collected from a sieve was then subjected to the centrifugal sugar flotation technique to separate eggs from other debris (Jenkins 1964). Eggs were enumerated with a nematode counting slide (Chalex LLC, Park City, UT, U.S.A.) under an inverted compound microscope (ZEISS Axio Vert.A1, Jena, Germany) at 40X magnification. To collect the J2s from the eggs, a Kimwipe tissue was placed over wire mesh, which was positioned above a bowl filled with water until the paper just touched the water's surface. The egg suspensions were then poured onto the Kimwipe tissue, and the entire hatching setup was placed in an incubator at a temperature of 25 ± 1°C. Emerging J2s were collected daily by passing the water through a 25 µm mesh sieve, and the freshly hatched juveniles retained on the sieve were collected and counted using an inverted microscope.

Chemicals used: Formulated products were used in all assays. The product used and the active ingredients in them are: fluopyram (Velum® Prime 41.5 %; Bayer CropScience, Research Triangle Park, NC, USA), fluensulfone (Nimitz®; 40 %; ADAMA Agricultural Solutions Ltd,

Raleigh, NC, USA), fluazaindolizine (Salibro™ 41.15 %; Corteva Agriscience, Indianapolis, IN, USA) and oxamyl (Vydate® L 24 %; Corteva Agriscience)

Microwell assays: To evaluate the effects of nematicide concentrations on nematode motility and egg hatching, assays were conducted using a 24-well microplate system. For the motility assay, wells were filled with 1 ml of nematicide solutions at varying concentrations: fluazaindolizine (10, 20.5, 41, 82, 164, 247, 330, 411 ppm), fluensulfone (10, 20, 40, 80, 160 ppm), fluopyram (0.2, 0.4, 0.8, 1.6, 3.3 ppm), oxamyl (6, 12, 24, 30, 36, 42, 48, 96 ppm), and water as a control in each nematicide assay. For each *Meloidogyne* species, J2s suspended in water were mixed with nematicide solutions in centrifuge tubes to achieve target concentrations. From these solutions, 1 ml containing at least 60 J2s was transferred into microplate wells, with each concentration and control replicated four times. Plates were incubated at room temperature for 24 hours, after which nematodes were categorized as active (moving, including abnormally) or inactive (straight and immobile) after viewing under the inverted compound microscope at 40X magnification. To assess whether the effects of the nematicides were reversible, J2s exposed to nematicide solutions for 24 hours in centrifuge tubes were rinsed with water, and solution of nematodes rinsed with water were transferred to wells as before. Following an additional 24-hour incubation, nematode activity was reassessed. Each assay was conducted twice.

Egg hatching experiments followed a similar protocol, with adjusted concentrations for fluazaindolizine (10, 20.5, 41, 82, 164, 330 ppm) and oxamyl (6, 12, 24, 48, 96 ppm), while fluopyram and fluensulfone concentrations remained unchanged. Sixty eggs in 50 µL of water were added to each well containing 950 µL nematicide solutions. Plates were incubated at room temperature for seven days, after which hatched J2s were counted. Each nematicide

concentration for each nematode species was replicated four times, and the entire egg hatching assay was conducted twice.

Reproduction assay: To assess whether pre-exposure to non-fumigant nematicides reduces the *in vivo* reproduction of nematodes, J2s of each *Meloidogyne* spp. were suspended in 2ml of water and treated with 7.5 ml of a nematicide solution in a centrifuge tube. Final exposure concentrations matched those used in the motility assay, with water-only controls included in all experiments. The centrifuge tubes were incubated at room temperature for 2 hours. After incubation, nematodes were rinsed with water for 5 to 10 seconds over a 25 µm mesh sieve and collected in centrifuge tubes. Second stage juveniles were then enumerated using an inverted compound microscope. Approximately 600 J2s of each nematode species were inoculated into 4-week-old tomato (*Solanum lycopersicum*) 'Rutgers' seedlings planted in 4-cm diameter pots containing sandy loam soil. Each nematicide concentration was replicated four times per nematode species, and the entire reproduction assay was repeated twice for each nematode–nematicide combination. The inoculated plants were arranged in a randomized complete block design within a greenhouse under long-day conditions (16-hour photo period). The greenhouse environment was maintained at an average daily temperature of 27 ± 5°C and relative humidity of 47 ± 6%. After eleven weeks, roots were harvested and gently washed to remove the adhering soil. Eggs were extracted using a 1% sodium hypochlorite solution for 4 minutes with shaking at 250 rpm, as before (to enhance efficiency compared to the 0.5% solution used previously) and enumerated using an inverted compound microscope. The reproduction factor (Rf) was calculated as the final number of eggs (Pf) divided by the initial inoculum level (Pi). To normalize differences in reproduction rates among *Meloidogyne* species, the Rf for each

treatment replicate was divided by the Rf ratio of the water control replicates for the respective species and experiment, and the result was expressed as a percentage:

Percentage Rf of Mean Control Rf = (Treatment replicate Rf/Mean Control Rf for experiment/species) ×100.

This ratio provided a relative measure of how well the nematodes reached their maximum reproductive potential (control levels) in the experiment, allowing for cross-treatment and cross-species comparisons (Wram and Zasada 2020).

Modeling microwell dose-response assay data: A log-logistic regression model was used to evaluate the dose-response relationships for the proportion of active *Meloidogyne* species J2s after 24-hour exposure to fluensulfone, fluopyram, and oxamyl. For fluopyram and oxamyl, a four-parameter log-logistic model was applied, while a three-parameter model was used for fluensulfone. In the four-parameter model, the parameters include: b, the coefficient representing the steepness of the dose-response curve; c and d, the lower and upper asymptotes of the response curve, respectively; and e, the EC_{50} value. In the three-parameter model, the lower asymptote (c) was fixed to 0. None of the log-logistic parameters were statistically significant for fluazaindolizine. Consequently, fluazaindolizine was excluded from modeling the dose-response data for the microwell assay.

Statistical analysis: Data from motility, egg hatch, and reproduction bioassay experiments were analyzed using the mixed linear model procedure, where experimental repetitions and treatment replications were modeled as random variables and treatments were modeled as fixed factors. Data were transformed using $\log_{10}(x + 1)$ in the case where data were not normally distributed. There was no ($P < 0.05$) experimental repetition (trial) by nematicide

treatment and/or timing interaction for any experiment; thus, only the main effects are reported for all experiments. Means were separated according to Tukey's honestly significant difference procedure at $\alpha = 0.05$. Median effective concentration (EC_{50}) values, based on J2s motility for each nematicide were calculated by using the R (R studio version 4.2.1) package *drc* (Ritz et al., 2015).

