LAYING DOWN THE GROUNDWORKS FOR PLANT-PARASTIC NEMATODE MANAGMENT IN SOUTH GEORGIA VEGETABLE PRODUCTION SYSTEMS

by

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ABSTRACT

Plant-parasitic nematodes (PPN) limit vegetable yield. Our aim was to assess the threat of PPNs on vegetables and investigate potential management tactics. In 2018, a surveillance study was conducted on 246 vegetable fields from 29 counties. Root-knot nematode (RKN) was the dominate PPN infesting two thirds of sampled fields. Patterns of PPN community suggest cropping systems have a greater effect on PPN population dynamics than the edaphic factors measured in this study. Five RKN species (*M. incognita, M. arenaria, M. javanica, M. floridensis* and *M. haplanaria*) from RKN-infested fields were identified by species-specific PCR and mitochondrial haplotyping. *Meloidogyne floridensis* and *M. haplanaria* were detected in Georgia and pose a threat by reproducing on *Mi*-resistant vegetable cultivars. Most fields were infested with *M. incognita* (91.9%) or *M. arenaria* (36.0%), with many fields mixed with both species (29%). Monthly deep-soil sampling found seasonal fluctuations in the vertical distribution of RKN and *Nanidorus* spp. PPN abundance increased from mid-spring to late winter as PPNs completed multiple life-cycles and then stopped reproduction in the winter fallow period. Patterns of seasonal vertical distribution suggest an illuvial zone barrier in the north region has a prominent role. Since a majority of RKN dwell at ≤ 30 cm during the summer, fumigants/nematicide application are suggested for this season to minimize risk of escapees. Cover crop-fallowing is an important cultural practice for PPN management. Cover crop greenhouse host-status trials found many cover crops with low root galling, yet females successfully produced egg masses. Based on egg mass index, bahiagrass, bermudagrass, marigold, millet, and velvetbean were either nonhosts or ranged from nonhosts to poor hosts. A field trial testing cover crop-fallow seasons and deep tillage on nematode suppression found sunn hemp suppresses *M. incognita* independent of tillage practice, whereas deep tillage suppresses *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Nanidorus minor*. Winter rye did not suppress RKN. Fall sunn hemp had higher biomass than spring sunn hemp and reduced root galling even in the second subsequent vegetable. These findings provide new insights for vegetable growers to develop programs of sustainable PPN management.

INDEX WORDS: diagnostics, molecular identification, crop rotation, host resistance, integrated pest management, deep soil sampling, ecology, sustainable agriculture, allelopathy

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Ad Maiorem Dei Gloriam

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Vegetable Production

Vegetables are important components to the human diet providing people with dietary fiber, low molecular weight carbohydrates, protein, vitamins, and minerals (Kevers et al. 2007), which are strongly associated with alleviating degenerative diseases (Kaur and Kapoor 2001; Kevers et al. 2007). As consumers become more health conscious, the consumer demand for minimally processed vegetables (Kevers et al. 2007; Pollack 2001; Rico et al. 2007) has increased in the past few decades (Ajlouni et al. 2006). Though most vegetables are perishable commodities and only available on a seasonal basis from the local growing region, modern transportation and post-harvest storage has made vegetables readily available throughout the global marketplace (Netscher and Sikora 1990; Pollack 2001). Fruit and vegetable consumption has been positively correlated with income, as the greatest consumption of fruits and vegetables come from high-income countries with a greater selection of fruits and vegetables from domestic production and global trade (Pollack 2001). In the past 30 years, vegetable production has increased rapidly especially in the subtropical and tropical regions of the world (Hallmann and Meressa 2018).

In Georgia, USA, the vegetable industry has also been growing in farmgate value by $459 million (USD) from 2011 to 2020 (Georgia Farm Gate Value Report 2020, University of Georgia, 2019), currently valued at $1.24 billion and ranked as the 4th highest commodity group in the state (AgSnapshots 2022, University of Georgia, 2022). Georgia produces about 33 different vegetable crops, in which the top 10 main crops with corresponding percentage of total vegetable farmgate value consists of: sweet corn (*Zea mays* var. *saccharate*; 13.97%), watermelon (*Citrullus lanatus*; 12.8%), onions (*Allium cepa*; 10.8%), bell peppers (*Capsicum annuum*; 10.8%), cucumber (*Cucumis sativus*; 6.4%), tomato (*Solanum lycopersicum*, 4.4%), yellow squash (*Cucurbita pepo*; 4.3%), cabbage (*Brassica oleracea* var. *capitate*; 4.0%), carrot (*Daucus carota subsp. sativus*, 3.8%), and collards (*Brassica oleracea* var. *viridis*; 3.0%; Georgia Farm Gate Value Report 2020, University of Georgia, 2022). Therefore, the vegetable industry is important for Georgia's economy and needs to be protected from yield-limiting factors like plant-parasitic nematodes (PPN), which are problematic pests for vegetables (Hallmann and Meressa 2018).

Root-Knot Nematode

Root-knot nematodes (RKN; *Meloidogyne* spp.) are the most important PPN in the warmer sub-tropical and tropical vegetable producing regions of the world (Collange et al. 2011; Netscher and Sikora 1990; Phatak 1992) causing significant crop damage typically reported as an average of 10% yield loss, which can be much greater depending on the local region, RKN population density, and the plant host (Collange et al. 2011). For example, RKN can cause 46-64% yield loss on cabbage, cauliflower, lettuce, onions and potatoes (Olthof and Potter 1972); 85% on tomatoes (Barker et al. 1976); 90% on carrot (Bélair 1992); 60% on cucumber (Ornat et al. 1997); 80% on bell pepper (Di Vito et al. 1985) and 30% on watermelon (Davis 2007). Although damage symptoms can differ between different host species, typical symptoms consist of stunting, wilting, chlorosis (Greco and Di Vito 2018), early die-back (Hallmann and Meressa 2018), and the diagnostic root-galling (Noling 2002). It has been argued that RKN are the world's most damaging agricultural pests in respect to the cumulative effect on all their hosts (Ntalli et al. 2016) since they have a broad host range (Nyczepir and Thomas 2009) that can parasitize nearly every species of vascular plants (Moens et al. 2004). The importance of RKN as a pathogen on vegetables can be attributed to 1) global distribution (Collange et al. 2011; Morris et al. 2016; Sikora and Fernandez 2005a), 2) completing multiple generations within a single growing season, 3) and high fecundity (Nyczepir and Thomas 2009). RKN can also compound the problem by forming disease complex with fungal pathogens (Bergeson 1972) like *Fusarium oxysporum* (Mai and Abawi 1987; Manzanilla-Lopez and Starr 2009) and *Rhizoctonia solani* on vegetable crops (Hallmann and Meressa 2018). RKN has also been observed to synergistically interact with *Pratylenchus* spp. (Shakeel et al. 2012).

RKN is an obligate sedentary plant endoparasite requiring the formation of giant cells to complete their lifecycle (Abad et al. 2009) which can be completed in as little as 20 days (Ploeg and Maris 1999). RKN first-stage juveniles develop within the egg and hatch by environmental cues, mainly temperature and moisture, and become an infective second-stage juvenile (J2) (Moens et al. 2004). The J2 finds its host through cues released by the plant roots and accumulate behind the root cap at the region of cell elongation or the apical meristems where lateral roots emerge. Although penetration typically occurs behind the root cap, J2 can penetrate at any site, but will migrate towards the root tip to circumvent the endodermis barrier after penetration (Karssen et al. 2013). The J2 penetrates the root by using their stylet and secreting cell-wall-degrading enzymes (Abad et al. 2003), migrating intracellular through the cortex apoplast (Abad et al. 2009) behind the root cap in the region of cell differentiation, until it finds protoxylem and protophloem vascular tissue (Karssen et al. 2013; Moens et al. 2009). The J2 will then form a permanent feeding site in which 2-12 specialized multinucleate giant cells are formed from phloem or adjacent parenchyma cells (Karssen et al. 2013). Each giant cell contains over 100 nuclei (Abad et al. 2009), in which each nucleus is polyploidy, containing up to 8 times more than the original number of chromosomes (Moens et al. 2009). As the RKN feed, they cause rapid cell division and growth among the surrounding cortex cells resulting in root gall formation from increased auxin and cytokinin production (Karssen et al. 2013), which are the primary symptoms of RKN infection on roots (Moens et al. 2009; Netscher and Sikora 1990), however, not a requirement for RKN development (Karssen et al. 2013). Within 2 weeks of feeding, the J2 will molt two more times and go through the J3 and J4 stage within 4-6 days. As the juvenile enters the J3 and J4 stage, it no long feeds and loses its functional stylet (Abad et al. 2009). At the J4 stage, the juvenile develops into a female, unless under limited food supply, which triggers male development in partheonogenetic species (Abad et al. 2009). As a female, the stylet reforms to resume feeding, while the reproduction organs develop to produce hundreds of eggs at a rate of 30-80 eggs per day (Karssen et al. 2013), which are deposited into an egg matrix of glycoprotein which protect against the environment and antagonistic microbes (Moens et al. 2004). RKN species in the tropical *M. incognita* group (MIG), *M. incognita*, *M. arenaria,* and *M. javanica*, are the most frequently occurring species on vegetables. Compared to other species, these species are known to have the broadest host range (Netscher and Sikora 1990) and a shorter life cycle due to obligatory mitotic parthenogenesis (Chitwood and Perry 2009).

Other plant-parasitic nematodes associated with vegetables

Other plant-parasitic nematodes that can affect vegetable production worldwide include *Rotylenchulus reniformis* on most vegetable crops*; Nacobbus aberrans*, *N. bolivianus* and *N. dorsalis* on cabbage, beans (*Phaseolus vulgaris*), turnip (*Brassica rapa* subsp. *rapa*), pepper, squash (*Cucurbita pepo*), gourd (*Lagenaria siceraria*), lettuce (*Lactuca sativa*), tomato, cucumber and carrot (Hallmann and Meressa 2018); *Globadera rostochiensis* and *G. pallida* on potato (*Solanum tuberosum*), tomato and eggplant (*Solanum melongena*); *Heterodera schachtii* and *H. Cruciferae* on brassicas (Zyl and Meyer 2000); *Ditylenchus dipsaci* and *D. destructor* on most vegetables (Hallmann and Meressa 2018); *Pratylenchus brachyurus, P. zeae* and *P. penetrans* on cucumber, tomato, pepper, and carrot (Anwar and McKenry 2012; Matthiessen and Kirkegaard 2006); *Radopholus similis* on carrot, beet (*Beta vulgaris*), bean, tomato, squash, maize, cowpea (*Vigna unguiculate*), pepper, okra (*Abelmoschus esculentus*), radish (*Raphanus raphanistrum* subsp. *sativus*), cantaloupe (*Cucumis melo* var. *cantalupensis*), cucumber, broccoli (*Brassica oleracea* var. *italica*) and watermelon (Anwar et al. 2013; Feder and Feldmesser 1957); *Belonolaimus longicaudatus* on carrot, pepper, cabbage, cucumber, eggplant, okra, pea (*Pisum sativum*), potato, celery (*Apium graveolens*), pumpkin (*Cucurbita pepo*), onion and tomato (Anwar and McKenry 2012); *Paratrichodorus allius* on sugar beet (*Beta vulgaris* subsp. *vulgaris* convar. *vulgaris* var. *altissima*) and potato in the pacific north west vectoring the tobacco rattle virus which causes corky ringspot dieasese (Ingham et al. 2000), *P. mirzai* on carrot, and *P. minor* on most vegetables; *Trichodorus viruliferus* on pepper; *Tylenchorhynchus brassicae* on brassicas (Hallmann and Meressa 2018); *Longidorus africanus* on lettuce and carrot (Huang and Ploeg 2001) and *L. vineacola* on celery (Cohn and Auscher 1970); and *Xiphinema ifacolum* on eggplant, tomato, pepper, onion, lettuce, carrot, broccoli, celery, cucumber, radish, potato and okra (*Abelmoschus esculentus*) (Hallmann and Meressa 2018). Other PPN that are commonly found associated with vegetable crops include *Helicotylenchus* spp., *Scutellonema* spp., *Hoplolaimus* spp., *Aorolaimus* spp., *Zygotylenchus* spp., *and Mesocriconema* spp. and could potentially be important for vegetable crops, but currently considered not economically important (Hallmann and Meressa 2018).

Root-Knot Nematode Management

Chemical control with synthetic nematicides has been the main mode of RKN control for intensive production systems with moderate to high-value crops for the 20th century (Nyczepir and

Thomas 2009) in which vegetable production represents 38% of the global nematicide market (Hallmann and Meressa 2018). However, growers have been forced to re-evaluate alternative approaches as many successful fumigants have been banned due to toxicity to non-targets (Sikora and Roberts 2018), starting with 1,2 dibromo-3-chloropropane (DBCP) in 1981 for all crops (Johnson and Feldmesser 1987), followed by a methyl bromide ban in the USA and Western Europe in January, 2005 with the exception of a few crops (Clean Air Act, 1990). The European union has also been phasing out 1,3-dichloropropene (1,3-D), by withdrawing authorization in 2008 (Colla et al. 2012). Some non-fumigant nematicides, like fenamiphos, have been voluntarily withdrawn from registration in the United States, as of May 2007, due to reports of killing birds, while others like aldicarb and carbofuran are under United States Environmental Protection Agency (US EPA) review for groundwater contamination or bird toxicity (Ferris 2019). Therefore, new fumigant (*i.e.* Allyl isothiocyanate and dimethyl disulfide) and non-fumigant (*i.e.* fluensulfone, fluopyram and fluazaindolizine) nematicides have been released or in the process of being released to offer chemical control of PPN for vegetable production (Hajihassani 2018). Unlike fumigants, non-fumigants, also known as contact nematicides (Taylor 2003), suppress nematodes through delaying nematode penetration by paralyzing nematodes via contact or systemic activity (Hallmann and Meressa 2018; Oka et al. 2009). These nematicides can be used after planting for enhancing fumigant control of PPN (Zasada et al. 2010).

Other approaches to PPN management consist of cultural practices of planting timing at cooler temperatures when RKN populations are lower; disrupting the RKN lifecycle with a fallow period; destroying infected roots after harvest; soil tillage; creating anaerobic environment by flooding; releasing toxic compounds and enhancing RKN antagonist by incorporating organic matter; heating the soil by solarization; biofumigation; and rotation of non-host, trap crops, and/or cover crops (Hallmann and Meressa 2018). PPN management typically varies among regions and crops to appropriately address the specific nematode challenges. Currently, there is a need to address regional PPN management of vegetables for the state of Georgia, especially of RKN.

Objectives and Hypothesis of the Studies

Although the vegetable industry has grown to be an important source of revenue for the state, there has been a lack of research on addressing the specific challenges of PPN management for Georgia. Majority of commercial vegetable production is localized in south Georgia represents 90.9% of the state's vegetable farm gate value (Georgia Farm Gate Value Report 2020, University of Georgia, 2022). The approach of this study is to examine PPN population dynamics in vegetable rotation cropping systems unique to southern Georgia. In addition, due to the growing interest in cover cropping, cover crop rotation in a typical vegetable cropping system will be evaluated in south Georgia's climate to determine its potential in PPN management. Below are the specific objectives of this study.

1) Assess plant-parasitic nematodes geospatial associations with edaphic factors and cultural practices
    a) Determine occurrence, incidence, and distribution of different PPN genera.
    b) Find any associations of PPN genera to different vegetable crops.
2) Identify root-knot nematode in vegetable crops.
    a) Find the occurrence, incidence, and distribution of RKN species.
    b) Disclose any associations of RKN species to different vegetable crops.
    c) Determine if any vegetable fields are infested with new RKN species.
    d) Understand the diversity of haplotypes within a single species of RKN.

3) Examine the seasonal fluctuations in vertical distribution of PPN and their interactions with edaphic factors.
    a) Assess seasonal fluctuation in population densities of PPN in south Georgia's vegetable plastic and bare-ground bed cropping systems.
    b) Evaluate if population dynamics differ in latitude and soil environment (soil texture, porosity, moisture, temperature, and pH).
    c) Determine if seasonal changes affect the vertical distribution of PPN.
4) Evaluate host status of cover crops for major species of root-knot nematodes (*M. incognita, M. arenara,* and *M. javanica*).
5) Assess suppression of *M. incognita* by cover crop rotation of sunn hemp (*crotalaria junciea*), wrens abruzzi rye (*Secale cereale*) and deep tillage for vegetable production systems.
    a) Find the optimal season to rotate a cover crop in a vegetable rotation in south Georgia for *M. incognita* suppression.
    b) Evaluate if deep tillage can enhanse cover crop-fallowing for *M. incognita* management.
    c) Determine if cover crop-fallowing and deep tillage affect other soilborne fungal pathogens and weed density and share any interactions with PPN.

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CHAPTER 2

THE INFLUENCE OF THE ENVIRONMENT AND VEGETABLE CROPPING SYSTEMS ON PLANT-PARASITIC NEMATODE COMMUNITIES IN SOUTHERN GEORGIA, USA[1]

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Abstract

Plant-parasitic nematodes (PPN) limit yields in vegetable production in the United States. During the spring and fall cropping seasons of 2018, 436 fields in bare ground and plastic bed cropping systems were randomly sampled from 29 counties in Southern Georgia. The incidence (%), mean and maximum relative abundance (nematodes per 100 cm^3 of soil) of the 10 different PPN genera detected in 32 vegetable crops in bare ground and plastic bed cropping systems include *Meloidogyne* spp. (67.3, 292, 14144), *Nanidorus* spp. (49.4, 6, 136), *Mesocriconema* spp. (39.6, 17, 340), *Helicotylenchus* spp. (31.6, 20, 1152), *Pratylenchus* spp. (20.1, 2, 398), *Rotylenchulus* spp. (5.9, 1, 116), *Hoplolaimus* spp. (12.6, 1, 78), *Heterodera* spp. (2.3, <1, 60), *Tylenchorhynchus* spp. (0.9, <1, 12) and *Xiphinema* spp. (0.2, <1, 2). A Non-metric Multidimensional Scaling analysis (NMS or NMDS) indicated that most environmental and geological factors (*i.e.* longitude, precipitation, soil moisture, sand + silt content and soil electrical conductivity) had no apparent relationship with nematode counts, except for latitude, soil pH and temperature. Multi-rank Permutation Procedure (MRPP) followed by Indicator Species Analysis (ISA) and non-parametric Kruskal-Wallis Analysis of Variance (KW ANOVA) indicated that *Meloidogyne* spp. was the predominant PPN associated with plastic beds in the South region sampled. The South region consisted mainly of commercial fields which rotated multiple vegetables crops through the same plastic beds. All other PPNs were associated with bare ground beds in the North region that are commonly rotated with row crops. This study validates that *Meloidogyne* spp. is the most important PPN in vegetable fields of Southern Georgia and suggests that cropping systems have a greater effect on PPN population dynamics than the environment.

Keywords: detection, plant-parasitic nematodes, root-knot nematode, *Meloidogyne* spp., vegetable, Georgia, Non-metric Multidimensional Scaling

Introduction

Georgia is among the leading vegetable producers of the United States, and is ranked 3rd or 4th in utilized vegetable production in the last three years after California, Arizona and Florida (USDA-NASS 2021). The vegetable industry in Georgia is considered the 4th top agricultural commodity valued at $1.13 billion (Wolfe and Stubbs 2019). Georgia produces at least 33 different vegetable crops (Kane 2021), in which the top 10 main crops consist of: sweet corn (*Zea mays* var. *saccharata*), onions (*Allium cepa*), bell peppers (*Capsicum annuum*), watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), carrot (*Daucus carota subsp. sativus*), cabbage (*Brassica oleracea* var. *capitate*), yellow squash (*Cucurbita pepo*) and zucchini (*C. pepo*) (Wolfe and Stubbs 2019).

Plant-parasitic nematodes (PPNs) are one of the major causes of crop losses. Because of their worldwide distribution and broad host range, root-knot nematodes (RKNs; *Meloidogyne* spp.), are an important PPN of vegetable crops and cause substantial yield reductions (Collange et al. 2011; Davis 2007; Sikora and Fernandez 2005b). Major RKN species that threaten vegetable production include *M. incognita, M. arenaria*, *M. javanica*, *M. hapla*, *M. enterolobii* and *M. floridensis* (Hallmann and Meressa 2018; Koenning et al. 1999b; Mitkowski et al. 2002). Other important PPNs that threaten worldwide vegetable production include *Belonolaimus longicaudatus*, *Rotylenchulus* spp., *Pratylenchus* spp., *Heterodera* spp., *Nanidorus* spp. (syn. *Paratrichodorus* spp.), *Trichodorus* spp., *Globodera* spp., *Ditylenchus* spp., *Radopholus similis*, *Tylenchorhynchus* spp. and *Xiphinema* spp. (Anwar et al. 2013; Anwar and McKenry 2012; Hallmann and Meressa 2018; Robinson et al. 1987; Zyl and Meyer 2000).

Our understanding of the occurrence, incidence, and geographical distribution of PPNs in vegetable fields of Southern Georgia and the influence edaphic factors and cultural practices have on PPNs is lacking and needs to be pursued to assess their potential in causing economic damage to vegetables and predict high risk conditions. The last statewide surveys conducted on RKN were on fields of row crops, peanut (*Arachis hypogaea*) and cotton (*Gossypium* spp.), and are decades old (Baird et al. 1996; Motsinger et al. 1976a). Moreover, many of these surveys did not include the main vegetable producing regions of the state. Currently, the incidence of PPNs on vegetable crops of Southern Georgia have not been investigated, despite many PPNs found in the United States to cause crop losses to specific vegetables. For example, *D. dipsaci* was found to be an important pest on garlic and onion in California (Miyagawa and Lear 1970) and on garlic in Minnesota (Mollov et al. 2012), Ohio (Testen et al. 2014) and New Mexico (Testen et al. 2014); *R. reniformis* on cucurbits, legumes and sweet potato in Florida, Alabama, Arkansas, Louisiana, Mississippi, Texas and Hawaii (Koenning et al. 1999a); *P. penetrans* on potato in Ohio, Missouri, Illinois and Indiana (Westphal et al. 2018); *H. schachtii* on crucifers sugar beets in Utah, California, Colorado, and Idaho (Harveson and Jackson 2008)*; H. cruciferae* on crucifers in California (Hallmann and Meressa 2018); *H. carotae* on carrots in Michigan; *H. glycines* on legumes in Michigan, Montana and North Carolina; *H. goettingiana* on legumes in Washington (Koenning et al. 1999a); *G. pallida* in New York and *G. rostochiensis* in Idaho (Dandurand et al. 2019), *B. longicaudatus* on cucurbits in Florida (Khanal and Desaeger 2020); *Mesocriconema* spp. on legumes and onion in Washington; *Trichodorus* spp. on onion in California; *Tylenchorhynchus* spp. on lettuce in California; *N. allius* on onion and sweet corn in Washington (Koenning et al. 1999a) and *N. allius* on sugar beet and potato in the Pacific North West vectoring the tobacco rattle virus which causes corky ringspot disease (Ingham et al. 2000).

Because most PPNs dwell in the soil, edaphic factors can influence their distribution, incidence, and population densities (Norton 1979). Temperature and soil moisture are argued as the most important edaphic factors affecting nematode physiology and behavior (Barbercheck and Duncan 2004; Norton 1979). Temperature affects hatching (Curtis et al. 2009), vertical migration, respiration (Moens and Vincx 2000), and size (Barbercheck and Duncan 2004) of the nematodes. Field capacity, the maximum water holding capacity after gravitational water has drained, is optimal for nematodes (Wallace 1966), maintaining both sufficient soil moisture and continuous channels of air for respiratory gases and plant volatiles to diffuse at long-distance gradients (Curtis et al. 2009). Soil texture may be the most extensively studied edaphic factor (Barbercheck and Duncan 2004; Norton 1979). For example, higher severity of root-knot infestation has frequently been reported in soils with high sand content (Di Vito et al. 1985), while *R. reniformis* reproduction is greater in soil with higher clay content (Koenning et al. 1996). Though lower pH (~5.2) can inhibit *M. javanica* hatching (Wallace 1966), pH effects are minimal for most nematodes under normal pH conditions (Barbercheck and Duncan 2004). Within the state of Georgia, the majority of the vegetables are grown in Southern Georgia. However, this part of Georgia contains nine different biogeographical ecoregions, which have different underlying geological histories, and may have different edaphic conditions that could influence PPN populations.

This study was aimed at assessing PPN associated with temperate vegetable crops (*e.g.* sweet, watermelon, cucumber, tomato, carrot, cabbage, yellow squash and zucchini) in the intensive vegetable producing region of Southern Georgia to expand our understanding of the incidence, abundance and geographic distribution of different PPN genera, and identify any potential relationships of PPN genera with different edaphic factors and cropping systems.

Material and Methods

Soil sampling and processing. The survey consisted of 436 vegetable fields of both bare ground (n= 194 fields) and plastic beds (n= 242 fields) in 29 counties of Southern Georgia, sampled in the spring and fall cropping seasons from May to December, 2018 (Fig. 1). A total of 32 vegetable crops representing 9 crop families, 14 crop genera and 19 crop species were sampled (Fig. 1, Table S1). Crops sampled only in the spring cropping season (May through August) included onion, watermelon, cantaloupe, hard squash, gourd, butter bean, Southern pea, tomatillo, potato and carrots. Crops sampled only in the fall cropping season (October to December) consisted of broccoli, cauliflower and napa cabbage. Crops sampled in both cropping seasons included mustard greens, cabbage, collard greens, kale, sweet potato, cucumber, acorn squash, butternut squash, pumpkin, spaghetti squash, yellow squash, zucchini, snap beans, okra, sweet corn, bell pepper, chili pepper, tomato and eggplant.

A total of 9 ecoregions (Atlantic Southern Loam plains, Bacon Terraces, Coastal Marsh, Coastal Plain Red Up, Dougherty Plains, Okefenokee Plains, Sand Hills, Tifton Upland and Valdosta Limesink) comprised the Southern Georgia vegetable growing region (Fig. 1). The counties represented by our sampling included Appling, Ben Hill, Brooks, Bulloch, Colquitt, Cook, Crisp, Crawford, Decatur, Dooly, Echols, Grady, Jeff Davis, Lee, Lowndes, Macon, McIntosh, Mitchell, Peirce, Sumter, Tattnall, Telfair, Thomas, Tift, Toombs, Turner, Ware, Wayne, Wilcox, and Worth. Each field location was marked by GPS coordinates retrieved by a mobile phone app: GPS Waypoints Navigator Version 9.14 (DS Software, Las Cruces, NM).

For fields that were > 0.4 ha, 30 soil cores (2.54 cm-diam.) were collected and mixed to form a composite-bulk sample to represent a single field from a random location in the field. Each soil core was sampled to a depth of approximately 20 cm sampling the crop rhizosphere at about every 3-4 m across planting-bed-rows of the field through a diagonal transect in a zig-zag pattern. For smaller fields (< 0.4 ha), 30 cores were collected in a W-like pattern. Soil samples were stored in individual zip-lock plastic bags in a cooler at ambient temperature before they were delivered to the laboratory where they were stored at room temperature (21 ± 1 °C) within 1-2 weeks of sampling until processing. Because the sampled vegetable crops varied widely in planting and harvesting timings, coordinating root sampling was not logistically possible.

Nematodes were extracted by centrifugal sugar flotation technique (Jenkins 1964) from 100 cm^3 of soil with the addition of 0.5 g of Kaolin (Merck KGaA, Darmstadt, Germany) before centrifugation in the sugar solution to assist in pellet formation (Julius and Ilvo 2013). PPNs were identified to the genus based on stylet, head, tail, and other morphological features (Mai and Mullin 1996; Smart and Nguyen 1988) using a ZEISS Axio Vert.A1 (Oberkochen, Germany) inverted microscope at a 10-40x magnification and counted.

Measurement of soil properties. All soil samples were subjected to texture, pH, and electrical conductivity (EC) characterizations. Soil pH was based on guidelines from McLean (1982) and was measured by mixing 10 g of soil (wet wt.) with 10 mL of water in a 50 mL conical tube, letting the solution incubate overnight before measuring. The supernatant was poured into a 100 mL cup and measured with a pH meter to the nearest tenth (Oakton Instruments Waterproof pHTestr 20, Vernon Hills, IL). Soil texture was determined through the Bouyoucos hydrometer method (Bouyoucos 1962) from 50 g of soil, while EC was determined through a salt meter from 50 g of soil by Waters Agricultural Laboratories (Camilla, GA, USA). Meteorological data (precipitation and air temperature) and edaphic factors (soil temperature and soil moisture) were collected from the University of Georgia Weather Network (http://www.georgiaweather.net/) from the nearest weather station. Soil temperature was measured at the 10 cm depth with a 109 temperature probe (Cambell Scientific, Logan, UT), while volumetric soil moisture was measured with 616 30-cm water content reflectometer (Cambell Scientific, Logan, UT). Precipitation was measured with a 20-cm funnel orifice rain gage (Cambell Scientific, Logan, UT). Weather data was represented as a 30-day mean of the day of sampling and the preceding 29 days before sampling.

Statistical Analysis. Because nematode counts were severely over-dispersed and log transformations did not improve normality, we used a Wilcoxon-Mann-Whitney Test for two-sample comparisons, while Kruskal-Wallis Analysis of Variance (KW ANOVA) were used to make multi-sample comparisons of median nematode relative abundance (*i.e.* population density). PROC NPAR1WAY in SAS® 9.4 (SAS Inc. Cary, NC) was used for the Wilcoxon-Mann-Whitney Test. Pairwise, post-hoc differences between groups after KW ANOVA were assessed with a Dunn-Bonferroni test using NPTESTS in SPSS statistics version 26 (IBM Corporation, Armonk, NY). To compare incidence between PPNs, incidence data (0=absence and 1=present) were fit to a binomial distribution with each field as a random effect and subjected to one-way ANOVA using PROC GLIMIX in SAS® 9.4 (SAS Inc. Cary, NC) followed by means separation with Tukey's Honest significant difference test at α<0.05. To determine differences of environmental variables between the North and South region sampled (Fig. 1), meteorological data (precipitation and air temperature) and edaphic factors (soil EC, pH, texture, temperature and moisture) were normally distributed, or else, log transformed and subjected to one-way ANOVA with season as a random effect followed by means separation with Tukey's least squares means test at $\alpha < 0.05$. Prominence values for each nematode genus was calculated with the following equation:

$$PV = \frac{Population\ density \times Frequency\ of\ occurrence}{10} \tag{1}$$

Relative prominence value was calculated as follows:

$$RPV = 100 \times \frac{PV}{Total\ sum\ of\ PV\ of\ each\ genus} \qquad (2)$$

To understand how Southern Georgia vegetable PPN communities may be related to weather, edaphic factors, crop, season and ecoregions, we used Non-metric Multidimensional Scaling (NMS or NMDS ordination) (Clarke 1993) to account for all variables simultaneously and visualize the associated patterns of nematode species occurrence and abundance in sampled fields with environmental variables. The NMS analysis was based on two data matrices, a main matrix containing the number of nematodes counted in each genus and a secondary matrix consisting of environmental variables [categorical variables = crop family; crop genus; crop species; month sampled; season sampled; ecoregion sampled; soil texture class; North and South regions and bare ground and plastic bed cropping systems; quantitative variables = precipitation (mm), soil moisture (v/v; %), soil temperature (°C), sand + silt content (%), pH and EC (mmhos/cm)]. Variables from the secondary matrix were used as an overlay onto the main matrix ordination solution of nematode abundance. Because the main nematode abundance matrix was comprised of many undetected taxa (zeroes) and the count data spanned several orders of magnitude between samples, all data were *ln* (x+1) transformed. Adding one to all samples enabled us to *ln*-transform all values (*ln* of 0 is undefined but *ln* of 1 = 0), and log transformation preserves the relative abundance of each nematode taxon while generating a multivariate normal distribution (an assumption of NMS). NMS analysis was run with Gower's distance and under the "slow and thorough" autopilot mode in PC-ORD 7 (McCune and Mefford 2016).

To investigate the effects edaphic factors had on PPN relative abundance, Multi-rank Permutation Procedure (MRPP) was conducted with the original transformed matrices to determine statistical differences between relative abundance of PPN genera among the environmental groups (sand + silt content, soil pH, soil EC). Since MRPP only tests for group membership, we used Indicator Species Analysis (ISA) to determine if any PPN genera are statistically associated with any environmental group by producing indicator species values (Severns and Sykes 2020). These values ranged from 100, for perfect association, to 0, for no association (Dufrêne and Legendre 1997). Probability values were produced through 5,000 randomizations with PC-ORD 7.

To determine if cultural practices of cropping systems affect nematode's associations in the North and South region, fields were divided between bare ground and plastic bed cropping systems. MRPP analysis followed by a non-parametric Kruskal-Wallis test was conducted to investigate if any nematode genus were associated with the North or South region in either bare ground or plastic bed cropping systems.

Results

The distribution, incidence and abundance of plant-parasitic nematodes infesting vegetable fields of Southern Georgia. A total of 92.4% of the vegetable fields were infested with at least one of the 10 PPN genera which included: root-knot (*Meloidogyne* spp.), stubby-root (*Nanidorus* spp.), ring (*Mesocriconema* spp.), spiral (*Helicotylenchus* spp.), root-lesion (*Pratylenchus* spp.), reniform (*Rotylenchulus* spp.), lance (*Hoplolaimus* spp.), cyst (*Heterodera* spp.), stunt (*Tylenchorhynchus* spp.) and dagger (*Xiphinema* spp.) nematodes. Incidence between PPNs differed from each other ($F = 71.61$, d.f. = 9, $P < 0.0001$). *Meloidogyne* spp. incidence exceeded ($P < 0.05$) other PPN genera, infesting nearly two thirds of the fields sampled (Fig. 2, Table 2). This is also reflected in rank prominence, with *Meloidogyne* spp. representing 92.2% of the total PPNs recorded (Table 2). *Nanidorus* spp. had a greater incidence ($P < 0.05$) than *Helicotylenchus* spp., infesting nearly half the fields, though not statistically different from

Mesocriconema spp., and these were followed by *Pratylenchus* spp. and *Hoplolaimus* spp.; *Rotylenchulus* spp., *Tylenchorhynchus* spp. and *Xiphinema* spp. Incidence of *Heterodera* spp. did not statistically differ from *Rotylenchulus* spp., *Tylenchorhnychus* spp. and *Xiphinema* spp. (Table 2, Fig. 2).

Meloidogyne spp. had a median of 8 nematodes/100 cm^3 of soil, while all other nematode genera had median of 0 nematodes/100 cm^3 of soil (Table 2). Though other genera had a median of 0, the sample populations ranked differently which can be observed by the 5th and 95th quantiles. Median relative abundance significantly differed between PPN genera ($\chi^2 = 1197$, d.f. = 9, $P < 0.0001$). *Meloidogyne* spp. median relative abundance was significantly greater ($P < 0.05$) than all other PPN followed by *Helicotylenchus* spp.; *Nanidorus* spp.; *Pratylenchus* spp. and *Hoplolaimus* spp.; *Rotylenchulus* spp., *Tylenchorhynchus* spp. and *Xiphinema* spp. However, the median number of *Mesocriconema* spp. was not statistically different from *Helicotylenchus* spp. and *Nanidorus* spp., and *Heterodera* spp. from *Rotylenchulus* spp. and *Tylenchorhynchus* spp. (Table 2).

When relative abundance and distribution of PPNs were mapped according to field location, *Meloidogyne* spp. incidence and relative abundance appeared to be greater in the South than the North region (Fig. 2). However, *Helicotylenchus* spp. and *Mesocriconema* spp. relative abundance appeared to be greater in the North than South region. The high incidence of *Nanidorus* spp. can be observed in the distribution map (Fig. 2D). Though there were only 26 fields infested with *Rotylenchulus* spp., they appeared to be greater in the western than eastern region (Fig. 2F). All other PPNs did not have any clear pattern in their distribution (data not shown).

Associations of environmental variables with plant-parasitic nematode communities. Three environmental variables with the greatest r^2 values consisted of latitude (17%), soil pH (12.5%) and soil temperature (8.5%) (Fig. 3). All other environmental variables (sand + silt content, EC, pH, Longitude, precipitation, soil moisture and soil temperature) explained less than 8% of the variation in aggregate. The vector representing latitude was oriented in the opposite direction from the vectors representing soil pH and soil temperature, mainly along axis 1, suggesting that axis 1 represented a combination of geographic location and soil characteristics (Fig 3). Centroids of the joint plot indicated that *Meloidogyne* spp. and *Heterodera* spp. occurred in ordination space associated with increasing soil pH and temperature, while *Helicotylenchus* spp., *Hoplolaimus* spp., *Rotylenchulus* spp. and *Tylenchorhynchus* spp. consistently occurred in ordination space associated with increasing latitude for all axes (Fig. 3). However, *Mesocriconema* spp., *Nanidorus* spp. and *Pratylenchus* spp. did not have any clear relationship with environmental variables nor confined to any part of the ordination space.

MRPP indicated that nematode counts did not statistically differ between soil texture classes (loamy sand, sand, sandy clay loam and sandy loam; $P = 0.45401057$, $T = 0.061138528$, $A = -0.00011868$); however, nematode counts differed between crop families (Amaryllidaceae, Brassicaceae, Convolvulaceae, Cucurbitaceae, Fabaceae, Malvaceae, Poaceae, Solanaceae and Umbellifers; $P < 0.00000001$, $T = -16.456639$, $A = 0.06170223$). ISA found *Meloidogyne* spp. ($P = 0.0202$) and *Rotylenchulus* spp. ($P = 0.0306$; Table 3) as a significant indicator of Solanaceous and leguminous crops, respectively. *Nanidorus* spp., *Helicotylenchus* spp., *Pratylenchus* spp., *Mesocriconema* spp., *Hoplolaimus* spp., *Rotylenchulus* spp., *Heterodera* spp. and *Tylenchorhynchus* spp. were not statistically significant indicators for any crops.

The Influence of latitude on plant-parasitic nematodes. Because latitude was the most influential environmental variable on nematodes, fields were divided into North and South regions at the midpoint in latitude (31.647469°; Fig. 1). The North region consisted of 14 counties (Appling, Bulloch, Crawford, Crisp, Dooly, Jeff Davis, Lee, Macon, Sumter, Tattnall, Telfair, Toombs, Turner and Wilcox) and 155 vegetable fields, while the South region consisted of 14 counties (Brooks, Colquitt, Cook, Decatur, Echols, Grady, Lee, Lowndes, McIntosh, Mitchell, Peirce, Thomas, Tift, Ware, Wayne and Worth) and 281 vegetable fields. Mann-Whitney Tests indicated that the nematode counts differed between the North and South region for 8 nematode genera ($P < 0.05$). *Meloidogyne* spp. relative abundance was greater ($Z = -5.1988$, $P < 0.0001$) in the South region than North, while all other 7 nematode genera had significantly greater relative abundance in the North than South region [*Nanidorus* spp. ($Z = 2.3138$, $P = 0.0211$), *Helicotylenchus* spp. ($Z = 9.1807$, $P < 0.0001$), *Pratylenchus* spp. ($Z = 5.2459$, $P < 0.0001$), *Mesocriconema* spp. ($Z = 5.0383$, $P < 0.0001$), *Hoplolaimus* spp. ($Z = 7.1723$, $P < 0.0001$), *Rotylenchulus* spp. ($Z = 5.0166$, $P < 0.0001$) and *Heterodera* spp. ($Z = 2.2973$, $P = 0.0221$)].

To determine if meteorological data and edaphic factors were associated with latitude, comparisons were made between the North and South region. Clay, silt and sand content did not statistically differ between the regions, while soil EC ($T = -2.08$, d.f. = 330, $P = 0.0381$) and pH ($T = -6.57$, d.f. = 330, $P < 0.0001$) were lower in the North than South region. When season was considered a random effect, precipitation ($T = -6.82$, d.f. = 434, $P < 0.0001$) and air temperature ($T = -3.54$, d.f. = 434, $P = 0.0004$) were greater in the South than North region, while soil moisture was greater ($T = 7.54$, d.f. = 434, $P < 0.0001$) in the North than South region.

The influence of plastic and bare ground cropping systems on plant-parasitic nematode communities. When PPN counts were categorized in NMS analysis as originating from bare ground (black pyramid) or plastic beds (green pyramid), bare ground samples dominated the right portion of the ordination, while samples from plastic beds were mostly on the left side of the ordination (Fig. 4). MRPP indicated that the nematode counts differed between bare ground and plastic bed cropping systems ($P < 0.00000001$, $T = -55.319247$, $A = 0.07558361$).