Results

Nematicide impacts on nematode motility: Fluazaindolizine significantly reduced nematode motility at higher concentrations. For *M. enterolobii*, motility reductions were observed at concentrations ≥ 247 ppm, while reductions for *M. floridensis*, *M. haplanaria*, and *M. incognita* were observed at minimum concentrations of ≥ 82 ppm, ≥ 10 ppm, and ≥ 20.5 ppm, respectively (Supplemental Table 4.1). Fluazaindolizine exhibited nematicidal properties in the short-term assay, as J2s motility did not recover after treatment, even when replaced with water (Fig. 4.1).

Fluensulfone significantly reduced motility across all nematode species at concentrations ≥ 10 ppm (Supplemental Table 4.2). Fluensulfone demonstrated strong nematicidal activity, as treated J2s did not recover motility after being washed with water (Fig. 4.1). The EC_{50} values of fluensulfone for *M. enterolobii*, *M. floridensis*, *M. haplanaria*, and *M. incognita* were recorded at 56.5 ppm, 26.6 ppm, 21.1 ppm, and 20.2 ppm, respectively (Table 4.5). After rinsing with water, the EC_{50} values of fluensulfone for *M. enterolobii*, *M. floridensis*, *M. haplanaria*, and *M. incognita* were recorded at 30.4, 11.9, 13.0, and 10.7 ppm respectively.

The efficacy of fluopyram in reducing the motility was less effective against *M. enterolobii*, showing significant reductions compared to the untreated control only at

concentrations ≥ 0.8 ppm. In contrast, significant reductions in motility were observed for *M. incognita* and *M. haplanaria* at concentrations ≥ 0.2 ppm, and for *M. floridensis* at concentrations ≥ 0.4 ppm (Supplemental Table 4.3). The EC_{50} values of fluopyram for *M. enterolobii*, *M. floridensis*, *M. haplanaria*, and *M. incognita* were 2.2 ppm, 0.9 ppm, 0.9 ppm, and 0.4 ppm, respectively (Table 4.5). Fluopyram demonstrated nematostatic behavior at lower concentrations, as motility was partially reversible when the compound was replaced with water (Fig. 4.1).

Oxamyl significantly reduced motility of *M. haplanaria*, *M. floridensis*, and *M. incognita* at concentrations ≥ 24 ppm, and of *M. enterolobii* at concentrations ≥ 30 ppm (Supplemental Table 4.4). Oxamyl exhibited nematicidal activity as non-motile J2 were unable to recover and move after being washed with water (Fig. 4.1). The EC_{50} values of oxamyl for *M. enterolobii*, *M. incognita*, *M. floridensis*, and *M. haplanaria* were recorded at 39.5 ppm, 29.4 ppm, 24.2 ppm, and 30.7 ppm, respectively (Table 4.5).

Nematicide impacts on egg hatch rate: Nematicides had a significant effect on the egg hatch rate of all nematode species. Fluazaindolizine significantly reduced egg hatch only at the two highest tested concentrations (247 and 330 ppm) for *M. incognita* and *M. floridensis*. For *M. enterolobii* and *M. haplanaria*, significant reductions in egg hatch rates were observed at concentrations ≥ 20.5 ppm and ≥ 41 ppm, respectively (Supplemental Table 4.5). Fluensulfone significantly reduced egg hatch rates for all nematode species at concentrations ≥ 20 ppm compared to the untreated control (Supplemental Table 4.6). Fluopyram at concentrations ≥ 0.8 ppm significantly reduced egg hatch for all nematode species, and for *M. incognita*, a concentration of fluopyram ≥ 0.4 ppm was sufficient to cause a significant reduction in egg hatch

rate (Supplemental Table 4.7). Oxamyl, at concentrations ≥ 24 ppm, significantly reduced egg hatch rates for all *Meloidogyne* species (Supplemental Table 4.8).

Nematicides impacts on nematode reproduction: Fluazaindolizine significantly reduced reproduction in all species at concentrations ≥ 10 ppm. *Meloidogyne haplanaria* and *M. floridensis* were the most sensitive, with their reproduction levels reaching only 29 % and 22 % of the untreated control at 10 ppm, respectively. In contrast, *M. enterolobii* and *M. incognita* exhibited reproduction levels reaching 51 % and 46 % of the untreated control, respectively. Even at concentrations above 10 ppm, *M. enterolobii* showed less sensitivity, consistently exhibiting reproductive potential greater than all other species tested (Table 4.1).

Fluensulfone was effective at reducing reproduction in all nematode species at concentrations ≥ 10 ppm. At 10 ppm, *M. enterolobii* and *M. haplanaria* exhibited reproduction levels reaching 41.9 % and 38.8 % of the untreated control, respectively, which were at least three times higher than those of *M. incognita* and *M. floridensis*. At concentrations exceeding 25 ppm, *M. enterolobii* consistently exhibited the less sensitivity, maintaining higher reproductive potential than all other species tested (Table 4.2).

Fluopyram significantly reduced reproduction across all nematode species at concentrations ≥ 0.2 ppm. However, sensitivity to fluopyram varied among the *Meloidogyne* species. At all concentrations, *M. enterolobii* consistently exhibited higher reproductive potential than all other species. This difference was most pronounced at lower concentrations. For instance, at 0.2 ppm, *M. enterolobii* exhibited reproduction levels reaching 44 % of the untreated control, whereas *M. floridensis*, *M. incognita*, and *M. haplanaria* exhibited reproduction levels reaching only 6.7 %, 5 %, and 17.7 % of the untreated control, respectively. Similar trends were

observed at higher concentrations, where *M. enterolobii* consistently exhibited greater reproductive potential than the other species (Table 4.3).

Oxamyl effectively reduced reproduction in all nematode species at concentrations ≥ 12 ppm. *M. enterolobii* was less sensitive among the species tested, with reproductive potential consistently greater than that of all other species across all concentrations. Conversely, *M. haplanaria* was the most sensitive, particularly at lower concentrations (6 ppm, 12 ppm, 24 ppm, and 30 ppm), where it exhibited reproductive potential lower than all other species tested (Table 4.4).

Discussion

The phase-out and regulation of traditional fumigant and non-fumigant nematicides in the U.S. have driven the development of newer options like fluazaindolizine, fluensulfone, and fluopyram, which offer reduced toxicity to humans and non-target organisms. The effectiveness of these non-fumigant nematicides is influenced by different soil factors such as soil texture, moisture content, and organic matter, which create inconsistent and low concentrations of nematicide over the application area, in which nematodes may minimize exposure (Morris et al. 2018; Wram and Zasada 2019). Successful management of nematodes depends on their sensitivity to varying concentrations of nematicides present in the soil. Although previous studies have examined the responses of *M. incognita* to non-fumigant nematicides, there have been limited to no studies on the recently identified species in Georgia including *M. enterolobii, M. floridensis,* and *M. haplanaria*.

In our short-term assay, fluopyram was the only nematicide that showed a nematostatic effect on *Meloidogyne* juveniles. The non-motile J2s after fluopyram treatment were able to

recover after washing with water. Even the low concentrations of fluopyram were able to reduce the motility and the infectivity of all *Meloidogyne* species. The nematostatic effect of fluopyram was previously reported (Faske and Hurd 2015; Wram and Zasada 2020). In a study conducted by Watson (2022), sublethal concentrations of fluopyram were able to reduce the motility and infectivity of *M. enterolobii* and *M. incognita* similar to our study. However, there was no difference in the infectivity among *M. enterolobii* and *M. incognita*, unlike our study, where *M. enterolobii* was the less sensitive with reproduction levels being at least 15 % of the untreated control until 0.8 ppm, which was more than 2 times the reproduction levels of other species in the study at 0.8 ppm concentration.

In contrast to fluopyram, fluensulfone acted as a true nematicide, as nematodes were unable to recover after treatment with this compound. Notably, the percentage of J2s that became immotile increased even after rinsing with water, suggesting a persistent and potentially cumulative impact on nematode health following fluensulfone exposure. The nematicidal properties of fluensulfone have been documented in several studies (Oka and Soraya 2019; Wram and Zasada 2019). In our study, fluensulfone reduced the motility and infectivity of all *Meloidogyne* species tested, even at 10 ppm. Among the species, *M. enterolobii* exhibited the less sensitivity in terms of motility compared to the other species. Differences in sensitivity among *Meloidogyne* species have been previously reported. Oka and Soraya (2019) observed that *M. javanica* was less sensitive to fluensulfone, with a higher EC_{50} value compared to *M. incognita*. Similar to our study, fluensulfone effectively reduced the infectivity of *M. javanica* and *M. incognita* when pretreated with sublethal concentrations (Oka and Soraya 2019).

No reversibility was observed in the non-motile J2s after treatment with oxamyl and rinsing with water. Similar effects of oxamyl were reported by Wram and Zasada (2019) and Watson (2022). However, McGarvey et al. (1984) showed that recovery was time-dependent, with motility decreasing from 100% to 30% between 10 and 40 minutes at 8000 ppm. These findings suggest that both exposure duration and concentration play critical roles in determining whether oxamyl acts in a nematostatic or nematicidal manner. Although there was minimal difference in the *in vitro* sensitivity of *Meloidogyne* species to oxamyl, with EC_{50} values ranging from 24.2 ppm to 39.5 ppm across all tested species, differences in infectivity were evident. *Meloidogyne enterolobii* demonstrated higher reproductive potential at all tested concentrations compared to other species.

Fluazaindolizine also behaved as a true nematicide, as nematodes were unable to recover after treatment. The nematicidal properties of fluazaindolizine have been documented in several studies (Thoden and Wiles 2019; Wram and Zasada 2019; Wram and Zasada 2020). In our *in vitro* assays, higher concentrations were required to significantly reduce motility compared to controls. However, even 10 ppm was sufficient to significantly reduce the reproduction of all *Meloidogyne* species tested. This suggests that motile nematodes observed after fluazaindolizine treatment lose their potential to infect plants. The reduction in the infectivity of *Meloidogyne* species, even at lower concentrations of fluazaindolizine, was reported by Thoden and Wiles (2019), who observed a significant reduction in infectivity at just 5 ppm. Consistent with the other nematicide evaluations we present in this manuscript, *M. enterolobii* was less sensitive species, exhibiting greater reproductive potential than all other species tested at all concentrations. Differences in sensitivity among *Meloidogyne* species to fluazaindolizine were

also reported by Wram and Zasada (2019), who observed that *M. chitwoodi* was less sensitive than *M. hapla* and *M. incognita*, with *M. chitwoodi* showing greater reproductive potential at all sublethal doses tested in their study.

There have been several recent studies evaluating the efficacy of fluorinated non-fumigant nematicides on *M. incognita* in different cropping systems under field, greenhouse, and microplot conditions. In a greenhouse trial, fluazaindolizine, fluensulfone and fluopyram significantly reduced the number of eggs per gram of root and galling severity in tomato plants when treated with the recommended dose (Silva et al. 2019). Another greenhouse study reported that fluazaindolizine, fluensulfone, and fluopyram when applied at the full recommended dose, significantly reduced populations of *M. enterolobii* and *M. incognita* in sweet potato (Watson 2022). In a microplot study, fluazaindolizine, fluensulfone, and fluopyram effectively reduced populations of *M. incognita* at inoculation densities of 1,000, 5,000, 10,000, and 20,000 J2s per microplot (Hajihassani et al. 2019). Similarly, a field trial demonstrated that fluazaindolizine, fluensulfone and fluopyram, along with oxamyl, reduced infestations and populations of *M. incognita* in double-cropped squash (Nnamdi et al. 2020). However, studies on the efficacy of fluorinated non-fumigant nematicides against other RKN species remain limited. In a field study, all fluorinated nematicides, fluazaindolizine, fluensulfone and fluopyram applied at recommended doses failed to consistently reduce *M. enterolobii* populations in sweet potato fields across multiple years, whereas fumigation with 1,3-dichloropropene was effective (Grabau et al. 2024). Most field research on nematicide efficacy against *M. enterolobii* has been limited to single-trial reports from North Carolina, often involving mixed populations with *M. incognita*. These trials have shown inconsistent results for fluopyram and fluazaindolizine in managing

Meloidogyne populations or symptoms and improving yield (Collins et al. 2017, 2018, 2019; Huckaba et al. 2023; Jeffreys et al. 2022; Malter et al. 2023). In a study by Poudel et al (2024), fluazaindolizine applied as a drench did not reduce *M. floridensis* populations in a tomato-planted microplot study. This contrasts with our findings, where *M. floridensis* populations were significantly reduced compared to the control, even at 10 ppm. Notably, this is the only study to date investigating the efficacy of non-fumigant nematicides against *M. floridensis*. Further research under diverse environmental conditions is necessary to better understand the efficacy of nematicides on *M. floridensis*.

Multiple factors can influence the efficacy of non-fumigant nematicides on *Meloidogyne* species, including soil conditions, application methods, and initial nematode pressure. But one key factor contributing to the varying effects of nematicides on different *Meloidogyne* species is the difference in sensitivity of these species to nematicides, as observed in our study. Our findings demonstrated that the reproduction of all *Meloidogyne* species was suppressed following pre-exposure to non-fumigant nematicides, although the extent of suppression varied among species. The mechanisms underlying this variation in sensitivity of *Meloidogyne* species to these nematicides remain unclear, as the mode of action of most of these nematicides is unknown.