To determine differences in nematode communities between bare ground and plastic bed cropping systems among the North and South region, a KW ANOVA was performed. A total of 53 fields had plastic beds and 102 fields had bare ground beds in the North region, while the South had 189 fields with plastic beds and 92 beds with bare ground beds. According to KW ANOVA, 8 nematode genera (*Meloiodogyne* spp., *Helicotylenchus* spp., *Mesocriconema* spp., *Nanidorus* spp., *Pratylenchus* spp., *Rotylenchulus* spp., *Hoplolaimus* spp. and *Heterodera* spp.) had significant differences in relative abundance between the bare ground or plastic bed cropping systems in the North and South region (Table 4). Relative abundance of *Meloidogyne* spp. was greater ($P < 0.05$) in plastic beds than in the bare ground beds of the South region and both the bare ground and plastic beds in the North region. All other PPNs with significant differences were higher ($P < 0.05$) in relative abundance in bare ground beds of the North region than plastic beds of the South region (Table 4). Relative abundance of *Helicotylenchus* spp. was greatest in plastic beds in the North region followed by bare ground beds in the North region, bare ground beds in the South region and plastic beds in the South region. *Nanidorus* spp. had no difference between the North and South region, but bare ground beds had greater ($P < 0.05$) relative abundance than plastic beds in both regions. *Rotylenchulus* spp. and *Hoplolaimus* spp. had no significant difference between bare ground and plastic beds, but the North region had significantly greater relative abundance compared to the South region. Relative abundance of *Mesocriconema* spp. and *Pratylenchus* spp. in plastic beds of the South region were less ($P < 0.05$) than bare ground beds in the South region and bare ground and plastic beds in the North region (Table 4). Relative abundance of *Heterodera* spp. in bare ground beds of the North region were only greater ($P < 0.05$) than relative abundance in plastic beds of the South region (Table 4).

Discussion

PPN associated with vegetable crops in Southern Georgia. This is the first comprehensive study on PPNs' associations with vegetable crops in Southern Georgia, providing valuable insights into its endemic PPN community and varied interactions with unique edaphic conditions, mainly of sandy soil in a humid subtropical climate with high precipitation and short winters. As expected, *Meloidogyne* spp. were found to be the predominant PPNs in Southern Georgia vegetable fields, infesting over two-thirds of the fields sampled. Root-knot nematode is associated globally with vegetable production (Hallmann and Meressa 2018) and is one of the major soilborne pathogens of vegetables in Southeastern United States (Hajihassani 2018; McSorley et al. 1999). *Nanidorus* spp. were the second most frequently detected PPNs in vegetable fields of South Georgia, yet the frequency of these nematodes may not necessarily indicate association with vegetables crops, since they are known to be widely distributed in Georgia (Hajihassani et al. 2018a). Moreover, vegetable fields maintained much lower relative abundance of *Nanidorus* spp. in comparison to *Meloidogyne* spp. *Nanidorus minors* is commonly neglected as a threat to vegetable production because of their indistinct symptoms compared to *Meloidogyne* spp. (Hallmann and Meressa 2018), yet it is a known pathogen of tomato (Netscher 1970), cowpea and eggplant (Baujard and Martiny 1995). In Georgia, *N. minor* is a pest on sweet onion seedling nurseries used for transplant production in the Vidalia onion growing region (Hajihassani et al.

2018b) and on field corn throughout Southern Georgia (Davis and Timper 2000a; Timper et al. 2007). *Mesocriconema* spp. and *Helicotylenchus* spp. were also detected frequently in the vegetable fields; yet, these nematodes are mainly a problem on perennial crops, *e.g.* peach, blueberry, muscadine grapes and turf (Hajihassani et al. 2018a; Jagdale et al. 2013; Jagdale et al. 2019). The relative abundance of *Helicotylenchus* spp. was second only to *Meloidogyne* spp.; however, the economic importance of *Helicotylenchus* spp. on vegetable crops has not been determined (Hallmann and Meressa 2018). In plastic polytunnel vegetable production systems in the Caribbean, *Helicotylenchus* spp. are found but associated with weeds, especially those of the Asteraceae family (Queneherve et al. 1995).

Pratylenchus spp. and *R. reniformis* are considered important PPNs for vegetables (Hallmann and Meressa 2018); yet, these PPNs were not prevalent nor in high relative abundance (Table 2). *Pratylenchus penetrans* is commonly found on legumes, cucurbits, sweet corn and solanaceous crops in Northern United States (Koenning et al. 1999a) and Ontario, Canada (Olthof and Potter 1973), but has not yet been reported in Georgia. In South Georgia, *P. vulnus, P. zeae* and *P. brachyurys* are found on peach (Fliegel 1969; Nyczepir and Pinochet 2001); *P. hexincisus* on giant miscanthus (Mekete et al. 2011) and *P. brachyurus* on peanut (Minton et al. 1970) and corn (Davis and Timper 2000b). *Pratylenchus brachyurus* and *P. zeae* can be detected at high population densities in vegetable roots (Hallmann and Meressa 2018); however, population density of *P. brachyurus* was found to have an antagonistic relationship with *Meloidogyne* spp. when tomato was the host (Gay and Bird 1973). This phenomenon may explain the low incidence and relative abundance of *Pratylenchus* spp., as other vegetable crops in Southern Georgia may also share this antagonism when concomitant populations of *Meloidogyne* spp. and *Pratylenchus* spp. are present. The lack of root samples in our study may have also underestimated populations of

Pratylenchus in vegetable roots. *Rotylenchulus reniformis* is considered second to *Meloidogyne* spp. in affecting vegetable crops (Hallmann and Meressa 2018), yet, it was not prevalent in the present study and likely related to the high sand content of South Georgia, as *R. reniformis* is known to associated with finer soil textures (Heald and Robinson 1990; Robinson et al. 1987; Starr et al. 1993).

In our study, the low prevalence of *Hoplolaimus* spp. suggests that this nematode is not a major threat to vegetable production in Southern Georgia. *Hoplolaimus* spp. have been reported to cause 5-10% yield loss on leguminous crops in Alabama (Koenning et al. 1999a) and survive on vegetables (Fassuliotis 1974), yet, it is not clear if this nematode causes significant damage to vegetables (Hallmann and Meressa 2018). In Georgia, *H. colombus* is mainly a problem for cotton and soybean (Hajihassani et al. 2018a), which are common row crops rotated in Southern Georgia.

Heterodera spp., *Tylenchorhynchus* spp. and *Xiphinema* spp. were rarely detected in this study and are probably not a threat to vegetable production in Southern Georgia. Though *H. schachtii* can cause significant losses for brassicas (Hallmann and Meressa 2018), it has not been reported in Georgia. Furthermore, only 6 out of 56 fields with brassicas were infested with *Heterodera* spp. The low prevalence of *Heterodera* spp. suggests that this nematode is not associated with vegetable crops of Southern Georgia and is probably *H. glycines* or *H. cyperi*, which have been reported in Georgia on soybean and yellow nutsedge, respectively (Hajihassani et al. 2018c; Motsinger et al. 1976b). *Xiphinema* spp. was the rarest PPN to detect and was found in only one collard field in Colquitt county. Species of *Xiphinema* spp. including *X. ifacolum* have been found to be a pest on cowpea, okra, eggplant and tomato (Lamberti et al. 1992), while *X. americanum* was associated with lettuce, broccoli and onion (Pedroche et al. 2013). The low prevalence of *Xiphinema* spp. in Georgia suggest that it is most likely not associated with vegetable production nor a threat to the vegetable industry in Georgia. The sting nematode, *B. longicaudatus,* is reported as an important pest of cucurbits, legumes and potato in Florida (Crow et al. 2000; Koenning et al. 1999a), yet, none were found in vegetables of South Georgia.

PPN associations with meteorological and edaphic factors. PPN distribution, incidence and relative abundance can be influenced by edaphic factors and their relationship to a plant host (Norton 1979). However, our NMS ordination suggested that despite some variation in environmental variables, most variables were not major contributors to the variations in nematode counts. NMS suggested that soil temperature, pH and latitude were the only major variables accounting for variation in nematode counts, with latitude explaining the most variation, while other environmental variables did not appear to have any statistical or biological relevant associations. Variation in temperature and pH may be indirectly related to the gradation in latitude, as air temperature and pH were found to be greater in the South than the North region. Though soil temperature may have a minor effect on nematode relative abundance and incidence in Southern Georgia, only explaining 8.5% of the variance, the NMS ordination could be reflecting the effect of warmer temperatures associated with lower latitude shortening the life cycle of *Meloidogyne* spp. (Tyler 1933), therefore enhancing the nematode's fecundity. Warmer temperatures may also be associated with fields that have a longer period to grow vegetables and allow *Meloidogyne* spp. reproduction. Though the ordination suggested a negative association of soil pH with latitude, it is not clear if cultural practices (*i.e.* fertilizing and liming), that affect pH (Kissel et al. 2009), are associated with changes in latitude. Precipitation was greater in the South region than the North, which have been reported to raise pH readings due to leaching of salts (Kissel et al. 2009); however on the contrary, EC was higher in the South than the North region, suggesting that leaching of salts are not likely to be greater in the South region.

Despite many PPNs found more frequently in higher abundance and causing more severe symptoms on host crops with certain soil textures (Heald and Robinson 1990; Norton 1979; Robinson et al. 1987; Starr et al. 1993); no associations were evident in our dataset. Sand and silt content only explained 3.3% of the variance in the NMS analysis. MRPP also suggested that PPNs in this study did not have any strong or statistically detectable associations with soil texture classes. The lack of associations could be related to limited variation in soil texture, which were all high in sand content and low in clay content. Furthermore, association of *R. reniformis* with fine textured soils could be related to specific interactions with cotton as a host (Starr et al. 1993) and not necessarily vegetable crops. The lack of variation in soil texture may also explain why the 9 different ecoregions of Southern Georgia did not have any clear associations with PPN communities.

PPN geospatial associations. Since NMS ordination suggested that latitude had the greatest effect on PPN communities, we decided to further investigate the effect of latitude between the North and South region in which we found differences between North and South regions. Mann-Whitney test results suggested that *Meloidogyne* spp. were associated with the South region, while other PPNs like *Heliotylenchus* spp., *Mesocriconema* spp., *Hoplolaimus* spp. and *Pratylenchus* spp., had greater prevalence in the North region. Distribution maps of each nematode genus also show this pattern against latitude. Though the environmental variables only minimally affect nematode abundance and incidence, other factors like the vegetable crop and cultural practices that differ along latitude are potential biological-relevant factors that may have a greater influence on relative abundance and incidence of PPNs found in the North and South regions.

PPN and geospatial associations with cropping systems. Crop host is an important factor that impacts the frequency and abundance of PPN communities (Greco and Di Vito 2009; Norton 1979) and may be the most important biological-relevant factor affecting PPN incidence and abundance in this study as most environmental variables other than latitude and those associated with latitude (*i.e.* soil temperature and pH) did not significantly affect PPN communities. Our MRPP analysis supports this type of dependence as there were community differences between crops. Additionally, there may be predictable interactions, as ISA showed that *Meloidogyne* spp. was associated with solanaceous crops, while *R. reniformis* was associated with leguminous crops. These associations may also be due to different cultural practices associated with each crop. Specifically, solanaceous crops like tomato, eggplant and pepper are typically grown on plastic beds, while leguminous crops like southern pea, snap beans and butter beans are grown in bare ground beds. When samples were grouped into fields with and without plastic beds, a clear separation in the PPN community was observed in the NMS ordination. Perhaps more importantly, the samples from bare ground beds were associated with increasing latitude (*i.e.* North region), while samples from the plastic bed cropping system were associated with decreasing latitude (*i.e.* South region) and increasing soil temperature and pH. *Meloidogyne* spp. was found as the dominate PPN in plastic beds of the South region than plastic beds in the North region and bare ground beds in both regions. In fields of the South region, many growers use plastic beds, in which a series of 2-4 vegetable crops are replanted in the same bed, while those in the North region typically rotate watermelon and other vegetables (*i.e.* cucumber, squash, brassicas, snap beans and cowpeas) on plastic or bare ground with a row crop, usually cotton, corn or peanut, depending on the root-knot species present. The higher relative abundance of PPNs, other than *Meloidogyne* spp., from fields of bare ground beds in the North region suggests that these PPNs are not necessarily associated with vegetable production, but potentially weeds and/or other row crops (*i.e.* corn, soybean, peanut and cotton) related with a bare ground cropping system. For example, many weed species commonly found in vegetable fields are host of *Helicotylenchus* spp. (Queneherve et al. 1995), while other PPNs are known pest of row crops such as *P. brachyurus* on peanut (Minton et al. 1970) and corn (Davis and Timper 2000b), *H. colombus* on cotton and soybean (Hajihassani et al. 2018a) and *H. glycines* on soybean (Motsinger et al. 1976b).

Conclusion

This study has confirmed *Meloidogyne* spp. as the most dominate PPN in vegetable fields of Southern Georgia with an exceptionally higher abundance and incidence compared to any other PPN detected. Though many other PPNs were common in vegetable fields, there were no strong statistically detectable associations with intensive vegetable production in plastic beds in the South region as there was for *Meloidogyne* spp. Though soil pH and temperature likely affect nematode population dynamics in Southern Georgia, latitude had the strongest association with PPN community differences. This phenomenon may be related to cultural practices of plastic and bare ground bed cropping systems that differ between the North and South regions of Southern Georgia. Therefore, cultural practices and crop associated with plastic and bare ground bed cropping systems may play a more important role than the environmental variables collected in this study (precipitation, soil temperature, soil moisture, soil texture, soil pH and soil EC). Specifically, *Meloidogyne* spp. were more associated at lower latitudes in the South region of Southern Georgia, where plastic bed vegetable cropping systems are more frequent. Therefore, we conclude that plastic bed cropping systems are at a much higher risk of *Meloidogyne* spp. outbreak than bare ground bed cropping systems. Other PPN (*i.e. Nanidorus* spp., *Mesocriconema* spp., *Helicotylenchus* spp., *Pratylenchus* spp., *Rotylenchulus* spp., *Hoplolaimus* spp. and *Heterodera* spp.) associations with bare ground cropping systems in the North region suggest that these PPNs may not necessarily be associated with vegetables, but instead be associated with other row crops rotated in these vegetable fields and/or weeds associated with bare ground beds.

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Tables

Table 1. Summary of environmental variables of sampled vegetable fields.

Environmental variables	Range	Mean[y]
Meteorological variables[z]		
Precipitation (mm)	0-27.1	6.4 ± 0.3
Soil moisture (v/v; %)	7-27.9	17 ± 0.3
Soil temperature (°C)	10.4-31.9	23.9 ± 0.4
Edaphic factors		
Sand + silt content (%)	71.6-100	92.8 ± 0.3
pH	5.1-7.6	6.8 ± 0.02
EC (mmhos/cm)	0.03-0.88	0.19 ± 0.01

[z] Meteorological variables were represented as the month average starting from the day of sampling and 29 days following sampling. [y] Mean followed by standard error.

Table 2. The relative abundance, incidence, prominence value, and relative prominence values of plant-parasitic nematode genera in vegetable fields of Southern Georgia.

Nematode genus	**No. of infested fields**	**Mean**[z]	**Nematodes/100 cm^3 of soil**		**Incidence**		
			5th quantile, median and 95th quantile[y]	**Range**	**%**[x]	**PV**[w]	**RPV**[v]
Root-knot (*Meloidogyne* spp.)	294	292 ± 53	0, 8, 1228 a	0-14144	67.3 ± 2.2 a	19.6331	92.1584
Spiral (*Helicotylenchus* spp.)	138	20 ± 4	0, 0, 118 b	0-1152	31.6 ± 2.2 c	0.61886	2.9049
Ring (*Mesocriconema* spp.)	173	17 ± 2	0, 0, 110 bc	0-340	39.6 ± 2.3 bc	0.68830	3.2309
Stubby-root (*Nanidorus* spp.)	216	6 ± 1	0, 0, 30 c	0-138	49.4 ± 2.4 b	0.2897	1.3602
Lesion (*Pratylenchus* spp.)	88	2 ± 1	0, 0, 8 d	0-398	20.1 ± 1.9 d	0.0482	0.2262
Lance (*Hoplolaimus* spp.)	55	1 ± 0	0, 0, 6 d	0-78	12.6 ± 1.6 d	0.01578	0.0740
Reniform (*Rotylenchulus* spp.)	26	1 ± 0	0, 0, 4 e	0-116	5.9 ± 1.1 e	0.0086	0.0405
Cyst (*Heterodera* spp.)	10	<1	0, 0, 0 ef	0-60	2.3 ± 0.7 ef	0.0009	0.0043
Stunt (*Tylenchorhynchus* spp.)	4	<1	0, 0, 0 f	0-12	0.9 ± 0.5 f	3.7702	0.0002
Dagger (*Xiphinema* spp.)	1	<1	0, 0, 0 f	0-2	0.2 ± 0.2 f	1.0472	4.9160

[z] Relative abundance is based on mean nematode counts from 100 cm^3 of soil derived from infested fields or total number of fields sampled (N = 436) followed by standard error. [y] Nematode genus with the same letters are not significantly different ($P > 0.05$) according to Kruskal-Wallis Analysis of Variance followed by Dunn-Bonferroni test. Minimum counts other than zero were 2 in all infested fields for all genera. [x] Incidence of total fields sampled in which nematode genus with the same letters are not significantly different ($P > 0.05$) according to Tukey's honesty test. [w] PV = prominence value (mean x incidence/10), and [v] RPV=relative prominence value (100 x PV/Total sum of PV of each genus).

Table 3. Associations of plant-parasitic nematode genera detected in vegetable fields of Southern Georgia with crop families by Indicator Species Analysis.

Nematode genus	**Indicator group**	**Relative abundance index value**^{z}	**Relative frequency index value**	**Indicator index value**	***P*-value**
Root-knot (*Meloidogyne* spp.)	Solanaceae	20	96	18.8	0.0202
Stubby-root (*Nanidorus* spp.)	Poaceae	19	88	16.4	0.1018
Spiral (*Helicotylenchus* spp.)	Malvaceae	15	57	8.7	0.4867
Lesion (*Pratylenchus* spp.)	Malvaceae	27	57	15.6	0.0558
Ring (*Mesocriconema* spp.)	Fabaceae	17	65	10.9	0.4061
Lance (*Hoplolaimus* spp.)	Amaryllida	24	33	8.1	0.2444
Reniform (*Rotylenchulus* spp.)	Fabaceae	65	23	14.8	0.0306
Cyst (*Heterodera* spp.)	Amaryllida	33	17	5.6	0.3383
Stunt (*Tylenchorhynchus* spp.)	Malvaceae	77	14	11	0.0516

^{z}All index values range from a scale of 0-100 indicating no association to perfect association. Probability values are derived from 5,000 randomizations.

Table 4. The number of infested fields, median, 5th quantile, 95th quantile and range of nematode abundance in the North and South region in plastic or bare ground vegetable beds.

Nematode genus	**No. of infested fields**				**5th quantile, median and 95th quantile of nematodes/100 cm^3 of soil**[z]					**Range of nematodes/100 cm^3 of soil**			
	North		**South**		**North (n=155)**		**South (n=283)**			**North**		**South**	
	Plastic	Bare ground	Plastic	Bare ground	Plastic (n=53)	Bare ground (n=102)	Plastic (n=189)	Bare ground (n=92)	ANOVA	Plastic	Bare ground	Plastic	Bare ground
Root-knot (*Meloidogyne* spp.)	27	65	143	58	0, 2, 246[z] b	0, 4, 262 b	0, 54, 2672 a	0, 4, 596 b	χ=42.1; p<0.0001	0-386	0-650	0-14144	0-1214
Spiral (*Helicotylenchus* spp.)	38	52	23	24	0, 10, 432 a	0, 2, 164 b	0, 0, 14 d	0, 0, 28 c	χ=91.2; p<0.0001	0-1152	0-250	0-752	0-152
Ring (*Mesocriconema* spp.)	26	59	28	59	0, 0, 240 a	0, 4, 126 a	0, 0, 18 b	0, 6, 122 a	χ=88.9; p<0.0001	0-340	0-266	0-196	0-240
Stubby-root (*Nanidorus* spp.)	23	68	67	57	0, 0, 8 b	0, 4, 32 a	0, 0, 20 b	0, 4, 46 a	χ=33.9; p<0.0001	0-28	0-50	0-128	0-138
Lesion (*Pratylenchus* spp.)	21	32	12	23	0, 0, 6 a	0, 0, 10 a	0, 0, 2 b	0, 0, 16 a	χ=44.1; p<0.0001	0-12	0-138	0-32	0-398
Reniform (*Rotylenchulus* spp.)	9	12	5	0	0, 0, 18 a	0, 0, 14 a	0, 0, 0 b	0, 0, 0 b	χ=27.3; p<0.0001	0-66	0-116	0-60	0-0
Lance (*Hoplolaimus* spp.)	14	29	10	2	0, 0, 18 a	0, 0, 12 a	0, 0, 2 b	0, 0, 0 b	χ=50.8; p<0.0001	0-78	0-36	0-66	0-4
Cyst (*Heterodera* spp.)	0	7	1	2	0, 0, 0 ab	0, 0, 2 a	0, 0, 0 b	0, 0, 0 ab	χ=13.4; p=0.004	0-0	0-32	0-60	0-22
Stunt (*Tylenchorhynchus* spp.)	0	0	1	3	0, 0, 0 a	0, 0, 0 a	0, 0, 0 a	0, 0, 0 a	χ=7.2; p=0.065	0-0	0-0	0-2	0-12
Dagger (*Xiphinema* spp.)	0	0	0	1	0, 0, 0 a	0, 0, 0 a	0, 0, 0 a	0, 0, 0 a	χ=3.7; p=0.295	0-0	0-0	0-0	0-2

[z] 5th quantile followed by the median and 95th quantile. Same letters along the rows among plastic and bare ground beds of both the North and South region for each nematode genus are not significantly different according to Kruskal-Wallis ANOVA followed by Dunn-Bonferroni test for pairwise comparisons (*P* < 0.05).

Figures

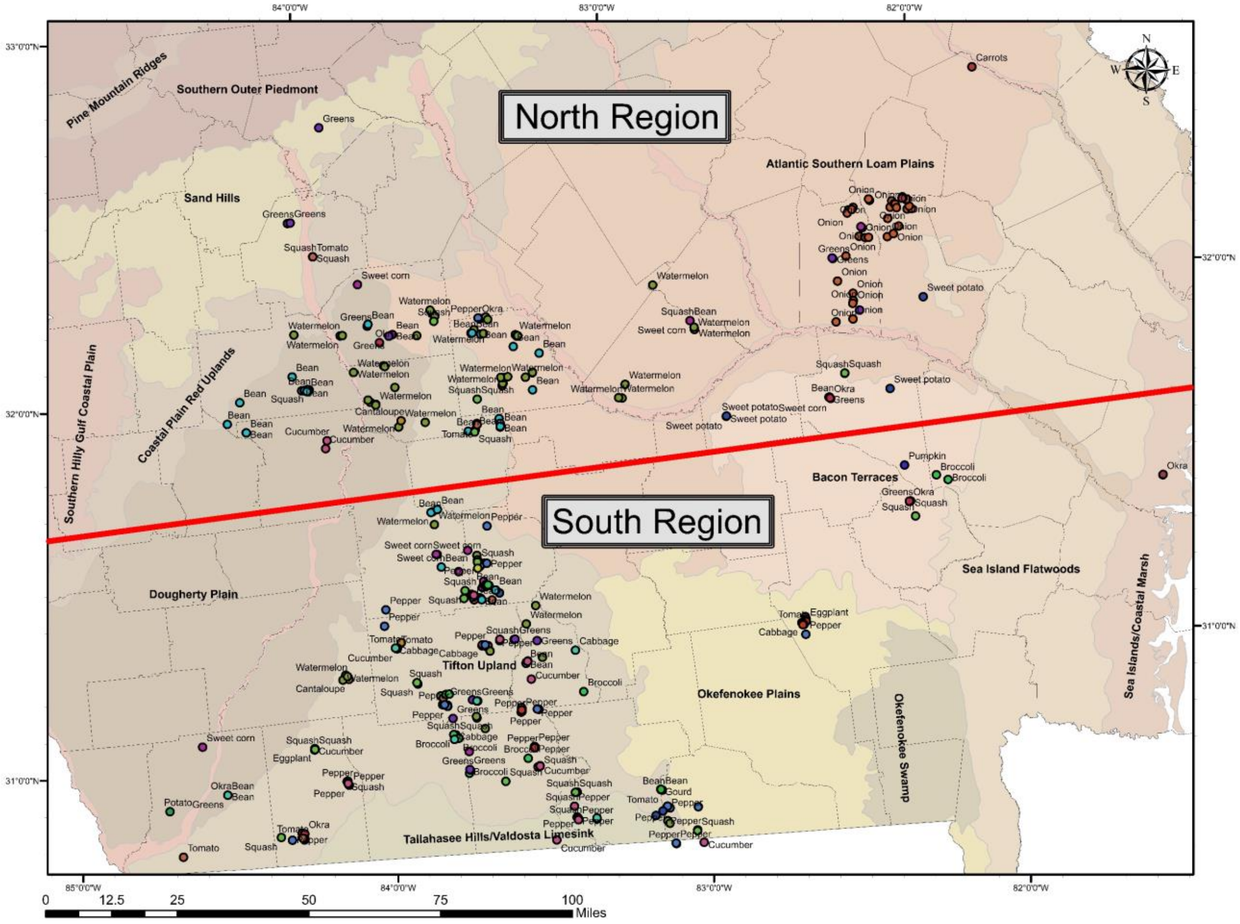

Fig. 1. Commercial vegetable fields located in 8 different ecoregions of Southern Georgia sampled in the spring and fall, 2018. The different colored regions indicate the different ecoregions while the grey lines represent the county borders.

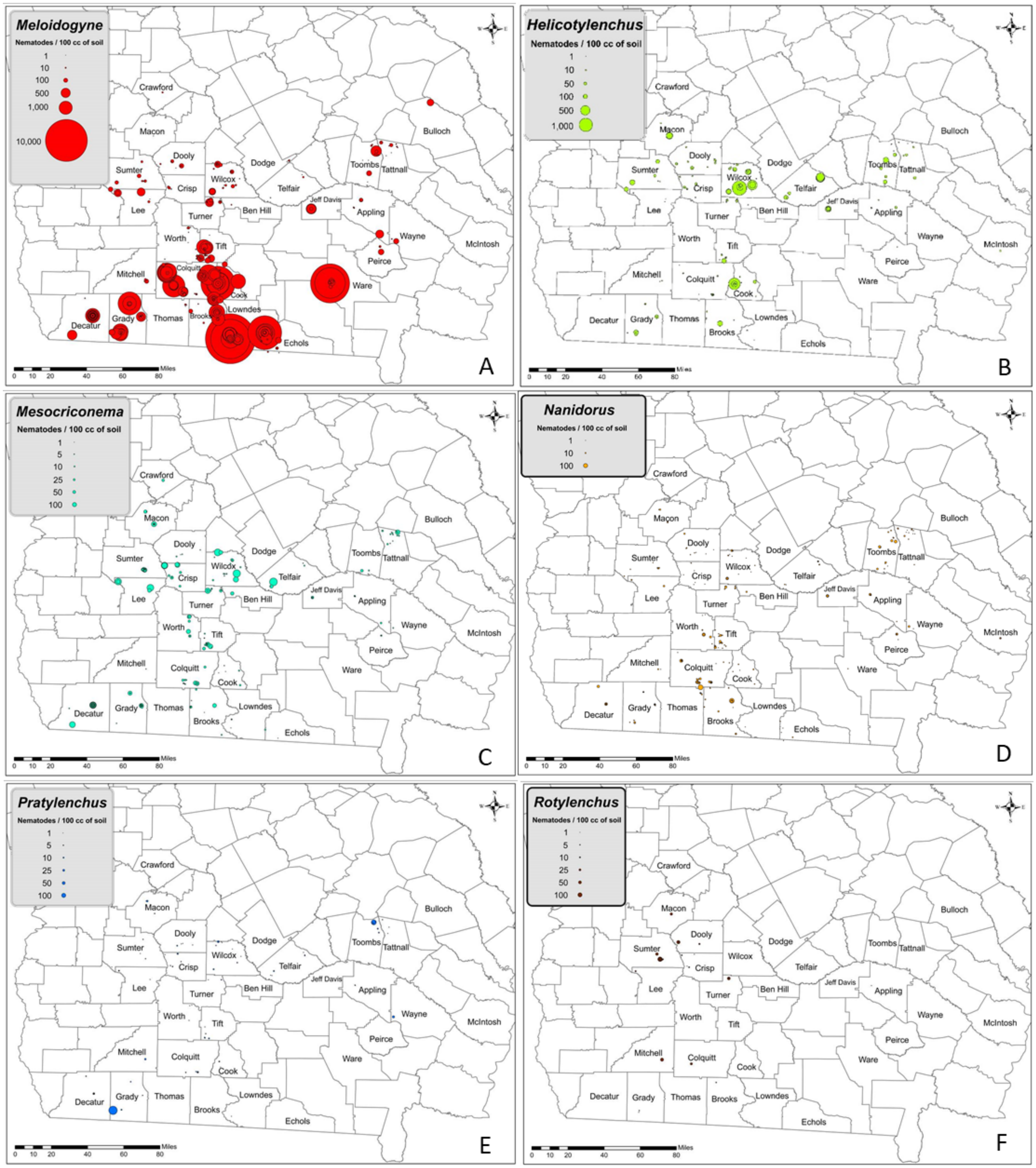

Fig. 2. The distribution and relative abundance of 10 PPN genera detected in vegetable fields: A) *Meloidogyne* spp., B) *Helicotylenchus* spp., C) *Mesocriconema* spp., D) *Nanidorus* spp., E) *Pratylenchus* spp., and F) *Rotylenchulus* spp. Spatial graphics were produced with ArcGIS (esri, Redlands, CA).

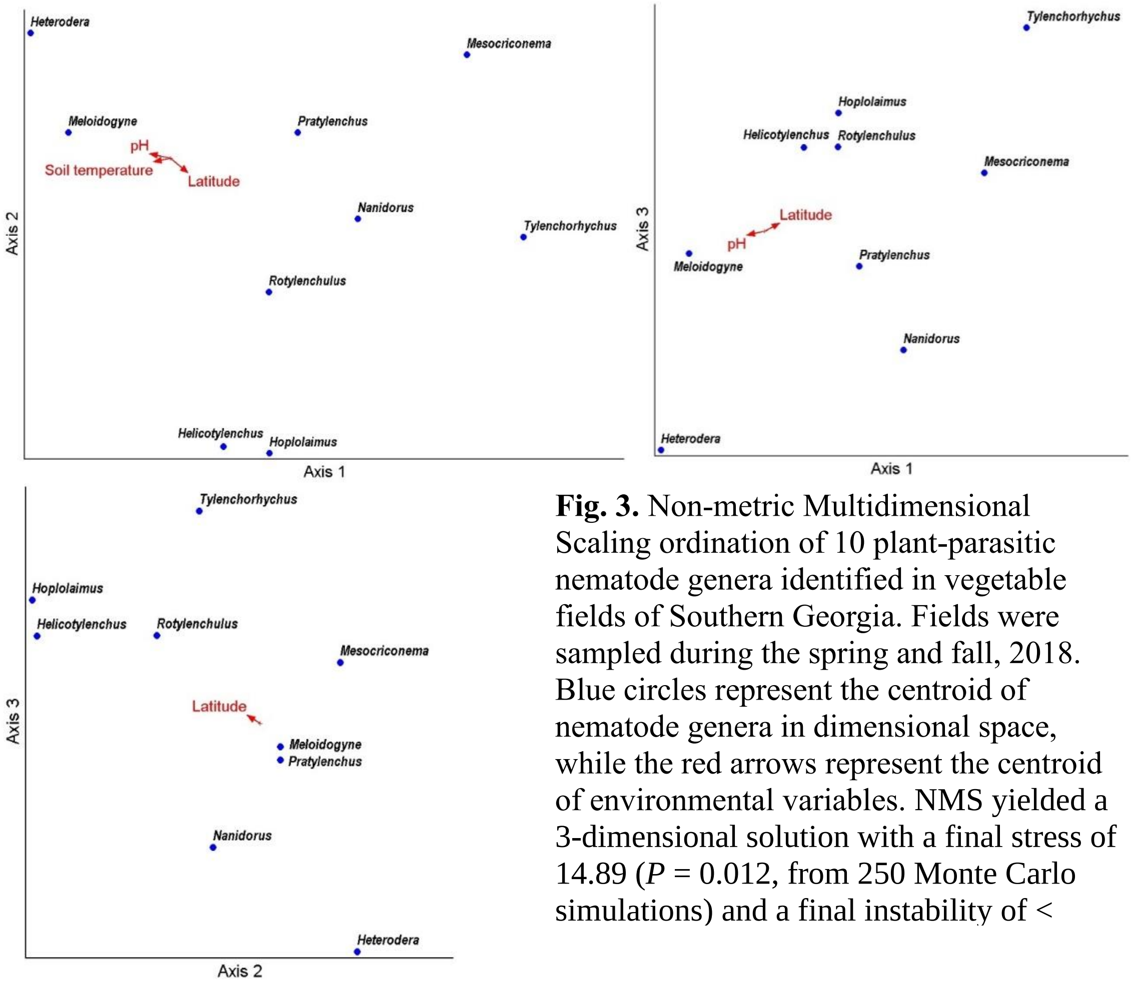

Fig. 3. Non-metric Multidimensional Scaling ordination of 10 plant-parasitic nematode genera identified in vegetable fields of Southern Georgia. Fields were sampled during the spring and fall, 2018. Blue circles represent the centroid of nematode genera in dimensional space, while the red arrows represent the centroid of environmental variables. NMS yielded a 3-dimensional solution with a final stress of 14.89 (*P* = 0.012, from 250 Monte Carlo simulations) and a final instability of <

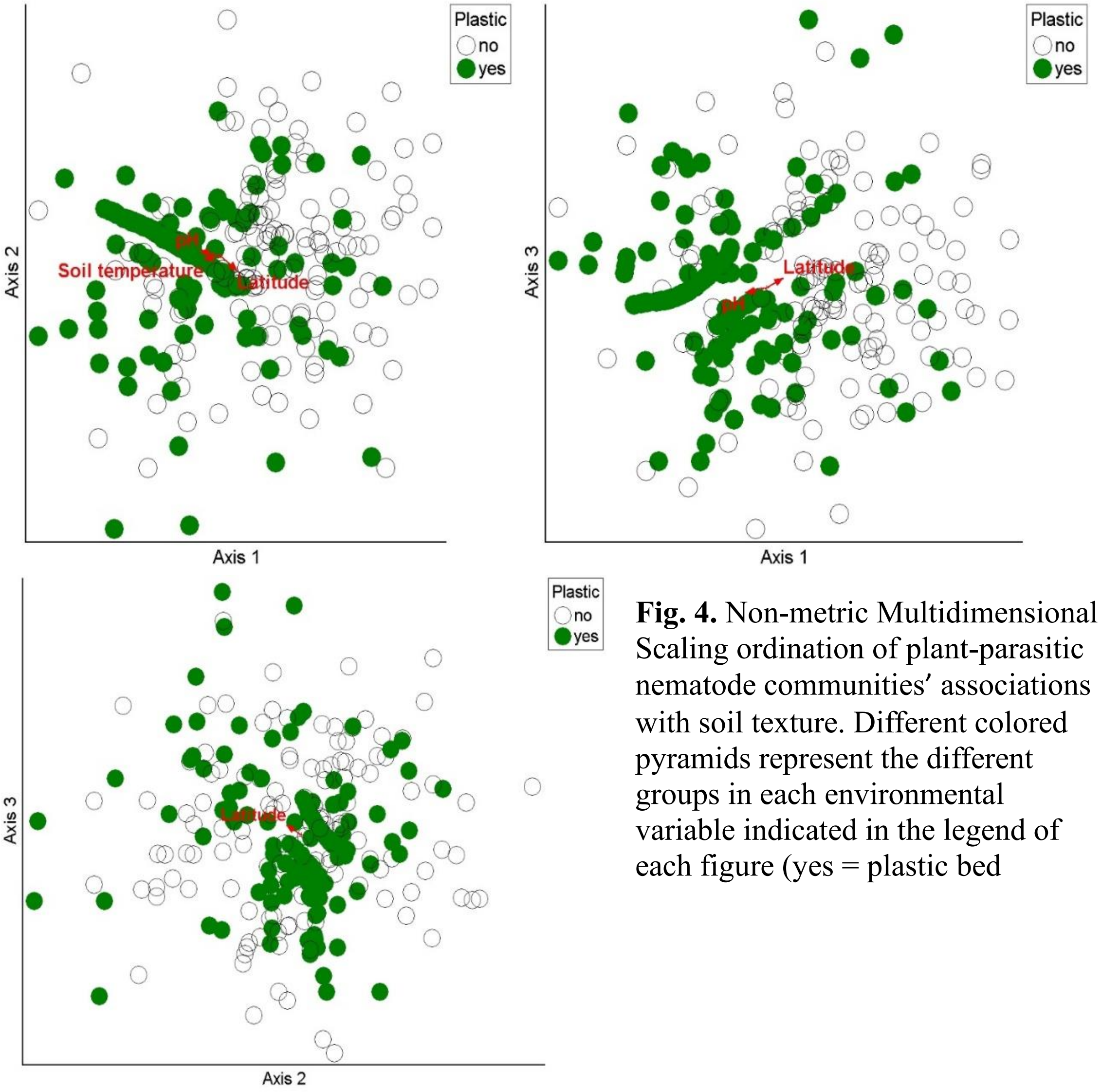

Fig. 4. Non-metric Multidimensional Scaling ordination of plant-parasitic nematode communities′ associations with soil texture. Different colored pyramids represent the different groups in each environmental variable indicated in the legend of each figure (yes = plastic bed

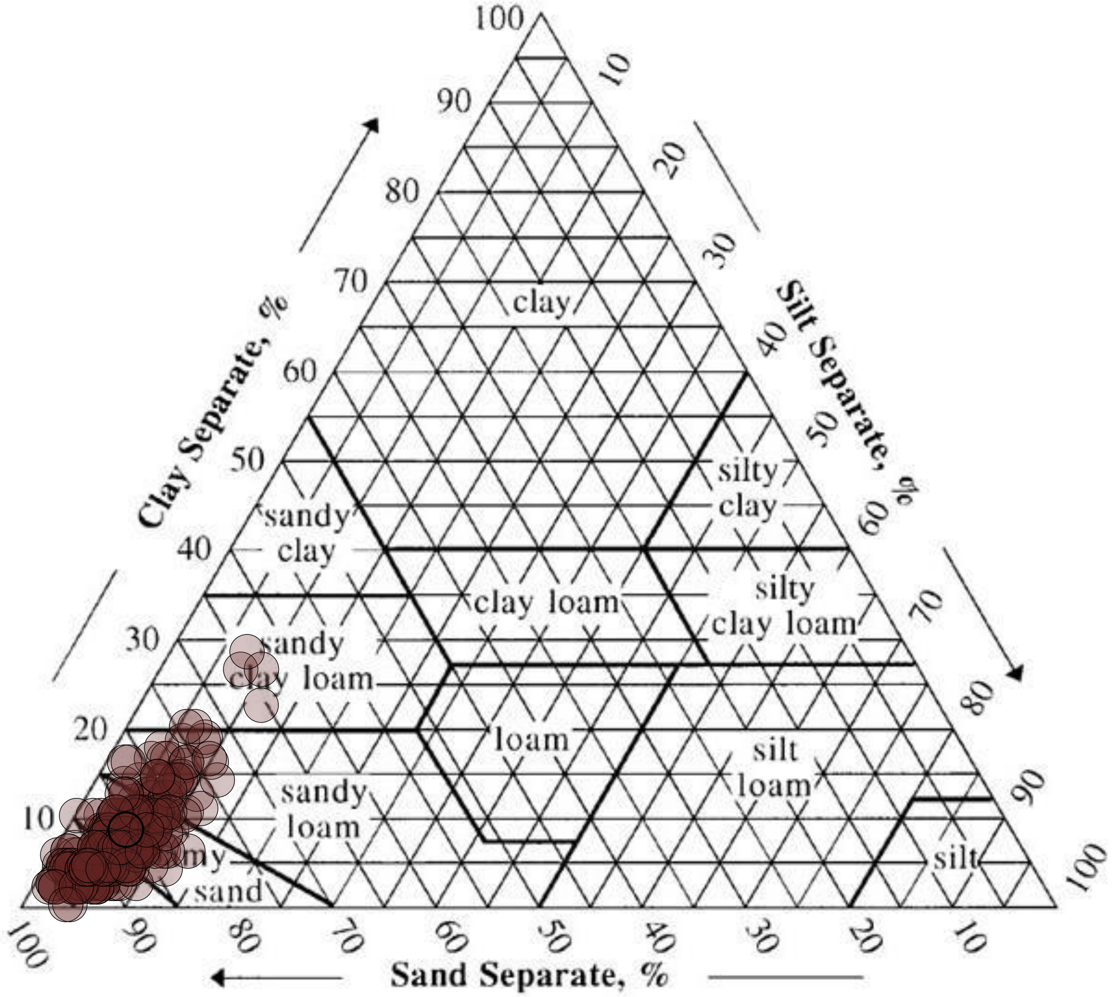

Fig. 5. Soil texture of vegetable fields on a soil textual triangle. The brown circles represent the individual soil samples collected from vegetable fields. The soil texture classes are based on United State Department of Agriculture soil classification taxonomy. Soil texture triangle was produced by Microsoft Excel Macro-enabled spreadsheet provided by United States Department of Agriculture Natural Resources Conservation Service Soils (USDA-NRCS).

Supplemental table

Table S1. Number of fields infested with each plant-parasitic nematode genus by crop genus, crop species, and crop common name.