In field conditions, various factors-such as soil moisture, organic matter content, and soil texture-can influence the availability and distribution of nematicides in the soil, potentially altering the actual concentrations encountered than those applied. A key consideration is whether the concentration of nematicide present in the soil is sufficient to impair nematode infectivity. Our findings suggest that the sub-lethal dose required to suppress reproduction may vary among

Meloidogyne species. Therefore, future research should evaluate the efficacy of non-fumigant nematicides across different *Meloidogyne* species under field conditions. Such studies will be critical for refining application rates to minimize economic losses and sustain crop productivity across diverse agroecosystems.

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Table 4.1. Reproduction of different *Meloidogyne* species on tomato 'Rutgers' after a 24 h preexposure of second-stage juveniles to the varying concentrations of the fluazaindolizine

Fluazaindolizine concentrations (ppm)	*Meloidogyne enterolobii*[1,2]		*Meloidogyne floridensis*[1,2]		*Meloidogyne haplanaria*[1,2]		*Meloidogyne incognita*[1,2]	
	Rf[3]	Rf ratio[4]	Rf[3]	Rf ratio[4]	Rf[3]	Rf ratio[4]	Rf[3]	Rf ratio[4]
0	35.1 (6.9) a	100.0 (19.8) a	36.2 (6.7) a	100.0 (18.6) a	30.8 (10.5) a	100.0 (34.2) a	33.6 (12.7) a	100.0 (37.8) a
10	19.4 (4.6) b	55.3 (13.2) ab	10.6 (1.9) b	29.3 (5.4) b	9.1 (3.18) b	29.8 (10.3) b	16.7 (3.7) b	49.8 (11.2) b
20.5	16.5 (6.4) b	47.1 (18.5) bc	9.9 (2.4) b	27.4 (6.6) b	4.8 (2.0) b	15.6 (6.6) b	10.0 (4.0) bc	29.9 (11.9) bc
41	11.8 (2.4) bc	33.6 (6.9) bc	4.0 (1.7) c	11.2 (4.8) c	3.5 (1.6) c	11.3 (5.3) c	5.6 (1.7) cd	16.7 (5.1) cd
82	7.3 (2.3) c	21.0 (6.5) c	2.3 (1.7) c	6.5 (4.9) c	2.3 (1.5) c	7.7 (5.0) c	3.8 (1.1) cd	11.5 (3.5) cd
164	1.8 (1.5) d	5.3 (4.0) d	2.2 (0.7) c	6.0 (1.9) c	0.6 (0.5) d	2.0 (1.7) d	1.0 (0.4) d	2.9 (1.3) d
P value								
Treatment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Trial	0.62	0.70	0.67	0.97	0.98	0.95	0.58	0.62
Treatment × Trial	0.14	0.19	0.96	0.80	0.13	0.18	0.56	0.52

[1]Values are the mean of eight replications from two trials. Numbers in parentheses represent standard deviations.
[2]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).
[3]Rf (Reproduction factor) was determined as the ratio of the initial inoculum density of *Meloidogyne* spp. over the final egg population density.
[4]Rf ratio: Rf was normalized for the variable reproduction rates of the different *Meloidogyne* spp. by dividing the Rf of the treatment by the Rf of the control for each species within an experiment replicate

Table 4.2. Reproduction of different *Meloidogyne* species on tomato 'Rutgers' after a 24 h preexposure of second-stage juveniles to the varying concentrations of the fluensulfone

Fluensulfone concentrations (ppm)	*Meloidogyne enterolobii*[1,2]		*Meloidogyne floridensis*[1,2]		*Meloidogyne haplanaria*[1,2]		*Meloidogyne incognita*[1,2]	
	Rf[3]	Rf ratio[4]	Rf[3]	Rf ratio[4]	Rf[3]	Rf ratio[4]	Rf[3]	Rf ratio[4]
0	35.0 (11.0) a	100.0 (31.5) a	31.6 (10.5) a	100.0 (33.1) a	28.8 (14.2) a	100.0 (49.2) a	36.8 (11.5) a	100.0 (31.1) a
10	16.8 (4.0) b	48.1 (11.5) b	6.3 (1.6) b	20.1 (5.3) b	11.6 (2.8) b	40.5 (9.9) b	5.0 (2.4) b	13.7 (6.7) b
20	9.2 (2.8) c	26.4 (8.0) c	1.5 (0.9) c	4.7 (2.9) c	2.5 (0.8) c	8.6 (2.7) c	1.4 (0.5) c	3.9 (1.3) c
40	4.7 (2.2) cd	13.5 (6.4) d	1.0 (0.2) c	3.1 (0.8) cd	0.7 (0.4) d	2.6 (1.6) d	1.1 (1.0) c	3.2 (2.8) c
80	2.6 (1.0) cd	7.5 (3.1) d	0.8 (0.5) cd	2.5 (1.8) cd	0.6 (0.7) d	2.3 (2.4) d	0.8 (0.4) c	2.3 (1.2) cd
160	0.5 (0.4) d	1.6 (1.4) e	0.4 (0.4) d	1.3 (1.2) d	0.3 (0.3) d	1.0 (0.9) d	0.5 (0.5) c	1.5 (1.5) d
P value								
Treatment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Trial	0.69	0.76	0.25	0.49	0.79	0.86	0.69	0.77
Treatment × Trial	0.68	0.79	0.62	0.84	0.90	0.51	0.78	0.75

[1]Values are the mean of eight replications from two trials. Numbers in parentheses represent standard deviations.
[2]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).
[3]RF (Reproduction factor) was determined as the ratio of the initial inoculum density of *Meloidogyne* spp. over the final egg population density.
[4]Rf ratio: Rf was normalized for the variable reproduction rates of the different *Meloidogyne* spp. by dividing the Rf of the treatment by the Rf of the control for each species within an experiment replicate.

Table 4.3. Reproduction of different *Meloidogyne* species on tomato 'Rutgers' after a 24 h preexposure of second-stage juveniles to the varying concentrations of the fluopyram

Fluopyram concentrations (ppm)	*Meloidogyne enterolobii*[1,2]		*Meloidogyne floridensis*[1,2]		*Meloidogyne haplanaria*[1,2]		*Meloidogyne incognita*[1,2]	
	Rf[3]	Rf ratio[4]	Rf[3]	Rf ratio[4]	Rf[3]	Rf ratio[4]	Rf[3]	Rf ratio[4]
0	36.1(12.9) a	100.0 (35.8) a	32.8 (11.6) a	100.0 (35.4) a	31.0 (8.6) a	100.0 (27.8) a	29.5 (4.6) a	100.0 (15.7) a
0.2	16.8 (3.0) b	46.7 (8.3) b	8.0 (2.0) b	24.4 (6.2) b	6.6 (1.9) b	21.3 (6.2) b	5.8 (1.8) b	19.9 (6.3) b
0.4	13.8 (1.6) b	36.4 (4.6) b	3.5 (1.2) bc	10.8 (3.7) bc	3.4 (1.4) bc	11.0 (4.5) c	3.9 (1.5) c	13.3 (5.3) b
0.8	5.6 (2.7) c	15.8 (7.7) c	1.8 (1.3) cd	5.7 (4.2) cd	1.4 (1.0) bc	4.6 (3.5) d	1.3 (0.6) d	4.6 (2.3) c
1.6	3.6 (1.5) c	10.2 (4.2) cd	1.4 (0.5) cd	4.3 (1.7) cd	1.3 (0.5) bc	4.4 (1.8) d	1.0 (0.2) de	3.3 (0.9) cd
3.3	1.7 (1.6) d	4.8 (4.4) d	0.7 (0.7) d	2.2 (2.1) d	0.7 (0.2) c	2.4 (0.8) d	0.5 (0.4) e	1.9 (1.4) d
P value								
Treatment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Trial	0.64	0.69	0.96	0.97	0.87	0.95	0.66	0.78
Treatment × Trial	0.18	0.05	0.56	0.60	0.10	0.07	0.05	0.06

[1]Values are the mean of eight replications from two trials. Numbers in parentheses represent standard deviations.
[2]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).
[3]RF (Reproduction factor) was determined as the ratio of the initial inoculum density of *Meloidogyne* spp. over the final egg population density.
[4]Rf ratio: Rf was normalized for the variable reproduction rates of the different *Meloidogyne* spp. by dividing the Rf of the treatment by the Rf of the control for each species within an experiment replicate.

Table 4.4. Reproduction of different *Meloidogyne* species on tomato 'Rutgers' after a 24 h preexposure of second-stage juveniles to the varying concentrations of the oxamyl

Oxamyl concentrations (ppm)	*Meloidogyne enterolobii*[1,2]		*Meloidogyne floridensis*[1,2]		*Meloidogyne haplanaria*[1,2]		*Meloidogyne incognita*[1,2]	
	Rf[3]	Rf ratio[4]	Rf[3]	Rf ratio[4]	Rf[3]	Rf ratio[4]	Rf[3]	Rf ratio[4]
0	31.4 (8.2) a	100.0 (26.3) a	29.6 (9.4) a	100.0 (31.8) a	34.0 (7.1) a	100.0 (21.1) a	31.1(9.8) a	100.0 (31.6) a
6	28.5(10.2) ab	90.7 (32.6) a	24.0 (6.5) a	81.2 (22.2) ab	20.1 (3.0) b	52.5 (8.8) a	23.9 (7.0) ab	76.9 (22.8) a
12	22.8 (2.8) b	72.6 (8.9) ab	11.9 (4.1) b	40.3 (13.9) bc	7.6 (3.7) c	19.8 (11.1) b	14.1 (6.9) b	45.6 (22.4) ab
24	11.6 (1.9) c	37.0 (6.1) bc	6.9 (2.3) bc	23.4 (7.7) cd	4.6 (1.8) cd	9.0 (3.2) bc	5.9 (4.6) c	19.0 (14.8) bc
30	10.3 (3.0) cd	32.8 (9.6) cd	5.3 (1.5) c	18.1 (5.2) cd	2.6 (1.3) d	7.9 (2.9) cd	3.6 (2.0) cd	11.6 (6.4) cd
36	9.6 (2.7) cd	30.4 (8.6) cd	3.4 (1.3) cd	11.4 (4.6) de	1.4 (0.6) d	9.0 (1.9) d	2.5 (1.0) cde	8.0 (3.4) cde
42	4.8 (0.8) cde	15.3 (2.6) de	2.0 (1.1) d	6.7 (3.9) e	1.2 (0.5) d	4.3 (1.6) d	1.6 (1.3) def	5.2 (4.2) def
48	3.3 (1.7) de	10.7 (5.6) e	1.9 (1.3) d	6.5 (4.1) e	1.2 (0.4) d	2.5 (1.3) d	0.9 (0.7) ef	3.0 (2.3) ef
96	1.3 (1.2) e	4.1 (3.9) f	1.5 (1.2) d	5.2 (4.6) e	0.5 (0.5) d	1.1 (1.0) e	0.7 (0.4) f	2.4 (1.9) f
P value								
Treatment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Trial	0.14	0.27	0.56	0.68	0.89	0.92	0.45	0.59
Treatment × Trial	0.12	0.10	0.09	0.06	0.15	0.19	0.97	0.95

[1]Values are the mean of eight replications from two trials. Numbers in parentheses represent standard deviations.
[2]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).
[3]RF (Reproduction factor) was determined as the ratio of the initial inoculum density of *Meloidogyne* spp. over the final egg population density.
[4]Rf ratio: Rf was normalized for the variable reproduction rates of the different *Meloidogyne* spp. by dividing the Rf of the treatment by the Rf of the control for each species within an experiment replicate.

Table 4.5. Log-logistic dose-response curve model parameters for different *Meloidogyne* spp. motility exposed to different non-fumigant nematicides 24 h after exposure followed by a water rinse and another 24 h incubation.

Nematicides	*Meloidogyne* spp.	Model Parameter			
		b[w]	c[x]	d[y]	e[z]
Fluensulfone (24 h)	*M. enterolobii*	-1.0 $P < 0.001$	Fixed	130.8 $P < 0.001$	56.5 $P < 0.001$
	M. floridensis	-0.9 $P < 0.001$	Fixed	125.1 $P < 0.001$	26.6 $P < 0.001$
	M. haplanaria	-1.0 $P < 0.001$	Fixed	108.2 $P < 0.001$	21.1 $P < 0.001$
	M. incognita	-0.9 $P < 0.001$	Fixed	105.11 $P < 0.001$	20.2 $P < 0.001$
Fluensulfone (24 h after water rinse)	*M. enterolobii*	-1.76 $P < 0.001$	Fixed	124.5 $P < 0.001$	30.4 $P = 0.01$
	M. floridensis	-0.8 $P < 0.001$	Fixed	109.4 $P < 0.001$	11.9 $P < 0.001$
	M. haplanaria	-1.0 $P < 0.001$	Fixed	111.7 $P < 0.001$	13.0 $P < 0.001$
	M. incognita	-0.9 $P < 0.001$	Fixed	115.1 $P < 0.001$	10.7 $P < 0.001$
Fluopyram (24 h)	*M. enterolobii*	-1.9 $P = 0.002$	3.3 $P = 0.12$	112.5 $P < 0.001$	2.2 $P = 0.006$
	M. floridensis	-2.0 $P < 0.001$	9.6 $P = 0.98$	102.8 $P < 0.001$	0.9 $P < 0.001$
	M. haplanaria	-1.0 $P < 0.001$	1.2 $P = 0.69$	105.3 $P < 0.001$	0.9 $P = 0.016$
	M. incognita	-1.5 $P < 0.001$	-0.09 $P < 0.001$	99.2 $P < 0.001$	0.4 $P < 0.001$
Fluopyram (24 h after water rinse)	*M. enterolobii*	-2.63 $P = 0.006$	3.64 $P < 0.01$	82.4 $P = 0.008$	2.5 $P = 0.005$
	M. floridensis	-2.2 $P = 0.001$	5.7 $P = 0.006$	65.4 $P < 0.001$	1.5 $P < 0.001$
	M. haplanaria	-2.3 $P = 0.02$	3.5 $P = 0.24$	87.2 $P < 0.001$	1.7 $P < 0.001$
	M. incognita	-1.19 $P < 0.001$	-0.08 $P = 0.97$	86.12 $P < 0.001$	0.8 $P < 0.001$
Oxamyl (24 h)	*M. enterolobii*	-8.67 $P < 0.001$	7.9 $P < 0.001$	89.8 $P < 0.001$	39.5 $P < 0.001$
	M. floridensis	-6.9 $P < 0.001$	5.5 $P < 0.001$	94.9 $P < 0.001$	29.4 $P < 0.001$
	M. haplanaria	-3.2 $P < 0.001$	4.2 $P < 0.001$	93.0 $P < 0.001$	24.2 $P < 0.001$
	M. incognita	-5.6 $P < 0.001$	10.8 $P < 0.001$	90.8 $P < 0.001$	30.7 $P < 0.001$
Oxamyl (24 h after water rinse)	*M. enterolobii*	-7.2 $P < 0.001$	5.2 $P < 0.001$	88.0 $P < 0.001$	39.3 $P < 0.001$
	M. floridensis	-4.0 $P < 0.001$	7.67 $P < 0.001$	91.8 $P < 0.001$	30.7 $P < 0.001$
	M. haplanaria	-3.0 $P < 0.001$	4.07 $P < 0.002$	96.7 $P < 0.001$	25.5 $P < 0.001$
	M. incognita	-6.4 $P < 0.001$	7.7 $P < 0.001$	92.5 $P < 0.001$	29.4 $P < 0.001$