Crop family		Crop genus		Crop species		Common name												
Name	N	Name	N	Name	N	Name	N	Season	*Meloidogyne* spp.	*Helicotylenchus* spp.	*Mesocriconema* spp.	*Nanidorus* spp.	*Pratylenchus* spp.	*Rotylenchulus* spp.	*Hoplolaimus* spp.	*Heterodera* spp.	*Tylenchorhynchus* spp.	*Xiphinema* spp.
Amaryllidaceae		*Allium*	29	*cepa*	29	Onion	29	Spring	19	12	14	18	7	0	8	3	0	0
Brassicaceae		*Brassica*	56	*juncea*	5	Mustard greens	5	Spring - Fall	1	2	4	4	2	0	2	0	0	0
				oleracea	50	Broccoli	20	Fall	11	2	14	16	3	0	1	0	1	0
						Cabbage	11	Spring - Fall	8	3	3	4	2	0	0	0	1	0
						Cauliflower	1	Fall	1	0	1	0	0	0	0	0	0	0
						Collard greens	13	Spring - Fall	7	4	7	10	5	0	3	1	0	1
						Kale	5	Spring - Fall	3	2	3	2	2	0	0	0	0	0
				rapa	1	Napa Cabbage	1	Fall	1	1	1	1	0	0	0	0	0	0
Convolvulaceae		*Ipomoea*	12	*batatas*	12	Sweet potato	12	Spring - Fall	8	6	7	8	0	0	1	1	0	0
Cucurbitaceae		*Citrullus*	66	*lanatus*	66	Watermelon	66	Spring	33	40	33	21	22	9	18	0	0	0
		Cucumis	60	*melo*	9	Cantaloupe	9	Spring	5	0	2	5	1	0	0	0	0	0
				sativus	51	Cucumber	51	Spring - Fall	38	10	11	14	4	2	0	1	0	0
		Cucurbita	53	*pepo*	53	Acorn squash	5	Spring - Fall	4	0	1	3	0	0	0	0	0	0
						Butternut squash	3	Spring - Fall	2	1	1	1	1	0	0	0	0	0
						Hard squash	1	Spring	0	0	0	0	0	0	0	0	0	0
						Pumpkin	2	Spring - Fall	2	0	1	1	0	0	0	0	0	0
						Spaghetti squash	2	Spring - Fall	2	0	0	1	0	0	0	0	0	0
						Yellow squash	28	Spring - Fall	19	11	11	14	5	1	4	0	0	0
						Zucchini	12	Spring - Fall	8	2	5	6	4	2	0	0	0	0
		Lagenaria	1	*siceraria*	1	Gourd	1	Spring	1	0	0	0	0	0	0	0	0	0
Fabaceae		*Phaseolus*	38	*vulgaris*	38	Butter bean	5	Spring	3	1	3	1	2	0	1	0	0	0
						Snap Beans	21	Spring - Fall	17	11	15	19	5	6	6	1	0	0
						Southern pea	12	Spring	9	5	5	3	7	1	2	0	0	0
Malvaceae		*Abelmoschus*	7	*esculentus*	7	Okra	7	Spring - Fall	6	4	4	5	4	0	2	0	1	0
Poaceae		*Zea*	20	*mays*	20	Sweet corn	20	Spring - Fall	14	8	9	18	6	0	3	2	1	0
Solanaceae		*Capsicum*	57	*annuum*	57	Bell pepper	40	Spring - Fall	31	3	7	10	1	1	2	1	0	0
						Chili pepper	17	Spring - Fall	15	0	2	4	0	0	0	0	0	0
		Physalis	1	*philadelphica*	1	Tomatillo	1	Spring	0	0	0	1	0	0	0	0	0	0
		Solanum	31	*lycopersicum*	16	Tomato	16	Spring - Fall	10	5	4	11	2	2	1	0	0	0
				melongena	13	Eggplant	13	Spring - Fall	13	3	1	10	2	1	1	0	0	0
				tuberosum	2	Potato	2	Spring	1	0	2	1	1	0	0	0	0	0
Umbellifers		*Daucus*	3	*carota*	3	Carrots	3	Spring	1	1	1	2	0	0	0	0	0	0

^{z}N = number of fields for each crop family, genus or species. Red color represents the numeric value in the number of fields for each plant-parasitic nematode genus.

CHAPTER 3

IDENTIFICATION, DIVERSITY, AND DISTRIBUTION OF MELOIDOGYNE SPP. IN VEGETABLE FIELDS OF SOUTH GEORGIA, USA[2]

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[2] Marquez, Josiah and Hajihassani, Abolfazl. Accepted by Phytopathology. Reprinted here with permission of publisher.

Abstract

Root-knot nematode (RKN; *Meloidogyne* spp.) is the most prevalent plant-parasitic nematode in vegetable fields of Georgia, with an incidence of 67.3%. Since aggressive RKN species are reported in the southeastern United States, molecular-based identification of RKN species was conducted on soil samples taken from a nematode surveillance study in 2018 from 292 RKN-infested vegetable fields in South Georgia. The RKN-infested soil was potted with tomato cv. Rutgers and individual nematode females were isolated from galled roots and subjected to species-specific PCR and mitochondrial haplotype-based RKN species identification. The incidence (%), mean, and maximum relative abundance (second-stage juveniles per 100 cm^3 of soil) of the 5 RKN species identified consisted of *M. incognita* (91.9, 486, 14144), *M. arenaria* (36.0, 707, 14144), *M. floridensis* (2.2, 909, 5264), *M. javanica* (5.5, 352, 1488), and *M. haplanaria* (0.7, 8, 14). A large proportion of fields (29%) had mixed populations of *M. incognita* and *M. arenaria* which may reflect the region's long history of cotton and peanut cultivation. For unknown reasons, mixed populations of *M. incognita* and *M. arenaria* were associated with higher population densities. *Meloidogyne incognita* is the most important RKN species in vegetable fields followed by *M. arenaria*, therefore, pure or mixed populations of these species should be addressed in nematode management programs. Although at a lower incidence, the newly detected species, *M. floridensis* and *M. haplanaria*, have the potential to become a major threat since they reproduce on vegetables with *Mi*-resistant genes.

Introduction

Over recent years, the state of Georgia has consistently been ranked among the top 4 vegetable-producing states in fresh market vegetable production (USDA-NASS 2021). Root-knot nematode is the most important plant-parasitic nematode (PPN) for vegetable crops, especially in the subtropical and tropical regions of the world (Netscher and Sikora 1990). In Georgia, RKN has the greatest frequency and abundance over other PPNs, infesting 66.7% of vegetable fields (Marquez et al. 2021a). The most prevalent RKN species worldwide are in the *M. incognita* group (MIG) consisting of polyphagous tropical species: the southern (*M. incognita*), peanut (*M. arenaria*), and Javanese (*M. javanica*) RKN (Netscher and Sikora 1990). These species reproduce by obligatory mitotic parthenogenesis and are associated with a short life cycle and high fecundity (Moens et al. 2009).

Meloidogyne incognita is the most economically important PPN for vegetable crops; yet new emerging RKN species (*e.g.*, *M. enterolobii, M. floridensis*, and *M. haplanaria*) threaten the vegetable industry by reproducing on RKN-resistant cultivars to the tropical MIG species (Bendezu et al. 2004; Stanley et al. 2009; Ye et al. 2013). These new species have been recently reported in neighboring states of Georgia. For example, *M. enterolobii* has been reported on ornamental nurseries in Florida (Brito et al. 2004); cotton and soybean in North Carolina (Ye et al. 2013); sweet potato in South Carolina (Rutter et al. 2019); and sweet potato propagules in Louisiana sourced from North Carolina (Overstreet et al. 2019). This species has been considered the most damaging (Koutsovoulos et al. 2020), being highly aggressive and virulent with a broad host range (Collett et al. 2021). *Meloidogyne floridensis* has been reported in 12 counties in Florida (Brito et al. 2015). Recently, this species has been found in two counties of California on almond trees (Westphal et al. 2019) and one County in South Carolina on peach trees (Reighard et al.

2019). Although initially reported on peach, *M. floridensis* can reproduce on many vegetable crops, including cucumber, eggplant, tomato, snap bean, and squash (Stanley et al. 2009) and *M. incognita*-resistant cultivars of tomato (containing *Mi-1* gene), pepper (*N* gene), corn cv. Mp-710, and tobacco cv. NC 95 (Stanley et al. 2009). *Meloidogyne haplanaria* was first detected in Texas on peanut (Bendezu et al. 2004; Eisenback et al. 2003) and later found in Arkansas in 6 counties on tomato, peanut, okra, elm, ash, Indian hawthorn, and willow tree (Khanal et al. 2016; Ye et al. 2019), Florida on tomato (Joseph et al. 2016), and in California on American pitcher plants (Subbotin 2021). This species can overcome the resistance of tomato *Mi-1* gene (Bendezu et al. 2004), but not of pepper carrying the resistant genes *N*, *Me1*, or *Me3* (Hajihassani et al. 2019).

RKN species that have been reported in Georgia consist of: *M. incognita, M. arenaria*, and *M. javanica* on corn (Davis and Timper 2000), *M. hapla* on peanut (Motsinger et al. 1976), *M. partityla* on pecan (Nyczepir et al. 2002), *M. graminicola* on purple nutsedge (Minton et al. 1987), and *M. marylandi* on bermudagrass (Jagdale et al. 2019). *Meloidogyne incognita* is widespread in cotton (Baird et al. 1996), while *M. hapla* and *M. arenaria* are found on peanut (Motsinger et al. 1976) in Georgia. However, *Meloidogyne* spp. associated with vegetable fields have not yet been investigated. Moreover, an extensive surveillance study using molecular tools for RKN species identification has not been attempted in Georgia.

Traditional methods of identification based on morphology, morphometrics, isozyme phenotype, and differential host test (Moens et al. 2009) are time-intensive (Powers and Harris 1993), vary with environmental conditions and hosts (Baidoo et al. 2016), and are not reliable for new species of RKN. Molecular approaches in identification have increasingly become the standard practice in the past 20 years, as it is fast, sensitive, less subjective, and effective at identifying at any life stage (Baidoo et al. 2016; Ye et al. 2019). Species-specific primers have been developed for rapid identification of the MIG species; however, it is not a standalone approach for identification due to difficulties with ambiguous results, low sensitivity, and lack of reproducibility between laboratories (Janssen et al. 2016). For example, ambiguous negative results from species-specific primers can indicate either a failed reaction, different species, or a variant of the targeted species (Joseph et al. 2016). Hence, there is a growing interest in diagnostic DNA-barcoding as it can either detect unforeseen RKN threats with unknown lineages or assess their genetic diversity (Janssen et al. 2016). Although nuclear ribosomal RNA genes are effective in differentiating species of many organisms, they are often unreliable in differentiating RKN species (Kiewnick et al. 2014; Subbotin 2021), especially for the MIG species, as copies of the rDNA gene within an individual nematode can have a greater variation than between species (Joseph et al. 2016). However, the mitochondrial DNA (mtDNA) spanning the cytochrome c oxidase subunit II (COX2), large subunit 16S rDNA (l-rRNA) gene, and the NADH dehydrogenase subunit 5 (NAD5) gene have been proven to be dependable for RKN species identification due to a high mutation rate with divergence limited by uni-parental inheritance (Janssen et al. 2016; Joseph et al. 2016; Subbotin 2021).

This study aims to utilize species-specific PCR followed by DNA-sequence based mitochondrial haplotyping to detect RKN species in vegetable crops of South Georgia and assess their threats to Georgia’s vegetable industry by determining the occurrence, prevalence, and distribution of RKN species. In addition, we are interested in detecting newly described species of RKN infesting vegetable fields, understanding the intra- and inter-species diversity of RKN populations in South Georgia, and determining the feasibility of mitochondrial haplotyping with primers currently available.

Material and Methods

Sampling and culturing of root-knot nematode. Soil samples consisted of RKN-infested vegetable fields from a PPN survey study in South Georgia (Marquez et al. 2021a). Briefly, each field was represented by thirty 2.5-cm soil cores mixed as one composite bulk sample. Each core was sampled approximately 20 cm deep every 3 to 4 m across vegetable beds in a diagonal transect of the field. Samples were collected from a total of 35 vegetables (acorn squash, bell pepper, bell pepper, broccoli, butter beans, butter beans, butternut squash, cabbage, cantaloupe, carrots, cauliflower, chili pepper, collards, collards, cucumber, eggplant, gourd, kale, mustard greens, napa cabbage, okra, onion, pepper, potato, pumpkin, snap beans, cowpea, spaghetti squash, sweet corn, sweet potato, tomato, vegetable garden, watermelon, yellow squash, yellow squash, and zucchini) representing 18 different vegetable species (Table S1) from 28 counties (Appling, Brooks, Bulloch, Colquitt, Cook, Crawford, Crisp, Decatur, Dooly, Echols, Grady, Jeff Davis, Lee, Lowndes, Macon, Mitchell, Peirce, Sumter, Tattnall, Telfair, Thomas, Tift, Toombs, Turner, Ware, Wayne, Wilcox, and Worth) during the growing seasons of spring and fall, 2018. Due to their proximity, neighboring fields with different crops shared one GPS coordinate. These fields were sampled to address the effect of different host preferences among neighboring vegetable crops. Infested samples were confirmed by centrifugal sugar floatation from 100 cm^3 of soil (Jenkins 1964), followed by counting of second-stage juveniles (J2) under the inverted compound microscope (ZEISS Axio Vert.A1, Oberkochen, Germany). RKN-infested soil samples (~2 kg of soil) were then potted into 15-cm diam. polyethylene pots containing 2-week-old seedlings of tomato (*Solanum lycopersicum* cv. Rutgers) for rearing female RKN which were used for extracting genomic DNA samples. Females were reared to reduce the time sensitivity of processing DNA samples from J2s, as J2s die without a host within a few months. Plants were incubated in the greenhouse at 28 ± 3°C for at least 8 weeks before roots were washed to visually assess galls. A portion of roots with galls was then stored in the refrigerator at 4 °C with 0.9% NaCl solution and streptomycin (0.025%; w/v) in 50-mL conical tubes before individual females were isolated for extraction of genomic DNA.

DNA extraction. Three to four genomic DNA samples from individual female RKN were made from tomato roots grown in each soil sample. Females were used due to being more reliable and easier to extract DNA than J2s due to their larger size. If all DNA samples for a particular soil sample had negative results for species-specific PCR or could only make one DNA sample from tomato roots, another set of 3 to 4 DNA samples was made from the same soil sample after replanting with the tomato. A genomic DNA sample consisted of an individual RKN female isolated from galled roots using a scalpel under the dissecting microscope (Meiji EMZ, MEIJI Techno co. LTD., Tokyo, Japan). An individual female was crushed against the wall of the 1.5-mL Eppendorf tube using a 10 µL pipette tip containing 20 µL of PCR (polymerase chain reaction) grade water (ACS Reagent Grade Water, Teknova). After crushing, pipetting was performed to homogenize nematode tissue. The resulting homogenate was frozen at -20˚C overnight and thawed on a heat block set at 95˚C for 1 min before being used as a DNA template for PCR.

Species identification with species-specific PCR. PCR with species-specific primers (Table 1) was performed with 10 µL of 2.0X Taq red or clear master mix kit (Genesee Scientific, San Diego, CA), 6.4 µL of double deionized water, 0.8 µL of primer, and 2 µL of DNA template per reaction for a total reaction volume of 20 µL of PCR product at a final primer concentration of 0.4 µM (8 pmol). To reduce false negatives, a secondary PCR was performed on the negative samples using diluted DNA at a 1:10 dilution in order to prevent crude DNA contaminants (lipids, proteins, chemicals, etc.) from inhibiting PCR amplification. Genomic DNA samples of *M.*

incognita, M. arenaria, M. javanica, M. hapla, and *M. enterolobii* (*M. hapla* and *M. enterolobii* DNA were obtained from W. Ye, North Carolina Department of Agriculture & Consumer Services, Raleigh, NC) and were used as positive controls for species-specific PCR. Multiple *M. incognita*-specific PCR primers were initially tested in developing an efficient protocol for identification of this species (Table 1). The final protocol consisted of a duplex PCR assay using primers SEC-1F/SEC-1R and Mi2F4/Mi1R1 described in (Hajihassani et al. 2022). Two *M. incognita*-specific primers were used for detection due to false negatives from individual primer sets. The first assay consisted of MiF4/Mi1F1 and Far/Rar primer sets for codetection of *M. incognita* and *M. arenaria*, while the second consisted of SEC-1F/SEC-1R and Fjav/Rjav primer sets for codetection of *M. incognita* and *M. javanica*. The C100 Touch Thermal cycler (BioRad, Hercules, CA) was used for PCR amplification with the following conditions: initial denaturation at 95°C for 5 min, followed by 45 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 60 sec, and extension at 72°C for 90 sec, with a final extension at 72°C for 5 min. Five µL of PCR product was loaded into a 1% agarose gel stained with GelRed (Biotium, Fremont, CA) for 30 minutes at 130 V and visualized with a UV gel doc (AnalytikJena, Jena, Germany).

Species identification with mitochondrial haplotyping. All DNA samples with negative species-specific PCR results were subjected to PCR with universal primers targeting genes in the mitochondrial genome (Table 2). Specifically, C2F3/1108 targets portions of the COX2, intergenic spacer (IGS), tRNA-His, and the l-rRNA genes; MORF/MITHS targets portion of IGS and tRNA-His; and TRNAH/MRH106 targets a portion of the tRNA-His and l-rRNA genes. PCR of each of the three primer sets was attempted on DNA samples, but not all were successfully amplified. To minimize excessive sequencing, only the best PCR product from one primer set was used for identification; however, if sequence quality was low, new DNA samples and PCR products were made and sequenced. Amplification conditions for primer sets MORF/MITHS and TRNAH/MRH106 included an initial denaturation at 94°C for 6 min, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 68°C for 1 min, with a final extension at 68°C for 10 min. Conditions for primer set C2F3/1108 include an initial denaturation at 95°C for 5 min, followed by 44 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 1 min, and extension at 72°C for 2 min with a final extension at 72°C for 5 min.

PCR products were visualized by loading 5 µL on a 1% agarose gel. A 100-bp DNA ladder (Biotium, Fremont, CA) was used to determine the size of the PCR product after being separated with electrophoresis at 130 V for 25 min. DNA bands were observed under a UV transilluminator (UVsolo TS stand-alone gel documentation system, Irvine, CA). Amplified DNA was purified by spin columns with DNA clean & concentrator 5 kit (Zymo Research, Irvine, CA) or enzymatic cleanup with ExoSAP-IT™ Express (ThermoFish scientific, Waltham, MA). After PCR purification, DNA concentration was estimated by NanoDrop UV-Vis spectrophotometer (ThermoFisher Scientific Inc., Waltham, MA) and diluted to 2 ng/µL for premixing before sanger DNA sequencing. Premixing consisted of 10 µL of diluted DNA (~20 ng) and 5 µL of either forward or reverse primers at 5 µM. DNA samples were then submitted to Genewiz LLC (South Plainfield, NJ) for Sanger DNA sequencing. Raw reads were then trimmed, edited, and aligned with Geneious prime 2022.1.1 (Biomatters Ltd., Auckland, New Zealand). RKN species were identified from the highest match(s) based on pairwise identity and query coverage with DNA sequences from known RKN species in GenBank from the National Center for Biotechnology Information (Bethesda, MD) using Basic Local Alignment Search Tool (BLAST) in Geneious prime 2022.1.1.

Statistical analysis. Since nematode counts are over-dispersed, a non-parametric Kruskal-Wallis Analysis of Variance (KW ANOVA) was used to make multi-sample comparisons between median nematode relative abundance (*i.e.*, population density). Post-hoc pairwise comparisons between medians were assessed by a Dunn-Bonferroni test at $\alpha < 0.05$ using NPTESTS in SPSS statistics version 26 (IBM Corporation, Armonk, NY). To compare prevalence between RKN species, predictors from prevalence data (0=absence and 1=present) were linked with a binomial distribution with each field as a random effect and subjected to a one-way ANOVA using PROC GLIMIX in SAS® 9.4 (SAS Inc. Cary, NC), followed by post-hoc pairwise comparisons with Tukey's Honest significant difference test at $\alpha < 0.05$.

Phylogenetic analysis. DNA references were retrieved from Genbank and selected based on the top 50 best *Meloidogyne* spp. matches from published studies with Georgia populations based on pairwise identity and query coverage. This was done by exporting BLAST analysis results with Geneious prime 2022.1.1. DNA sequences and associated features were uploaded with Biopython (Cock et al. 2009). Other references were selected from other studies (Eisenback et al. 2019; Ye et al. 2019). DNA sequences were trimmed and edited with Geneious prime 2022.1.1 and aligned with Clustal Omega (EMBL-EBI, Cambridgeshire, UK) as a plugin. The best nucleotide substitution model for DNA sequence data was tested against 24 different models with Mega 10.0.4. The General Time Reversible (GTR+G) model using a discrete Gamma distribution for invariable sites was the best model for aligned DNA references and samples of C2F3/1108 PCR amplified product of COX2 - l-rRNA gene fragment. Hasegawa-Kishino-Yano (HKY+G) model using a discrete Gamma distribution for invariable sites was the best tested models for aligned DNA references and samples of MORF/MTHIS and TRNAH/MRH106 PCR amplified product of IGS and tRNA-His - l-rRNA gene, respectively. Bayesian analysis ran the chain for

1,100,000 generations and set the ‘burnin’ at 100,000 with MrBayes 3.2.7 as a plugin to Geneious prime 2022.1.1. Markov Chain Monte Carlo (MCMC) methods were used to estimate posterior probabilities of a phylogenetic consensus tree with a 50% majority-rule. All trees were rooted with *M. mali* (KC112913) from Japan as the outgroup taxa since it had the most distinct RKN mtDNA sequence (Eisenback et al. 2019). Trees were visualized using R package ggtree (Yu et al. 2017). Preliminary DNA alignments and trees consisted of all DNA samples from the study and references. However, due to excessive size, redundant references and samples were removed. Only one reference was selected to represent each RKN species and county or U.S. state. Representative DNA sequences from this study were selected based on groups formed in preliminary trees.

Results

RKN species distribution, abundance, and prevalence. Out of a total of 292 RKN-infested soil samples collected from vegetable fields, 280 samples (95.9%) successfully reproduced on tomato roots and produced galls for identification. A total of 1095 DNA samples were made from individual RKN females from these tomato roots. RKN species were identified from 1067 DNA samples (97.4% out of the total DNA samples) representing 272 RKN-infested vegetable fields by the combined approach of species-specific PCR and mitochondrial haplotyping. The combined approach identified 272 fields of soil samples that were successfully cultured (97.1% out of a total of 280 soil samples). Five RKN species (*M. incognita, M. arenaria, M. javanica, M. floridensis* and *M. haplanaria*) were identified with the combined approach of species-specific PCR and mitochondrial haplotyping (Table 3, Fig. 1). In most cases (94 fields) where mitochondrial haplotyping was required for species identification, mitochondrial haplotyping identified the same species identified by species-specific PCR of DNA samples from the same field, except for 2 neighboring fields (Tu1 and Tu2) in which mitochondrial haplotyping identified *M. haplanaria*, five fields (C8, Do12, Tu3, Wa8, and Wi21) which identified *M. floridensis*, and five fields (Col9, Col14, Co7, Lo5, and Te7) which identified *M. incognita*. However, neighboring fields of Col9, Co7, and Lo5, had *M. incognita* were identified with species-specific PCR. Twelve fields identified *M. incognita* with only mitochondrial haplotyping. Among these *M. incognita* infested fields, almost all DNA sequences from these fields had > 99% pairwise identity to *M. incognita* references in GenBank with the exception of one DNA replicate from field Tu8 (98.3%). However, the other two replicates of this field had a pairwise identity of 99.7 and 99.9% identity to *M. incognita* references.

From all DNA samples identified, *M. incognita* was the most prevalent species infesting 91.9% of identified infested fields. *Meloidogyne arenaria* was the second most prevalent species infesting 36% of identified fields followed by *M. javanica* (5.5%) and then by *M. haplanaria* (0.7%). The prevalence of *M. floridensis* (2.2%) did not differ between *M. javanica* and *M. haplanaria* (Table 3). However, 36.4% of the fields contained mixed populations of RKN species. Mixed populations of *M. incognita* and *M. arenaria* were more frequent than any other mixed populations, infesting 29% of total fields (272) identified (Table 4). Furthermore, mixed populations of *M. incognita* and *M. arenaria* had a higher prevalence ($P < 0.05$) than populations of exclusively *M. arenaria,* which infested only 5.1% of total fields identified. However, fields infested with only *M. incognita* were more prevalent (57.7%) than any other group of species (Table 4). Among mixed and single-species populations that infested more than 2 fields, J2 abundance was greater ($P < 0.05$) in the mixed population of *M. incognita* and *M. arenaria,* than in populations exclusively *M. incognita* or *M. arenaria* (Table 4).

Most neighboring fields had the same species present, but in some cases neighboring fields showed variations in species detected (Table S3). For example, D4-D18 are neighboring fields in which most fields are infested with mixed populations of *M. incognita* and *M. arenaria*, but only *M. incognita* was detected in a field of sweet corn (D6) and tomato (D10), whereas only *M. arenaria* was detected in the collard field (D17). Lo4-Lo6 are neighboring fields, mixed populations of *M. incognita* and *M. arenaria* were found for fields Lo4 and Lo5, but Lo6 (bell pepper field) had a mixed population of *M. incognita* and *M. javania*. Lo24-28 are neighboring fields, however, mixed populations of *M. incognita* and *M. arenaria* were detected from a gourd field, *M. incognita* and *M. javanica* from a cantaloupe field, exclusively *M. incognita* from a yellow squash and cucumber field, and exclusively *M. arenaria* from a watermelon field.

Meloidogyne incognita was exclusively identified from 30 different vegetable (acorn squash, bell pepper, bell pepper, broccoli, butter beans, butternut squash, cabbage, carrots, cauliflower, chili pepper, collards, collards, cucumber, eggplant, kale, napa cabbage, okra, onion, pepper, pumpkin, snap beans, cowpea, spaghetti squash, sweet corn, sweet potato, tomato, watermelon, yellow squash, yellow squash, and zucchini; Table S1). *Meloidogyne arenaria* was exclusively identified from same vegetables as *M. incognita* except for 15 of the crops (acorn squash, broccoli, butter beans, butternut squash, cabbage, carrots, cauliflower, kale, napa cabbage, okra, pepper, pumpkin, cowpea, spaghetti squash, and zucchini). *Meloidogyne javanica* was exclusively identified from only a cucumber field (B3), whereas *M. floridensis* was exclusively identified from only a cowpea field (Do14). Mixed populations of *M. incognita* and *M. arenaria* were identified from 27 different vegetable fields (acorn squash, bell pepper, broccoli, butter beans, butternut squash, cabbage, cantaloupe, chili pepper, cucumber, eggplant, gourd, kale, mustard greens, okra, onion, pepper, potato, pumpkin, snap beans, cowpea, sweet corn, sweet potato, tomato, watermelon, yellow squash, yellow squash, and zucchini). Mixed populations of *M. incognita and M. javanica* were identified from 9 different vegetable fields (bell pepper, cantaloupe, chili pepper, eggplant, onion, cowpea, watermelon, yellow squash, and zucchini). Mixed populations of *M. incognita* and *M. floridensis* were identified from collard, cucumber, and tomato fields (Do12, Wa8, and Wi21, respectively). Mixed populations of *M. arenaria* and *M. javanica* were identified from a sweet corn field (T5 and W1). Mixed populations of *M. javanica and M. haplanaria* were found in a cowpea field (Tu2).

Meloidogyne incognita was exclusively identified in all counties infested with RKN, while *M. arenaria* was exclusively identified in Brooks, Decatur, Lee, Lowndes, Sumter, Tattnall, Tift, Toombs, and Turner counties. Also *M. javanica* was exclusively identified in Brooks county and *M. floridensis* in Dooly county (Table S2). Mixed populations of *M. incognita* and *M. arenaria* were identified in most counties except Bulloch, Crawford, Lee, Macon, Peirce, Thomas, Turner, Ware, Wayne, and Worth counties. Mixed populations of *M. incognita* and *M. javanica* were identified in Brooks, Crisp, Echols, Lowndes, Toombs, and Worth counties. Mixed populations of *M. incognita* and *M. floridensis* were identified in Dooly, Ware, and Wilcox counties. Mixed populations of *M. arenaria* and *M. javanica* were identified in Toombs and Worth county. Mixed population of *M. arenaira* and *M. floridensis* were identified in Crisp and Turner county, whereas a mixed population of *M. arenaria* and *M. haplanaria* were identified in Turner county. A mixed population of *M. javanica* and *M. floridenis* was also found in Turner county.

Identification by PCR of species-specific primers. Species-specific PCR successfully identified 888 out of a total of 1095 DNA samples (81.1%), identifying RKN species in 262 fields out of the total

280 soil samples (93.6%) (Table S3). Only the three major MIG species (*M. incognita*, *M. arenaria*, and *M. javanica*) were identified with species-specific PCR. There were no positive PCR reactions from MH0F/MH1R (*M. hapla*) and Me-F/Me-R (*M. enterolobii*) primers. Positive results from PCR with Finc/Rinc primer sets produced a 1200 bp product, while Finc-F0/FincR1 produced a 400 bp product, inc-k14-F/inc-k14-R produced a 400 bp product, Mi2F4/Mi1R1 produced a 300 bp product, Sec-1F/Sec-1R produced a 500 bp product, Far/Rar produced a 420 bp product, and Fjav/Rjav produced a 670 bp product (Fig. S1). Species-specific PCR failed to identify 210 DNA samples. *Meloidogyne incognita*-specific duplex PCR reactions amplified DNA in 53 samples. Yet only 30.2% of positive samples were amplified by both primer sets. The remaining positive samples were exclusively amplified with either Mi2F4/Mi1R1 primers (26.4%) or Sec-1F/Sec-1R primers (43.4%). Samples with negative results for species-specific PCR were subjected to mitochondrial haplotyping.

Identification by DNA sequencing of mitochondrial genes. A total of 201 (95.7%) of samples with failed species-specific PCR reactions were identified by mitochondrial haplotyping. This method identified *M. incognita*, *M. arenaria*, *M. floridensis* and *M. haplanaria* in 120 different fields with an average of 99.5 ± 0.1% (92.9-100%) pairwise identity and 98.1 ± 0.5% (55.3-100%) query coverage. NCBI accessions that matched with DNA samples include KP001571, MK460551, MK460549, MH152333, KM881685, KF993635, KJ476151, MH152335, LN864824, MT796124, FJ159631, JX100437, KP001570, FJ159614, KP001568, FJ159616, MZ066384, KX214347, KP001567, MK033432, JX100438, KP306536, MK102802, MK460552, AY942853, MH152332, MK033430, FJ159619, LC547506, KP306537, MF043913, FJ159624, MK033434, MT212718, MH152334, AY635611, MK861920, MK102799, MK033431, MN156469, LN864824, MK102798, KM100872, MK033429, MT066217,

KP001569, MK460550, MN103539, KP306535, EF183527, MK033435, MT212719, FJ159626, MN166622, MN097146, MK102801, and MK033433 for *M. incognita*; MK033428, FJ159615, KX983450, MH332685, EU364879, MK033427, KP202350, MH332686, KU841772, JQ446377, MW315989, AY635610, and KX280643 for *M. arenaira*; DQ228697, MT787563, KP732361, and KP732360 for *M. floridensis*; and OL893014, KM881682, KT783539, OL893015, MK102795, MK102794, MZ081011, MK102793, and AY757905 for *M. haplanaria*. Since *M. floridensis* and *M. haplanaria* lacked species-specific primers, they could only be identified by mitochondrial haplotyping. All DNA sequences used for identification were annotated and deposited into GenBank with their accession numbers and matches in Table S3. Most (171 from a total of 203 samples; 84.2%) of the DNA sequences matched with only one species of RKN but with more than one assession in GenBank with equal pairwise identity and query coverage. Specifically, C2F3/1108 (COX2 - l-rRNA gene) primers identified 46 DNA samples matching with DNA sequences of *M. incognita* with 99.3 ± 0.3% (100-92.9 %) pairwise identity and 91.1 ± 3.0% (100-55.3%) query coverage, *M. arenaria* with 99.2 ± 0.3% (100-97.2%) pairwise identity and 99.5 ± 0.5% (100-93.4%) query coverage, *M. floridensis* with 98.6% pairwise identity and 100% query coverage, and *M. haplanaria* with 98.8 ± 1.2% (100-97.5%) pairwise identity and 100% query coverage. MORF/MTHIS (IGS - tRNA-His gene) identified 132 samples and match with DNA sequences of *M. incognita* only with 99.6 ± 0.1% (100-94.3%) pairwise identity and 99.4 ± 0.4% (100-61.9%) query coverage. TRNAH/MRH106 (IGS – lrRNA gene) identified 24 samples and match with DNA sequences of *M. incognita* with 99.7 ± 0.2% (100-98.2%) pairwise identity and 100% query coverage, *M. floridensis* with 99.6 ± 0.3% (100-95.9%) pairwise identity and 95.7 ± 2.9% (100-73.7%) query coverage, and *M. haplanaria* with 99.6% pairwise identity and 100% query coverage. Low query coverage for DNA fragments amplified by C2F3/1108 and

MORF/MTHIS primers was due to large gaps (304-775 bp) in the IGS region. Species identification of samples with less than 99% pairwise identity matched with other replicates with ≥ 99% pairwise identity or with species-specific PCR.

The COX2 gene fragment was highly conserved among twenty seven *M. incognita* and *M. arenaria* samples encoding the polypeptide fragment FGWITLFDFFKLNLLTNWLFYFCWSKSKY, while *M. haplanaria* encoded the polypeptide fragment LVEVPLFDFFKLNLLTN. No fragment of the COX2 gene was amplified from *M. floridensis*. Instead, only the AT-rich intergenic region and fragment of the l-rRNA gene was amplified.

The other 34 DNA samples failed to be identified, resulting in 6 unidentified fields. This resulted from either poor sample quality resulting in a failed PCR reaction, producing low quality DNA sequence reads, or containing minimal sequence variation to differentiate among RKN species. The latter was the case for two (OM523657 and OM523691) of the DNA sequences (IGS – l-rRNA gene) that were 99.7% and 93.8% identical to 36 and 9 DNA sequences with equal pairwise identity and query coverage among sequences of *M. arenaria*, *M. incongita*, and *M. javanica* and *M. incognita*, *M. arenaria, M. ethiopica,* and *M. paranaensis*, respectively (Table S3). By the time resampling was a consideration for these samples, these field cultures were lost. However, other replicates were identified with species-specific PCR as a mixed population of *M. arenaria* and *M. javanica* (W1) and single-species population of *M. incognita* (Lo12).

Phylogenetic relationships. The phylogenetic trees based on mtDNA of the COX2 - l-rRNA gene place the RKN populations into 6 to 7 distinct groups consisting of Clades I-IV and subclades Ia-Ic (Fig. 2-4). A total of 23 countries (Australia, Brazil, Burkina Faso, China, Costa Rica, French West Indie, Greece, India, Japan, Kenya, Korea, Netherlands, Nicaragua, Peru, Portugal, Serbia, Slovenia, South Africa, Thailand, The Ivory Coast, The Netherlands, Turkey, and USA) and 16 USA states (Arizona, Arkansas, California, Connecticut, Florida, Georgia, Hawaii, New Mexico, New York, North Carolina, Oregon, Pennsylvania, Rhode Island, South Carolina, Texas, Washington, and Wyoming) and 25 RKN species were represented in each tree. Trees of this study placed *M. arabicida, M. arenaria, M. enterolobii, M. ethiopica, M. floridensis, M. haplanaria, M. hispanica, M. incognita, M. javanica, M. lopezi, M. luci, M. morocciensis*, and *M. paranaensis* into Clade I; *M. hapla* and *M. partityla* into Clade II; *M. aegracyperi, M. chitwoodi, M. exigua, M. fallax, M. graminicola,* and *M. minor,* and *M. naasi* into Clade III; and *M. graminis* and *M. marylandi* into Clade IV. Clade III and IV were consistently placed as a basal taxon to all RKN species in trees with 93-100% support. *Meloidogyne haplanaria, M. enterolobii,* and *M. floridensis* formed separate monophyletic subclades with 99-100% support within Clade I referred to as subclade Ia, subclade Ib, and subclade Ic, respectively.

A phylogenetic tree based on DNA fragments of the COX2 - l-rRNA gene from C2F3/1108 primers placed RKN populations into 6 distinct groups (Fig. 2), totaling 83 DNA sequences from 77 references and 6 Georgia isolate representatives. Phylogenetic tree of the IGS – tRNA-His region from MORF/MTHIS primers placed RKN populations into 7 distinct groups (Fig. 3) totaling 77 DNA sequences from 72 references and 5 representative samples from this study. A phylogenetic tree based on l-rRNA gene from TRNAH/MRH106 primers placed RKN populations into 6 distinct groups (Fig. 4), totaling 75 DNA sequences from 68 references and 7 representative samples from this study.

Georgia's *M. incognita* and *M. arenaria* isolates were in Clade I with 99-100% support, yet some showed divergence within Clade 1. For example, a representative isolate of *M. incognita* from Lowndes County (OM523655) formed a monophyletic clade with *M. incognita* isolates from

Arkansas (MK102798, MK102801) with 100% support from the COX2 – l-rRNA gene region (Fig. 2). This subclade is also a sister clade with the *M. floridensis* Clade 1c. *Meloidogyne arenaria* representative from Decatur county (OM523685) formed a monophyletic clade with *M. arenaria* isolates from Tukey (KX983450) and Arkansas (KM881687). Georgia's isolates in a phylogenetic tree of the IGS – tRNA-His region (Fig. 3) formed a subclade within Clade 1 with 100% support.

Meloidogyne haplanaria formed a subclade Ia within Clade I in all trees with 99- 100% support with *M. haplanaria* isolates from Arkansas (KM881682, MK102793), California (MZ081011), and Florida (KT783539). It also formed a sister clade to the large monophyletic clade of other tropical obligate mitotic parthenogenic species (*i.e., M. arabicida, M. arenaria, M. ethiopica, M. floridensis, M. hispanica, M. incognita, M. javanica, M. lopezi, M. luci, M. morocciensis,* and *M. paranaensis*) with 98-100% support among all trees. Facultative meiotic parthenogenetic *M. floridensis* was also found in Clade I for all three trees and formed a separate subclade in trees of COX2 – l-rRNA genes and IGS – tRNA-His region with 100% support with isolates from Florida (KP732358, DQ228697) and California (MH729182), respectively.

Discussion

The occurrence, prevalence, and distribution of RKN species. This is the first widespread surveillance study using molecular techniques for identification of RKN species in Georgia. Accurate species identification of RKN and understanding their genetic diversity is the first critical step in developing an appropriate management program. As with many other mid-south and southeast U.S. states (Subbotin and Chitambar 2018), *M. incognita* was the most prominent RKN species. *Meloidogyne arenaria* was also found at a high frequency and is most likely related to the long history and widespread cultivation of peanuts in Georgia, in which this species is most frequently found (Timper et al. 2018). By replicating nematode DNA samples from a single field, mixed populations of RKN species were possible to detect. Multiple RKN species are commonly found in field populations (Carrillo-Fasio et al. 2021). Since many newly described species do not have species-specific primers for detection, identifying samples with negative results from species-specific PCR with mitochondrial haplotyping is critical for newly described species detection. In this study, most of the fields infested with the newly described species, *M. haplanaria* and *M. floridensis*, were from mixed populations. If identification were halted after one positive result from a replicate, *M. haplanaria* and *M. floridensis* would not have been detected in most of the infested fields. Regardless of their proximity, some neighboring fields with different vegetable crops also had different species, indicating that neighboring fields can show species diversity. However, it is possible that host preferences could have affected the species detected in these fields.

The co-infestation of *M. incognita* and *M. arenaria* was the largest mixed-species population. This may reflect Georgia's history of tobacco cultivation commonly found with concomitant populations of *M. incognita* and *M. arenaria* (Ngambi et al. 1995) and the rotation of cotton and peanut in these vegetable fields. For unknown reasons, fields with co-infestation of *M. incognita* and *M. arenaria* had higher J2 population densities in the soil. Currently, there are no clear patterns in the interactions of mixed populations of RKN species. There have been observations of concomitant inoculations of *M. arenaria* and *M. incognita* having slightly lower population densities than single species inoculations (Ngambi et al. 1995). A pathogenicity trial comparing *M. arenaria* and *M. incognita* as mixed inoculum and separate inoculum found the mixed population to have an intermediate egg production from that of exclusively *M. arenaria* and *M. incognita* on soybean (Ibrahim and Lewis 1985). It is also reported that one species of RKN can overcome host resistance for another species. For example, resistant tobacco cv. NC95 to *M.*

incognita race 1 inoculated simultaneously with *M. arenaria* race 2 was also susceptible to *M. incognita* (Ibrahim 1987). Conversely, a split-root study on resistant tobacco cv. NC95 found *M. arenaria* did not systemically break down host resistance to *M. incognita* (Baum et al. 1995).