[w]b the coefficient that represents dose-response curve steepness

[x]c the lower asymptote of the response curve

[y]d the upper asymptote of the response curve

[z]e the effective dose needed to kill 50% of the nematode population

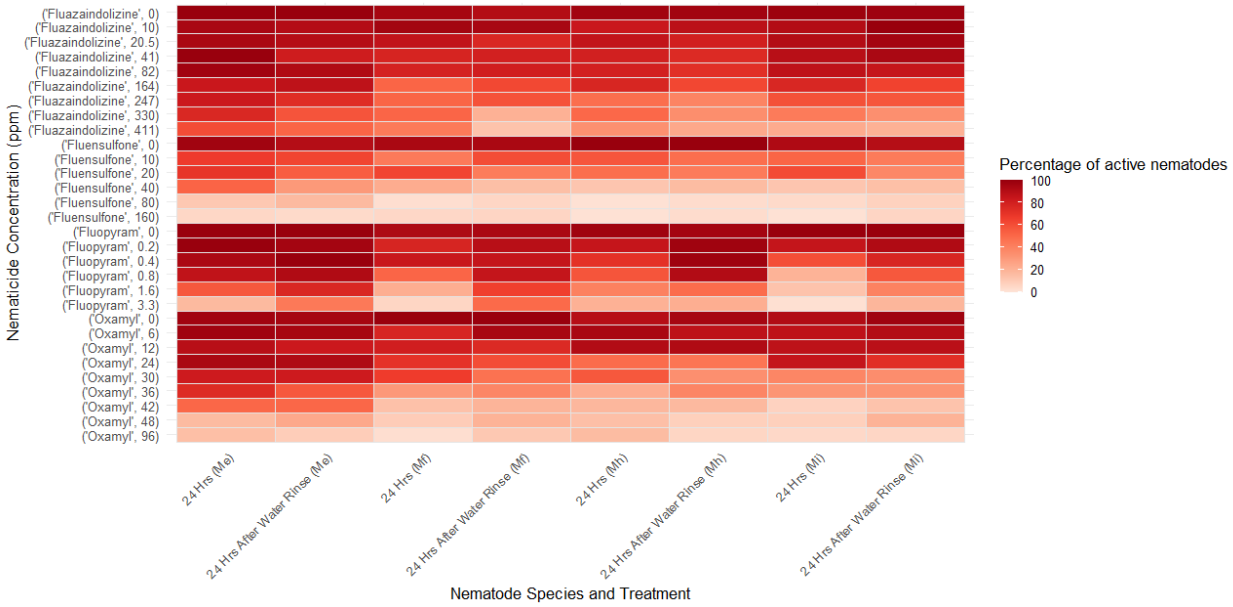

Figure 4.1. Heatmap of second-stage juveniles of *Meloidogyne* spp. activity after exposure to nematicides for 24 h. Nematodes were exposed to different concentrations of fluazaindolizine, fluensulfone, fluopyram and oxamyl in a microwell assay for 24 h and their activity was observed. After 24 h, the J2s in microwell was replaced with another set of nematicide-treated juveniles rinsed with water. After another 24 h, activity was again observed. The heatmap displays the percentage of motile nematodes for each species at each dosage and measurement time period. Lighter colors indicate that the *Meloidogyne* spp. was less active after exposure to nematicides. Me: *Meloidogyne enterolobii*, Mf: *Meloidogyne floridensis*, Mh: *Meloidogyne haplanaria* and Mi: *Meloidogyne incognita*

Supplemental Table 4.1. Percentage of motile second-stage juveniles of different *Meloidogyne* species after 24 h exposure to varying concentrations of fluazaindolizine.

Fluazaindolizine concentrations (ppm)	*Meloidogyne enterolobii*[1,2]	*Meloidogyne floridensis*[1,2]	*Meloidogyne haplanaria*[1,2]	*Meloidogyne incognita*[1,2]
0	99.1 (1.5) a	94.9 (2.3) a	97.3 (0.6) a	99.0 (1.2) a
10	97.0 (3.4) a	96.3 (0.4) a	86.4 (2.6) b	94.1 (1.8) ab
20.5	95.8 (2.2) a	89.4 (3.9) a	86.1 (3.8) b	89.4 (4.3) bc
41	98.1 (2.1) a	93.0 (8.3) a	81.3 (2.4) bc	88.6 (3.8) bc
82	97.1 (2.0) a	73.3 (4.2) b	78.4 (3.7) bcd	86.2 (3.2) c
164	92.2 (4.4) ab	65.6 (8.1) b	75.6 (7.3) cd	73.2 (5.8) d
247	82.7 (6.2) bc	55.8 (7.0) c	71.4 (10.6) d	61.3 (3.2) e
330	68.5 (6.2) c	46.6 (6.4) d	50.4 (7.6) e	40.4 (6.8) f
411	52.0 (7.9) d	40.4 (4.2) d	28.7 (5.3) f	28.1 (3.5) g
P value				
Treatment	<0.001	<0.001	<0.001	<0.001
Trial	0.58	0.84	0.97	0.47
Treatment × Trial	0.06	0.09	0.20	0.25

[1]Values are the mean of eight replications from two trials. Numbers in parentheses represent standard deviations.