Meloidogyne javanica, M. floridensis, and *M. haplanaria* were at low prevalence. It is possible that low prevalence is related to the frequent rotations of crops that are poor hosts or nonhosts to these species and susceptible to *M. incognita* and *M. arenaria*. For example, cotton is frequently rotated in these fields and is an excellent host to *M. incognita* yet it serves as a nonhost to *M. haplanaria* (Eisenback et al. 2003) and a poor host to *M. floridensis* (Handoo et al. 2004). Peanut, susceptible to *M. arenaria*, can serve as a poor host to *M. javanica* (Abdel-Momen and Starr 1997) and a nonhost to *M. floridensis* (Handoo et al. 2004). Moreover, in the case of *M. haplanaria,* some vegetable crops like cowpea cv. Iron Clay, pepper, and watermelon are also nonhosts (Eisenback et al. 2003; Hajihassani et al. 2019; Marquez and Hajihassani, 2022). Resistant peanut cultivars like NemaTam to *M. arenaria* and *M. javanica* have also been shown to be resistant to *M. haplanaria* (Bendezu et al. 2004). The MIG species have also been shown to outcompete *M. haplanaria* as this species egg production was 20% less than *M. incognita* and *M. arenaria* on susceptible tomato (Eisenback et al. 2003). *Meloidogyne haplanaria* was also found to be less aggressive than *M. arenaria* and *M. javanica* on peanut (Abdel-Momen and Starr 1997). Further investigation found Georgia's *M. haplanaria* isolate to not reproduce on vegetable crops grown at the time of sampling (*i.e.*, cowpea and watermelon) (Marquez and Hajihassani 2022), therefore the threat of this species on vegetable production in Georgia is low.

This study also detected *M. floridensis* in Georgia for the first time at population densities as high as 5,264 J2/100 cm^3 of soil in Ware County. This population was isolated as a single egg mass culture and further characterized and confirmed as *M. floridensis* by DNA sequence-based identification with NAD5 gene (Marquez et al. 2021b) and showed virulence towards *Mi*-resistant tomato (Marquez and Hajihassani, accepted). Most fields infested with *M. floridensis* were detected in counties that have had a history of peach cultivation (Crisp, Turner, Wilcox, and Dooly). It is possible that *M. floridensis* was introduced by the trade of peach trees from Florida. Yet, *M. floridensis* could also be an endemic species overlooked as *M. incognita* race 3 (Handoo et al. 2004).

Despite the large sample size of 436 fields (Marquez et al. 2021a), *M. enterolobii* was not detected in vegetable fields. Recently, it has been detected in sweet potato fields of Tattnall and Lowndes counties (Hajihassani et al., unpublished), suggesting that sweet potato plant trade is at high risk of spreading *M. enterolobii* in vegetable fields of Georgia. Since peanut is a nonhost (Yang and Eisenback 1983), frequent rotations of peanut could suppress *M. enterolobii* reproduction. However, there has been a report from South Africa of a *M. enterolobii* population reproducing on peanut (Rashidifard et al. 2019).

Although *M. hapla* has been detected in Georgia, this study could not detect this species in vegetable fields. *Meloidogyne hapla* is adapted to more temperate climates and was expected to be rare. The probability of detecting this species may have been improved if this study increased sampling of fields with crops grown in the colder months of the year and in regions of higher elevations and latitudes. It is also possible that concomitant infections of *M. incognita* interfered with the ability of *M. hapla* to infect and reproduce (Ogallo and McClure 1995).

Not all RKN-infested fields were able to be detected in this study due to lost cultures. It is possible that these lost cultures are species like *M. marylandi* and *M. pratityla* which cannot reproduce on tomato plant (Faske and Starr 2009; Starr et al. 1996). Although these species may be present in vegetable fields, they are not a major threat to vegetables as these species have a limited host range to either the grasses for *M. marylandi* (Oka et al. 2003) or woody trees in the Juglandaceae family (*i.e.*, hickory, pecan, and walnut) for *M. partityla* (Starr et al. 1996). Although resampling could have confirmed RKN species for lost cultures, we believe it would not have been a cost-effective decision, given the number of samples that were identified.

Genetic diversity of RKN species. Understanding the inter- and intra- specific diversity of RKN populations can help assess the adaptation potential of these populations to survive in an agroecosystem. Consistent with others (Alvarez-Ortega et al. 2019; Castagnone-Sereno et al. 2013), interspecific genetic variations in this study had no association with geographic origin, despite including taxa from 23 countries representing all 6 contents with arable land. Instead, taxa are grouped mainly by mode of reproduction. Specifically, RKN species were grouped into Clades I, II, III, and IV sensu Alvarez-Ortega et al. (2019) and generally agree with multigene trees from Castagnone-Sereno et al. (2013) and Kiewnick et al. (2014).

Obligate mitotic parthenogenic species, including the MIG species, were grouped into Clade I, known as the tropical RKN complex (Alvarez-Ortega et al. 2019). Due to their mode of reproduction, these species lack interspecific variations to cluster into different clades with strong support, making it difficult to correlate genetic variations with variations in aggressiveness, virulence, host adaptation, and race (Kaloshian and Teixeira 2019). There was evidence of intraspecific genetic diversity among Georgia's isolates. For example, *M. incognita*-specific duplex PCR indicated that not all of Georgia's *M. incognita* populations carry the targeted DNA sequences of these species-specific primers (*i.e.*, putative esophageal gland protein and RAPD marker). In addition, some of Georgia's *M. incognita* and *M. arenaria* populations in the COX2 – l-rRNA gene fragment and IGS – tRNA-His gene fragment diverged and formed a monophyletic subclade within Clade I. However, species-specific PCR screening of Georgia's populations may have been biased against divergent populations that are not commonly amplified by these primers. Further investigation confirmed that some of the populations have divergent phenotypes (*i.e.*, breaking resistance to *Mi-1.2* gene) (Hajihassani et al. 2022). Since the mitochondrial genome of MIG species has the lowest divergence levels among RKN species (Humphreys-Pereira and Elling 2015), accurately characterizing the genetic divergence may require multiple genes, microsatellites, or whole-genome sequencing.

Within Clade I, *M. enterolobii, M. haplanaria,* and *M. floridensis* diverged into separate subclades. Consistent with other studies (Alvarez-Ortega et al. 2019; Subbotin 2021), *M. haplanaria* populations were also part of Clade I but separated from other obligate mitotic parthenogenic species with strong support. This is also true for *M. enterolobii,* which is another tropical mitotic parthenogenic species in Clade I (Janssen et al. 2016) but is clearly divergent from other species in Clade I. Conversely, the facultative meiotic parthenogenetic *M. floridensis* belongs to the large tropical mitotic monophyletic subclade of Clade I. It has been hypothesized that the speciation of *M. floridensis* was a hybrid event between *M. incognita* and an unknown RKN species (Lunt et al. 2014).

The challenges of species-specific identification and the feasibility of mitochondrial haplotyping. Species-specific primers did not successfully identify all of Georgia's RKN populations sampled in this study, including many populations from MIG species. Species-specific primers could only detect MIG species in 80.8% of the samples, possibly due to poor DNA quality, lack of sensitivity, or variations in DNA targets. Most samples that failed to be identified with species-specific primers were identified with mitochondrial haplotyping as MIG species, suggesting that currently available species-specific primers for MIG species are limited in targeting all MIG populations. This study suggests a need for developing more reliable primers for the MIG species with improved target scope and sensitivity. Gene-specific primers targeting species-specific genes could offer improved detection. With advances in characterizing species-specific effectors (Jagdale et al. 2021), it is increasingly more feasible to use gene-specific primers to target species-specific effectors for the detection of the MIG species.

Mitochondrial haplotyping was reliable with a success rate of 95.7%. Almost all samples subjected to mitochondrial haplotyping could be differentiated to a species of RKN except 2 samples (OM523657 and OM523691) that were ambiguous in species differentiation. This is not unusual (Ye et al. 2019) and is a weakness of this method. Coupling species-specific primers with mitochondrial haplotyping effectively identified most samples, with a high success rate of 96.9% compared to other similar studies with only mitochondrial haplotyping (Khanal et al. 2016). However, due to the large sample size, mitochondrial haplotyping was limited to only one region, COX2 – l-rRNA gene fragment.

Although multigene approaches improve resolution, this region has been proven to be robust and reliable in RKN species differentiation (Janssen et al. 2016) and is used as a single marker for RKN species differentiation (Khanal et al. 2016). Studies that use multiple marker genes have verified identification based on COX2 – l-rRNA gene fragment is consistent with other nuclear ribosomal genes (Ye et al. 2019).

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Tables

Table 1. Species specific primers for *Meloidogyne* spp. identification.

Name	*Meloidogyne* spp.	Fragment size	Primer sequence 5'-3'	Direction	Source
FincF0	*incognita*	400 bp	AACGGCCACTCTTTGGTGGTGTAT	F	Wemin Ye
FincR1			AGGTTGTTGTTGCTGTAATGCTGC	R	
SEC-1F	*incognita*	502 bp	GGGCAAGTAAGGATGCTCTG	F	Tesařová et al. (2003)
SEC-1R			GCACCTCTTTCATAGCCACG	R	
inc-K14-F	*incognita*	399 bp	GGGATGTGTAAATGCTCCTG	F	Randig et al. (2002)
inc-K14-R			CCCGCTACACCCTCAACTTC	R	
Finc	*incognita*	1200 bp	CTCTGCCCAATGAGCTGTCC	F	Zijlstra et al. (2000)
Rinc			CTCTGCCCTCACATTAAG	R	
Mi2F4	*incognita*	300 bp	ATGAAGCTAAGACTTTGGGCT	F	Kiewnick et al. (2013)
Mi1R1			TCCCGCTACACCCTCAACTTC	R	
Far	*arenaria*	420 bp	TCGGCGATAGAGGTAAATGAC	F	Zijlstra et al. (2000)
Rar			TCGGCGATAGACACTACAACT	R	
Fjav	*javanica*	670 bp	GGTGCGCGATTGAACTGAGC	F	Zijlstra et al. (2000)
Rjav			CAGGCCCTTCAGTGGAACTATAC	R	
MH0F	*hapla*	960 bp	CAGGCCCTTCCAGCTAAAGA	F	Williamson et al. (1997)
MH1R			CTTCGTTGGGGAACTGAAGA	R	
Me-F	*enterolobii*	236 bp	AACTTTTGTGAAAGTGCCGCTG	F	Hu et al. (2011)
Me-R			TCAGTTCAGGCAGGATCAACC	R	

Table 2. Universal primers for *Meloidogyne* spp. identification.

Name	Gene position^z	Fragment size	Primer sequence 5'-3'	Direction	Source
C2F3	COX2, IGS, tRNA-His, and l-rRNA	500-1000 bp	GGTCAATGTTCAGAAATTTGTGG	F	Powers et al. (2018)
1108			TACCTTTGACCAATCACGCT	R	
MORF	IGS and tRNA-His	200-800 bp	ATCGGGGTTTAATAATGGG	F	Hugall et al. (1994)
MTHIS			AAATTCAATTGAAATTAATAGC	R	
TRNAH	tRNA-His and l-rRNA	500-800 bp	TGAATTTTTTATTGTGATTAA	F	Stanton et al. (1997)
MHR106			AATTTCTAAAGACTTTTCTTAGT	R	

^{z}Mitocondrial genes consist of cytochrome c oxidase subunit II (COX2), intergenic spacer (IGS), tRNA-His, and large subunit 16S rDNA (l-rRNA).

Table 3. The overall prevalence, prominence value, and relative prominence values of each RKN species infesting vegetable fields of South Georgia.

RKN species	Infested fields	Prevalence		
		Prevalence (%)	PV	RPV
M. incognita	250	91.9 ± 1.7 a	45	60.31
M. arenaria	98	36.0 ± 2.9 b	25	34.37
M. javanica	15	5.5 ± 1.4 c	1.9	2.614
M. floridensis	6	2.2 ± 0.9 cd	2	2.7
M. haplanaria	2	0.7 ± 0.5 d	0	0.008

Mean (± SE) prevalence with the same letters are not significantly different according to ANOVA followed by Tukey HSD test (α = 0.05). Prevalence = Percentage calculated by the number of fields infested with species divided by the total number of *Meloidogyne* identified infested (272 fields), PV = prominence value (mean abundance x incidence/10), and RPV = Relative prominence value (100 x PV/Total sum of PV of each RKN species).

Table 4. Prevalence and relative abundance of pure and mixed populations of *Meloidogyne* spp. in infested vegetable fields.

	Prevalence				Relative abundance (second-stage Juvenile/100 cm^3 of soil)	
RKN species groups	Infested fields	Prevalence (%)[z]	PV	RPV	25th quantile, median, and 75th quantile	Range
M. incognita	157	57.7 ± 3.0 a	16.214	35.816	6, 38, 262 b	2 - 14144
M. incognita and *M. arenaria*	79	29.0 ± 2.8 b	24.824	54.836	20, 208, 724 a	2 - 14144
M. arenaria	14	5.1 ± 1.3 c	0.316	0.698	8, 25, 84 b	4 - 1488
M. incognita and *M. javanica*	11	4.0 ± 1.2 c	1.656	3.658	8, 18, 856 ab	2 - 5264
M. incognita and *M. floridensis*	3	1.1 ± 0.6 c	1.953	4.313	4, 56, 5264 ab	2 - 14144
M. arenaria and *M. javanica*	2	0.7 ± 0.5 c	0.242	0.533	40, 345, 650	4 - 5264
M. arenaria and *M. floridensis*	2	0.7 ± 0.5 c	0.022	0.049	2, 32, 62	4 - 1488
M. floridensis	1	0.4 ± 0.4 c	0.026	0.057	-	40 - 650
M. javanica	1	0.4 ± 0.4 c	0.011	0.025	-	2 - 62
M. javanica and *M. haplanaria*	1	0.4 ± 0.4 c	0.006	0.012	-	14 - 14
M. arenaria and *M. haplanaria*	1	0.4 ± 0.4 c	0.001	0.002	-	2 - 2

Mean (± SE) prevalence with the same letters is not significantly different according to ANOVA followed by Tukey HSD test (α = 0.05). Median relative abundance with the same letters is not significantly different according to Kruskal-Wallis Analysis of Variance (KW-ANOVA) followed by Dunn-Bonferroni test (α = 0.05). KW-ANOVA limited to species groups with ≥ 3 fields. [z]Percentage calculated by the number of fields divided by the total number of *Meloidogyne* infested fields in which *Meloidogyne* spp. that were identified (278 fields). PV = prominence value (mean abundance x incidence/10), and RPV = Relative prominence value (100 x PV/Total sum of PV of each RKN species group).

Figures

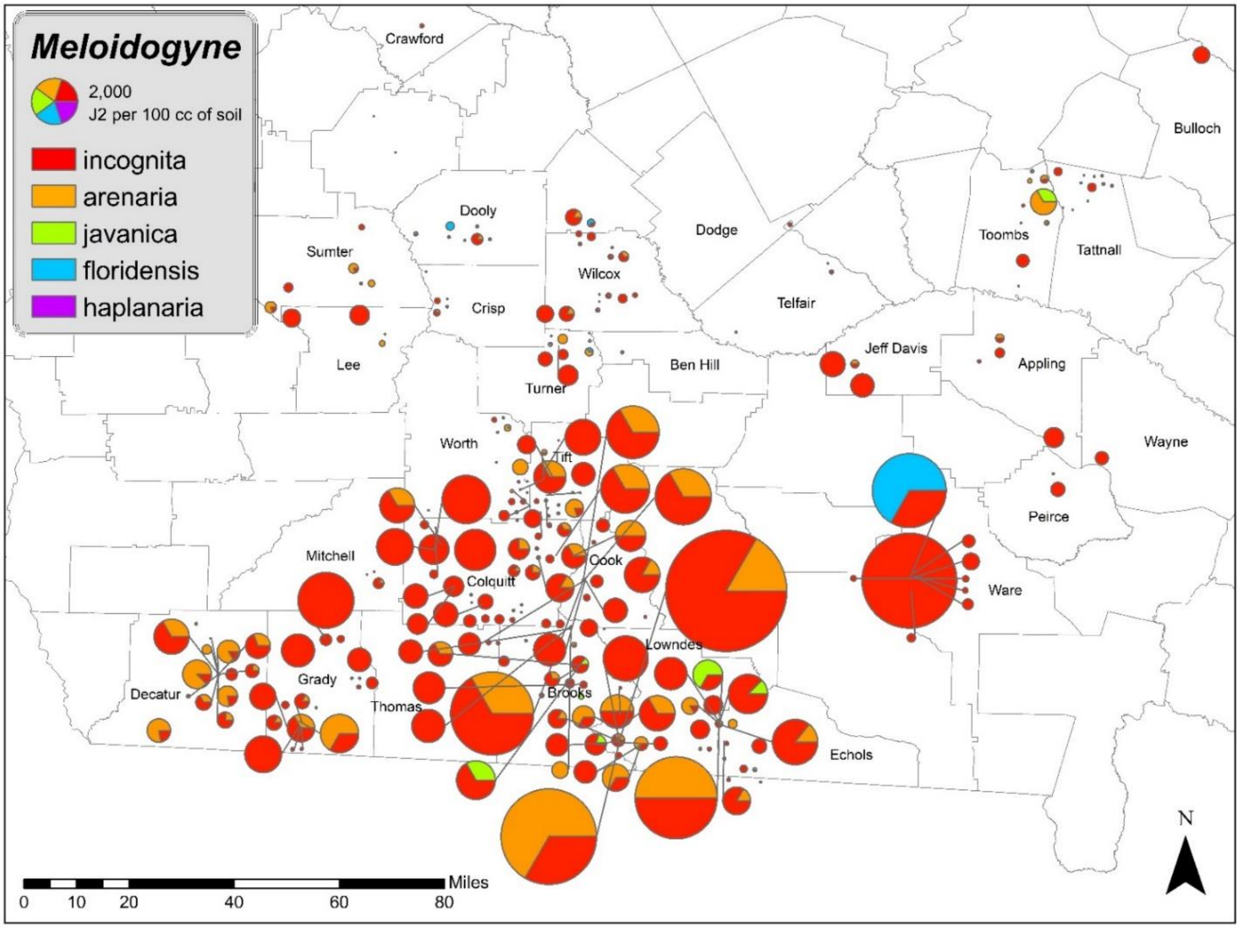
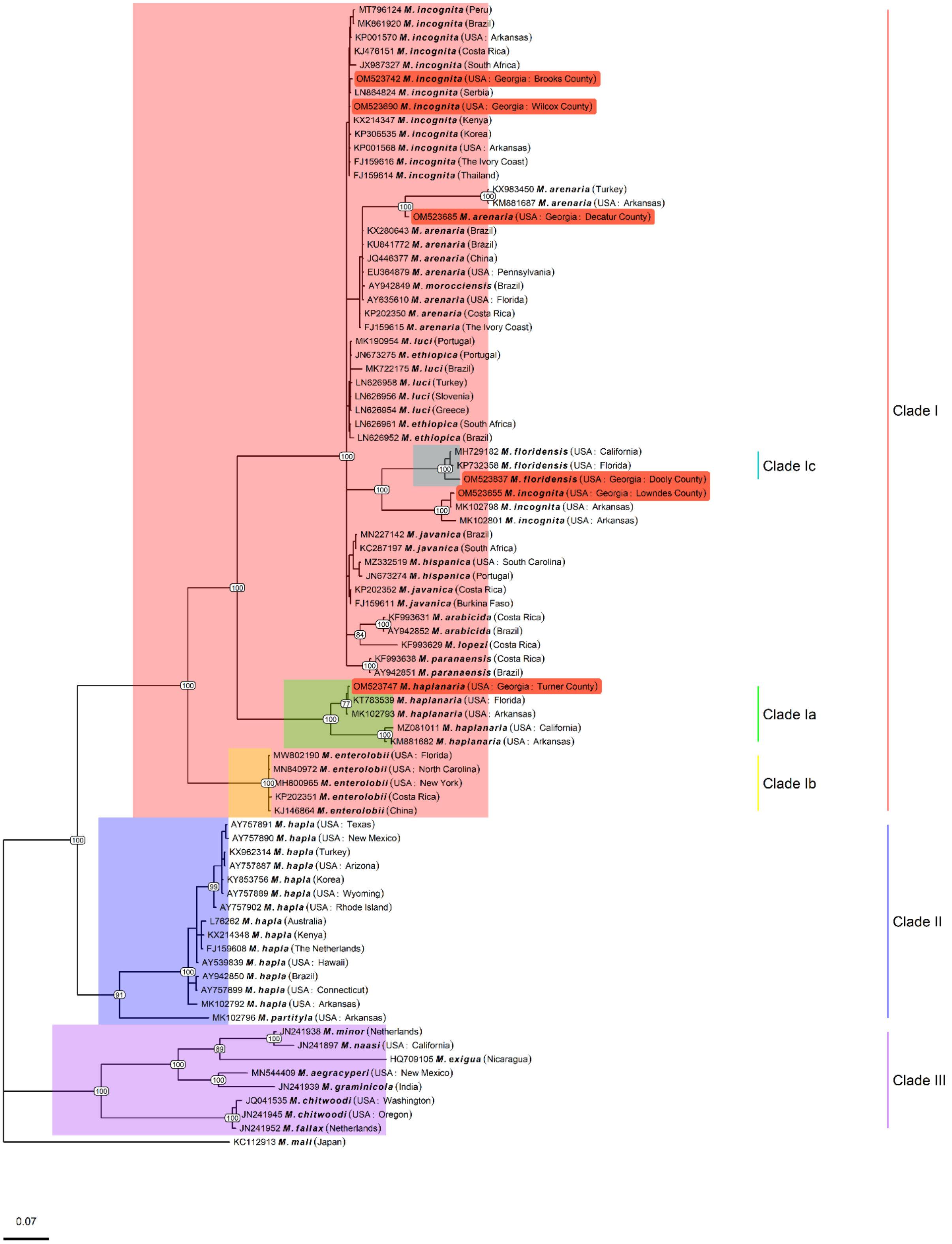


Fig. 1. The distribution, abundance, and proportion of root-knot nematode species in vegetable fields of Georgia, 2018. Spatial graphics were made with ArcGIS (esri, Redlands, CA).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JX987327 M. incognita (South Africa)
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LN864824 M. incognita (Serbia)
OM523690 M. incognita (USA: Georgia: Wilcox County)
KX214347 M. incognita (Kenya)
KP306535 M. incognita (Korea)
KP001568 M. incognita (USA: Arkansas)
FJ159616 M. incognita (The Ivory Coast)
FJ159614 M. incognita (Thailand)
KX983450 M. arenaria (Turkey)
100
KM881687 M. arenaria (USA: Arkansas)
100
OM523685 M. arenaria (USA: Georgia: Decatur County)
KX280643 M. arenaria (Brazil)
KU841772 M. arenaria (Brazil)
JQ446377 M. arenaria (China)
EU364879 M. arenaria (USA: Pennsylvania)
AY942849 M. morocciensis (Brazil)
AY635610 M. arenaria (USA: Florida)
KP202350 M. arenaria (Costa Rica)
FJ159615 M. arenaria (The Ivory Coast)
MK190954 M. luci (Portugal)
JN673275 M. ethiopica (Portugal)
MK722175 M. luci (Brazil)
LN626958 M. luci (Turkey)
LN626956 M. luci (Slovenia)
LN626954 M. luci (Greece)
LN626961 M. ethiopica (South Africa)
LN626952 M. ethiopica (Brazil)
MH729182 M. floridensis (USA: California)
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KP732358 M. floridensis (USA: Florida)
100
OM523837 M. floridensis (USA: Georgia: Dooly County)
100
OM523655 M. incognita (USA: Georgia: Lowndes County)
MK102798 M. incognita (USA: Arkansas)
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MK102801 M. incognita (USA: Arkansas)
MN227142 M. javanica (Brazil)
KC287197 M. javanica (South Africa)
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AY942852 M. arabicida (Brazil)
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KF993629 M. lopezi (Costa Rica)
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AY942851 M. paranaensis (Brazil)
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100
KM881682 M. haplanaria (USA: Arkansas)
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MN840972 M. enterolobii (USA: North Carolina)
100 MH800965 M. enterolobii (USA: New York)
KP202351 M. enterolobii (Costa Rica)
KJ146864 M. enterolobii (China)
100
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AY757887 M. hapla (USA: Arizona)
KY853756 M. hapla (Korea)
99
AY757889 M. hapla (USA: Wyoming)
AY757902 M. hapla (USA: Rhode Island)
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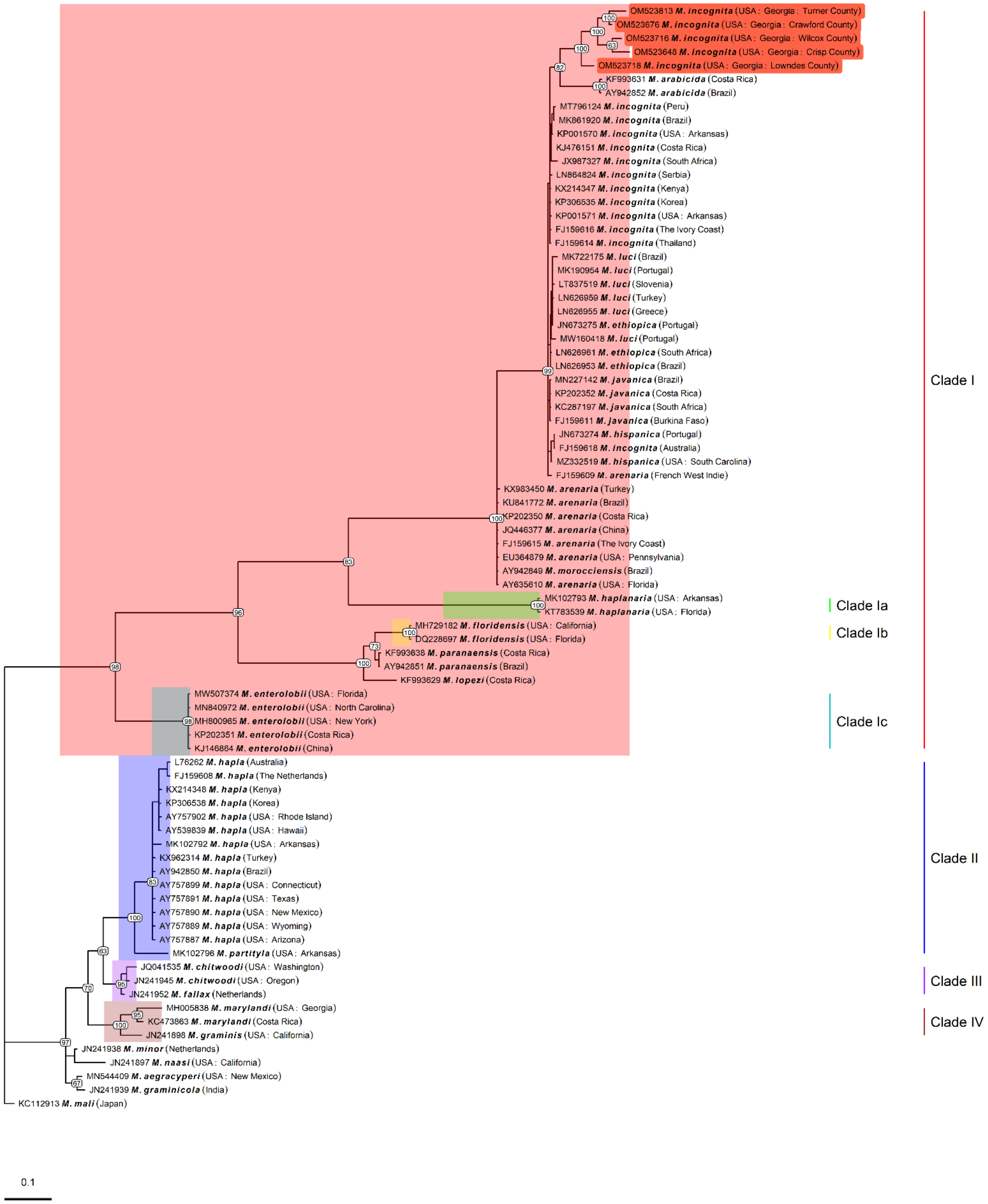
AY942850 M. hapla (Brazil)
91
AY757899 M. hapla (USA: Connecticut)
MK102792 M. hapla (USA: Arkansas)
MK102796 M. partityla (USA: Arkansas)
JN241938 M. minor (Netherlands)
100
JN241897 M. naasi (USA: California)
89
HQ709105 M. exigua (Nicaragua)
100
MN544409 M. aegracyperi (USA: New Mexico)
100
JN241939 M. graminicola (India)
100
JQ041535 M. chitwoodi (USA: Washington)
JN241945 M. chitwoodi (USA: Oregon)
100
JN241952 M. fallax (Netherlands)
KC112913 M. mali (Japan)
Clade I
Clade Ic
Clade Ia
Clade Ib
Clade II
Clade III
0.07

Fig. 2. Bayesian 50% majority-rule consensus tree phylogenetic analysis of *Meloidogyne* species from mitochondrial DNA fragment of cytochrome c oxidase subunit II to large subunit 16S rRNA genes from C2F3/1108 PCR. *Meloidogyne* species are represented by different colored branch tips. Branch support values (posterior probability values) are shown as node labels. Branch lengths means of posterior distribution. GenBank accession number proceed species and origin. Taxa highlighted in red are Georgia isolates.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FJ159614 M. incognita (Thailand)
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MK190954 M. luci (Portugal)
LT837519 M. luci (Slovenia)
LN626959 M. luci (Turkey)
LN626955 M. luci (Greece)
JN673275 M. ethiopica (Portugal)
MW160418 M. luci (Portugal)
LN626961 M. ethiopica (South Africa)
LN626953 M. ethiopica (Brazil)
MN227142 M. javanica (Brazil)
KP202352 M. javanica (Costa Rica)
KC287197 M. javanica (South Africa)
FJ159611 M. javanica (Burkina Faso)
JN673274 M. hispanica (Portugal)
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MZ332519 M. hispanica (USA: South Carolina)
FJ159609 M. arenaria (French West Indie)
KX983450 M. arenaria (Turkey)
KU841772 M. arenaria (Brazil)
KP202350 M. arenaria (Costa Rica)
JQ446377 M. arenaria (China)
FJ159615 M. arenaria (The Ivory Coast)
EU364879 M. arenaria (USA: Pennsylvania)
AY942849 M. morocciensis (Brazil)
AY635610 M. arenaria (USA: Florida)
MK102793 M. haplanaria (USA: Arkansas)
KT783539 M. haplanaria (USA: Florida)
MH729182 M. floridensis (USA: California)
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KP202351 M. enterolobii (Costa Rica)
KJ146864 M. enterolobii (China)
L76262 M. hapla (Australia)
FJ159608 M. hapla (The Netherlands)
KX214348 M. hapla (Kenya)
KP306538 M. hapla (Korea)
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MK102792 M. hapla (USA: Arkansas)
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AY942850 M. hapla (Brazil)
AY757899 M. hapla (USA: Connecticut)
AY757891 M. hapla (USA: Texas)
AY757890 M. hapla (USA: New Mexico)
AY757889 M. hapla (USA: Wyoming)
AY757887 M. hapla (USA: Arizona)
MK102796 M. partityla (USA: Arkansas)
JQ041535 M. chitwoodi (USA: Washington)
JN241945 M. chitwoodi (USA: Oregon)
JN241952 M. fallax (Netherlands)
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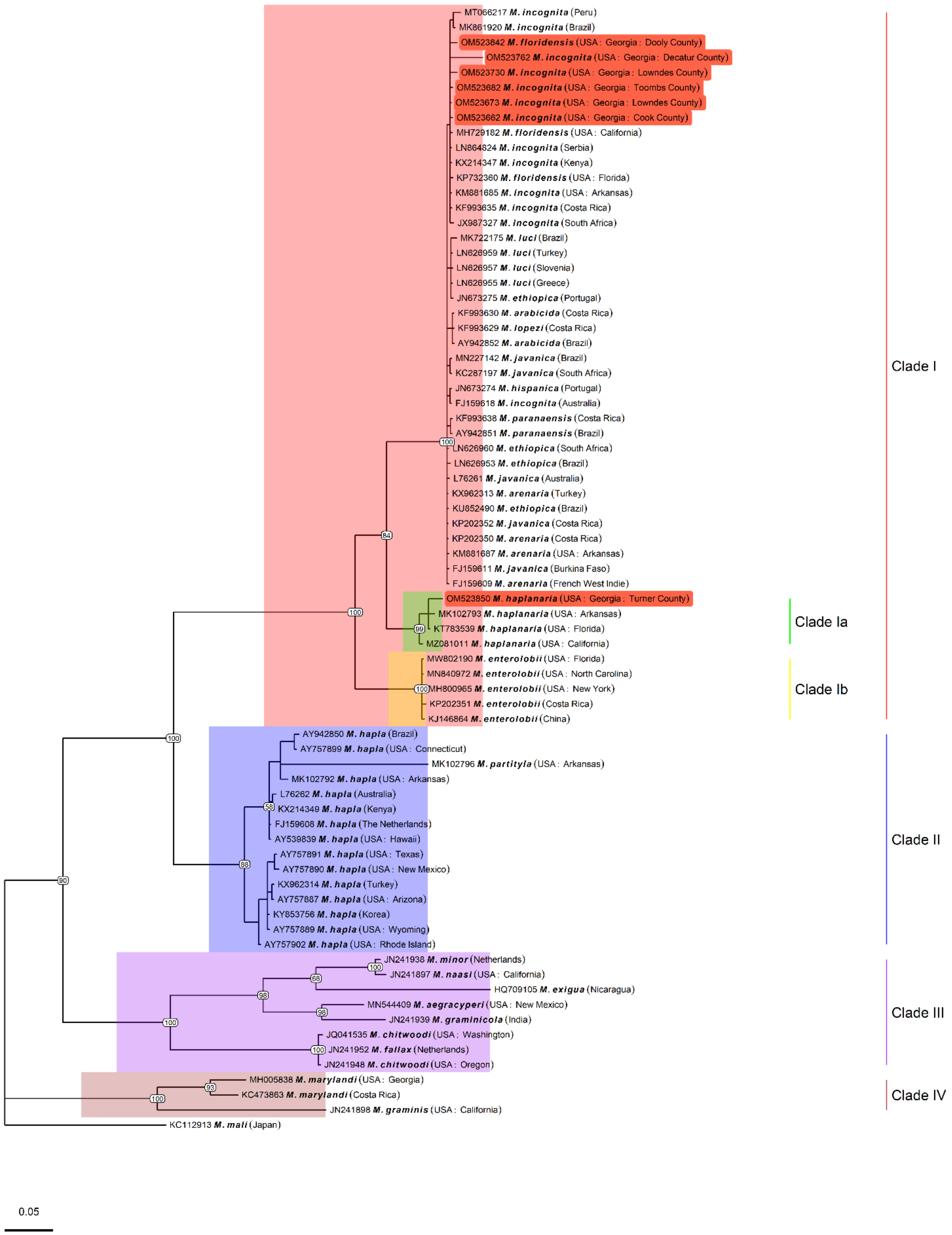
JN241898 M. graminis (USA: California)
JN241938 M. minor (Netherlands)
JN241897 M. naasi (USA: California)
MN544409 M. aegracyperi (USA: New Mexico)
JN241939 M. graminicola (India)
KC112913 M. mali (Japan)
100
100
63
100
82
100
99
100
83
100
96
100
73
100
98
98
83
100
63
95
70
95
100
97
67
Clade I
Clade Ia
Clade Ib
Clade Ic
Clade II
Clade III
Clade IV
0.1

Fig. 3. Bayesian 50% majority-rule consensus tree phylogenetic analysis of *Meloidogyne* species from mitochondrial DNA fragment of intergenic spacer to tRNA-His genes from MORF/MTHIS PCR. *Meloidogyne* species are represented by different colored branch tips. Branch support values (posterior probability values) are shown as node labels. Branch lengths means of posterior distribution. GenBank accession number proceed species and origin. Taxa highlighted in red are Georgia isolates.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JX987327 M. incognita (South Africa)
MK722175 M. luci (Brazil)
LN626959 M. luci (Turkey)
LN626957 M. luci (Slovenia)
LN626955 M. luci (Greece)
JN673275 M. ethiopica (Portugal)
KF993630 M. arabicida (Costa Rica)
KF993629 M. lopezi (Costa Rica)
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MN227142 M. javanica (Brazil)
KC287197 M. javanica (South Africa)
JN673274 M. hispanica (Portugal)
FJ159618 M. incognita (Australia)
KF993638 M. paranaensis (Costa Rica)
AY942851 M. paranaensis (Brazil)
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KU852490 M. ethiopica (Brazil)
KP202352 M. javanica (Costa Rica)
KP202350 M. arenaria (Costa Rica)
KM881687 M. arenaria (USA: Arkansas)
FJ159611 M. javanica (Burkina Faso)
FJ159609 M. arenaria (French West Indie)
84
OM523850 M. haplanaria (USA: Georgia: Turner County)
MK102793 M. haplanaria (USA: Arkansas)
100
99
KT783539 M. haplanaria (USA: Florida)
MZ081011 M. haplanaria (USA: California)
MW802190 M. enterolobii (USA: Florida)
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KJ146864 M. enterolobii (China)
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AY942850 M. hapla (Brazil)
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AY757902 M. hapla (USA: Rhode Island)
JN241938 M. minor (Netherlands)
100
JN241897 M. naasi (USA: California)
68
HQ709105 M. exigua (Nicaragua)
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MN544409 M. aegracyperi (USA: New Mexico)
98
JN241939 M. graminicola (India)
100
JQ041535 M. chitwoodi (USA: Washington)
100
JN241952 M. fallax (Netherlands)
JN241948 M. chitwoodi (USA: Oregon)
MH005838 M. marylandi (USA: Georgia)
93
KC473863 M. marylandi (Costa Rica)
100
JN241898 M. graminis (USA: California)
KC112913 M. mali (Japan)
Clade I
Clade Ia
Clade Ib
Clade II
Clade III
Clade IV
0.05

Fig. 4. Bayesian 50% majority-rule consensus tree phylogenetic analysis of *Meloidogyne* species from mitochondrial DNA fragment of partial l-rRNA gene from TRNAH/MRH106 PCR. *Meloidogyne* species are represented by different colored branch tips. Branch support values (posterior probability values) are shown as node labels. Branch lengths means of posterior distribution. GenBank accession number proceed species and origin. Taxa highlighted in red are Georgia isolates.

Supplemental tables

Table S1. Number of fields infested with *Meloidogyne* spp. for each vegetable sampled.

Crop genus	Crop species	Crop common name	*M. incognita* (Mi)	*M. arenaria* (Ma)	*M. javanica* (Mj)	*M. floridensis* (Mf)	Mi and Ma	Mi and Mf	Mi and Mj	Ma and Mf	Ma and *M. haplanaria* (Mha)	Ma and Mj	Mj and Mha	Total
Allium	*cepa*	Onion	12	2	0	0	3	0	1	0	0	0	0	18
Brassica	*juncea*	Mustard greens	0	0	0	0	1	0	0	0	0	0	0	1
	oleracea	Broccoli	8	0	0	0	1	0	0	0	0	0	0	9
		Cabbage	4	0	0	0	3	0	0	0	0	0	0	7
		Cauliflower	1	0	0	0	0	0	0	0	0	0	0	1
		Collards	3	0	0	0	0	0	0	0	0	0	0	3
		Collards	1	1	0	0	0	1	0	0	0	0	0	3
		Kale	2	0	0	0	1	0	0	0	0	0	0	3
	rapa	Napa cabbage	1	0	0	0	0	0	0	0	0	0	0	1
Ipomoea	*batatas*	Sweet potato	3	1	0	0	3	0	0	0	0	0	0	7
Citrullus	*lanatus*	Watermelon	18	1	0	0	7	0	2	2	1	1	0	32
Cucumis	*melo*	Cantaloupe	0	0	0	0	4	0	1	0	0	0	0	5
	sativus	Cucumber	25	1	1	0	8	1	0	0	0	0	0	36
Cucurbita	pepo	Acorn squash	3	0	0	0	1	0	0	0	0	0	0	4
		Butternut squash	1	0	0	0	1	0	0	0	0	0	0	2
		Pumpkin	1	0	0	0	1	0	0	0	0	0	0	2
		Spaghetti squash	2	0	0	0	0	0	0	0	0	0	0	2
		Yellow squash	1	1	0	0	1	0	0	0	0	0	0	3
		Yellow squash	8	1	0	0	4	0	2	0	0	0	0	15
		Zucchini	6	0	0	0	1	0	1	0	0	0	0	8
Lagenaria	siceraria	Gourd	0	0	0	0	1	0	0	0	0	0	0	1
Phaseolus	vulgaris	Butter beans	1	0	0	0	0	0	0	0	0	0	0	1
		Butter beans	0	0	0	0	3	0	0	0	0	0	0	3
		Snap beans	7	1	0	0	3	0	0	0	0	0	0	11
		Cowpea	1	0	0	1	3	0	1	0	0	0	1	7
Abelmoschus	esculentus	Okra	2	0	0	0	2	0	0	0	0	0	0	4
Zea	mays	Sweet corn	6	1	0	0	6	0	0	0	0	1	0	14
Capsicum	*annuum*	Bell pepper	1	0	0	0	0	0	0	0	0	0	0	1
		Bell pepper	20	1	0	0	5	0	1	0	0	0	0	27
		Chili pepper	7	1	0	0	6	0	1	0	0	0	0	15
		Pepper	1	0	0	0	1	0	0	0	0	0	0	2
Solanum	*lycopersicum*	Tomato	4	1	0	0	4	1	0	0	0	0	0	10
	melongena	Eggplant	7	1	0	0	4	0	1	0	0	0	0	13
	tuberosum	Potato	0	0	0	0	1	0	0	0	0	0	0	1
Daucus	carota	Carrots	1	0	0	0	0	0	0	0	0	0	0	1

Table S2. Number of counties infested with *Meloidogyne* spp. for each crop present in field.

County	*M. incognita* (Mi)	*M. arenaria* (Ma)	*M. javanica* (Mj)	*M. floridensis* (Mf)	Mi and Ma	Mi and Mf	Mi and Mj	Ma and Mf	Ma and *M. haplanaria* (Mha)	Ma and Mj	Mj and Mha	Total
Colquitt	21	0	0	0	11	0	0	0	0	0	0	32
Lowndes	10	2	0	0	13	0	4	0	0	0	0	29
Tift	16	3	0	0	8	0	0	0	0	0	0	27
Brooks	12	1	1	0	3	0	3	0	0	0	0	20
Grady	15	0	0	0	5	0	0	0	0	0	0	20
Wilcox	9	0	0	0	6	1	0	0	0	0	0	16
Decatur	2	2	0	0	11	0	0	0	0	0	0	15
Cook	9	0	0	0	5	0	0	0	0	0	0	14
Tattnall	8	1	0	0	1	0	0	0	0	0	0	10
Toombs	4	1	0	0	3	0	1	0	0	1	0	10
Worth	7	0	0	0	0	0	1	0	0	1	0	9
Ware	8	0	0	0	0	1	0	0	0	0	0	9
Turner	4	2	0	0	0	0	0	1	1	0	1	9
Echols	6	0	0	0	1	0	1	0	0	0	0	8
Dooly	1	0	0	1	4	1	0	0	0	0	0	7
Sumter	4	1	0	0	2	0	0	0	0	0	0	7
Crisp	2	0	0	0	1	0	1	1	0	0	0	5
Telfair	2	0	0	0	2	0	0	0	0	0	0	4
Appling	2	0	0	0	1	0	0	0	0	0	0	3
Peirce	3	0	0	0	0	0	0	0	0	0	0	3
Michell	2	0	0	0	1	0	0	0	0	0	0	3
Jeff Davis	2	0	0	0	1	0	0	0	0	0	0	3
Thomas	2	0	0	0	0	0	0	0	0	0	0	2
Lee	1	1	0	0	0	0	0	0	0	0	0	2
Crawford	2	0	0	0	0	0	0	0	0	0	0	2
Wayne	1	0	0	0	0	0	0	0	0	0	0	1
Bulloch	1	0	0	0	0	0	0	0	0	0	0	1
Macon	1	0	0	0	0	0	0	0	0	0	0	1

Table S3. Root-knot nematode from vegetable fields identified with species-specific PCR or mitochondrial haplotyping.

County	GPS coordinates[z]	Field ID	Crop	Species[y]	Primers[x]	Field No.
Appling	31.82756, -82.359002	A1	Yellow squash	*M. incognita*	Mi2F4/Mi1R1	1
	31.828408, -82.35861	A2	Yellow squash	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	2
	31.764725, -82.415511	A4	Sweet corn	*M. incognita*	Sec-1 F/R	3
Brooks	30.844703, -83.620788	B1	Yellow squash	*M. incognita* and *M. javanica* + *M. incognita*	inc-k14-F/R, Mi duplex, Fjav/Rjav, Finc-F0/R1, and SEC-1F/SEC-1R + MORF/MTHIS (OM523833)	4
	30.8727385, -83.5121682	B3	Cucumber	*M. javanica*	Fjav/Rjav	5
	30.875175, -83.5051935	B4	Acorn squash	*M. incognita*	Finc-F0/R1	6
	30.8740133, -83.505222	B5	Cucumber	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R + MORF/MTHIS (OM523808)	7
	30.9281054, -83.5152779	B6	Chili pepper	*M. incognita* and *M. arenaria* + *M. incognita*	Far/Rar, Mi2F4/Mi1R1, and Finc/Rinc + C2F3/1108 (OM523742)	8
		B7	Eggplant	*M. incognita* and *M. javanica*	Fjav/Rjav and Finc-F0/R1	9
	30.926852, -83.5123442	B8	Chili pepper	*M. incognita* + *M. incognita*	Finc/Rinc + C2F3/1108 (OM523779 and OM523820)	10
	30.9295267, -83.5148994	B9	Chili pepper	*M. incognita* and *M. javanica* + *M. incognita*	Sec-1 F/R, Fjav/Rjav, Finc/Rinc, and Finc-F0/R1 + MORF/MTHIS (OM523709)	11
	30.9256717, -83.5153601	B10	Eggplant	*M. incognita* and *M. arenaria* + *M. incognita*	inc-k14-F/R, Far/Rar, and Finc-F0/R1 + MORF/MTHIS (OM523788)	12
	31.0288161, -83.4852593	B11	Bell pepper	*M. incognita* + *M. incognita*	Mi duplex and Sec-1 F/R + MORF/MTHIS (OM523796)	13
	31.0276632, -83.5429271	B14	Chili pepper	*M. incognita*	inc-k14-F/R	14
	31.0292011, -83.540111	B15	Yellow squash	*M. arenaria*	inc-k14-F/R, Far/Rar, and Mi2F4/Mi1R1	15
	31.0289425, -83.5412554	B16	Chili pepper	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	16
	31.0369869, -83.5383959	B17	Cucumber	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R + MORF/MTHIS (OM523763)	17
	31.0400366, -83.5400122	B18	Cucumber	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523674)	18
	30.995849, -83.6639	B20	Watermelon	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R + MORF/MTHIS (OM523829, OM523828, and OM523830)	19
	30.9387568, -83.7245351	B23	Sweet corn	*M. incognita*	Mi2F4/Mi1R1	20
	31.030394, -83.490309	B26	Bell pepper	*M. incognita*	Mi duplex and Sec-1 F/R	21
	31.029939, -83.54052	B29	Chili pepper	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523795 and OM523794)	22
	31.03334, -83.540284	B30	Eggplant	*M. incognita*	Mi duplex and Sec-1 F/R	23
Bulloch	32.60816, -81.80321	Bu1	Carrots	*M. incognita*	Finc-F0/R1	24
Colquitt	31.222539, -83.64004	Col2	Cabbage	*M. incognita* + *M. incognita*	Finc/Rinc + C2F3/1108 (OM523688, OM523686, and OM523687)	25
	31.225079, -83.6391	Col3	Cucumber	*M. incognita* and *M. arenaria* + *M. incognita*	Mi duplex, Far/Rar, Mi2F4/Mi1R1, and Finc-F0/R1 + MORF/MTHIS (OM523724)	26
	31.220807, -83.629818	Col4	Cucumber	*M. incognita* and *M. arenaria*	SEC-1F/SEC-1R, Far/Rar, and Finc-F0/R1	27
	31.220173, -83.628829	Col5	Cucumber	*M. incognita* and *M. arenaria* + *M. incognita*	Mi duplex, Far/Rar, and Sec-1 F/R + MORF/MTHIS and C2F3/1108 (OM523697, OM523696, and OM523656)	28

	31.2208249, -83.628446	Col6	Cucumber	*M. incognita* and *M. arenaria* + *M. incognita*	Mi duplex and Far/Rar + MORF/MTHIS (OM523666 and OM523665)	29
	31.220541, -83.620793	Col7	Cucumber	*M. incognita*	SEC-1F/SEC-1R and Mi duplex	30
	31.224024, -83.62962	Col9	Bell pepper	*M. arenaria* + *M. incognita*	Far/Rar + MORF/MTHIS (OM523721 and OM523720)	31
	31.229964, -83.581618	Col10	Cucumber	*M. incognita* and *M. arenaria* + *M. incognita*	Far/Rar and Finc-F0/R1 + MORF/MTHIS (OM523704)	32
	31.228258, -83.534705	Col12	Yellow squash	*M. incognita* and *M. arenaria*	Mi duplex and Far/Rar	33
	31.228717, -83.531855	Col13	Kale	*M. incognita* and *M. arenaria* + *M. incognita*	Far/Rar and Finc-F0/R1 + MORF/MTHIS (OM523708)	34
	31.259843, -83.89635	Col14	Cantaloupe	*M. arenaria* + *M. incognita*	Far/Rar + MORF/MTHIS and TRNAH/MRH106 (OM523711 and OM523712)	35
	31.244989, -83.915464	Col17	Eggplant	*M. incognita*	Mi2F4/Mi1R1	36
	31.140927, -83.860593	Col18	Bell pepper	*M. incognita*	Mi2F4/Mi1R1	37
	31.076238, -83.790107	Col20	Sweet potato	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	38
	31.144397, -83.862328	Col21	Zucchini	*M. incognita*	MORF/MTHIS (OM523825, OM523826, and OM523827)	39
	31.100463, -83.792361	Col22	Broccoli	*M. incognita* and *M. arenaria* + *M. incognita*	SEC-1F/SEC-1R, Far/Rar, and Mi2F4/Mi1R1 + MORF/MTHIS (OM523760)	40
	31.094039, -83.785077	Col23	Tomato	*M. incognita*	Mi duplex	41
	31.074528, -83.785077	Col25	Bell pepper	*M. incognita*	SEC-1F/SEC-1R	42
	31.073058, -83.774479	Col28	Broccoli	*M. incognita*	SEC-1F/SEC-1R	43
	31.070343, -83.7754401	Col30	Bell pepper	*M. incognita* + *M. incognita*	Sec-1 F/R + TRNAH/MRH106 (OM523667)	44
	31.10262, -83.775057	Col32	Broccoli	*M. incognita*	SEC-1F/SEC-1R	45
	31.07863, -83.692796	Col34	Kale	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R + MORF/MTHIS (OM523761)	46
	31.074239, -83.678871	Col35	Napa Cabbage	*M. incognita*	SEC-1F/SEC-1R	47
	31.074264, -83.678587	Col36	Collards	*M. incognita*	SEC-1F/SEC-1R and Finc/Rinc	48
	31.073663, -83.676654	Col37	Kale	*M. incognita*	SEC-1F/SEC-1R	49
	31.310133, -83.943406	Col39	Bell pepper	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523798)	50
	31.245298, -83.912537	Col40	Cucumber	*M. incognita*	Mi duplex	51
	31.24632, -83.911181	Col41	Eggplant	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R and Finc-F0/R1 + C2F3/1108 (OM523768)	52
	31.244136, -83.911785	Col42	Cucumber	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523783)	53
	31.245032, -83.914961	Col43	Cucumber	*M. incognita*	SEC-1F/SEC-1R	54
	31.246695, -83.917276	Col44	Cabbage	*M. incognita*	SEC-1F/SEC-1R, Mi duplex, and Finc-F0/R1	55
	31.144951, -83.86285	Col45	Zucchini	*M. incognita*	MORF/MTHIS (OM523792 and OM523793)	56
Cook	31.114038, -83.496635	Co1	Cucumber	*M. incognita*	SEC-1F/SEC-1R	57
	31.164594, -83.499894	Co3	Bell pepper	*M. incognita*	Mi2F4/Mi1R1	58
	31.160214, -83.50602	Co4	Cowpea	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	59
	31.160214, -83.506025	Co5	Butter Beans	*M. incognita* and *M. arenaria*	Mi duplex and Far/Rar	60
	31.159179, -83.506448	Co6	Mustard greens	*M. incognita* and *M. arenaria*	Mi duplex, Far/Rar, and Finc-F0/R1	61
	31.161636, -83.505258	Co7	Bell pepper	*M. arenaria* + *M. incognita*	Far/Rar + MORF/MTHIS (OM523723)	62

	31.176735, -83.343738	Co9	Cabbage	*M. incognita* and *M. arenaria* + *M. incognita*	SEC-1F/SEC-1R, Far/Rar, and Finc-F0/R1 + MORF/MTHIS (OM523707)	63
	31.266165, -83.489429	Co10	Watermelon	*M. incognita*	Mi2F4/Mi1R1	64
	31.312837, -83.451784	Co11	Watermelon	*M. incognita*	SEC-1F/SEC-1R	65
	31.061089, -83.335104	Co13	Broccoli	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS and TRNAH/MRH106 (OM523661 and OM523662)	66
	31.159029, -83.506792	Co14	Zucchini	*M. incognita*	SEC-1F/SEC-1R and Finc/Rinc	67
	31.158907, -83.50642	Co15	Chili pepper	*M. incognita*	SEC-1F/SEC-1R and Mi duplex	68
	31.163972, -83.501032	Co16	Cucumber	*M. incognita*	SEC-1F/SEC-1R and Finc/Rinc	69
	31.216734, -83.462063	Co17	Chili pepper	*M. incognita*	SEC-1F/SEC-1R and Mi duplex	70
Crawford	32.688907, -83.950428	Cr1	Collards	*M. incognita*	MORF/MTHIS (OM523676, OM523677, and OM523678)	71
	32.340345, -84.023131	Cr2	Vegetables	*M. incognita*	C2F3/1108 (OM523782)	72
Crisp	31.924598, -83.897024	C2	Watermelon	*M. incognita* and *M. arenaria*	Far/Rar, Mi2F4/Mi1R1, and Finc-F0/R1	73
	31.931401, -83.903615	C3	Watermelon	*M. incognita* + *M. incognita*	Mi duplex, Mi2F4/Mi1R1, and Finc-F0/R1 + MORF/MTHIS (OM523648, OM523823, OM523843, and OM523824)	74
	32.011638, -83.939025	C6	Watermelon	*M. incognita*	Mi duplex	75
	31.917264, -83.880229	C8	Watermelon	*M. arenaria* + *M. floridensis*	Far/Rar + TRNAH/MRH106 (OM523765 and OM523764)	76
	31.915386, -83.880775	C9	Watermelon	*M. incognita* and *M. javanica* + *M. incognita*	SEC-1F/SEC-1R, Fjav/Rjav, Mi2F4/Mi1R1, and Finc-F0/R1 + MORF/MTHIS (OM523750)	77
Decatur	30.74785, -84.674915	D1	Tomato	*M. incognita* and *M. arenaria* + *M. incognita* and *M. arenaria*	Mi duplex and Far/Rar + C2F3/1108 (OM523737, OM523738, and OM523835)	78
	31.041928, -84.572096	D3	Sweet corn	*M. incognita* and *M. arenaria*	Mi duplex, Mi2F4/Mi1R1, Far/Rar, Sec-1 F/R, and SEC-1F/SEC-1R	79
	30.90262, -84.510353	D4	Cowpea	*M. incognita* and *M. arenaria*	Far/Rar and Finc-F0/R1	80
		D5	Potato	*M. incognita* and *M. arenaria* + *M. arenaria*	Far/Rar and Sec-1 F/R + C2F3/1108 (OM523726, OM523685, OM523684, and OM523683)	81
		D6	Sweet corn	*M. incognita* + *M. incognita*	Mi duplex, Sec-1 F/R, and Finc-F0/R1 + MORF/MTHIS (OM523729 and OM523836)	82
		D9	Eggplant	*M. arenaria* + *M. arenaria*	Far/Rar + C2F3/1108 (OM523839 and OM523725)	83
		D10	Tomato	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523822)	84
		D11	Pepper	*M. incognita* and *M. arenaria* + *M. incognita*	inc-k14-F/R, Mi duplex, Finc-F0/R1, Far/Rar, and Sec-1 F/R + MORF/MTHIS (OM523651 and OM523731)	85
		D12	Okra	*M. incognita* and *M. arenaria* + *M. incognita*	inc-k14-F/R, Mi duplex, Finc-F0/R1, Far/Rar, and Sec-1 F/R + MORF/MTHIS (OM523732)	86
		D13	Butter Beans	*M. incognita* and *M. arenaria* + *M. incognita*	Sec-1 F/R, Mi duplex, Far/Rar, and Mi2F4/Mi1R1 + TRNAH/MRH106 (OM523762)	87
		D14	Yellow squash	*M. incognita* and *M. arenaria*	inc-k14-F/R, Mi duplex, Finc-F0/R1, Far/Rar, and Sec-1 F/R	88
		D15	Zucchini	*M. incognita* and *M. arenaria* + *M. incognita*	Sec-1 F/R, Mi duplex, Far/Rar, and Mi2F4/Mi1R1 + MORF/MTHIS (OM523751)	89
		D16	Cucumber	*M. incognita* and *M. arenaria*	Far/Rar, Mi2F4/Mi1R1, and Finc-F0/R1	90

		D17	Collards	*M. arenaria* + *M. arenaria*	Far/Rar + C2F3/1108 (OM523727 and OM523728)	91
		D18	Cabbage	*M. incognita* and *M. arenaria* + *M. arenaria*	Sec-1 F/R, Far/Rar, and Mi2F4/Mi1R1 + C2F3/1108 (OM523744 and OM523743)	92
Dooly	32.101194, -83.798152	Do9	Butter Beans	*M. incognita* and *M. arenaria* + *M. incognita*	SEC-1F/SEC-1R, Far/Rar, and Finc-F0/R1 + MORF/MTHIS (OM523710)	93
		Do10	Cowpea	*M. incognita* and *M. arenaria*	Mi duplex, Far/Rar, and Sec-1 F/R	94
		Do11	Sweet corn	*M. incognita* and *M. arenaria*	SEC-1F/SEC-1R and Far/Rar	95
	32.09801, -83.810281	Do12	Collards	*M. incognita* + *M. floridensis*	Sec-1 F/R + C2F3/1108 and TRNAH/MRH106 (OM523838, OM523837, and OM523848)	96
	32.132763, -83.876	Do13	Collards	*M. incognita*	SEC-1F/SEC-1R	97
	32.137478, -83.87255	Do14	Cowpea	*M. floridensis*	TRNAH/MRH106 (OM523844, OM523842, and OM523849)	98
	32.115783, -83.967251	Do15	Watermelon	*M. incognita* and *M. arenaria*	inc-k14-F/R and Far/Rar	99
Echols	30.705089, -83.020763	E1	Bell pepper	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R + C2F3/1108 (OM523754)	100
	30.712261, -83.110909	E3	Bell pepper	*M. incognita*	Finc-F0/R1	101
	30.718161, -83.117906	E4	Bell pepper	*M. incognita* and *M. arenaria* + *M. incognita*	inc-k14-F/R, Far/Rar, and Finc-F0/R1 + TRNAH/MRH106 (OM523735)	102
	30.670505, -83.117556	E6	Watermelon	*M. incognita* + *M. incognita*	Sec-1 F/R, Mi duplex, and Mi2F4/Mi1R1 + MORF/MTHIS (OM523834)	103
	30.612856, -83.105732	E7	Bell pepper	*M. incognita*	Finc-F0/R1	104
	30.606031, -83.017563	E9	Cucumber	*M. incognita*	Finc-F0/R1	105
	30.642196, -83.032772	E10	Bell pepper	*M. incognita* + *M. incognita*	Finc-F0/R1 + TRNAH/MRH106 (OM523703)	106
	30.639915, -83.033433	E12	Zucchini	*M. incognita* and *M. javanica*	SEC-1F/SEC-1R and Fjav/Rjav	107
Grady	30.89815, -84.12456	G2	Bell pepper	*M. incognita*	Mi duplex	108
	30.891769, -84.121786	G4	Bell pepper	*M. incognita* and *M. arenaria*	Far/Rar, Mi2F4/Mi1R1, and Finc-F0/R1	109
	30.774251, -84.288809	G5	Eggplant	*M. incognita* and *M. arenaria*	inc-k14-F/R and Far/Rar	110
	30.771397, -84.280681	G6	Bell pepper	*M. incognita*	Mi duplex	111
	30.77121, -84.28133	G7	Bell pepper	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523670 and OM523671)	112
	30.771397, -84.280681	G8	Okra	*M. incognita* and *M. arenaria*	inc-k14-F/R, Mi duplex, Far/Rar, and Finc-F0/R1	113
	30.754632, -84.283597	G9	Tomato	*M. incognita* and *M. arenaria* + *M. incognita*	Far/Rar, Mi2F4/Mi1R1, and Finc/Rinc + C2F3/1108 (OM523739)	114
	30.769285, -84.356467	G10	Yellow squash	*M. incognita* and *M. arenaria*	Far/Rar, Mi2F4/Mi1R1, and Finc-F0/R1	115
	30.8986, -84.124406	G11	Yellow squash	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523803)	116
		G12	Pepper	*M. incognita* + *M. incognita*	Mi duplex + TRNAH/MRH106 (OM523800)	117
	30.997597, -84.214126	G14	Acorn squash	*M. incognita* + *M. incognita*	Finc-F0/R1 + MORF/MTHIS (OM523781)	118
	30.75859, -84.284175	G15	Eggplant	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R + MORF/MTHIS (OM523799)	119
	30.757628, -84.281873	G16	Bell pepper	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R + MORF/MTHIS and TRNAH/MRH106 (OM523801 and OM523802)	120
	30.758124, -84.322287	G17	Chili pepper	*M. incognita*	MORF/MTHIS and C2F3/1108 (OM523659, OM523660, and OM523658)	121

	31.000608, -84.215028	G18	Cucumber	*M. incognita*	MORF/MTHIS (OM523773, OM523772, and OM523771)	122
	31.000351, -84.214802	G19	Eggplant	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523786 and OM523787)	123
	30.997941, -84.214783	G21	Butternut squash	*M. incognita* + *M. incognita*	Mi duplex and Finc-F0/R1 + MORF/MTHIS (OM523790)	124
	31.891918, -84.122224	G22	Cucumber	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R + MORF/MTHIS (OM523806 and OM523807)	125
	30.893843, -84.122712	G23	Cucumber	*M. incognita*	SEC-1F/SEC-1R and Mi duplex	126
	30.759987, -84.288356	G24	Tomato	*M. incognita*	Mi duplex, Sec-1 F/R, and Finc-F0/R1	127
Jeff Davis	31.756715, -82.758928	JD1	Sweet potato	*M. incognita*	Mi2F4/Mi1R1, Finc/Rinc, and Finc-F0/R1	128
	31.756913, -82.75849	JD2	Sweet potato	*M. incognita*	Mi2F4/Mi1R1	129
	31.757444, -82.757917	JD3	Sweet potato	*M. incognita* and *M. arenaria* + *M. incognita*	SEC-1F/SEC-1R, Far/Rar, and Mi2F4/Mi1R1 + MORF/MTHIS (OM523752)	130
Lee	31.835172, -84.052296	L1	Cucumber	*M. incognita*	SEC-1F/SEC-1R	131
	31.814019, -84.060002	L2	Cucumber	*M. arenaria*	inc-k14-F/R and Far/Rar	132
Lowndes	31.789636, -83.398467	Lo1	Yellow squash	*M. incognita* and *M. javanica*	Fjav/Rjav and Finc-F0/R1	133
	30.7894472, -83.4039212	Lo2	Yellow squash	*M. incognita*	MORF/MTHIS (OM523668)	134
	30.752093, -83.412843	Lo4	Acorn squash	*M. incognita* and *M. arenaria*	Far/Rar and Finc-F0/R1	135
		Lo5	Butternut squash	*M. arenaria* + *M. incognita*	Far/Rar + C2F3/1108 (OM523655)	136
		Lo6	Bell pepper	*M. incognita* and *M. javanica*	Mi2F4/Mi1R1, Mi duplex, Fjav/Rjav, and Finc-F0/R1	137
	30.752093, -83.41284	Lo7	Cucumber	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	138
	30.721629, -83.408813	Lo8	Yellow squash	*M. incognita*	SEC-1F/SEC-1R and Mi2F4/Mi1R1	139
	30.721241, -83.409707	Lo9	Chili pepper	*M. incognita* and *M. arenaria* + *M. incognita*	Far/Rar and Finc-F0/R1 + C2F3/1108 (OM523715)	140
	30.717761, -83.405985	Lo10	Chili pepper	*M. incognita* and *M. arenaria* + *M. incognita*	Mi duplex and Far/Rar + MORF/MTHIS and TRNAH/MRH106 (OM523845 and OM523730)	141
	30.717509, -83.407029	Lo11	Chili pepper	*M. incognita* and *M. arenaria* + *M. incognita*	SEC-1F/SEC-1R and Far/Rar + MORF/MTHIS (OM523719 and OM523718)	142
	30.717115, -83.406699	Lo12	Bell pepper	*M. incognita* + *M. incognita* and *M. incognita*, *M. arenaria*, M. paranaensis, and M. ethiopica	SEC-1F/SEC-1R and Finc/Rinc + C2F3/1108 (OM523691 and OM523692)	143
	30.716727, -83.406334	Lo13	Yellow squash	*M. incognita*	Mi duplex	144
	30.715506, -83.406573	Lo14	Bell pepper	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	145
	30.713723, -83.40618	Lo15	Cucumber	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS and TRNAH/MRH106 (OM523673 and OM523672)	146
	30.709145, -83.346734	Lo16	Cucumber	*M. incognita*	Mi2F4/Mi1R1	147
	30.711981, -83.346668	Lo17	Cucumber	*M. incognita* and *M. arenaria*	SEC-1F/SEC-1R and Far/Rar	148
		Lo18	Cabbage	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	149
	30.763899, -83.127447	Lo20	Tomato	*M. incognita* and *M. arenaria* + *M. incognita*	SEC-1F/SEC-1R, Mi duplex, and Far/Rar + C2F3/1108 (OM523701 and OM523700)	150
		Lo21	Chili pepper	*M. incognita* and *M. arenaria*	inc-k14-F/R, Far/Rar, and Mi2F4/Mi1R1	151
		Lo22	Butter Beans	*M. incognita*	Finc-F0/R1	152

	30.76506, -83.13111	Lo23	Cowpea	*M. incognita* and *M. javanica*	Fjav/Rjav and Finc-F0/R1	153
	30.766307, -83.131436	Lo24	Gourd	*M. incognita* and *M. arenaria*	inc-k14-F/R, Mi duplex, and Far/Rar	154
		Lo25	Cantaloupe	*M. incognita* and *M. javanica* + *M. incognita*	SEC-1F/SEC-1R, Fjav/Rjav, and Finc-F0/R1 + MORF/MTHIS (OM523680, OM523679, and OM523681)	155
		Lo26	Yellow squash	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R + C2F3/1108 (OM523694 and OM523693)	156
		Lo27	Cucumber	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R and Finc-F0/R1 + MORF/MTHIS and C2F3/1108 (OM523698 and OM523699)	157
		Lo28	Watermelon	*M. arenaria*	Far/Rar	158
	30.766755, -83.132361	Lo29	Pumpkin	*M. incognita* and *M. arenaria* + *M. incognita*	SEC-1F/SEC-1R, Mi duplex, and Far/Rar + MORF/MTHIS (OM523753)	159
	30.696993, -83.158542	Lo30	Sweet potato	*M. arenaria*	Far/Rar	160
		Lo31	Sweet potato	*M. incognita*	Finc-F0/R1	161
Macon	32.439882, -84.082693	Ma5	Collards	*M. incognita*	Finc-F0/R1	162
Michell	31.177647, -84.078118	M2	Cantaloupe	*M. incognita* and *M. arenaria* + *M. incognita*	SEC-1F/SEC-1R, Mi duplex, and Far/Rar + MORF/MTHIS (OM523695)	163
	31.177679, -84.097927	M3	Watermelon	*M. incognita*	SEC-1F/SEC-1R	164
	31.185685, -84.081868	M5	Watermelon	*M. incognita*	SEC-1F/SEC-1R and Mi duplex	165
Peirce	31.412266, -82.198635	P1	Yellow squash	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523804)	166
	31.55495, -82.209892	P3	Pumpkin	*M. incognita*	Mi duplex and Sec-1 F/R	167
	31.454978, -82.211424	P5	Okra	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523789)	168
Sumter	31.913343, -84.366768	S2	Snap Beans	*M. incognita* and *M. arenaria*	inc-k14-F/R and Far/Rar	169
	32.020746, -84.138751	S5	Snap Beans	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	170
	31.979494, -84.089197	S7	Yellow squash	*M. arenaria*	Far/Rar	171
	32.134037, -84.116302	S8	Watermelon	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R and Finc-F0/R1 + MORF/MTHIS (OM523713 and OM523714)	172
	31.968015, -84.31799	S9	Snap Beans	*M. incognita* + *M. incognita*	Mi duplex and Sec-1 F/R + MORF/MTHIS (OM523791)	173
	31.883769, -84.309021	S10	Snap Beans	*M. incognita*	SEC-1F/SEC-1R	174
	31.979236, -84.105577	S14	Cucumber	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R and Finc-F0/R1 + MORF/MTHIS (OM523780)	175
Tattnall	32.181833, -82.158061	Ta1	Onion	*M. incognita*	Mi duplex	176
	32.206032, -82.11636	Ta2	Onion	*M. incognita*	Mi duplex and Sec-1 F/R	177
	32.260629, -82.134951	Ta5	Onion	*M. arenaria*	inc-k14-F/R and Far/Rar	178
	32.27719, -82.126851	Ta6	Onion	*M. incognita*	Mi2F4/Mi1R1	179
	32.287424, -82.198241	Ta7	Onion	*M. incognita*	inc-k14-F/R and Finc-F0/R1	180
	32.267009, -82.117177	Ta10	Onion	*M. incognita*	Finc-F0/R1	181
	32.248239, -82.066793	Ta12	Onion	*M. incognita*	MORF/MTHIS (OM523810, OM523809, and OM523811)	182
	32.25614, -82.073093	Ta13	Onion	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	183
	32.273922, -82.078761	Ta14	Onion	*M. incognita*	Mi duplex	184
	32.275855, -82.077012	Ta15	Onion	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523831)	185
Telfair	32.141261, -82.936732	Te1	Watermelon	*M. incognita* + *M. incognita*	Mi duplex and Mi2F4/Mi1R1 + MORF/MTHIS (OM523840, OM523734, and OM523841)	186

	32.030336, -82.832485	Te5	Sweet corn	*M. incognita* and *M. arenaria* + *M. incognita*	SEC-1F/SEC-1R, Far/Rar, and Mi2F4/Mi1R1 + MORF/MTHIS (OM523847 and OM523846)	187
	32.010979, -82.822246	Te7	Watermelon	*M. arenaria* + *M. incognita*	Far/Rar + MORF/MTHIS (OM523733, OM523653, OM523654, and OM523652)	188
	31.845504, -83.08504	Te8	Watermelon	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523816 and OM523815)	189
Thomas	30.988871, -83.761462	Th3	Broccoli	*M. incognita*	SEC-1F/SEC-1R	190
	30.990537, -83.766958	Th4	Broccoli	*M. incognita*	SEC-1F/SEC-1R	191
Tift	31.39635, -83.607681	Ti1	Sweet corn	*M. arenaria*	Far/Rar	192
	31.385225, -83.60856	Ti2	Snap Beans	*M. incognita* and *M. arenaria*	Far/Rar, Mi2F4/Mi1R1, and Finc-F0/R1	193
	31.381088, -83.614377	Ti3	Sweet corn	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	194
	31.390303, -83.608379	Ti4	Watermelon	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523675)	195
	31.45218, -83.615024	Ti7	Chili pepper	*M. arenaria*	Far/Rar	196
	31.44626, -83.59798	Ti9	Eggplant	*M. incognita* and *M. arenaria*	Mi duplex, Far/Rar, and Finc-F0/R1	197
	31.4455151, -83.589959	Ti10	Cucumber	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R and Finc-F0/R1 + MORF/MTHIS (OM523702)	198
		Ti11	Chili pepper	*M. incognita*	Finc-F0/R1	199
	31.35187, -83.623948	Ti12	Cowpea	*M. incognita*	Finc-F0/R1	200
	31.343594, -83.588669	Ti13	Tomato	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	201
	31.365637, -83.652263	Ti15	Bell pepper	*M. arenaria*	Far/Rar	202
	31.437931, -83.618123	Ti18	Cantaloupe	*M. incognita* and *M. arenaria*	Mi duplex, Far/Rar, and Finc-F0/R1	203
	31.439531, -83.613476	Ti19	Cantaloupe	*M. incognita* and *M. arenaria*	SEC-1F/SEC-1R and Far/Rar	204
	31.360071, -83.562009	Ti21	Sweet potato	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	205
	31.452251, -83.619601	Ti22	Yellow squash	*M. incognita*	SEC-1F/SEC-1R and Finc/Rinc	206
	31.435226, -83.621167	Ti23	Cauliflower	*M. incognita*	Mi duplex	207
	31.384489, -83.592447	Ti24	Broccoli	*M. incognita*	Mi duplex	208
	31.382551, -83.601457	Ti25	Broccoli	*M. incognita*	Finc/Rinc	209
	31.366613, -83.652405	Ti26	Zucchini	*M. incognita*	SEC-1F/SEC-1R	210
	31.362468, -83.648117	Ti27	Bell pepper	*M. incognita*	Mi2F4/Mi1R1	211
	31.357709, -83.648593	Ti28	Spaghetti squash	*M. incognita*	SEC-1F/SEC-1R	212
	31.3579045, -83.677918	Ti31	Yellow squash	*M. incognita* + *M. incognita*	Finc/Rinc + MORF/MTHIS (OM523758 and OM523759)	213
	31.369166, -83.572566	Ti32	Sweet corn	*M. incognita* and *M. arenaria* + *M. incognita*	Mi duplex and Far/Rar + MORF/MTHIS (OM523664 and OM523663)	214
	31.369364, -83.574049	Ti33	Snap Beans	*M. incognita*	SEC-1F/SEC-1R and Mi duplex	215
	31.363159, -83.645176	Ti34	Cucumber	*M. incognita*	SEC-1F/SEC-1R	216
	31.546772, -83.573841	Ti35	Bell pepper	*M. incognita*	Finc/Rinc	217
	31.39745, -83.60778	Ti37	Sweet corn	*M. incognita*	SEC-1F/SEC-1R	218
Toombs	32.19385, -82.24881	T1	Onion	*M. incognita* + *M. incognita*	inc-k14-F/R, Sec-1 F/R, and Finc-F0/R1 + MORF/MTHIS (OM523649 and OM523650)	219
	32.187968, -82.22948	T2	Onion	*M. incognita* and *M. arenaria*	inc-k14-F/R and Far/Rar	220
	32.187348, -82.21883	T3	Onion	*M. incognita* + *M. incognita*	Sec-1 F/R + MORF/MTHIS (OM523821)	221
	32.216179, -82.235235	T4	Sweet corn	*M. incognita* and *M. arenaria*	inc-k14-F/R, Mi duplex, Far/Rar, and Sec-1 F/R	222

	32.219388, -82.238362	T5	Sweet corn	*M. arenaria* and *M. javanica*	inc-k14-F/R, Far/Rar, and Fjav/Rjav	223
	32.27007, -82.269843	T7	Onion	*M. incognita* and *M. javanica*	Mi duplex and Fjav/Rjav	224
	32.261824, -82.276297	T8	Onion	*M. arenaria*	Far/Rar	225
	31.971872, -82.307152	T14	Onion	*M. incognita* + *M. incognita*	Mi2F4/Mi1R1, Finc/Rinc, and Finc-F0/R1 + C2F3/1108 and TRNAH/MRH106 (OM523745, OM523682, and OM523746)	226
	32.041801, -82.295119	T18	Onion	*M. incognita*	Mi2F4/Mi1R1	227
	32.14551, -82.300411	T19	Onion	*M. incognita* and *M. arenaria*	Mi duplex, Far/Rar, and Finc-F0/R1	228
Turner	31.811441, -83.5942	Tu1	Watermelon	*M. arenaria* + *M. haplanaria*	Far/Rar + C2F3/1108 (OM523748 and OM523747)	229
		Tu2	Cowpea	*M. javanica* + *M. haplanaria*	Fjav/Rjav + TRNAH/MRH106 (OM523850)	230
	31.81148, -83.48952	Tu3	Watermelon	*M. arenaria* + *M. floridensis*	Far/Rar + TRNAH/MRH106 (OM523736)	231
	31.834667, -83.489315	Tu4	Snap Beans	*M. incognita*	Mi duplex and Mi2F4/Mi1R1	232
	31.820955, -83.487862	Tu5	Snap Beans	*M. arenaria* + *M. arenaria*	Far/Rar + C2F3/1108 (OM523749)	233
	31.812127, -83.570917	Tu7	Snap Beans	*M. incognita*	SEC-1F/SEC-1R and Mi2F4/Mi1R1	234
	31.829349, -83.563178	Tu8	Tomato	*M. incognita*	MORF/MTHIS (OM523812, OM523814, and OM523813)	235
	31.826206, -83.562743	Tu9	Tomato	*M. arenaria*	Far/Rar and Mi2F4/Mi1R1	236
	31.8069, -83.574022	Tu10	Zucchini	*M. incognita*	SEC-1F/SEC-1R	237
Ware	31.181629, -82.595866	Wa1	Cucumber	*M. incognita* + *M. incognita*	Mi duplex and Sec-1 F/R + MORF/MTHIS (OM523832)	238
	31.174353, -82.594805	Wa2	Cucumber	*M. incognita* + *M. incognita*	Mi duplex and Finc-F0/R1 + MORF/MTHIS (OM523778)	239
	31.174401, -82.595264	Wa3	Cucumber	*M. incognita*	Mi duplex and Finc-F0/R1	240
	31.168361, -82.595826	Wa4	Eggplant	*M. incognita*	MORF/MTHIS (OM523818, OM523819, and OM523817)	241
	31.165826, -82.60799	Wa6	Cabbage	*M. incognita* + *M. incognita*	Mi duplex and Finc-F0/R1 + MORF/MTHIS (OM523797)	242
	31.166705, -82.610293	Wa7	Cabbage	*M. incognita*	SEC-1F/SEC-1R, Mi duplex, and Finc-F0/R1	243
	31.16408, -82.60792	Wa8	Tomato	*M. incognita* + *M. floridensis*	SEC-1F/SEC-1R + TRNAH/MRH106 (OM523784 and OM523785)	244
	31.160641, -82.607729	Wa9	Eggplant	*M. incognita*	Mi duplex	245
	31.133636, -82.602675	Wa10	Bell pepper	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523770 and OM523769)	246
Wayne	31.498331, -82.077741	Way1	Broccoli	*M. incognita*	Mi duplex and Sec-1 F/R	247
Wilcox	31.923354, -83.465877	Wi1	Watermelon	*M. incognita* and *M. arenaria* + *M. incognita*	Far/Rar and Finc/Rinc + C2F3/1108 (OM523690 and OM523689)	248
	31.929351, -83.461677	Wi2	Watermelon	*M. incognita* + *M. incognita*	Mi duplex + MORF/MTHIS (OM523669)	249
	31.937708, -83.387165	Wi5	Watermelon	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R, Finc/Rinc, and Finc-F0/R1 + MORF/MTHIS (OM523705)	250
	31.947125, -83.363151	Wi6	Watermelon	*M. incognita*	SEC-1F/SEC-1R and Mi duplex	251
	31.944914, -83.458819	Wi11	Watermelon	*M. incognita*	SEC-1F/SEC-1R and Mi duplex	252
	31.945423, -83.444001	Wi13	Watermelon	*M. incognita* and *M. arenaria*	SEC-1F/SEC-1R, Mi duplex, Far/Rar, and Finc-F0/R1	253
	32.112818, -83.507999	Wi18	Eggplant	*M. incognita* and *M. arenaria* + *M. incognita*	SEC-1F/SEC-1R and Far/Rar + MORF/MTHIS (OM523716 and OM523717)	254
	32.115136, -83.51173	Wi21	Cucumber	*M. incognita* + *M. floridensis*	Mi2F4/Mi1R1 + TRNAH/MRH106 (OM523741 and OM523740)	255

	32.115939, -83.51466	Wi22	Okra	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R + MORF/MTHIS (OM523766 and OM523767)	256
	32.115983, -83.514763	Wi23	Bell pepper	*M. incognita*	Finc-F0/R1	257
	32.108275, -83.483918	Wi25	Watermelon	*M. incognita*	Mi duplex and Finc-F0/R1	258
	32.056841, -83.401541	Wi26	Watermelon	*M. incognita*	Finc-F0/R1	259
	32.052657, -83.396975	Wi27	Watermelon	*M. incognita* and *M. arenaria* + *M. incognita*	SEC-1F/SEC-1R, Far/Rar, Finc/Rinc, and Finc-F0/R1 + C2F3/1108 (OM523722, OM523756, OM523755, and OM523757)	260
	32.053316, -83.394359	Wi28	Watermelon	*M. incognita* and *M. arenaria*	Far/Rar and Mi2F4/Mi1R1	261
	31.895495, -83.551749	Wi29	Zucchini	*M. incognita*	Mi duplex	262
		Wi30	Yellow squash	*M. incognita* and *M. arenaria*	SEC-1F/SEC-1R, Mi duplex, Far/Rar, and Finc-F0/R1	263
Worth	31.604832, -83.742641	W1	Watermelon	*M. arenaria* and *M. javanica* + *M. incognita*, *M. arenaria*, and *M. javanica*	Far/Rar and Fjav/Rjav + MORF/MTHIS (OM523657)	264
	31.569333, -83.741902	W2	Watermelon	*M. incognita* and *M. javanica*	Fjav/Rjav, Mi duplex, Mi2F4/Mi1R1, and Finc-F0/R1	265
	31.432678, -83.68387	W4	Sweet corn	*M. incognita*	Mi duplex	266
	31.378534, -83.671292	W6	Acorn squash	*M. incognita* + *M. incognita*	Finc-F0/R1 + MORF/MTHIS (OM523706)	267
		W7	Spaghetti squash	*M. incognita*	Mi duplex	268
	31.603644, -83.747628	W8	Snap Beans	*M. incognita*	Mi duplex	269
	31.609306, -83.725977	W9	Snap Beans	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R + MORF/MTHIS (OM523805)	270
	31.486924, -83.745261	W10	Sweet corn	*M. incognita* + *M. incognita*	SEC-1F/SEC-1R and Finc-F0/R1 + MORF/MTHIS (OM523777)	271
	31.354308, -83.932333	W13	Bell pepper	*M. incognita*	MORF/MTHIS (OM523776, OM523775, and OM523774)	272

[z]Fields with the same GPS coordinates are neighboring fields with different vegetable crops. [y]The plus symbol separates species identified with species-specific primers and those identified my mitochondrial haplotyping. [x]GenBank accession number of DNA sequences are in parentheses. Mi duplex = *M. incognita*-specific duplex PCR of SEC-1F/SEC-1R and Mi2F4/Mi1R1 primer sets.