[2]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).

Supplemental Table 4.2. Percentage of motile second-stage juveniles of different *Meloidogyne* species after 24 h exposure to varying concentrations of fluensulfone.

Fluensulfone concentrations (ppm)	*Meloidogyne enterolobii*[1,2]	*Meloidogyne floridensis*[1,2]	*Meloidogyne haplanaria*[1,2]	*Meloidogyne incognita*[1,2]
0	96.9 (2.3) a	95.3 (2.7) a	96.8 (3.0) a	96.2 (2.3) a
10	77.0 (5.6) b	54.9 (6.2) b	57.7 (4.5) b	54.0 (4.3) b
20	68.9 (3.2) c	51.3 (6.2) b	52.6 (4.6) b	52.1 (5.0) b
40	54.1 (2.1) d	27.5 (5.0) c	20.3 (6.3) c	19.3 (7.3) c
80	14.1 (3.3) e	7.4 (4.8) d	5.3 (2.2) d	5.6 (1.6) d
160	4.9 (2.0) f	3.5 (1.7) d	2.5 (2.5) d	2.3 (1.2) d
P value				
Treatment	<0.001	<0.001	<0.001	<0.001
Trial	0.62	0.78	0.49	0.18
Treatment × Trial	0.90	0.89	0.32	0.92

[1]Values are the mean of eight replications from two trials. Numbers in parentheses represent standard deviations.

[2]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).

Supplemental Table 4.3. Percentage of motile second-stage juveniles of different *Meloidogyne* species after 24 h exposure to varying concentrations of fluopyram.

Fluopyram concentrations (ppm)	*Meloidogyne enterolobii*[1,2]	*Meloidogyne floridensis*[1,2]	*Meloidogyne haplanaria*[1,2]	*Meloidogyne incognita*[1,2]
0	99.2 (2.2) a	91.7 (2.4) a	98.9 (1.2) a	100 (0) a
0.2	93.2 (8.6) a	85.3 (7.6) ab	80.4 (12.3) ab	75.6 (9.0) b
0.4	91.4 (8.0) ab	77.5 (5.3) b	69.5 (8.2) b	58.3 (15.6) c
0.8	84.5 (8.3) b	53.8 (10.0) c	51.4 (12.5) bc	22.2 (12.8) d
1.6	57.1 (3.7) c	20.3 (12.7) d	33.1 (7.1) c	19.6 (8.0) d
3.3	22.5 (6.3) d	4.3 (3.6) e	18.4 (9.0) d	2.27 (0.6) e
P value				
Treatment	<0.001	<0.001	<0.001	<0.001
Trial	0.59	0.71	0.73	0.90
Treatment × Trial	0.60	0.24	0.21	0.9

[1]Values are the mean of eight replications from two trials. Numbers in parentheses represent standard deviations.

[2]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).

Supplemental Table 4.4. Percentage of motile second-stage juveniles of different *Meloidogyne* species after 24 h exposure to varying concentrations of oxamyl.

Oxamyl Concentrations (ppm)	*Meloidogyne enterolobii*[1,2]	*Meloidogyne floridensis*[1,2]	*Meloidogyne haplanaria*[1,2]	*Meloidogyne incognita*[1,2]
0	94.9 (2.1) a	98.6 (0.5) a	95.9 (2.6) a	95.8 (2.6) a
6	92.9 (2.3) ab	86.8 (2) ab	92.6 (2.7) a	93.3 (2.8) a
12	91.6 (1.2) ab	87.5 (1.4) a	91.2 (2.3) a	91.2 (2.3) a
24	89.1 (3.4) ab	74.7 (2.0) b	46.9 (5.2) b	84.0 (2.7) b
30	83.4 (1.8) b	49.6 (4.7) c	40.5 (8.0) b	36.6 (5.1) c
36	65.3 (2.6) c	35.5 (3.8) d	29.4 (8.5) c	32.0 (5.8) c
42	46.7 (2.1) d	20.2 (3.5) e	18.3 (10.0) d	12.2 (8.5) d
48	16.7 (2.8) e	13.1 (1.8) e	12.0 (4.8) d	5.8 (5.2) de
96	12.6 (2.6) e	10.5 (1.9) e	8.9 (5.5) d	3.6 (2.7) e
P value				
Treatment	<0.001	<0.001	<0.001	<0.001
Trial	0.07	0.90	0.54	0.20
Treatment × Trial	0.23	0.62	0.97	0.80

[1]Values are the mean of eight replications from two trials. Numbers in parentheses represent standard deviations.

[2]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).

Supplemental Table 4.5. Hatch rate of different *Meloidogyne* species after seven days of exposure of eggs to different concentrations of fluazaindolizine.

Fluazaindolizine concentrations (ppm)	*Meloidogyne enterolobii*[1,2]	*Meloidogyne floridensis*[1,2]	*Meloidogyne haplanaria*[1,2]	*Meloidogyne incognita*[1,2]
0	63.6 (6.9) a	44.1 (8.8) a	54.5 (11.5) a	45.9 (10.8) a
10	57.9 (6.4) ab	39.1 (6.6) ab	51.1 (10.3) ab	34.8 (3.17) ab
20.5	47.6 (7.7) bc	40.2 (7.6) ab	43.9 (9.7) abc	39.6 (8.28) ab
41	48.5 (10.2) bc	36.6 (10.8) ab	40.8 (3.7) bcd	35.8 (7.3) ab
82	47.0 (9.8) bc	39.7 (3.9) ab	37.6 (7.2) cd	36.8 (6.0) ab
164	45.7 (7.6) c	39.3 (9.7) ab	35.8 (10.2) cd	28.9 (9.1) bc
330	39.1 (5.0) c	29.5 (5.2) b	30.1 (6.7) d	18.3 (8.7) c
P value				
Treatment	<0.001	0.04	<0.001	<0.001
Trial	0.67	0.95	0.5	0.06
Treatment × Trial	0.65	0.72	0.08	0.37

[1]Values are the mean of eight replications from two trials. Numbers in parentheses represent standard deviations.

[2]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).

Supplemental Table 4.6. Hatch rate of different *Meloidogyne* species after seven days of exposure of eggs to different concentrations of fluensulfone.