Supplemental figures

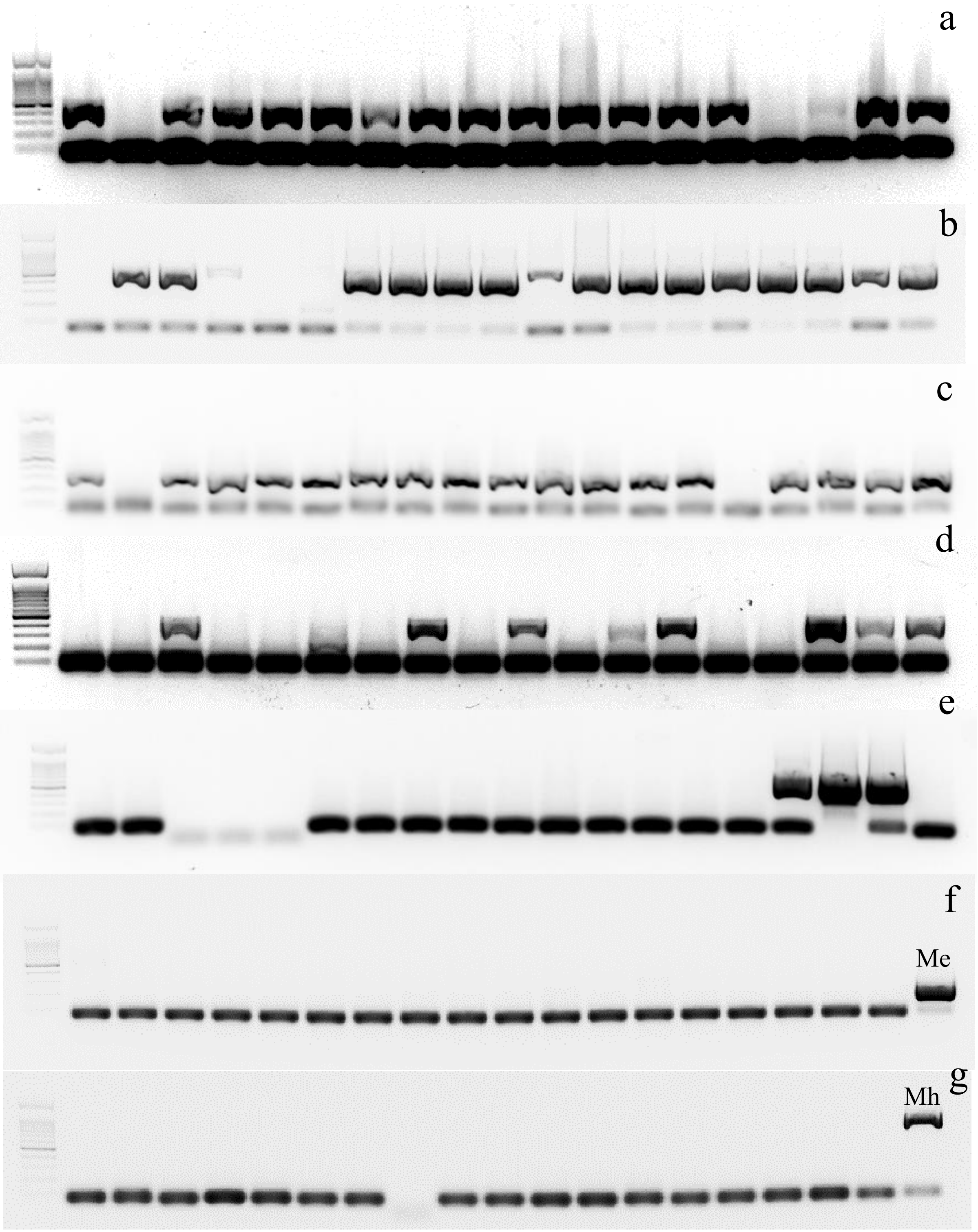

Fig. S1. Typical photographs of agarose gel electrophoresis of DNA samples subjected to PCR of root-knot nematode (*Meloidogyne* spp.) species-specific primer sets for diagnostic screening of species: *M. incognita* (a: FincF0/FincR1, b: SEC-1/SEC-1R and c: MIF4/MiR1), *M. arenaria* (d: Far/Rar), *M. javanica* (e: Fjav/Rjav), *M. enterolobii* (f: Me-F/Me-R, Me = positive control) and *M. hapla* (g: MH0F/MH1R, Mh = positive control).

CHAPTER 4

SEASONAL FLUCTUATIONS IN VERTICAL DISTRIBUTION OF PLANT-PARASITIC NEMATODES AND THEIR INTERACTIONS WITH EDAPHIC FACTORS IN VEGETABLE FIELDS OF SOUTH GEORGIA, USA[3]

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[3] Marquez, Josiah and Hajihassani, Abolfazl. To be submitted to Phytobiomes.

Abstract

Deep-dwelling plant-parasitic nematodes (PPNs) can contribute to nematode management and detection inconsistency. This study aimed to understand how seasonal fluctuation in edaphic factors associate with the vertical distribution of PPNs in south Georgia's vegetable cropping systems. Five-core composite soil samples were taken monthly (March 2020 to February 2022) at 3 random sites for each of the 6 vegetable fields. Fields represented 4 cropping systems (vegetable plasticulture; bare-ground cucumber; and plastic-bed watermelon rotation) and two regions (north and south) from five 15-cm strata. Only soil temperatures and precipitation had seasonal fluctuations, while the other edaphic factors varied across the soil profile. Latitude and stratum had the strongest associations with the nematode composition between fields in the north and south regions. Variations in soil texture, porosity, moisture, and PPN vertical distribution suggest the illuvial zone in the north region was the main factor in differences observed between regions and strata. Seasonal fluctuations in vertical distribution were found among RKN and *Nanidorus* spp. Higher abundances of deep-dwelling PPNs were limited to the south region in the winter for RKN and summer and winter for *Nanidorus* spp., probably due to the illuvial zone barrier in the north region. Since most RKN dwells at ≤ 30 cm during the summer, fumigants/nematicide application are suggested during this season to minimize the risk of escapees.

Keywords: Root-knot nematode, stubby-root nematode, population dynamics, deep soil sampling, ecology

Introduction

Plant-parasitic nematodes (PPNs) are a major threat to vegetable production, especially the root-knot nematode (RKN; *Meloidogyne* spp.) (Collange et al. 2011; Hajihassani et al. 2019; Marquez et al. 2021). PPN seasonal fluctuations in vertical distribution can complicate their management as the effectiveness of chemical fumigants and non-fumigant nematicides can be weakened by deep-dwelling PPNs that escape nematicide contact (Mojtahedi et al. 1991; Robinson et al. 2005a). PPNs in vegetable growing systems in Southern US are managed primarily by preplant soil fumigants that are typically shank injected at 10-35 cm under polyethylene mulch (Noling and Becker 1994; Zasada et al. 2010; Hajihassani 2018; Nnamdi et al. 2022). Since fumigants are costly and a risk to the environment (Desaeger et al. 2020), improving timing of application can allow growers to cut back on fumigant usage. The optimal time of application would be the time PPN populations primarily dwell at the depths of nematicide application. Accurate assessment of nematode populations is also critical for nematode management decision-making. However, over- and under-estimating of PPN abundance can occur by sampling at inappropriate depths (Pudasaini et al. 2006). Samplings at the optimal depths according to the season of the year and PPNs' vertical distribution can improve detection reliability and assist growers in accurate quantifications of PPN populations before a serious outbreak occurs, when more stringent and costly control measures are required.

Factors like the soil temperature, soil moisture, host roots, and soil texture are known to affect seasonal fluctuations in PPN vertical distribution (Norton 1979; O'Bannon et al. 1972; Prot and Vangundy 1981). Soil temperature is a major influencer (Curtis et al. 2009). Robinson (1994) found *M. incognita* to move towards heat irrespective of gravity. *Meloidogyne chitwoodi* infective second-stage juveniles (J2) migrated deeper in the soil profile when temperatures were either too high in summer or low in the winter (Curtis et al. 2009). *Rotylenchulus reniformis* migrate below 60 cm from the soil surface to survive the winter in cotton fields of Texas (Robinson et al. 2005a). A study in Florida found seasonal fluctuations in the vertical distribution of *M. incognita* populations as total J2s found in the upper 0-15 cm stratum increased from 12% to 80% as temperatures increased from the spring to fall season (McSorley and Dickson 1990).

Different nematodes have been known to be associated with varying soil textures (McSorley and Frederick 2002). Most PPN migrates better in sandy soils than heavy-textured soils (Prot and Vangundy 1981). *Meloidogyne* spp. are associated with sandy soils, as migration and penetration of host roots are hampered by increased clay content in natural soils (Mojtahedi et al. 1991; Prot and Vangundy 1981). Yet some clay particles are needed to assist migration, as pure silica sand prevents migration of *M. incognita* (Prot and Vangundy 1981). Soil porosity is affected by the size of soil particles (soil texture), aggregation, and compaction (Curtis et al. 2009). RKN also favors tilled soil with lower bulk density from no-till (Eo et al. 2007). Optimal soil moistures are believed to be at field capacity when restricted movement by low water potential is minimized, hatching and root growth conditions are optimal, and respiratory gases are diffused over large distances (Curtis et al. 2009).

Another important factor in vertical migration of PPNs is the root depth of the host plant. Vegetable roots can penetrate to depths of 1-1.5 meters (Ward 1964, 1967). This can allow *M. incognita* to be found at depths below 100 cm in tomato greenhouses of sandy loam soils in southwestern Ontario (Johnson and McKeen 1973). Yet, in Kentucky, tomato and pepper roots in a raised plastic bed typically reach 13 to 38 cm, with most roots (80-90%) dwelling at the top 15 cm (Coolong et al. 2012; Coolong et al. 2011). Other factors affecting vertical distribution include competition between species, earthworms, and the disruption of tillage (Boag and Yeates 2004).

Vegetable production in South Georgia is diverse consisting of over 30 vegetable crops in bare ground or plasticulture cropping systems, with plasticulture adopted in over half of the vegetable fields (Marquez et al. 2021). Plasticulture consists of raised beds covered with plastic mulch and fitted with drip irrigation (Nnamdi et al. 2022), in which 2 to 4 crops are rotated on the same plastic bed over multiple seasons. However, vegetable growers at higher latitudes mainly grow watermelon or legumes rotated with other row crops (*e.g.*, peanut and cotton) in bare ground systems (Marquez et al. 2021). Currently, information is limited on the population dynamics of PPNs and their association with edaphic factors in vegetable-producing systems in the region. Understanding the PPN seasonal fluctuation in vertical distribution among these different cropping systems and regions can assist growers on the most effective time to fumigate, apply non-fumigant nematicides, rotate vegetables with nonhost/poor host cover crops, fallow their field, and sample soil for PPN population assessment.

A previous survey (Marquez et al. 2021) looked at how edaphic factors affected PPN communities but failed to find any strong relationships as it did not address the seasonal fluctuations in edaphic factors or the vertical distribution of PPNs that could have affected population density estimates. This study takes a holistic approach to understanding how edaphic factors may affect the seasonal fluctuations in PPN vertical distribution in vegetable cropping systems *in situ*. Therefore, the objectives of this study are to determine if there are 1) fluctuations of edaphic factors among cropping systems, regions, seasons, and soil depth, 2) associations of

PPNs with variations in edaphic factors, and 3) seasonal fluctuations in vertical distribution of PPN.

Material and methods

Field selection. PPNs were monitored in 6 fields infested with RKN from 5 counties (Brooks, Crisp, Grady, Lowndes, and Sumter) that represent the variations of vegetable agroecosystems in south Georgia (Table 1), based on crop rotation, region, and type of vegetable bed. Since PPN communities in south Georgia's vegetable fields differed greatly by north and south regions (Marquez et al. 2021), Crisp and Sumter counties were selected to represent the north region (C15 and S24) and Brooks, Lowndes, and Grady to represent the south region (B38, B39, Lo8, and G30). Crisp and Brooks counties also represented plastic watermelon cropping systems at different latitudes (C15 and B38), while Sumter and Brooks represented cucumber bare ground vegetable cropping systems at different latitudes (S24 and B39). Grady and Lowndes counties were selected to represent plastic-bed all vegetable cropping systems in fields with different soil textures (*i.e.*, the field in Grady county has an alluvial zone, while in Lowndes county did not) but similar latitudes. Information on production practices such as previous crop, crop rotation, tillage, fumigation, and nematicide applications were recorded. Locations were selected based on the presence of RKN in vegetable fields from a preliminary sampling of 6-10 random field sites in early March 2020. RKN detection was confirmed by extracting nematodes from 100 cm^3 soil samples by decanting and sieving followed by centrifugal floatation (Jenkins 1964) and analyzing under the inverted microscope (ZEISS Axio Vert.A1, Oberkochen, Germany). Locations were marked by GPS coordinates retrieved with GPS Waypoints Navigator Version 9.14 (DS Software, Las Cruces, NM) mobile application.

Soil sampling. Three specified sites at a significant distance from each other (>100 m) in each field were sampled for two years (March 2020 to February 2022) at the end of each month. Each site was represented by 5 soil cores sampled 2 m apart in a zig-zag pattern across rows. Each soil core was taken ≤ 15 cm from the base of vegetable plants and divided into 5 different strata into 15 cm increments (0-15, 15-30, 30-45, 45-60, and 60-75 cm) with the assistance of the plastic liner. A slit was cut in the plastic liner to provide access to dividing soil core into 15-cm segments. Five soil cores from each stratum were homogenized into a single composite soil sample and stored in ziplocked bags in a cooler. Deep soil cores were taken by pounding a 5-cm diameter soil tube (Giddings machine company inc., Windsor, CO, USA) with a 2.7-kg sliding hammer. Implanted soil tube was recovered by hand or with the assistance of an apparatus consisting of a hand crank 680-kg capacity winch (Reese Towpower, Burnsville, Minnesota, USA) attached to a wood stud frame (Fig S1). Rope and 4 pullies were included in apparatus to increase torque of winch. When soil conditions were extremely wet, soil probe and plastic liner were washed with water and soap between samplings.

Soil analyses. March 2020 soil samples were subjected to soil texture and pH analysis for all 5 soil depths. Soil texture was measured from 50 g of soil with the Bouyoucos hydrometer method (Bouyoucos 1936), while soil pH was measured with 25 g of soil with a hydrogen probe at a 1:1 ratio of soil and water (McLean 1983). Bulk density was estimated as the total volume sampled over the total oven dry weight (g). Gravimetric soil moisture was estimated by drying 100 ± 5 g of wet soil at 70 °C for at least 48 hours. Total porosity was estimated by the equation, $1 - \frac{bulk\ density}{particle\ density}$, with a particle density of 2.65 g/cm^3. Water-filled porosity was estimated as product of volumetric soil moisture (%) and bulk density (g/cm^3), while air-filled porosity was estimated as the difference of total porosity and water-filled porosity. Precipitation, air temperature, soil temperature, and soil moisture were collected from the University of Georgia Weather Network (http://www.georgiaweather.net/) from the nearest weather station to each filed. Soil temperature was measured at the 5 and 20 cm depths with a 109 temperature probe (Cambell Scientific, Logan, UT). Volumetric soil moisture was measured with a 616 30-cm water content reflectometer (Cambell Scientific, Logan, UT) and precipitation with a 20-cm funnel orifice rain gauge (Cambell Scientific, Logan, UT). Weather data was converted into 30-day means from the day of sampling and the preceding 29 days before sampling based on the average life cycle period for RKN (Ploeg and Maris 1999). Soil taxonomy data was retrieved from USDA-NRCS web soil survey (https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm).

Nematode assays. Nematodes were extracted from the soil as mentioned above and counted under the inverted microscope at a 10-40x magnification. Nematodes were identified to the genus level based on variations of the stylet, head, tail, and other morphological features (Mai and Mullin 1996; Smart and Nguyen 1988). Representative soil samples from each field with high RKN counts were potted with eggplant cv. Black Beauty to rear RKN females for species identification using species-specific duplex PCR of primer sets SEC-1F/SEC-1R and Mi2F4/Mi1R1 described in Hajihassani et al. (2022).

Statistical analysis. To determine if there are variations in edaphic factor composition among the region, cropping system, season, and stratum, an exploratory principal component analysis (PCA) was conducted for longitude, latitude, precipitation (mm), maximum 5-cm soil temperature (°C), minimum 5-cm temperature, maximum 20-cm temperature, minimum 20-cm temperature, sand content (%), clay content (%), gravimetric soil moisture (%), volumetric soil moisture (%), bulk density (g/cm^3), total porosity (%), water-filled porosity (%), and air-filled porosity (%) with 'prcomp' function from *R* package stats (Team 2020). Bi-plots of the first two principal components were plotted with 'fviz_pca_biplot' function from *R* package factoextra (Kassambara and Mundt 2020). Soil samples were plotted by the cropping system, region, season, and stratum to find relationships with edaphic factor compositions.

A non-metric multidimensional scaling (NMS or NMDS) ordination (Clarke 1993) was conducted with Gower's distance and under the "slow and thorough" autopilot mode in PC-ORD 7 to visualize and determine if there were any associations between PPNs and edaphic factors, crop rotations, regions, seasons, and stratum (McCune and Mefford 2016). The NMS analysis was based on two data matrices; a main matrix containing nematode counts in each genus and a secondary matrix consisting of environmental variables [categorical variables = cropping system, region, county, field, field location, season, month, crop, clay layer, and texture class; quantitative variables = strata, latitude, longitude, 5-cm maximum soil temperature, 5-cm minimum soil temperature, 20-cm maximum soil temperature, 20-cm minimum soil temperature, sand content, clay content, gravimetric soil moisture, volumetric soil moisture, porosity, water-filled porosity, and air-filled porosity]. Variables from the secondary matrix were overlayed onto the main matrix ordination solution. Because the nematode counts were highly aggregated and not normally distributed, they were transformed with natural log transformation, *ln*(x+1).

To investigate the effects region and strata have on PPNs, a multi-rank permutation procedure (MRPP) was conducted with the original transformed matrices to determine statistical differences between nematode abundance among the environmental groups (soil depths and regions). Since MRPP only tests group membership, we used indicator species analysis (ISA) to determine if PPN genera are statistically associated with any environmental group by producing indicator species values (Severns and Sykes 2020). These values ranged from perfect association (100) to no association (0) (Dufrêne and Legendre 1997) with probability values generated after 5,000 randomizations with PC-ORD 7.

A 3-way analysis of variance (ANOVA) using a generalized linear mixed model with PROC GLIMMIX from SAS® 9.4 (SAS Inc. Cary, NC), compared region, time of year (month or season), and stratum as factors of PPN abundance and edaphic factors. Seasons consisted of spring (March to May), summer (June to August), fall (September to November), and winter (December to February). Region was considered a fixed effect, while trial, season, and soil depth were repeated measures of field location and treated as order-1 autoregressive covariance structure classes (Littell et al. 2000). For soil temperatures, trial and soil depths were repeated measures of Julian day, whereas Julian day was a repeated measure of the nearest weather station. If a 3-way interaction was significant, a post-hoc Tukey multiple comparison test was conducted to separate means by region, month, and soil depth at $\alpha = 0.05$, unless stated as $\alpha = 0.1$.

Results

Seasonal and vertical variations in edaphic factors. The first two principal components of the PCA explained 54.8% of the total variation in edaphic factors (Fig. 2A-D). The first principal component explained 30.1%, while the second principal component explained 24.7% of the total variation in edaphic factors. Along the first principal component, air-filled porosity explained the most variation (37.9%), whereas soil pH explained the least (2.8%). Along the second principal component, minimum 5-cm soil temperature explained the most variation (37.5%), whereas porosity explained the least (0.6%). The composition of edaphic factors cluster among different cropping systems, regions, seasons, and soil depths, but small differences in composition were found between cropping systems, with much overlap. Most edaphic factors were not associated with seasonal variations, but with soil depths and regions. For example, total porosity, air-filled porosity, longitude, and sand content were positively correlated with the shallowest stratum and south region. In contrast, latitude, clay content, volumetric soil moisture, gravimetric soil moisture, water-filled porosity, and bulk density were positively correlated with the deepest stratum and north region. Only the minimum and maximum soil temperatures were associated with seasonal variations. Soil temperatures positively correlated with the summer, while the winter season was negatively correlated. Variations in seasons and soil temperatures were perpendicular to variations in strata, regions, and other edaphic factors.

Plant-parasitic nematodes detected in vegetable fields. The overall incidence (%), mean, and maximum relative abundance (nematodes per 100 cm^3 of soil) of PPNs in all six fields over the course of sampling every month for two year are in order of greatest incidence consisting of *M. incognita* (47.3, 63, 26378), *Mesocriconema* spp. (54.0, 31, 912), *Nanidorus* spp. (30.1, 2, 82), *Helicotylenchus* spp. (19.5, 3, 331), *Hoplolaimus* spp. (7.7, 1, 220), *Pratylenchus* spp. (5.0, <1, 80), *Tylenchorhynchus* spp. (0.6, <1, 4), *Xiphinema* spp. (0.2, <1, 3), *Belonalaimus* spp. (0.4, <1, 16), and *Rotylenchulus reniformis* (0.1, <1, 2). After potting soil samples from February, 2021 (selected based on high RKN counts) from each field with eggplant cv. Black Beauty, RKN females from each field were identified as *M. incognita* with species-specific duplex PCR.

Associations between plant-parasitic nematodes and edaphic factors. NMS analysis was used to determine if PPNs have associations with edaphic factors. A three-dimensional solution was used for the NMS analysis with a final stress of 11.37 (P = 0.04, from 250 Monte

Carlo simulations) and a final instability of < 0.000001. Based on Pearson and Kendall correlations of environmental variables with ordination axes, all three axes explained 75.7% of the variation among variables with axis 1 to 3 explaining 10.2, 30.6, and 34.9%, respectively. When PPN abundances were grouped by region, cropping system, season, and stratum, samples separated best with the region (Fig. S3). Therefore, the first 3 NMS axes were plotted on 3 bi-plots with samples grouped by regions (Fig. 3). North and south region samples separated most along axis 3. Latitude and stratum had the strongest associations with the nematode community, explaining 19.9 and 12.1% of the variation of the NMS axes. All other environmental variables explained <10% of the variation. Specifically, volumetric soil moisture explained 7.3%, gravimetric soil moisture 6.5%, water-filled porosity 6.1%, longitude 4.1%, air-filled porosity 3.5%, minimum 5-cm soil temperature 2.9%, minimum 20-cm soil temperature 2.6%, clay 2.3%, maximum 5-cm soil temperature 2.2%, maximum 20-cm soil temperature 2.1%, soil pH 2.1%, total porosity 1.0%, sand content 0.7%, and precipitation 0.3%. Vectors representing latitude correlated with samples from north region. *Tylenchorhynchus* spp., *Hoplolaimus* spp., *Helicotylenchus* spp., and *Xiphinema* spp. were associated with latitude and the north region along axis 3, while *M. incognita* was associated with the south region and negatively associated with latitude. *Mesocriconema* spp., *Pratylenchus* spp., *Nanidorus* spp., and *Belonalaimus* spp. did not show any associations in the ordination space. *Mesocriconema* spp., *Xiphinema* spp., and *Tylenchorhynchus* spp. centroids were positively associated with stratum, whereas *M. incognita*, *Hoplolaimus* spp., *Nanidorus* spp., *Pratylenchus* spp., *Helicotylenchus* spp., and *Belonalaimus* spp. negatively associated with stratum along axis 2.

The MRPP indicated that PPN abundance significantly differed between regions ($P < 0.000000001$, $T = -111.87139$, $A = 0.03088338$, Table 3) and strata ($P < 0.000000001$, $T = -54.770693$, $A = 0.03026164$, Table 4). Indicator species analysis found *Mesocriconema* spp. and *M. incognita* as significant indicators of the south region, while all other PPNs were significant indicators of the north region. Indicator species analysis also found *M. incognita*, *Helicotylenchus* spp., *Hoplolaimus* spp., *Pratylenchus* spp., and *Belonalaimus* spp. as significant indicators of the 0-15-cm stratum, whereas *Nanidorus* spp. was a significant indicator of the 15-30-cm stratum and *Mesocriconema* spp. of the 30-45-cm stratum.

Edaphic factor variations among regions and strata. The south region had higher mean (±SE) soil temperatures (23.0 ± 0.3°C) than the north region (21.6 ± 0.6°C; $P < 0.05$). Overall, soil temperatures were highest in summer (29.4 ± 0.3°C) followed by spring (24.5 ± 0.4°C), fall (20.7 ± 0.5°C), and winter (13.6 ± 0.4°C) when soil temperatures from both regions were combined. Soil temperatures had a significant interaction between season × region and season × soil depth as seasonal temperature fluctuations depended on the region and stratum (Table S1). Soil temperatures were greatest in the summer followed by spring, fall, and winter in the north region; however, fall temperatures in the south region did not differ from the spring. Spring and fall temperatures at the 20-cm depth also did not differ. Soil temperatures did not reach below freezing (Fig. 4A and B). The north region had more days of minimal 5-cm and 20-cm soil temperatures below 10 °C than the south region ($P < 0.05$; Fig. 4C), while all other temperature ranges above 10 °C were comparable in frequencies between the north and south regions.

Sand content was greater ($P < 0.05$) in the south than the north region (Table 6). Silt was greater in the top 0-15 and 15-30-cm stratum than in the lowest 60-75-cm stratum. Clay content was lowest at the shallowest stratum and highest at the deepest stratum. Soil pH was highest at the 0-15 and 30-45 cm strata and lowest at the deepest stratum. Only clay content had a significant interaction between region and strata, as fluctuations in soil particle content among strata depended on the region. The north region had greater clay content at the 30-45, 45-60, and 60-75-cm stratum than the shallowest 0-15-cm stratum, whereas clay content did not differ among strata within the south region and with north region's shallowest 0-15-cm stratum.

Daily precipitation was greater in the south region than in the north region ($P < 0.05$; Table S2); however, differences in precipitation among regions depended on seasons ($P < 0.05$; Table S3). Overall, summer had the highest precipitation rate followed by winter and spring, with fall having the lowest (Table S2). In the south region, spring and summer had the highest precipitation, while in the north region spring had the lowest precipitation (Table S3). The lowest precipitation occurred in the spring for the north region and fall for the south region. Volumetric and gravimetric soil moistures and water-filled porosity were higher in the north region (Table S2). Soil moistures did not differ between seasons but increased with increasing soil depth. The shallowest stratum had the least soil moisture, and the deepest strata had the most soil moisture. Soil moistures between regions were dependent on seasons (Fig S2). Soil moisture did not differ in the north region between seasons but differed in the south region. Summer had the highest volumetric soil moisture, while fall and winter had the lowest. Lowest gravimetric soil moistures were found in the spring and fall of the south region. Gravimetric soil moisture between regions was also dependent on strata (Table S3). The south region had higher gravimetric soil moistures among strata above 30 cm, while the north region had higher gravimetric soil moistures below 30 cm.

Water-filled porosity was higher in the north region, but total and air-fill porosity did not differ between the regions (Table S2). Winter total and air-filled porosity were higher than any other seasons, while summer water-filled porosity was greater than any other seasons. Total porosity was greatest at the shallowest stratum than any other stratum, whereas the lowest total porosity was found at the 30-45-cm stratum, followed by the 45-60-cm stratum. The 15-30 and 60-75 cm stratum did not differ from the 30-45 or the 45-60-cm stratum. Water-filled porosity increased as soil depth increased, while air-filled porosity decreased. Both the 30-45 and 45-60-cm stratum were comparable in either water- or air-filled porosity. Only water-filled porosity between regions depended on seasons as the highest water-filled porosity was found in the summer of the south region, while all seasons in the north region did not differ from the summer in the south region. Lowest water-filled porosity was found in the south region's winter (Table S3). Soil porosities and gravimetric soil moisture between regions were dependent on strata ($P < 0.05$; Table S4). Total porosity was higher in the south region's 0-15 and 15-30-cm strata but was lower than the north region for the deeper 45-60 and 60-75-cm strata. Water-filled porosity was higher in the south region's 0-15 cm stratum, while air-filled porosity was lower. Water- and air-filled porosities did not differ between regions among the other strata. Gravimetric soil moisture was higher in the north region for strata below 30 cm, but did not differ between regions among strata above 30 cm.

Fluctuations in plant-parasitic nematode abundance. The south region had higher *M. incognita* and *Mesocriconema* spp. abundance than the north region, while *Helicotylenchus* spp. abundance was higher in the north region ($P < 0.05$; Table 6). *Meloidogyne incognita* abundance was lowest in the spring and summer and was higher fall, with winter having the highest abundance. *Mesocriconema* spp. abundance was highest in the winter followed by spring and lowest in the summer and fall. *Nanidorus* spp. abundance was highest in the spring and winter and lowest in the summer and fall. *Helicotylenchus* spp. abundance was highest in the winter, followed by fall and summer. *Helicotylenchus* spp. abundance in the spring did not differ between summer and fall. PPN abundance was highest in the shallowest stratum and decreased as soil depth increased. *Meloidogyne incognita* abundance was highest at the 0-15-cm stratum followed by the 15-30 cm and 30-45-cm stratum, with the lowest abundance found at the deepest stratum. *Mesocriconema* spp. highest abundance was found at the 0-15, 15-30, 30-45, and 45-60-cm stratum, while the lowest abundance was found at the deepest stratum. *Nanidorus* spp. highest abundance was found at both the 0-15 and 15-30-cm stratum followed by 30-45, and 45-60-cm stratum, with the lowest abundance found at the deepest stratum. *Helicotylenchus* spp. abundance was highest at 0-15-cm stratum followed by the 15-30 and 30-45-cm stratum, with the lowest abundance found at the deepest stratum. The 45-60-cm stratum did not differ in abundance of *Helicotylenchus* spp. from the 30-45 and 60-75-cm stratum. Only *M. incognita* abundance ($P < 0.05$) significant season × region and season × stratum interaction as abundance among season were dependent on region (Table S5) and stratum (Table S6). In the south region, abundance of *M. incognita* was highest during winter followed by the fall season, while the lowest abundance was in the summer and spring seasons. In the north region, *M. incognita* abundance in the winter was greater than the summer, however, the highest abundance found in the winter of the north region was comparable to the lowest abundance in the spring and summer of the south region. No difference in *M. incognita* abundance were found in the shallowest 0-15-cm stratum among seasons but were found from the other strata. In the 15-30-cm stratum, higher *M. incognita* abundance was found in the winter than in the spring and summer. The 30-45 and 45-60-cm strata had higher abundance in the winter than in the summer, whereas the lowest 60-75-cm stratum had higher abundance in the spring and winter than in the summer and fall. *Meloidogyne incognita* abundance between region was also dependent on stratum ($P < 0.05$; Table S7). Between the regions, *M. incognita* abundance was higher in the south than the north region for all stata above 45-60 cm. No differences in *M. incognita* abundance were found below 45 cm. *Meloidogyne incognita* ($P < 0.1$) and *Nanidorus* spp. ($P < 0.05$) abundance between regions were also dependent on season and stratum (Fig. 5). The highest abundance of *M. incognita* was limited to the top 0-15-cm stratum in the north region, while the abundance at the 30-45 and 45-60-cm stratum was comparable to the 0-15-cm stratum for the spring seasons in the south region. The highest abundance of *Nanidorus* spp. was limited to the top 0-15 and 15-30-cm strata in the north region, while the 45-60-cm stratum was comparable to the 0-15 and 15-30-cm strata for the summer and winter seasons in the south region.

Discussion

Seasonal, regional, and vertical fluctuations in edaphic factors. PCA suggested seasonal variations of edaphic factors were mainly represented by temperatures and precipitation, as there were no strong associations among the other edaphic factors (sand content, clay content, gravimetric soil moisture, volumetric soil moisture, bulk density, total porosity, water-filled porosity, and air-filled porosity). Moreover, temperatures and precipitation were perpendicular to the other edaphic factors, suggesting no clear associations. Soil moisture mainly remained constant throughout the year which may reflect the grower's ability to maintain constant soil moisture by irrigation. Only the south region had volumetric soil moisture greater in the summer than in the fall and winter, which may reflect high precipitation in the summer and low precipitation in the winter. Total porosity was only found to be enhanced in the winter. It is possible that the lack of tilling during the winter fallow period allows biogenic aggregate formation as crop residue decomposes (Ferreira et al. 2020).

Precipitation had little contribution to the edaphic factor composition but showed seasonal and regional fluctuations, with summer as the rainy season and fall as the dry season and the south region having more precipitation. Precipitation can be a better predictor of seasonal fluctuations in nematode populations throughout the year than temperature. For example, in a study conducted in Spain, *M. hapla* abundance reached a peak in a Kiwi orchard during the dry and unusually warm winter months and declined during the wet periods in the year (Pinochet et al. 1990). In this study, similar patterns in precipitation and PPN abundance were also observed; however, it was confounded by the seasonal fluctuations in soil temperature. For example, the warmest summer season had the highest precipitation and lowest PPN abundance. In addition, high precipitation did not consistently associate with low PPN abundance, as the higher precipitation rates in the south region did not correlate with lower PPN abundance.

Most edaphic factors fluctuated regionally and vertically, with higher sand content and porosity associated with the shallow strata in the south region and clay content, moisture, and bulk density associated with the deeper strata in the north region. This probably reflects the shallow illuvial zone (argillic and/or kandic Bt horizon) in the north region compared to the south region. Some of the fields in Brooks and Grady counties (B38, B38, and G30) in the south region have illuvial zones, but they were either not found at the lowest depth sampled (75 cm) or only had an illuvial zone consisting of only a kandic horizon (G30). This horizon does not require evidence of clay illuviation (Bockheim and Hartemink 2013). Clay content in the north region indicates evidence of clay illuviation. The illuvial zone of the north region was associated with higher gravimetric soil moistures at the lower strata below 30 cm and total porosity below 45 cm. This is expected as clayey soils retain more soil moisture due to more soil pores and higher specific surface area than sandy soils (Brady and Weil 2010).

Limited separation in the edaphic factor composition between cropping systems suggests these crop rotations and cultural practices have minimal effects on edaphic factors compared to region and stratum. NMS further confirmed region and soil depth as major factors in the fluctuations of edaphic factors, PPN abundance, and their associations. *Meloidogyne incognita* and *Mesocriconema* spp. were associated with the south region, while most other PPNs were associated with the north region as found previously in a nematode surveillance study on vegetable fields (Marquez et al. 2021).

Associations of PPN community with edaphic factors. NMS indicated that *Mesocriconema* spp., *Xiphinema* spp., and *Tylenchorhynchus* spp. associated with deeper soil strata. This was confirmed with ISA, as *Mesocriconema* spp. was a significant indicator of the deeper 45-60-cm stratum and *Nanidorus* spp. of the deeper 15-30-cm stratum. *Meloidogyne incognita*, *Helicotylenchus* spp., *Hoplolaimus* spp., and *Pratylenchus* spp. were significant indicators of the shallowest stratum (*i.e.*, 0-15 cm). Further investigation found PPN abundance to be vertically stratified with *M. incognita* and *Helicotylenchus* spp. decreasing in abundance as the soil depth increased, while *Mesocriconema* spp. and *Nanidorus* spp. abundance did not differ above 60 and 30 cm, respectively. McSorley and Dickson (1990) also found *Criconemella sphaerocephala* to be evenly distributed among the 0-15, 15-30, and 30-45 cm strata in north Florida's soybean fields. Ferris et al. (2004) found *Mesocriconema xenoplax* to have a higher abundance at soil depths below 30 and 60 cm. Nematode preferences to different soil depths can be related to its feeding behavior on host plants. For example, *M. hapla* in a grape vineyard was found concentrating near the drip-irrigation lines within the upper 61-cm stratum, as it feeds on fine roots, while *Xiphinema* spp. was found at depths of 122 cm, parasitizing other roots that dwell deeper in the soil (East et al. 2019).

As most nematodes are known to dwell in the uppermost 15-20 cm depth (Yeates 1980), all PPN abundances were found greater in the shallowest stratum (0-15 cm). Roots distribution could explain this pattern as the majority of vegetables dwell in this stratum (Coolong et al. 2012; Coolong et al. 2011). However, root distribution often does not correlate with the vertical distribution of PPNs. This has been the case for *B. longicaudatus*, *P. brachyurus*, and *N. minor* on soybean (Brodie 1976), *P. penetrans* on raspberry (Forge et al. 1998), and *B. longicaudatus* on turf (Mc Groary et al. 2009). The vertical stratification of edaphic factors may be an important confounding factor. For example, nematodes show distinct preferences to different soil textures (Brodie 1976). The higher sand content and total porosity associated with the shallowest soil depths are more favorable to RKN (Greco and Di Vito 2009; Starr et al. 1993; Di Vito et al. 1985). In addition, soil compaction (higher bulk densities/lower total porosity) and low soil water oxygen due to increased soil moistures (Curtis et al. 2009) at deeper depths could limit the ability of nematodes to dwell at deeper soil depths.

ISA also confirmed *M. incognita* as a significant indicator of the south region, while the rest of the PPNs were associated with the north region. Cropping systems are an important factor affecting PPNs between the north and south regions (Marquez et al. 2021). The majority of the fields in the south region were represented by vegetable plasticulture, except for one cucumber bare ground field. However, PCA and NMS analyses indicated that edaphic factors and PPN abundance between cropping systems overlapped greatly and did not separate as well as between region and stratum. Instead, the north region's distinct illuvial zone could be the main cause of regional and vertical variations, as its heavy texture and high soil moisture could form a barrier to many PPNs' vertical migration. There were limited vertical fluctuations in *M. incognita* and *Nanidorus* spp. within the shallower eluvial horizon (30 to 45 cm deep) in the north region. However, higher abundance at deeper stratum were found in the south region, suggesting that illuvial zone in the north region prevented these PPNs from dwelling deep.

Soil temperatures between the region may also provide an unfavorable environment for *M. incognita,* as soil temperatures in the north region reach below the minimal temperatures (base temperature = 10.1 °C) required for egg development (Ploeg and Maris 1999), but not in the south region. Deeper soil temperatures also showed signs of being more insulated from the air temperatures and providing a more favorable environment at temperature extremes. For example, the mean soil temperatures at the 20-cm soil depth did not differ between the spring and fall seasons, while they differed in the shallower 5-cm depth.

Seasonal fluctuations in vertical distribution of PPNs. PPNs complete multiple life cycles during crop rotations and stop reproduction during the winter fallow period (Wesemael and Moens 2008). Overall, most PPNs showed seasonal fluctuations as abundance increased from spring to winter, while populations dwindled over the late winter and into the early spring. Despite most fields being fumigated in March 2020, *M. incognita* reached very high abundance in the fall and winter seasons. Only *M. incognita* had significant seasonal fluctuations in vertical distribution, specifically, of higher abundance in the deeper strata of the winter season. Low PPN abundance in the spring is the reflection of the winter's suppressive effect on PPN development. However, despite winter soil temperatures being unfavorable for RKN reproduction, overall *M. incognita* abundance still peaked from December to January. This was also found in many studies, as RKN was found to peak during the early winter (Forge et al. 1998; Pinochet et al. 1990; Wesemael and Moens 2008). Starr and Jeger (1985) have suggested that *M. incognita* and *M. arenaria* J2 may also serve as an overwintering survival stage. Barker et al. (1969) found that extraction of actively mobile nematodes with Baermann funnel had the highest recovery of RKN J2s and *P. zeae* in the summer and fall, while extraction of immobile nematodes by sugar floatation had the highest recovery in the winter and spring in fields of eastern North Carolina. The high abundance found in the winter months of this study is probably inactive overwintering J2s extracted by the sugar floatation. These inactive overwintering J2s were confirmed to be still infective as soil samples collected in February 2021 were potted with eggplant for molecular identification resulting in plants with 25-100% root galling.

Conclusion

Seasonal fluctuations in the vertical distribution were found for *M. incognita* and *Nanidorus* spp. in the south region, in which higher abundance at deeper strata was limited to the late winter and early spring season. Regional and vertical variations in edaphic factors reflect the distinct illuvial zone in the north region. These results suggest deep-dwelling RKN is not a concern in the summer but should be considered by growers in the winter and early spring when sampling or applying nematicides. *Nanidorus* spp. can also dwell at high abundance in the deeper 45-60 cm stratum in both the summer and winter in the south region. We suggest the optimal time for fumigant/nematicide application would be the summer for RKN management, as most of the populations are above the common depth of application of 35 cm.