Fluensulfone concentrations (ppm)	*Meloidogyne enterolobii*[1,2]	*Meloidogyne floridensis*[1,2]	*Meloidogyne haplanaria*[1,2]	*Meloidogyne incognita*[1,2]
0	55.6 (10.4) a	36.5 (6.0) a	50.4 (12.0) a	32.2 (6.6) a
10	41.8 (6.8) b	24.6 (5.5) b	30.4 (10.2) b	24.4 (4.3) b
20	21.6 (11.9) c	15.1 (6.9) c	16.2 (6.5) c	12.8 (4.0) c
40	11.3 (5.1) cd	6.8 (3.1) d	12.1 (5.1) c	9.7 (3.6) c
80	8.9 (4.4) d	4.2 (3.0) d	13.1 (6.5) c	7.5 (3.8) c
160	5.7 (4.3) d	6.0 (5.1) d	10.0 (5.4) c	6.5 (2.2) c
P value				
Treatment	<0.001	<0.001	<0.001	<0.001
Trial	0.18	0.15	0.05	0.001
Treatment × Trial	0.69	0.18	0.33	0.08

[1]Values are the mean of eight replications from two trials. Numbers in parentheses represent standard deviations.

[2]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).

Supplemental Table 4.7. Hatch rate of different *Meloidogyne* species after seven days of exposure of eggs to different concentrations of fluopyram.

Fluopyram concentrations (ppm)	*Meloidogyne enterolobii*[1,2]	*Meloidogyne floridensis*[1,2]	*Meloidogyne haplanaria*[1,2]	*Meloidogyne incognita*[1,2]
0	55.6 (8.6) a	35.5 (3.4) a	55.2 (11.1) a	35.5 (6.2) a
0.2	52.8 (6.7) ab	35.3 (11.4) a	47.9 (12.8) ab	30.5 (2.7) ab
0.4	52.2 (5.0) ab	31.5 (9.4) ab	48.5 (24.6) ab	26.2 (3.7) b
0.8	44.5 (8.1) b	24.0 (5.5) b	31.6 (9.4) bc	18.8 (3.3) c
1.6	17.7 (7.1) c	5.7 (3.4) c	18.3 (7.7) cd	7.4 (5.2) d
3.3	7.6 (5.1) c	3.5 (2.6) c	13.9 (8.9) d	6.6 (5.1) d
P value				
Treatment	<0.001	<0.001	<0.001	<0.001
Trial	0.94	0.81	0.54	0.41
Treatment × Trial	0.09	0.50	0.19	0.28

[1]Values are the mean of eight replications from two trials. Numbers in parentheses represent standard deviations.

[2]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).

Supplemental Table 4.8. Hatch rate of different *Meloidogyne* species after seven days of exposure of eggs to different concentrations of oxamyl.

Oxamyl concentrations (ppm)	*Meloidogyne enterolobii*[1,2]	*Meloidogyne floridensis*[1,2]	*Meloidogyne haplanaria*[1,2]	*Meloidogyne incognita*[1,2]
0	52.7 (7.3) a	40.5 (6.6) a	52.8 (13.1) a	34.4 (8.7) a
6	48.2 (7.8) a	35.9 (7.0) a	52.0 (6.0) a	29.6 (4.2) a
12	45.0 (3.8) ab	28.9 (6.8) ab	45.4 (8.8) ab	25.3 (5.4) ab
24	34.7 (8.3) b	24.8 (3.6) b	40.7 (7.5) b	20.0 (4.3) b
48	21.2 (6.1) c	7.0 (6.2) c	30.6 (7.1) c	7.3 (4.5) c
96	10.4 (4.2) c	2.7 (2.5) c	14.2 (5.5) d	3.9 (2.3) c
P value				
Treatment	<0.001	<0.001	<0.001	<0.001
Trial	0.20	0.13	0.86	0.30
Treatment × Trial	0.87	0.47	0.86	0.32

[1]Values are the mean of eight replications from two trials. Numbers in parentheses represent standard deviations.
[2]Values followed by the same letter within a column are not significantly different according to Tukey's HSD test (α=0.05).

CHAPTER 5

CONCLUSION

Root-knot nematodes (*Meloidogyne* spp.) continue to pose a major threat to vegetable production in Georgia, one of the most significant vegetable-producing states in the U.S. The recent detection of aggressive species such as *M. enterolobii*, *M. floridensis*, and *M. haplanaria* (Marquez et al. 2021; Marquez and Hajihassani 2023; Hajihassani et al. 2023) signals an urgent need to re-evaluate current nematode management strategies. This study presents one of the first comprehensive evaluations of the host suitability of major vegetable crops grown in Georgia to these species, their relative reproductive potential, and their differential sensitivity to non-fumigant nematicides.

Our findings from chapter 2 show that *M. enterolobii* possesses a broader vegetable host range, greater reproductive potential, and a higher capacity to cause damage compared to *M. incognita* and *M. floridensis*. These characteristics not only enhance its ability to persist and spread in diverse vegetable cropping systems, but also suggest a competitive advantage to overcome the population of other *Meloidogyne* species. Furthermore, the significantly greater egg counts/gram of root in most of the vegetable crops indicates its greater threat to reduce yield in vegetable crops compared to *M. incognita* and *M. floridensis*.

Since *M. enterolobii* was first detected in 2021 in a sweet potato field in Tattnall County-an area where nearly 40% of Georgia's onion is produced and where sweet potato is commonly rotated with onion-it is likely that *M. enterolobii* could also infest onion crops (Hajihassani et al. 2023). Building upon this context, our findings from chapter 3 confirmed for the first time that

onion is a suitable host for *M. enterolobii*, with all tested onion type (Vidalia, red and white) and cultivars supporting nematode reproduction. The observed reductions in bulb and shoot weights in Vidalia and red onion cultivars further underscore the nematode's potential to cause significant yield suppression in this high-value crop.

Chemical nematicides remain one of the most reliable strategies for managing root-knot nematodes (RKNs). As alternatives to fumigant nematicides-which are expensive, environmentally hazardous, and require complex application procedures-several non-fumigant nematicides have recently been introduced, including fluazaindolizine, fluensulfone, and fluopyram. While these non-fumigant products have been extensively tested against M. incognita (Faske and Hurd 2015; Watson 2022; Wram and Zasada 2019), there is limited to no information on their efficacy against recently detected Meloidogyne species such as *M. enterolobii, M. floridensis*, and *M. haplanaria*. In chapter 4, we investigated both in vitro (motility, egg hatching) and in vivo (reproduction) responses of *M. enterolobii*, *M. floridensis*, *M. haplanaria*, and *M. incognita* to these non-fumigant nematicides. Our findings indicate that *M. enterolobii* exhibits reduced sensitivity to these nematicides, both in terms of motility inhibition and reproductive suppression, compared to the other species evaluated. These results underscore the importance of tailoring nematicide use to the target species and support the need for integrated management approaches that consider the chemical response variability among *Meloidogyne* species. Therefore, future research should evaluate the efficacy of non-fumigant nematicides across different *Meloidogyne* species under field conditions. Such studies will be critical for refining application rates to minimize economic losses and sustain crop productivity across diverse agroecosystems.

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