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Tables

Table 1. Selected vegetable fields of Southern Georgia.

County	Field	Fumigation[z]	Previous crop	Crops	Region	Bed	Cropping system	Soil series	Illuvial zone	Pi (J2/100 cm^3 of soil)[y]
Brooks	B39	1,3-D	Cotton	Cucumber, Cotton, Rye, Peanut, Fallow	South	Bare ground	Cucumber bare ground	Dothan	> 75 cm (Kandic)	424
Sumter	S24	1,3-D	Corn	Cucumber, Fallow, Squash, Cabbage	North	Bare ground	Cucumber bare ground	Tifton	≥ 30 cm (Argillic/Kandic)	1
Grady	G30	1,3-D, chlorpicrin (60/60%)	Corn	Pepper, Fallow, Butter bean	South	Plastic	Vegetable plasticulture	Dothan	≥ 30-45 cm (Kandic)	912
Lowndes	L8	Paladin/ chlorpicrin (79%/21%)	Squash	Pepper, Cucumber, Fallow, Squash	South	Plastic	Vegetable plasticulture	Lakeland	NA	28
Brooks	B38	1,3-D, chlorpicrin (60/60%)	Cotton	Watermelon, Fallow, Kale, Peanut	South	Plastic	Watermelon plasticulture/ bare ground	Alapaha, Stilson, and Tifton	> 75 cm (Albic/Argillic/Kandic)	12
Crisp	C15	Not fumigated	Cotton	Watermelon, Sorghum, Fallow, Cotton	North	Plastic	Watermelon plasticulture/ bare ground	Dothan, Fuquay, and Tifton	≥ 30 cm (Argillic/Kandic)	68

[z] 1,3-D = 1,3 dichloropropene. [y] Inital population of *M. incognita* sampled in early March 2020 before the study.

Table 2. Summary of environmental variables of sampled vegetable fields.

Data source	Environmental variables	Range	Mean (±SE)
^{z}Weather station (10)			
	Max air temperature (°C)	34.6 - 14.4	26.1 ± 0.2
	Min air temperature (°C)	23.2 - 4.0	14.9 ± 0.2
	Max 5 cm soil temperature (°C)	39.8 - 10.0	26.1 ± 0.2
	Min 5 cm soil temperature (°C)	27.9 - 3.3	19.1 ± 0.2
	Max 10 cm soil temperature (°C)	36.2 - 9.1	24.9 ± 0.2
	Min 10 cm soil temperature (°C)	28.2 - 4.6	19.8 ± 0.2
	Max 20 cm soil temperature (°C)	33.0 - 9.2	23.6 ± 0.2
	Min 20 cm soil temperature (°C)	28.6 - 7.0	21.0 ± 0.2
	Precipitation (mm)	85.6 - 0.0	2.5 ± 0.2
Soil core (8)			
	Sand (%)	97.6 - 6.4	80.8 ± 0.4
	Clay (%)	34.0 - 0.8	12.4 ± 0.3
	pH	6.8 - 4.0	5.7 ± 0.0
	Gravimetric soil moisture (%)	91.6 - 0.6	10.5 ± 0.1
	Volumetric soil moisture (%)	33.8 - 0.2	12.1 ± 0.1
	Bulk density (g/cm3)	1.8 - 0.0	1.2 ± 0.0
	Porosity (%)	99.3 - 31.4	56.0 ± 0.1
	Water filled porosity (%)	43.5 - 0.0	14.4 ± 0.1
	Air filled porosity (%)	99.3 - 0.0	41.6 ± 0.2

^{z}Weather station variables were represented as the month average starting from the day of sampling and 29 days following sampling.

Table 3. Associations of plant-parasitic nematode genera detected in vegetable fields of Southern Georgia with region by Indicator Species Analysis.

Nematode genus	Region	Relative abundance index value^{z}	Relative frequency index value	Indicator index value	*P*-value
Mesocriconema spp.	South	55	69	37.8	0.0052
Meloidogyne incognita	South	66	65	42.7	0.0002
Helicotylenchus spp.	North	81	47	38.0	0.0002
Nanidorus spp.	North	55	40	21.6	0.0468
Hoplolaimus spp.	North	95	27	26.0	0.0002
Pratylenchus spp.	North	62	8	5.1	0.016
Tylenchorhynchus spp.	North	83	2	1.4	0.0042
Xiphinema spp.	North	91	1	0.5	0.0496
Belonalaimus spp.	North	81	1	0.9	0.022

^{z}All index values range from a scale of 0-100 indicating no association to perfect association. Probability values are derived from 5,000 randomizations.

Table 4. Associations of plant-parasitic nematode genera detected in vegetable fields of Southern Georgia with soil depth (cm) by Indicator Species Analysis.

Nematode genus	Stratum	Relative abundance index value^{z}	Relative frequency index value	Indicator index value	P-value
Meloidogyne incognita	0-15	30	68	20.4	0.0002
Helicotylenchus spp.	0-15	41	39	16.2	0.0002
Hoplolaimus spp.	0-15	36	14	5	0.0012
Pratylenchus spp.	0-15	37	11	3.9	0.0008
Belonalaimus spp.	0-15	59	1	0.7	0.0794
Nanidorus spp.	15-30	32	52	16.7	0.0002
Mesocriconema spp.	45-60	22	73	15.9	0.0446
Tylenchorhynchus spp.	60-75	32	1	0.3	0.7173
Xiphinema spp.	60-75	52	1	0.4	0.2442

^{z}All index values range from a scale of 0-100 indicating no association to perfect association. Probability values are derived from 5,000 randomizations.

Table 5. The variations in soil particle content among regions, strata, and their interaction.

Factor 1		Factor 2					
Factor	Level	Factor	Level	Sand (%)	Silt (%)	Clay (%)	pH
Region							
	North			73.7 ± 3.1 b	7.3 ± 0.5 a	17.1 ± 1.9 a	5.6 ± 0.1 a
	South			84.3 ± 1.3 a	5.6 ± 0.8 a	10.1 ± 1.0 a	5.7 ± 0.1 a
Strata (cm)							
	0-15			84.5 ± 2.7 a	8.4 ± 2.1 a	7.1 ± 0.8 c	5.9 ± 0.1 a
	15-30			83.0 ± 2.4 a	7.3 ± 1.2 a	9.8 ± 1.5 bc	5.8 ± 0.1 ab
	30-45			82.0 ± 2.8 a	5.3 ± 0.9 ab	12.7 ± 2.2 ab	5.9 ± 0.1 a
	45-60			80.4 ± 2.8 a	5.5 ± 1.0 ab	14.2 ± 2.6 ab	5.6 ± 0.1 ab
	60-75			74.1 ± 4.8 a	4.3 ± 0.7 b	18.4 ± 2.5 a	5.4 ± 0.1 b
Region		Strata					
	North						
			0-15	86.7 ± 1.7 a	7.1 ± 1.0 a	6.2 ± 0.9 c	5.8 ± 0.2 a
			15-30	79.3 ± 2.1 a	8.3 ± 1.0 a	12.4 ± 1.7 abc	5.8 ± 0.2 a
			30-45	69.7 ± 5.1 a	9.1 ± 1.3 a	21.1 ± 4.2 ab	5.6 ± 0.2 a
			45-60	73.0 ± 5.2 a	5.6 ± 0.8 a	21.4 ± 5.0 ab	5.4 ± 0.2 a
			60-75	59.7 ± 11.7 a	6.3 ± 1.5 a	24.5 ± 3.9 a	5.3 ± 0.2 a
	South						
			0-15	83.4 ± 4.0 a	9.1 ± 3.1 a	7.5 ± 1.1 bc	5.9 ± 0.1 a
			15-30	84.8 ± 3.4 a	6.7 ± 1.8 a	8.5 ± 2.0 abc	5.7 ± 0.2 a
			30-45	88.1 ± 1.5 a	3.4 ± 0.8 a	8.5 ± 1.5 bc	6.0 ± 0.1 a
			45-60	84.0 ± 2.8 a	5.4 ± 1.5 a	10.5 ± 2.5 abc	5.7 ± 0.1 a
			60-75	81.3 ± 2.8 a	3.3 ± 0.5 a	15.4 ± 2.9 abc	5.4 ± 0.1 a

All means (±SE) of soil particle content across columns by each factor(s) with the same letter are not significantly different ($P < 0.05$) according to Tukey HSD test. All edaphic factors in table were collected at one time at the beginning of study.

Table 6. Fluctuations in plant-parasitic nematode abundance (counts/100 cm^3 of soil) among region, season, and stratum.

Factor	Level	*Meloidogyne incognita*	*Mesocriconema spp.*	*Nanidorus spp.*	*Helicotylenchus spp.*	*Hoplolaimus spp.*	*Pratylenchus spp.*
Region							
	North	7 ± 1 b	25 ± 2 b	2 ± 0 a	8 ± 1 a	3 ± 0 a	<1 ± <1 a
	South	91 ± 20 a	35 ± 2 a	1 ± 0 a	0 ± 0 b	0 ± 0 a	<1 ± <1 a
Season							
	Spring	18 ± 3 c	35 ± 3 b	3 ± 0 a	3 ± 0 bc	<1 ± <1 a	<1 ± <1 a
	Summer	30 ± 5 c	19 ± 2 c	1 ± 0 b	3 ± 0 c	1 ± 0 a	<1 ± <1 a
	Fall	127 ± 51 b	18 ± 1 c	1 ± 0 b	3 ± 0 b	1 ± 0 a	<1 ± <1 a
	Winter	76 ± 14 a	54 ± 4 a	2 ± 0 a	4 ± 0 a	<1 ± <1 a	<1 ± <1 a
Strata (cm)							
	0-15	204 ± 65 a	28 ± 3 a	3 ± <1 a	11 ± 1 a	3 ± <1 a	<1 ± <1 a
	15-30	75 ± 11 b	38 ± 4 a	3 ± <1 a	2 ± <1 b	<1 ± <1 a	<1 ± <1 a
	30-45	19 ± 4 c	37 ± 4 a	1 ± <1 b	1 ± <1 c	<1 ± <1 a	<1 ± <1 a
	45-60	7 ± 1 cd	33 ± 3 a	<1 ± <1 c	1 ± <1 bc	<1 ± <1 a	<1 ± <1 a
	60-75	9 ± 4 d	22 ± 3 b	<1 ± <1 d	<1 ± <1 d	<1 ± <1 a	<1 ± <1 a

All nematode abundance means (±SE) across columns by each factor with the same letter are not significantly different ($P < 0.05$) according to Tukey HSD test.

Figures

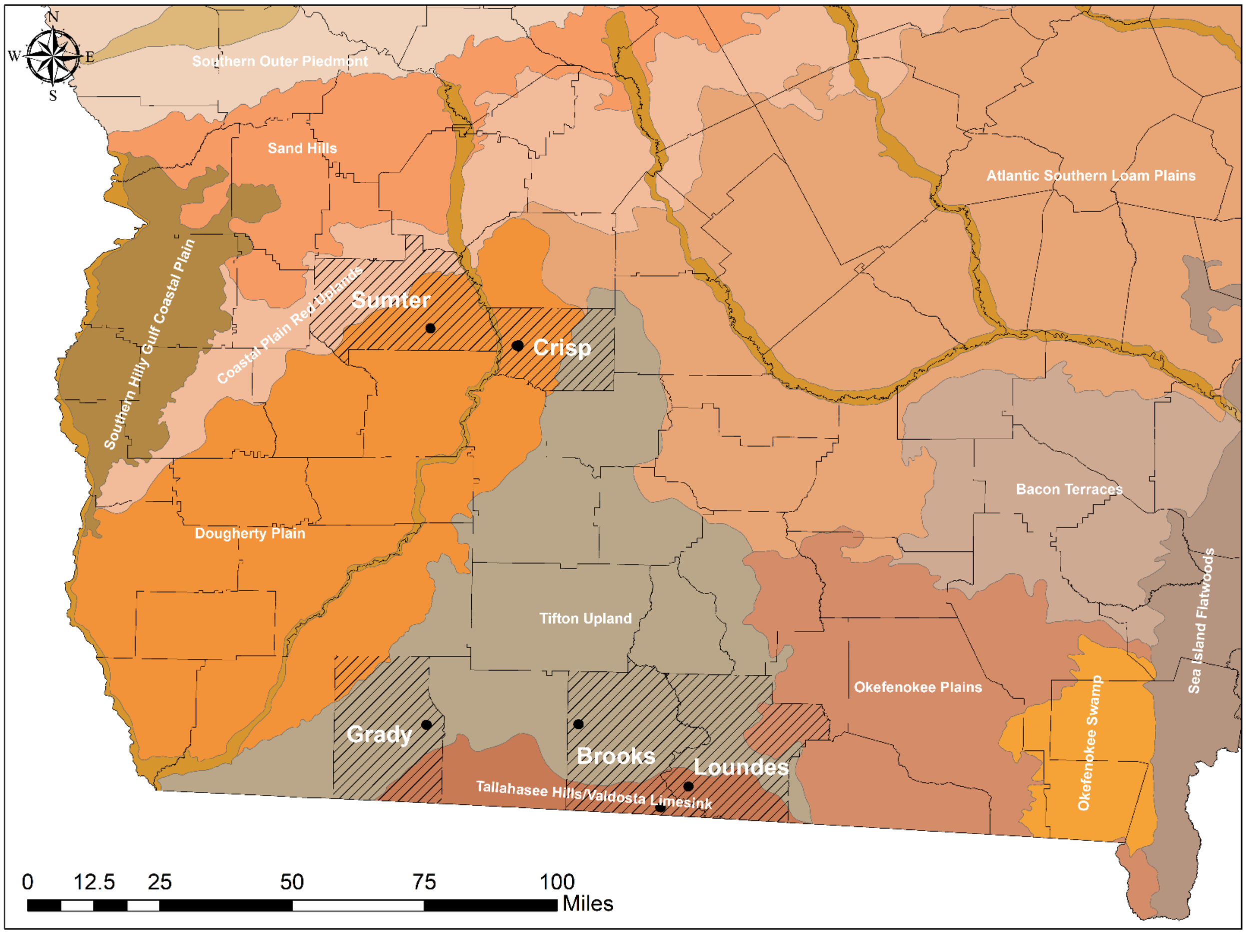

Fig. 1. Vegetable fields sampled in 6 counties of south Georgia from March 2020 to March 2021. Fields are indicated as black dotes. Colored polygons represent level IV ecoregions (Griffith et al. 2001).

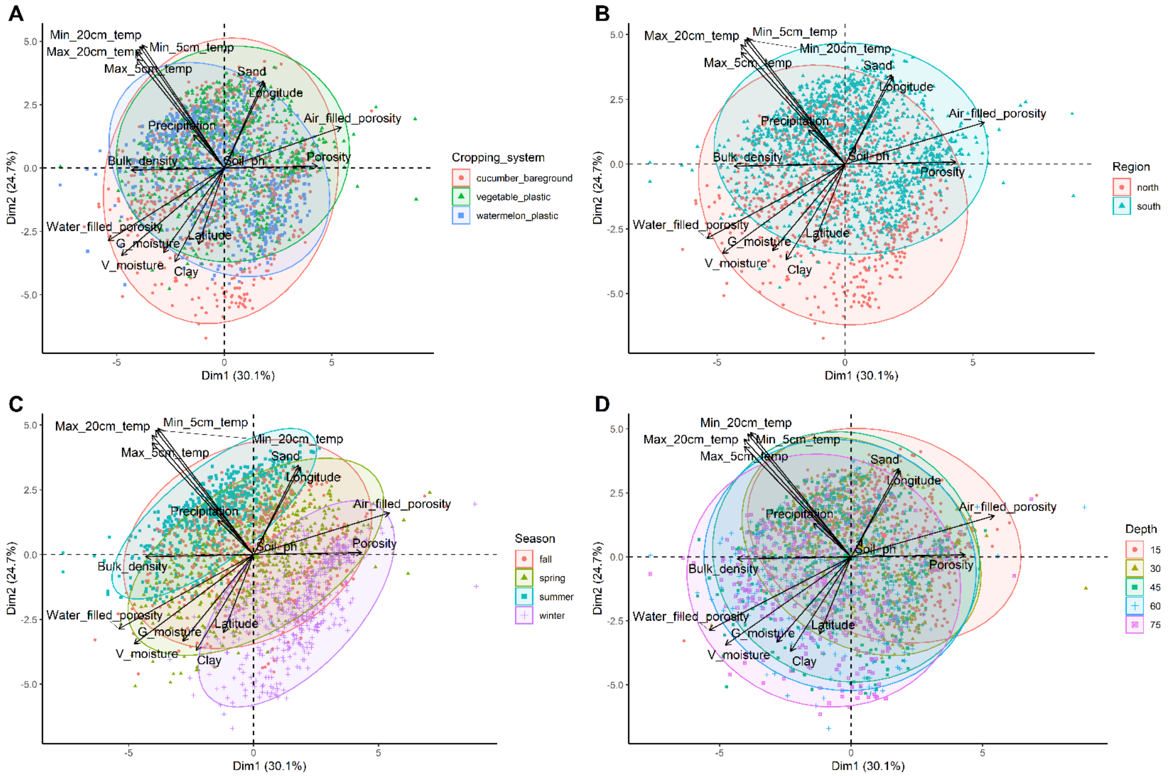

Fig 2. Principal component analysis of edaphic factors among different cropping systems (A), sampling regions (B), depths (C), and seasons (D) plotted on a bi-plot defined by the first two principal component axes (Dim1 and Dim2) which explained 69.2% of variation. Solid arrows indicate direction and weight of edaphic factor vector. V_moisture = volumetric soil moisture and G_moisture = gravimetric soil moisture.

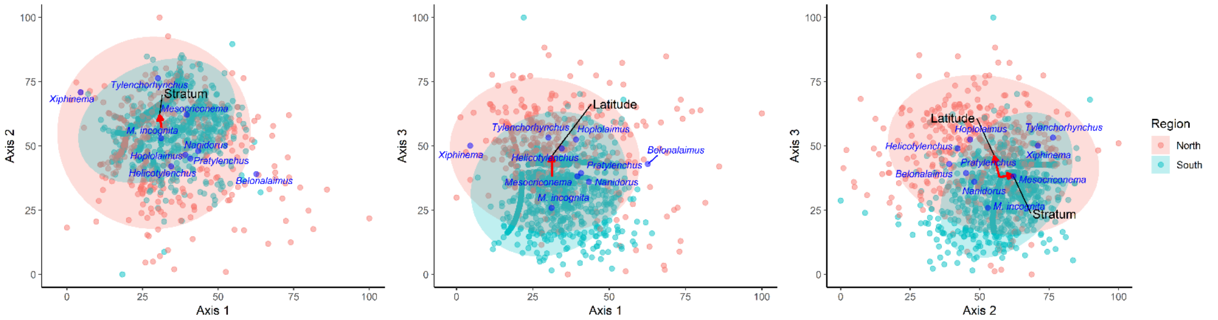

Fig 3. Non-metric multidimensional scaling (NMDS) bi-plot of nematode community among the north (indicated by green pyramid) and south (indicated by green pyramid) regions. Each dot represents a single sample. The ordination solution had a stress value of 11.37. Latitude and strata explained the most variation (>10%), with latitude explaining the most variation (19.9%).

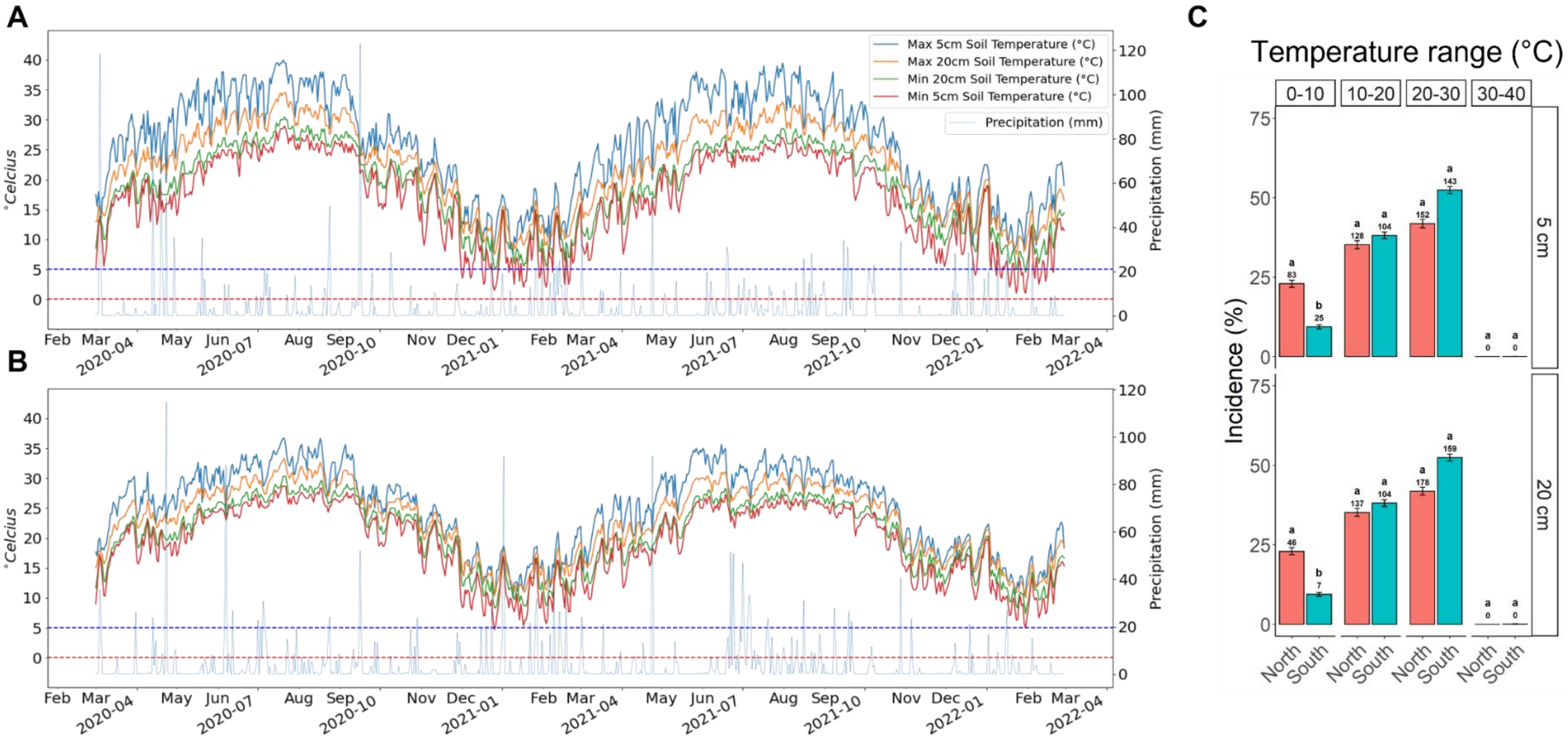

Fig. 4. The maximum and minimum daily soil temperature fluctuations at the 5 and 20 cm depth from the nearest weather station in the A) North and B) South regions during March 2020 to March 2022 and C) incidence of temperature ranges (0-10, 10-20,20-30, and 30-40 °C) between the North and South region at the 5 and 20 cm depths. Letters that are the same between regions are not significantly different according to Tukey's LSD (*P* < 0.05). Average number of days in a year are above the bars

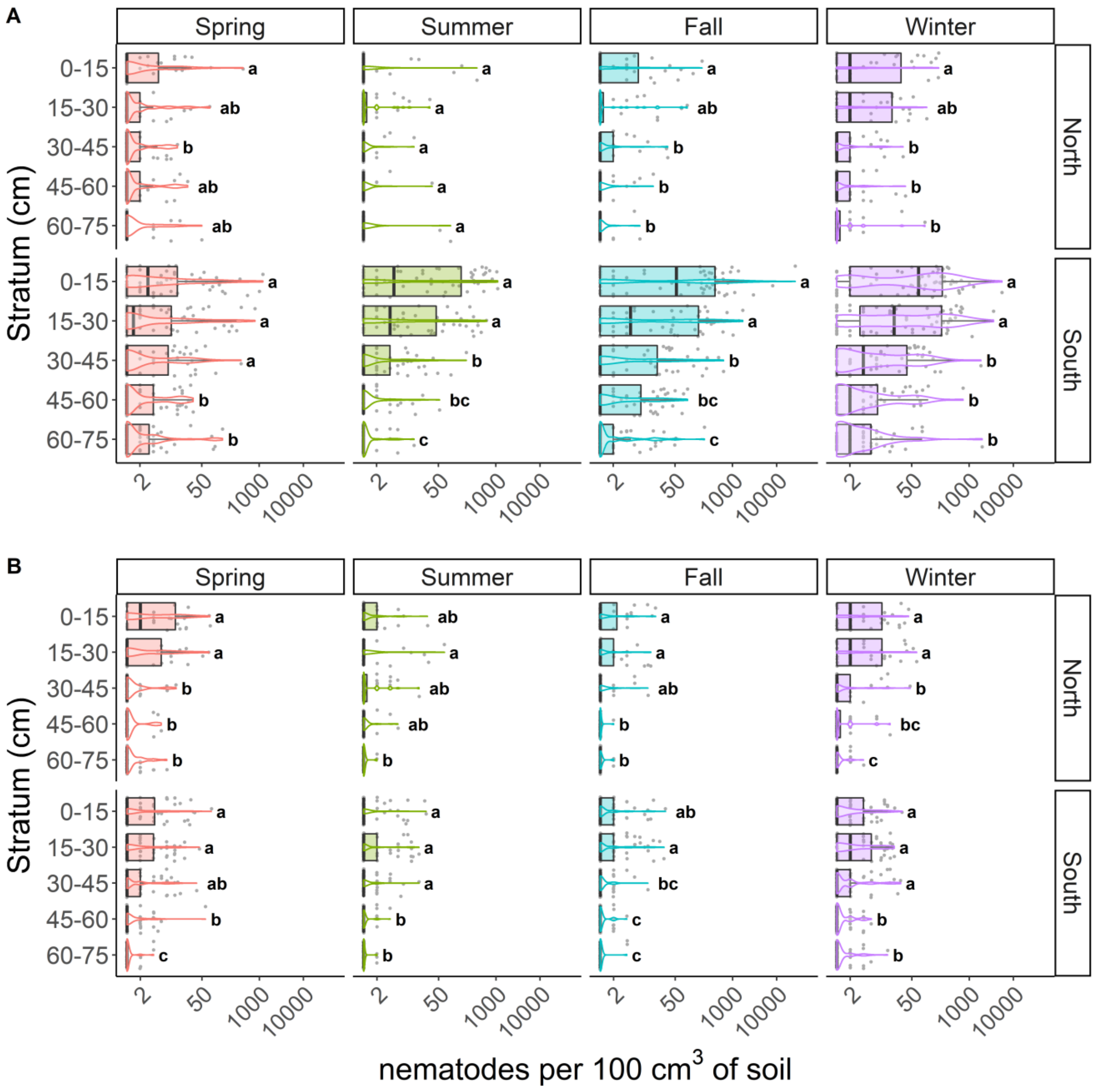

Fig. 5. The seasonal fluctuations in the vertical distribution of (A) *Meloidogyne incognita* and (B) *Nanidorus* spp. abundance between the north and south regions. Letters along seasons and stratum are not significantly different according to Tukey's LSD ($P < 0.05$).

Supplemental tables

Table S1. Seasonal fluctuations in soil temperatures differ between region and soil depth.

Season	Region	Temperature (°C)	Depth (cm)	Temperature (°C)
Spring				
	North	24.1 ± 0.8 b	5	25.2 ± 0.7 b
	South	24.7 ± 0.4 b	20	23.7 ± 0.4 bc
Summer				
	North	30.0 ± 0.6 a	5	29.9 ± 0.5 a
	South	29.1 ± 0.3 a	20	28.9 ± 0.2 a
Fall				
	North	18.6 ± 0.9 c	5	20.5 ± 0.7 d
	South	21.8 ± 0.5 bc	20	21.0 ± 0.5 cd
Winter				
	North	11.7 ± 0.7 d	5	13.5 ± 0.6 e
	South	14.6 ± 0.4 d	20	13.8 ± 0.4 e

All soil temperature means (±SE) across columns with the same letter are not significantly different ($P < 0.05$) according to Tukey HSD test.

Table S2. Fluctuations in precipitation, soil moisture, and porosity among region, season, and stratum.

			Soil moisture (%)		Porosity (%)		
Factor	Level	Precipitation^z (mm)	Volumetric (v/v)	Gravimetric (wt/wt)	Total	Water-filled	Air-filled
Region							
	North	1.0 ± 0.1 a (175)	13.2 ± 0.2 a	11.4 ± 0.1 a	56.0 ± 0.2 a	15.6 ± 0.2 a	40.4 ± 0.4 a
	South	3.3 ± 0.3 a (593)	11.6 ± 0.1 b	10.0 ± 0.1 b	55.9 ± 0.2 a	13.8 ± 0.2 b	42.1 ± 0.3 a
Season							
	Spring	3.5 ± 0.6 b (158)	12.0 ± 0.2 a	10.3 ± 0.1 a	55.9 ± 0.3 b	14.4 ± 0.3 ab	41.5 ± 0.5 b
	Summer	4.3 ± 0.3 a (191)	12.7 ± 0.2 a	10.8 ± 0.2 a	55.2 ± 0.2 b	15.3 ± 0.2 a	39.9 ± 0.4 b
	Fall	0.4 ± 0.1 c (19)	11.8 ± 0.2 a	10.0 ± 0.2 a	55.4 ± 0.3 b	14.1 ± 0.2 ab	41.3 ± 0.4 b
	Winter	1.9 ± 0.2 b (85)	12.1 ± 0.2 a	10.8 ± 0.2 a	57.3 ± 0.2 a	13.9 ± 0.2 b	43.5 ± 0.4 a
Strata (cm)							
	0-15	-	9.1 ± 0.2 e	8.4 ± 0.1 e	58.6 ± 0.4 a	10.2 ± 0.2 d	48.4 ± 0.5 a
	15-30	-	10.6 ± 0.1 d	9.1 ± 0.1 d	55.6 ± 0.2 bc	12.6 ± 0.2 c	43.0 ± 0.4 b
	30-45	-	12.3 ± 0.2 c	10.3 ± 0.1 c	54.8 ± 0.3 c	14.9 ± 0.2 b	39.9 ± 0.5 c
	45-60	-	13.5 ± 0.2 b	11.5 ± 0.1 b	55.6 ± 0.3 b	16.1 ± 0.3 b	39.5 ± 0.5 c
	60-75	-	15.2 ± 0.2 a	13.1 ± 0.2 a	55.3 ± 0.3 bc	18.2 ± 0.3 a	37.1 ± 0.5 d

All means (±SE) of edaphic factors across columns by each factor with the same letter are not significantly different ($P < 0.05$) according to Tukey HSD test. ^{z}Daily means (±SE) are before letters, while total precipitation is in parenthesis.

Table S3. Fluctuations of soil moisture and porosity between regions that depend on season.

			Soil moisture (%)		Porosity (%)		
Region	Season	Precipitation^z (mm)	Volumetric (v/v)	Gravimetric (wt/wt)	Total	Water-filled	Air-filled
North							
	Spring	0.2 ± 0.0 e (7)	13.3 ± 0.3 a	11.3 ± 0.3 ab	56.0 ± 0.5 a	15.9 ± 0.5 ab	40.1 ± 0.9 a
	Summer	1.8 ± 0.2 ab (81)	13.2 ± 0.3 abc	11.2 ± 0.2 abc	55.2 ± 0.4 a	15.8 ± 0.4 abc	39.3 ± 0.7 a
	Fall	1.1 ± 0.2 cd (51)	12.9 ± 0.3 abc	11.0 ± 0.3 abc	55.4 ± 0.5 a	15.3 ± 0.4 abc	40.1 ± 0.8 a
	Winter	0.8 ± 0.1 d (34)	13.6 ± 0.3 ab	12.2 ± 0.2 a	57.4 ± 0.3 a	15.4 ± 0.3 abc	42.1 ± 0.5 a
South							
	Spring	5.2 ± 0.9 bc (233)	11.4 ± 0.2 bc	9.7 ± 0.2 c	55.8 ± 0.4 a	13.7 ± 0.3 bc	42.2 ± 0.7 a
	Summer	5.5 ± 0.5 a (246)	12.4 ± 0.2 a	10.7 ± 0.3 ab	55.2 ± 0.3 a	15.0 ± 0.3 a	40.2 ± 0.6 a
	Fall	0.1 ± 0.0 e (3)	11.2 ± 0.2 c	9.5 ± 0.2 c	55.4 ± 0.3 a	13.5 ± 0.3 bc	41.9 ± 0.5 a
	Winter	2.5 ± 0.3 bcd (110)	11.4 ± 0.2 c	10.1 ± 0.2 bc	57.3 ± 0.3 a	13.1 ± 0.3 c	44.2 ± 0.5 a

All means (±SE) of edaphic factors across columns by region and season with the same letter are not significantly different ($P < 0.05$) according to Tukey HSD test. ^{z}total precipitation are in parenthesis.

Table S4. Fluctuations of soil moisture and porosity between regions that depend on depth.

		Soil moisture (%)		Porosity (%)		
Strata	Region	Volumetric (v/v)	Gravimetric (wt/wt)	Total	Water-filled	Air-filled
0-15						
	North	9.9 ± 0.3 a	8.6 ± 0.2 ef	56.8 ± 0.7 b	11.6 ± 0.5 f	45.2 ± 1.0 b
	South	8.8 ± 0.2 a	8.3 ± 0.2 f	59.5 ± 0.4 a	9.5 ± 0.2 g	50.0 ± 0.6 a
15-30						
	North	11.1 ± 0.2 a	9.2 ± 0.2 ef	54.2 ± 0.4 d	13.6 ± 0.4 de	40.6 ± 0.7 cd
	South	10.4 ± 0.2 a	9.0 ± 0.1 ef	56.3 ± 0.3 bc	12.1 ± 0.2 ef	44.1 ± 0.5 bc
30-45						
	North	13.9 ± 0.3 a	11.7 ± 0.2 bc	54.9 ± 0.4 cd	16.8 ± 0.5 bc	38.1 ± 0.8 de
	South	11.4 ± 0.2 a	9.6 ± 0.1 de	54.7 ± 0.3 d	13.9 ± 0.3 cd	40.8 ± 0.5 de
45-60						
	North	14.9 ± 0.3 a	13.1 ± 0.2 ab	57.2 ± 0.4 b	17.0 ± 0.4 bc	40.1 ± 0.7 de
	South	12.8 ± 0.2 a	10.7 ± 0.2 cd	54.8 ± 0.4 d	15.6 ± 0.3 b	39.1 ± 0.6 ef
60-75						
	North	16.4 ± 0.3 a	14.5 ± 0.3 a	56.9 ± 0.4 bc	18.9 ± 0.4 ab	38.0 ± 0.7 def
	South	14.6 ± 0.3 a	12.4 ± 0.3 b	54.5 ± 0.4 d	17.9 ± 0.4 a	36.6 ± 0.7 f

All means (±SE) of edaphic factors across columns by region and strata with the same letter are not significantly different ($P < 0.05$) according to Tukey HSD test.

Table S5. Fluctuations in plant-parasitic nematode abundance (counts/100 cm^3 of soil) between regions that depend on season.

Region	Season	*Meloidogyne incognita*	*Mesocriconema spp.*	*Nanidorus spp.*	*Helicotylenchus spp.*	*Hoplolaimus spp.*	*Pratylenchus spp.*
North							
	Spring	5 ± 2 cd	33.4 ± 8.0 a	3.8 ± 0.8 a	7.5 ± 2.0 a	2.0 ± 0.6 a	0.4 ± 0.2 a
	Summer	5 ± 2 d	15.9 ± 3.5 a	1.6 ± 0.5 a	8.7 ± 2.5 a	5.5 ± 1.6 a	0.1 ± 0.0 a
	Fall	5 ± 1 cd	9.8 ± 1.8 a	1.1 ± 0.2 a	7.5 ± 2.0 a	3.4 ± 1.1 a	0.1 ± 0.0 a
	Winter	11 ± 2 c	42.8 ± 7.6 a	4.4 ± 0.8 a	11.7 ± 2.1 a	1.7 ± 0.5 a	0.3 ± 0.1 a
South							
	Spring	24 ± 5 c	36.2 ± 4.3 a	2.7 ± 0.4 a	0.8 ± 0.2 a	0.0 ± 0.0 a	0.4 ± 0.2 a
	Summer	43 ± 7 c	21.4 ± 3.0 a	1.0 ± 0.2 a	0.5 ± 0.2 a	0.0 ± 0.0 a	0.1 ± 0.0 a
	Fall	188 ± 77 b	23.0 ± 2.7 a	1.1 ± 0.2 a	0.8 ± 0.2 a	0.0 ± 0.0 a	0.1 ± 0.0 a
	Winter	108 ± 21 a	59.8 ± 5.4 a	2.2 ± 0.2 a	1.1 ± 0.3 a	0.0 ± 0.0 a	0.2 ± 0.1 a

All nematode abundance means (±SE) across columns by region and season with the same letter are not significantly different ($P < 0.05$) according to Tukey HSD test.

Table S6. Fluctuations in plant-parasitic nematode abundance (counts/100 cm^3 of soil) between stratum that depend on season.

Stratum	Season	*Meloidogyne incognita*	*Mesocriconema spp.*	*Nanidorus spp.*	*Helicotylenchus spp.*	*Hoplolaimus spp.*	*Pratylenchus spp.*
0-15							
	Spring	46.1 ± 16.1 abc	15.7 ± 4.4 a	6.7 ± 1.4 a	9.8 ± 3.1 a	1.5 ± 0.7 a	0.6 ± 0.3 a
	Summer	102.0 ± 21.2 ab	21.5 ± 6.0 a	1.7 ± 0.5 a	11.5 ± 4.0 a	6.5 ± 2.6 a	0.2 ± 0.1 a
	Fall	498.3 ± 255.6 a	23.7 ± 5.6 a	2.0 ± 0.4 a	9.2 ± 3.1 a	4.0 ± 1.8 a	0.2 ± 0.1 a
	Winter	172.4 ± 53.9 a	52.0 ± 9.9 a	4.5 ± 0.8 a	15.3 ± 3.4 a	1.7 ± 0.8 a	0.6 ± 0.2 a
15-30							
	Spring	26.0 ± 9.2 cdef	36.6 ± 9.1 a	4.7 ± 1.0 a	2.6 ± 1.0 a	0.2 ± 0.1 a	0.2 ± 0.1 a
	Summer	41.7 ± 10.3 cdef	22.8 ± 5.6 a	2.8 ± 0.8 a	2.2 ± 0.9 a	1.5 ± 0.7 a	0.1 ± 0.0 a
	Fall	101.3 ± 25.2 bc	23.2 ± 4.4 a	2.4 ± 0.5 a	2.6 ± 1.3 a	1.0 ± 0.7 a	0.2 ± 0.1 a
	Winter	133.0 ± 37.0 ab	70.2 ± 12.4 a	5.9 ± 1.0 a	3.0 ± 0.7 a	0.4 ± 0.2 a	0.1 ± 0.1 a
30-45							
	Spring	9.3 ± 3.8 defg	48.9 ± 11.3 a	2.2 ± 0.5 a	1.2 ± 0.8 a	0.1 ± 0.1 a	0.2 ± 0.1 a
	Summer	5.6 ± 2.3 fgh	25.6 ± 6.5 a	1.1 ± 0.3 a	0.9 ± 0.4 a	0.6 ± 0.5 a	0.0 ± 0.0 a
	Fall	26.3 ± 8.3 cde	19.1 ± 3.5 a	0.7 ± 0.2 a	1.1 ± 0.5 a	0.3 ± 0.1 a	0.1 ± 0.0 a
	Winter	35.4 ± 17.3 cd	55.3 ± 8.5 a	2.8 ± 0.6 a	2.1 ± 0.7 a	0.2 ± 0.1 a	0.1 ± 0.1 a
45-60							
	Spring	3.6 ± 0.7 efgh	43.1 ± 9.1 a	1.2 ± 0.6 a	1.1 ± 0.6 a	0.3 ± 0.1 a	0.9 ± 0.7 a
	Summer	1.8 ± 0.6 gh	18.4 ± 4.5 a	0.3 ± 0.1 a	1.3 ± 0.8 a	0.3 ± 0.2 a	0.1 ± 0.1 a
	Fall	6.7 ± 1.5 efgh	15.8 ± 3.8 a	0.2 ± 0.1 a	1.3 ± 0.5 a	0.2 ± 0.1 a	0.1 ± 0.0 a
	Winter	16.1 ± 7.0 cde	56.3 ± 10.4 a	0.8 ± 0.2 a	2.2 ± 0.8 a	0.1 ± 0.1 a	0.5 ± 0.4 a
60-75							
	Spring	6.0 ± 2.0 cdef	32.2 ± 8.4 a	0.4 ± 0.1 a	0.3 ± 0.2 a	1.2 ± 0.8 a	0.1 ± 0.0 a
	Summer	1.8 ± 1.0 h	9.4 ± 2.8 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.3 ± 0.1 a	0.0 ± 0.0 a
	Fall	4.8 ± 2.3 gh	11.2 ± 3.6 a	0.1 ± 0.1 a	1.0 ± 0.5 a	0.2 ± 0.1 a	0.0 ± 0.0 a
	Winter	24.2 ± 18.3 cdef	36.9 ± 7.6 a	0.6 ± 0.2 a	0.6 ± 0.2 a	0.4 ± 0.2 a	0.0 ± 0.0 a

All nematode abundance means (±SE) across columns by stratum and season with the same letter are not significantly different (*P* < 0.05) according to Tukey HSD test.

Table S7. Fluctuations in plant-parasitic nematode abundance (counts/100 cm^3 of soil) between regions that depend on depth.

Stratum	Region	*Meloidogyne incognita*	*Mesocriconema* spp.	*Nanidorus* spp.	*Helicotylenchus* spp.	*Hoplolaimus* spp.	*Pratylenchus* spp.
0-15							
	North	21.9 ± 5.1 c	32.5 ± 7.1 a	5.3 ± 0.9 a	31.7 ± 4.7 a	10.3 ± 2.4 a	0.9 ± 0.2 a
	South	296.1 ± 98.5 a	26.1 ± 3.7 a	3.0 ± 0.4 a	1.4 ± 0.3 a	0.0 ± 0.0 a	0.2 ± 0.1 a
15-30							
	North	6.5 ± 1.4 de	31.8 ± 8.3 a	5.7 ± 1.1 a	6.9 ± 1.4 a	2.3 ± 0.7 a	0.2 ± 0.1 a
	South	110.0 ± 17.4 b	41.4 ± 5.0 a	3.0 ± 0.3 a	0.4 ± 0.1 a	0.0 ± 0.0 a	0.1 ± 0.0 a
30-45							
	North	2.1 ± 0.5 e	38.6 ± 8.8 a	1.6 ± 0.4 a	2.8 ± 0.9 a	1.0 ± 0.4 a	0.0 ± 0.0 a
	South	27.7 ± 7.4 c	36.5 ± 4.2 a	1.8 ± 0.3 a	0.6 ± 0.2 a	0.0 ± 0.0 a	0.1 ± 0.1 a
45-60							
	North	1.9 ± 0.5 de	15.7 ± 3.4 a	0.6 ± 0.2 a	2.1 ± 0.7 a	0.7 ± 0.2 a	0.0 ± 0.0 a
	South	9.6 ± 2.7 d	42.2 ± 5.4 a	0.7 ± 0.2 a	1.2 ± 0.4 a	0.0 ± 0.0 a	0.6 ± 0.3 a
60-75							
	North	2.9 ± 1.1 de	8.7 ± 2.6 a	0.3 ± 0.1 a	0.6 ± 0.2 a	1.5 ± 0.6 a	0.0 ± 0.0 a
	South	12.3 ± 6.9 de	29.3 ± 4.4 a	0.3 ± 0.1 a	0.4 ± 0.2 a	0.0 ± 0.0 a	0.0 ± 0.0 a

All nematode abundance means (±SE) across columns by each factor with the same letter are not significantly different ($P < 0.05$) according to Tukey HSD test.

Supplemental figures

Fig. S1. Apparatus used to pull soil probe consisting of a hand winch, rope, and pullies.

Fig. S2. The variations of soil texture along the soil profile among 6 fields selected in south Georgia.

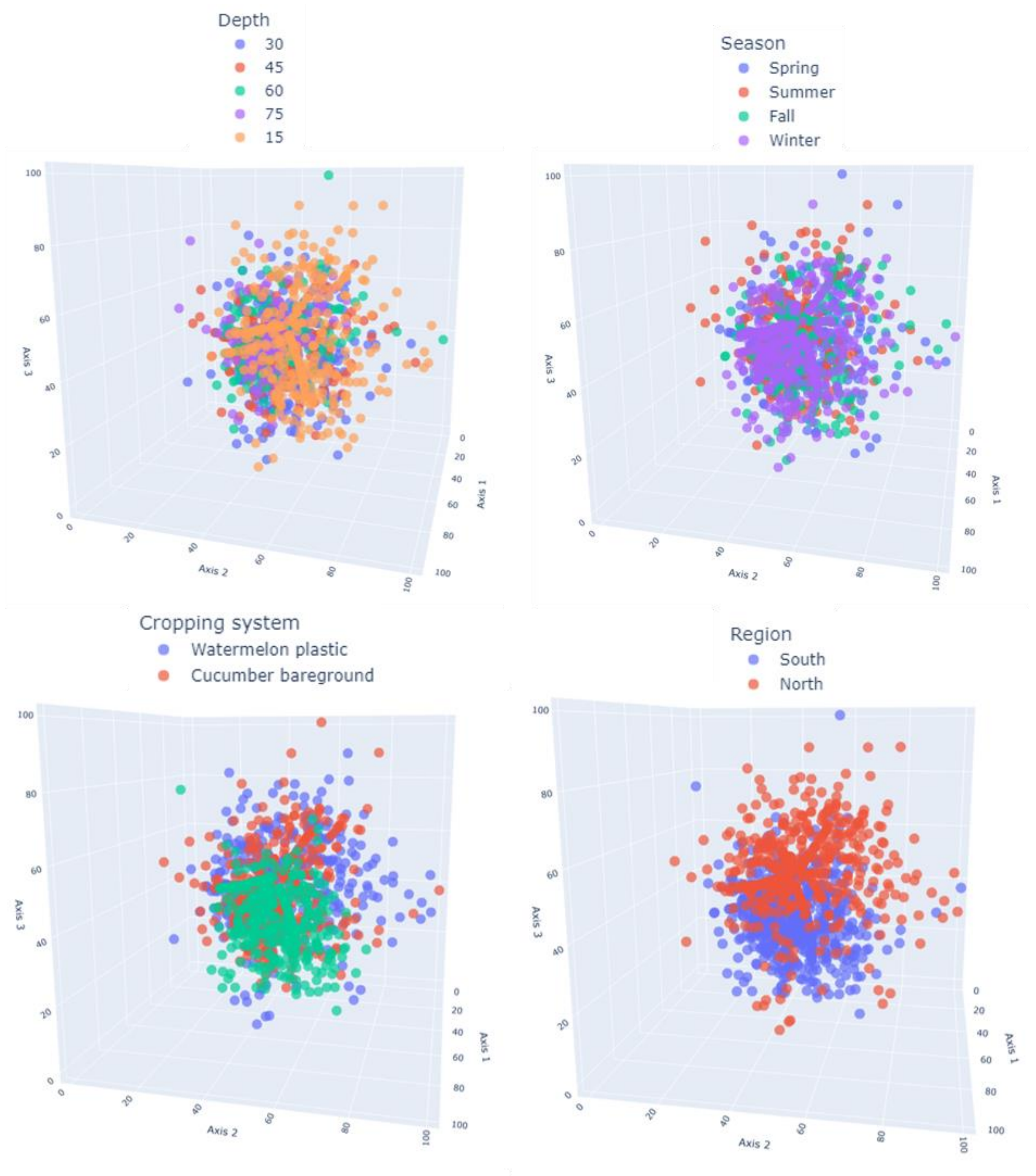

Fig. S3. Three-dimensional Non-metric Multidimensional Scaling ordination of plant-parasitic nematode communities' associations with soil depth, season, cropping system, and region. Different colored dotes represent the different groups in each environmental variable indicated in the legend of each figure.

CHAPTER 5

EVALUATION OF SUMMER AND WINTER COVER CROPS FOR VARIATIONS IN HOST SUITABILITY FOR *M. INCOGNITA*, *M. ARENARIA*, AND *M. JAVANICA*[4]

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[4] Marquez, Josiah, Hajihassani, Abolfazl, and Davis , Richard F. Accepted by Nematology (https://doi.org/10.1163/15685411-bja10172). Reprinted here with permission of publisher.

Abstract

Root-knot nematode (RKN) is an important pathogen on vegetables; therefore, planting a non- or poor host cover crop following a susceptible vegetable is a promising management option. This study builds upon previous studies and evaluates the variations in host status of cover crop candidates for reducing the reproduction of RKN populations in Georgia (*Meloidogyne incognita*, *M. arenaria*, and *M. javanica*) to shed light on previous inconsistencies regarding the host status of cover crops and effectiveness in the field. Two greenhouse trials tested the host status of 14 plant species and 18 cultivars plus susceptible tomato cv. Rutgers. Sixty days after inoculation, roots were evaluated for galling (GI) and egg mass index (EI). Gall formation was not a reliable indication of RKN reproduction for many cover crops, which had higher EI than GI ($P < 0.05$). Based on GI, all cover crops were either nonhosts, ranging from nonhosts to poor hosts or poor hosts to all three RKN species, except blue lupine and hairy vetch, which were susceptible to all three RKN species and had a GI and EI equal to the susceptible tomato control. Based on EI, only bahiagrass, bermudagrass, marigold, millet, and velvetbean were either nonhosts or ranged from nonhosts to poor hosts. Eleven cover crops varied in host status to the three RKN species screened, ranging from either nonhosts to poor hosts or poor hosts to susceptible, which could explain inconsistencies in greenhouse and field trials.

Key words: crop rotation, host resistance, host status, integrated pest management, non-parametric test, root-knot nematode, sustainable agriculture, vegetables

Introduction

Since the 1950s, vegetable production in the United States has been heavily dependent on nematicides to mitigate damage and reduce population densities of plant-parasitic nematodes (PPNs). However, with restrictions increasing on soil fumigants due to the harmful effects on non-target organisms (Abawi & Widmer, 2000) and stratospheric ozone (Noling & Becker, 1994), there is a growing need for alternative nematode management approaches.

The practice of incorporating cover crops in a crop rotation has been suggested for vegetable cropping systems to manage soil health, pests, diseases, and weeds (Abawi & Widmer, 2000). Cover crops grown between cash crops during a fallow season (Snapp *et al.*, 2005) are usually incorporated into the soil as green manure (Wang *et al.*, 2001) or remain as surface organic mulch (Quintanilla-Tornel *et al.*, 2016) to serve as a soil amendment. Cover crops have been recommended for vegetable cropping systems, especially in the late summer season for the southeastern region of the United States, to suppress high weed, insect, and disease pressures that peak in the late summer season (Wang *et al*., 2008; McSorley, 2011). Common cover crops grown in Georgia include cowpea (*Vigna unguiculate* cv. Iron clay) and the cool season cover crops, cereal rye (*Secale cereal* cv. Wrens Abruzzi) and crimson clover (*Trifolium incarnatum*) (Gaskin *et al.*, 2018). Though the practice of incorporating cover crops in rotations of peanut (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), and vegetables have been promoted for the many benefits they provide, including the management of soil-borne pathogens (Abawi & Widmer, 2000; Gaskin *et al.*, 2018), the risk of increased root-knot nematode (RKN; *Meloidogyne* spp.) population densities is often overlooked, especially in organic systems.

Root-knot nematode is a major soil-borne pathogen of vegetable crops worldwide and the most frequently found PPN in vegetable fields of Georgia, infesting nearly two-thirds of the fields

(Marquez *et al.*, 2021). *Meloidogyne incognita* is the dominant RKN species in southern Georgia's vegetable fields, followed by *M. arenaria* and *M. javanica* (Marquez, unpublished). These three RKNs are obligatory mitotic parthenogenetic nematodes and are known as the *M. incognita* group (MIG) of species. Despite the availability of vegetable cultivars with resistance to RKN, for example, tomato with the *Mi*-1.1 or *Mi*-1.2 gene (Milligan *et al.*, 1998) and pepper with the *N* or *Me* gene (Barbary *et al.*, 2015), most growers prefer susceptible cultivars due to superior horticultural traits in quality and yield. Although fallowing can curtail RKN population density for the subsequent susceptible vegetable crop, cover cropping is an alternative approach that benefits the soil. For example, cover cropping protects the soil from erosion, reduces soil compaction, increases organic matter contents, amends soil with nitrogen via nitrogen fixation, reduces leaching of nitrogen via nitrogen scavenging (Dabney *et al.*, 2001), and suppresses weeds (Osipitan *et al.*, 2019).

Growing cover crops can adversely impact PPN population densities in the following ways: 1) being a nonhost (no reproduction) or poor host (limited reproduction), 2) producing toxic secondary metabolites, 3) enhancing antagonistic flora and fauna, and 4) serving as a trap crop (Hokkanen, 1991; Halbrendt, 1996; Wang *et al.*, 2002; Hamid & Hajihassani, 2020). This study focuses on cover crops serving as a nonhost or poor host that lower initial RKN population densities for the subsequent susceptible crop. The level of RKN reproduction is also dependent on the length of the growing season. In the warmer summer and fall seasons, the life cycle of *M. incognita* is 27 days at 25 °C (Ploeg & Maris, 1999). However, the warmer winters in south Georgia allow *M. incognita* to complete 2 life cycles in roots of a susceptible host grown from January to May (Timper *et al.*, 2006). The potential duration of the cover crop growing season may vary from 5 months over the winter (Timper *et al.*, 2006) to only 30 days in the summer (Wauters *et al.*, 2021).

Vegetables in south Georgia can be rotated throughout the year in the spring, summer, fall, and winter seasons in bare ground or plastic bed cropping systems (Marquez *et al.*, 2021). Although cover crops cannot be utilized in plastic beds that span multiple vegetable crop seasons, growers will typically have a fallow period between plastic bed installations that offers an opportunity for cover cropping. Cover cropping could be implemented in any season between removal of used plastic and installation of new plastic beds. However, some vegetables like beans (*Phaseolus vulgaris*), squash (*Cucurbita pepo*), and cabbage (*Brassica olerace*) that are commonly grown in bare ground cropping systems or watermelon (*Citrullus lanatus*) which is grown in one-season plastic beds may allow higher flexibility in rotating a cover crop.

Although many studies have evaluated the host status of common cover crops in the United States, some cover crops have not been adequately tested, and reports of their host status for RKN are inconsistent. For example, blue lupine (*Lupinus angustifolius*) has been reported to maintain low population densities of *M. incognita* and *M. hapla* in their roots (Dobosz & Krawczyk, 2019), yet higher population densities of *M. incognita* were found in plots with blue lupine preceding susceptible corn (Wang *et al.*, 2004). Cereal rye cv. Wrens Abruzzi has been reported as a poor host (Timper *et al.*, 2006), but other studies found *M. incognita*, *M. arenaria*, and *M. javanica* to reproduce sufficiently and fail to reduce RKN population densities in the field (Timper *et al.*, 2011; Wang *et al.*, 2004). The reported host status of cowpea (*Vigna unguiculata*), jointvetch (*Aeschynomene americana*), and hairy vetch (*Vicia villosa*) also have been inconsistent (Kokalis-Burelle & Rosskopf, 2012; Timper *et al.*, 2006; Wang *et al.*, 2008). Furthermore, seeds of some cultivars tested in previous greenhouse studies are currently unavailable or in limited supply (*e.g.*, sunn hemp [*Crotalaria juncea*] cvs. AU Golden and Tropic Sun), whereas RKN host status has not been tested on other cover crops that are more readily available (*e.g.*, sunn hemp from India [cv. unspecified] and black oat cv. Soil Saver). In addition, some cover crops, including bahiagrass (*Paspalum notatum*), hairy vetch, rye (*Secale cereale*), blue lupine, and browntop millet (*Panicum ramosum*), have not been tested with all three MIG species.

This study is in response to the growing interest in utilizing cover crop rotation for vegetable crops in south Georgia for sustainable management of RKN populations and builds upon previous studies on the host status of cover crops by including currently available cover crops that have not been evaluated for all three MIG species. These include bahiagrass cv. Pensacola, bermudagrass cv. Wrangler, black oat cv. Soil Saver, blue lupine, browntop millet, hairy vetch, and sorghum-sudangrass (*Sorghum bicolor* X *S. sudanense*) cvs. Sweet Leaf, Tall One, and Kandy Graz. We also included the species for which conflicting reports regarding their host suitability are available (*i.e*., blue lupine, cowpea cv. Iron Clay, hairy vetch, rye cv. Wrens Abruzzi, jointvetch, and sesame [*Sesamum indicum*]). This study also attempts to determine whether some cover crops vary in host status to RKN species, which may explain past inconsistencies in reducing RKN population densities. Therefore, 14 species and 18 different cultivars were evaluated for the level of reproduction by endemic Georgia populations of the MIG species to identify cover crops that could be utilized in the region.

Materials and Methods

Cover crop species and cultivars. Greenhouse trials were conducted from November 2018 to June 2019 at the University of Georgia-Tifton campus to test the host status of 14 cover crop species and 18 cultivars (Table 1). Cultivars were selected based on inconsistent or insufficient reporting of host status or reduction in RKN reproduction, lack of tests on all MIG species, adaptability to Georgia’s climate, and seed availability. Others were selected based on well-supported evidence of resistance to RKN species to use in comparisons with those that are uncertain. Cover crops selected were bahiagrass cv. Pensacola (Handcock Seed Company, Dade, FL); bermudagrass cv. Wrangler (Handcock Seed Company, Dade, FL); black oat cv. Soil Saver (Jimmy Carter Plant Materials Center, Americus, GA); cowpea cv. Iron Clay (Handcock Seed Company, Dade, FL); Hairy Vetch (Siegers Seed Company, Moultrie, GA), jointvetch (Handcock seed company, Dade, FL); marigold cv. Crackerjack (Kauffman seeds Inc., Hutchinson, KS); rye cv. Wrens Abruzzi (Jimmy Carter Plant Materials Center, Americus, GA); sesame cv. Benne (Southern Exposure Seeds Exchange, Mineral, VA); Sorghum-sudangrass cv. Sweet Leaf, Tall One, and Kandy Graz (Kauffman Seeds Inc., Hutchinson, KS); sunn hemp cv. AU golden and Tropic Sun (Jimmy Carter Plant Materials Center, Americus, GA); sunn hemp cv. Common (Petcher Seeds LLC, Fruitdale, AL); velvetbean (Petcher Seeds LLC., Fruitdale, AL); blue lupine (Handcock Seed Company, Dade, FL); and millet cv. Browntop (Handcock Seed Company, Dade, FL). A susceptible host, tomato cv. Rutgers (Clifton Seeds Company, Faison, NC) was grown along with cover crops to serve as a susceptible control (Hamidi & Hajihassani, 2020).

Plant propagation. Plants were germinated in 128 seed plug polystyrene trays (Speedling Company, Ruskin, FL) filled with promix biofungicide + mycorrhizae potting mix (Mark’s Greenhouses, Albany, GA) and transplanted into deepots (D40L, 5cm dia. × 25.4 cm deep, Stuewe & Sons Inc, Tangent, Oregon) after 2 weeks, except for the bermudagrass and bahaiagrass, which required at least 4 weeks to reach 5 cm in length before transplanting. Each deepot was filled with a 2:1 (soil:sand v/v) mixture of pasteurized soil (sand 85%, silt 11%, and clay 4%) and washed beach sand. The soil was pasteurized by steam-sterilization at 100°C for 2 continuous days. Deepots were placed on stands spaced at every other hole to reduce contamination and provide space for plant growth. Different species of cover crops were mixed within each stand during randomization. Plants were then fertilized once, 1 week after transplanting, with 5 g of osmocote smart-release fertilizer (15-9-12, The Scotts Company, Marysville, OH). Plants were then watered daily and sprayed as needed to control spider mites (Malathion, Spectracide; and sulfur dust, Lilly Miller). Plants were grown for 60 days at 28 ± 3°C. All cultivars were replicated 6 times in an 18 x 3 factorial arrangement of treatments in a completely randomized design in two trials (November 2018 to February 2019 and March to June 2019).

Nematode inoculation. Inoculum of *M. incognita*, *M. arenaria*, or *M. javanica* came from pure cultures maintained in the greenhouse on eggplant (*Solanum melongena* cv. black beauty). Second-stage juveniles (J2) were extracted in a mist chamber located in a greenhouse (28 ± 3°C) and then collected one to two times within 10 days on nested sieves (100 mesh [149 µm] over 500 mesh [25 µm]). The nematode suspension was cleaned using the centrifugal sugar floatation technique (Jenkins, 1964) for estimating the total J2 number. One day after transplanting, plants were inoculated with 1000 fresh J2 per pot divided between two 2 cm-deep holes that were covered with soil immediately after inoculation. Pots were watered before inoculation, and they were not watered again until one day after inoculation to reduce the loss of J2s through water seepage.

Plant evaluation. Sixty days after inoculation, roots were evaluated for gall index (GI), egg mass index (EI), and root biomass. Roots were first rinsed under running water and dried briefly with a paper towel before weighing. Roots were then stained by boiling the roots for 30 sec in a 1% acid fuchsin solution (Byrd *et al.*, 1983) to assist in counting egg masses using a dissecting microscope. The GI and EI values were based on gall or egg mass counts that were then converted based on a scale of 0-5 in which 0 = none, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 ≥ 100 galls or egg masses (Taylor & Sasser, 1978).

Statistical analysis. To determine if there were differences in GI and EI between cover crops and RKN species, GI and EI data were subjected to a two-way analysis of variance (ANOVA) using the program PROC GLIMMIX with SAS® (v. 9.4, SAS Inc. Cary, NC) in which the two trials were considered a random effect. If a significant interaction was found between cover crops and RKN species, a Dunnett's test served as a post hoc analysis to compare cover crops to the susceptible tomato control ($P < 0.05$) for each RKN species.

Host status characterization from GI and EI was based on a scale modified from Sasser *et al.* (1984) with 0 as nonhost (immune), 1 or 2 as poor host (host resistance), and ≥ 3 as susceptible ($P < 0.05$). Due to noticeable differences between GI and EI, a student's two-sample t-test was conducted on each cover crop by RKN species combination to determine if GI and EI differed ($P < 0.05$). Because GI and EI rank scales are ordinal data, a one-sample non-parametric Wilcoxon signed-rank test was performed to determine the host status of cover crops by testing the host sample population against integers of the ordinal scale for GI and EI (*i.e.*, 0 to 5). This approach allows variations in host suitability to be reflected in ranges among different host-status groups (*e.g.*, nonhost to poor host and poor host to susceptible).

Results

Cover crop host status. Differences in host status were found between using GI and EI. Based on GI alone, all cover crops were either nonhosts, ranging from nonhosts to poor hosts or poor hosts to all three MIG species (Table 2; $P < 0.05$), except hairy vetch which was susceptible to all three RKN species and had a GI and EI equal to the susceptible tomato control (Fig. 1). However, based on EI alone, only marigold, millet, and velvetbean were nonhosts for all three MIG species. Both GI and EI indicated that millet, marigold, bahiagrass, sesame, velvetbean, and bermudagrass were nonhosts to *M. incognita*; millet, marigold, sesame, and velvetbean were nonhosts to *M. arenaria*; and jointvetch, millet, marigold, sunn hemp cv. Tropic Sun, velvetbean, and bermudagrass were nonhosts to *M. javanica* (Table 2).

Only the unspecified cultivar of sunn hemp was consistently a poor host to *M. incognita* based on using either GI and EI, while all other cover crops varied in their host status between GI and EI (Table 2). Egg index indicated sorghum-sudangrass cv. Sweet Leaf, sunn hemp cvs. AU Golden and unspecified as either poor hosts or ranging from poor hosts to susceptible or from nonhosts to poor hosts for all three major MIG species. Specifically, jointvetch and Sunn hemp cv. unspecified were poor hosts of *M. incognita*; jointvetch, sorghum-sudangrass cv. Kandy Graz, and sunn hemp cv. Tropic Sun were poor hosts of *M. arenaria*; and, rye, sesame, and sorghum-sudangrass cv. Tall One, sunn hemp cvs. AU Golden and unspecified were poor hosts to *M. javanica*. There were no cover crops that ranged from nonhosts to poor hosts to *M. incognita*, but bahiagrass and bermudagrass ranged from nonhosts to poor hosts to *M. arenaria*, and bahiagrass and sorghum-sudangrass cv. Sweet Leaf ranged from nonhosts to poor hosts to *M. javanica.*

Egg index indicated that blue lupine, hairy vetch, black oat, and cowpea were either susceptible or ranged from poor host to susceptible to all three RKN species (Table 2). Specifically, blue lupine, black oat, cowpea, rye, hairy vetch, sorghum-sudangrass cv. Kandy Graz, and cv. Tall One were susceptible, whereas sorghum-sudangrass cv. Sweet Leaf, sunn hemp cv. AU Golden, and cv. Tropic Sun ranged from poor hosts to susceptible to *M. incognita*. Cowpea, hairy vetch, and sorghum-sudangrass cv. Tall One were susceptible, whereas blue, black oat, rye, sorghum-sudangrass cv. Sweet Leaf, sunn hemp cvs. AU Golden and unspecified ranged from poor hosts to susceptible to *M. arenaria*. Cowpea and hairy vetch were susceptible, whereas blue lupine, black oat, and sorghum-sudangrass cv. Kandy Graz ranged from poor hosts to susceptible to *M. javanica*.

Gall and egg index comparisons among *Meloidogyne* spp. The GI and EI differed among cover crops and RKN species, and differences among cover crops depended on the RKN species (Cover Crop × RKN interaction, $P < 0.001$). *Meloidogyne incognita* and *M. arenaria* had greater GI than *M. javanica* ($P < 0.05$), whereas *M. incognita* had greater EI than *M. arenaria* followed by *M. javanica* ($P < 0.05$). When the GI and EI of cover crops were compared to the susceptible control within an RKN species (Fig. 1), hairy vetch had GI and EI comparable to the susceptible tomato control for all RKN species. Blue lupine shared similar susceptibility, however, a lower ($P < 0.001$) GI than the susceptible control was found for *M. javanica,* and lower ($P < 0.001$) EI was found for *M. arenaria*. Cowpea EI was comparable to the susceptible control when infected with *M. javanica*.

When GI and EI were compared to each other within cover crops and RKN species (Fig. 2), black oat, cereal rye, cowpea, and all cultivars of sorghum-sudangrass consistently had a greater EI than GI across all RKN species, whereas bahiagrass, blue lupine, hairy vetch, marigold, browntop millet, tomato, and velvetbean consistently had no difference between GI and EI. Other cover crops showed higher EI than GI for a particular RKN species. Specifically, *M. incognita* caused higher EI than GI for black oat, cereal rye, cowpea, all cultivars of sorghum-sudangrass, sunn hemp cvs. AU Golden and Tropic Sun; *M. arenaria* had higher EI than GI for the same cover crops as *M. incognita* with the addition of bermudagrass; and *M. javanica* had a higher EI than GI for the same cover crops as *M. incognita* with the addition of cowpea and jointvetch. No cover crop had a higher GI than EI.

Discussion

Unlike other studies that only use non-parametric analyses for comparisons between crops, this study improves on conventional methods of determining the host status (based on numeric means) by determining the probabilistic frequencies of a cover crop being no different from a host status criterion (0 as nonhost, 1 or 2 as poor host, and ≥ 3 as susceptible host). Moreover, by testing the host sample population against integers of the ordinal scale for GI and EI, variations in host suitability can be reflected in ranges among different host status groups that would otherwise be masked if only numeric means were used for evaluations. This is especially important for host sample populations with large variations and could help explain why some cover crops perform inconsistently in the field. Many cover crops that were ranked intermediate in susceptibility varied in host status (black oat, blue lupine, rye, sorghum-sudangrass cv. Sweet Leaf, sorghum-sudangrass cv. Kandy Graz, and sunn hemp cultivars), which may suggest segregation in host resistance and/or frequency of virulence alleles being expressed in the RKN population (Koutsovoulos *et al.*, 2020).

Most of the cover crops tested in this study had little or no visible galling, however, they allowed reproduction of RKN. Gall formation is not a reliable indication of RKN reproduction and susceptibility/resistance for some plant species (Hajihassani *et al.*, 2020). Since the lack of galls is not an indication of host status for most of the cover crops tested, using GI for determining host status (Sasser *et al.*, 1984) is not appropriate. Many of the cover crops had significantly higher EI than GI; therefore, the number of nonhost cover crops was inflated when GI was used to determine host status. In this study, many cover crops characterized as nonhosts based on GI were poor hosts according to EI. Cover crops with GI comparable to EI were either highly susceptible (hairy vetch and blue lupine) or nonhosts to the MIG species (Bahiagrass, bermudagrass, marigold, millet, and velvetbean).

Since blue lupine and hairy vetch were found to be highly susceptible to the MIG species, these should be avoided in RKN-infested fields. Blue Lupine has been recommended to reduce the population densities of *M. incognita* and *M. hapla* (Dobosz & Krawczyk, 2019), yet blue lupine was found to be susceptible to all three MIG species. Hairy vetch is an effective cover crop to suppress weeds (Mangan *et al.*, 1995) and serves as excellent green manure due to its high nitrogen content (Guertal *et al.*, 1998), yet many field studies suggest that population densities of *M. incognita*, *M. arenaria*, and *M. javanica* have increased after cover cropping with hairy vetch (Guertal *et al.*, 1998; Timper *et al.*, 2006; Wang *et al.*, 2004). Timper *et al.* (2006) found hairy vetches to be excellent hosts for *M. incognita* race 3 with a reproduction factor of ≥ 20. Hairy vetch has also been found to be a host for *Rotylenchulus reniformis* (Guertal *et al.*, 1998; Jones *et al.*, 2006), another important PPN that infects and damages many crops including vegetables.

Black oat, rye, and all cultivars of Sorghum-sudangrass were found susceptible to *M. incognita*. Some black oat cultivars have been found to be resistant to the MIG species (Hamidi & Hajihassani, 2020; Uesugi *et al.*, 2021); however, cv. Soil Saver was susceptible to *M. incognita*, and varied from a poor host to susceptible to *M. arenaria* and *M. javanica*. Borges *et al.* (2009) found five cultivars of black oat to all be susceptible to *M. incognita* race 4. Therefore, we conclude that black oat should be avoided if the field is infested with RKN, especially for *M. incognita*.

The host status and effectiveness of rye cv. Wrens Abruzzi in reducing population densities of RKN in the field is inconsistent (Timper *et al.*, 2006; Timper *et al.*, 2011; Wang *et al.*, 2004). Johnson & Motsinger (1989) found cv. Wrens Abruzzi to be a host for all three MIG species when temperatures ranged from 21-34°C. In this study, cv. Wrens Abruzzi was susceptible to *M. incognita*, ranged from poor host to susceptible to *M. arenaria*, and was a poor host for *M. javanica* based on EI. Although some of the winter cover crops show susceptibility to RKN species, the risk of RKN reproduction may be lowered in a cooler field environment during the winter season than in the greenhouse (28 ± 3°C). Nevertheless, it has been suggested that *M. incognita* can complete

2 life cycles on a susceptible host grown for 5 months in the warmer winters of south Georgia (Timper *et al.*, 2006), which should be comparable to this study (two life cycles in 60 days at 28 ± 3°C). This may result in population densities above the economic threshold for the susceptible cash crop in the spring. Allelopathic compounds produced by rye include aliphatic organic acids (McBride *et al.*, 2000) and benzoxainoids (Meyer *et al.*, 2009) that have nematicidal properties and may contribute to lower population densities of RKN despite reproduction in roots of this cover crop. However, to produce sufficient levels of allelopathic compounds for a reduction in nematode population density, high levels of rye biomass (>4,500 kg/ha) may be required, resulting in more input costs (Timper, 2017).

Sorghum-sudangrass is known to synthesize nematicidal allelopathic compounds including cyanogenic glucoside dhurrin (CGD), which degrades into hydrogen cyanide useful for biofumigation (Dutta *et al.*, 2019). Yet, sorghum-sudangrass often fails to reduce population densities of RKN when rotated in a field, possibly due to lower concentrations of CDG as the crop ages; however, sorghum-sudangrass cultivars can differ greatly in host status. The three cultivars tested in this study were either susceptible, a poor host, or ranged from poor hosts to susceptible to the MIG species. Since all three cultivars were susceptible to *M. incognita*, they would need to be avoided in *M. incognita*-infested fields. It is also important to note that sorghum-sudangrass can increase population densities of other PPNs such as *Paratrichodorus minor*, *Belonolaimus longicaudatus*, *Mesocriconema* spp., *Pratylenchus* spp., and *Tylenchorhynchus* spp. (Crow *et al.*, 2001; McSorley & Dickson, 1995).

Jointvetch was a nonhost to poor host for the MIG species. Although jointvetch has been regarded as resistant to RKN (McSorley, 1999), galling and egg production has been observed in all three MIG species (Kokalis-Burelle & Rosskopf, 2012). In this study, jointvetch was a poor host to *M. incognita* and *M. arenaria* and nonhost to *M. javanica* without any variation in host status. The lack of reduction in population densities of *M. incognita* and *M. arenaria* by jointvetch on the subsequent susceptible soybean crop compared to a soybean monoculture (Rodriguez-Kabana *et al.*, 1990) may reflect jointvetch's poor-host status.

All sunn hemp cultivars varied in host status from poor hosts to susceptible for at least one of the MIG species, only cv. Tropic Sun was a nonhost to *M. javanica*. Most host status studies found low levels of reproduction of RKN species in the roots of sunn hemp cultivars (McSorley, 1999; Mosjidis *et al.*, 2013). Since sunn hemp produces the allelopathic compounds, pyrrolizidine alkaloids (PAs) (Thoden & Boppre, 2010), biomass production plays an important role along with host status in reducing RKN population densities. In most field studies, sunn hemp was successful at suppressing RKN (Wang *et al.*, 2002), yet failure to reduce RKN population densities in the field does occur (Khanal *et al.*, 2021) and may be the result of low levels of RKN reproduction as a poor host over a long cropping season and/or low biomass production.

Cowpea cv. Iron Clay is reported to be resistant to all MIG species (McSorley, 1999) and carries the *Rk* resistance gene, yet some populations of *M. incognita* and *M. javanica* can reproduce well in roots of plants with the *Rk* gene (Roberts *et al.*, 2005). This study found cv. Iron Clay is susceptible to all MIG species based on EI; however, the EI for *M. incognita* and *M. arenaria* was lower than the susceptible tomato. Our results showed that *M. javanica* may cause more injury to cv. Iron Clay, which was consistent with other studies (Wang *et al.*, 2008; Wang *et al.*, 2006). However, GI for cowpea was nearly zero for all RKN species, unlike what was reported by Kokalis-Burelle *et al.* (2013), who reported cowpea roots to be galled by *M. arenaria,* despite low J2 counts from the soil. Cowpea has performed inconsistently in reducing nematode population densities (Roberts *et al.*, 2005; Wang *et al.*, 2008), which could be related to the variation of virulence in RKN populations being tested.

M. incognita and *M. arenaria* are at a higher risk of reproducing on the cover crops tested in this study than *M. javanica*. Although *M. javanica* can also be an aggressive species on some cover crops (Hamidi & Hajihassani, 2020), most of the cover crops in this study were nonhosts for these species. Since *M. incognita* and *M. arenaria* are more prevalent than *M. javanica* in south Georgia's vegetable fields (Marquez *et al*., unpublished), the risk of increasing populations of *M. incognita* and *M. arenaria* is of greater concern.

Selecting cover crops with resistance to multiple RKN species may be more advantageous, as many fields are infested with mixed populations (Marquez *et al*., unpublished). It may be important to consider the synthesis of nematicidal allelopathic compounds among cover crops that allow low levels of RKN reproduction; however, cover crops with the additional benefit of allelopathic effects have not shown improvement in reducing population densities from starving J2s by fallowing or growing a nonhost (McSorley, 2011).

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Tables

Table 1. Cover crop cultivars compared in greenhouse screening trials.

					Resistance or reduction in population density		Susceptibility	
Species common name	Species scientific name	Cultivar	Family	Season	*Meloidogyne* spp.	References	*Meloidogyne* spp.	References
Bahiagrass	*Paspalum notatum*	Pensacola	Grass	Perennial	*M. incognita* and *M. arenaria*	Rodriguez-Kabana *et al.* (1994)[2]; Sumner *et al.* (1999)[2]		
Bermudagrass	*Cynodon dactylon*	Wrangler	Grass	Perennial	*M. incognita* and *M. arenaria*	Johnson *et al.* (1995)[2]; Rodriguez-Kabana *et al.* (1994)[2]		
Black oat	*Avena strigosa*	Soil saver	Grass	Winter				
Blue lupine	*Lupinus pilosus*	VNS	Pedaliaceae	Summer		USDA		
Cowpea	*Vigna unguiculata*	Iron Clay	Legume	Summer	*M. incognita*, *M. arenaria*, and *M. javanica*	McSorley (1999)[1]	*M. enterolobii*, *M. incognita*, *M. arenaria*, and *M. javanica*	Bui & Desaeger (2021)[1]; Dareus *et al.* (2021)[1]; Wang *et al.* (2008)[2]
Hairy vetch	*Vicia villosa*	Unspecified	Legume	Winter	*M. arenaria* and *M. hapla*	Dobosz & Krawczyk (2019)[1]; Mosjidis *et al.* (1994)[1]	*M. incognita*, *M. arenaria*, and *M. javanica*	Guertal *et al.* (1998)[1,2]; Johnson *et al.* (1992)[2]
Jointvetch	*Aeschynomene americana*	Unspecified	Legume	Summer	*M. incognita*, *M. arenaria*, and *M. javanica*	McSorley (1999)[1]	*M. incognita*, *M. arenaria*, and *M. javanica*	Kokalis-Burelle & Rosskopf (2012)[1]
Marigold	*Tagetes erecta*	Crackerjack	Asteraceae	Summer	*M. incognita*, *M. arenaria*, and *M. javanica*	El-Hamawi *et al.* (2004)[2]; Wang *et al.* (2007a)[3]		
Browntop millet	*Panicum ramosum*	Unspecified	Grass	Summer	*M. incognita*	Brodie & Murphy (1975)[2]		
Cereal rye	*Secale cereale*	Wrens Abruzzi	Grass	Winter	*M. incognita*	Timper *et al.* (2006)[2]	*M. incognita*, *M. arenaria*, and *M. javanica*	Johnson & Motsinger (1989)[1]; Timper *et al.* (2011)[2]
Sesame	*Sesamum indicum*	Benne	Pedaliaceae	Summer	*M. arenaria*	Rodriguez-Kabana *et al.* (1988)[2]		
Sorghum-sudangrass	*Sorghum bicolor x S. sudanense*	Sweet leaf	Grass	Summer				
Sorghum-sudangrass	*Sorghum bicolor x S. sudanense*	Tall one	Grass	Summer				
Sorghum-sudangrass	*Sorghum bicolor x S. sudanense*	Kandy Graz	Grass	Summer				
Sunn hemp	*Crotalaria juncea*	AU Golden	Legume	Summer	*M. incognita*, *M. arenaria*, and *M. javanica*	Mosjidis *et al.* (2013)[3]		
Sunn hemp	*Crotalaria juncea*	Tropic Sun	Legume	Summer	*M. incognita*, *M. arenaria*, and *M. javanica*	McSorley (1999)[1]	*M. incognita*	Khanal *et al.* (2021)[2]
Sunn hemp	*Crotalaria juncea*	Unspecified	Legume	Summer				
Velvetbean	*Mucuna pruriens*	Unspecified	Legume	Summer	*M. incognita*, *M. arenaria*, and *M. javanica*	McSorley *et al.* (1994)[1]		

[1] Resistance or susceptibility based on greenhouse host status test, [2] Reproduction of *Meloidogyne* population based on population densities of second-stage juveniles in field trial, and [3] resistance reported in extension articles.

Table 2. Host suitability of cover crops to *M. incognita* race 3, *M. arenaria* race 1, and *M. javanica* based on egg mass or gall index.

		Egg mass index			Gall index		
Crop species	Cultivar	*M. incognita*	*M. arenaria*	*M. javanica*	*M. incognita*	*M. arenaria*	*M. javanica*
Tomato (*Solanum lycopersicum*)	Rutgers^z	S (5.0 ± 0.0)^y	S (5.0 ± 0.0)	S (5.0 ± 0.0)	S (5.0 ± 0.0)	S (5.0 ± 0.0)	S (5.0 ± 0.0)
Bahiagrass (*Paspalum notatum*)	Pensacola	N (0.2 ± 0.2)	N-P (0.3 ± 0.3)	N-P (0.5 ± 0.3)	N (0.0 ± 0.0)	N (0.0 ± 0.0)	N (0.0 ± 0.0)
Bermudagrass (*Cynodon dactylon*)	Wrangler	N (0.0 ± 0.0)	N-P (0.6 ± 0.2)	N (0.4 ± 0.2)	N (0.0 ± 0.0)	N (0.0 ± 0.0)	N (0.0 ± 0.0)
Black oat (*Avena strigosa*)	Soil Saver	S (4.0 ± 0.3)	P-S (2.8 ± 0.5)	P-S (1.9 ± 0.5)	P (0.8 ± 0.2)	N-P (0.5 ± 0.2)	N (0.1 ± 0.1)
Blue lupine (*Lupinus pilosus*)	VNS	S (4.7 ± 0.3)	P-S (2.7 ± 0.6)	P-S (4.5 ± 0.3)	S (4.4 ± 0.6)	S (3.9 ± 0.3)	P-S (4.0 ± 0.6)
Rye (*Secale cereale*)	Wrens Abruzzi	S (3.0 ± 0.3)	P-S (2.6 ± 0.3)	P (1.8 ± 0.4)	N (0.2 ± 0.1)	N-P (0.3 ± 0.3)	N (0.0 ± 0.0)
Cowpea (*Vigna unguiculata*)	Iron Clay	S (3.5 ± 0.2)	S (3.2 ± 0.3)	S (4.2 ± 0.3)	N (0.1 ± 0.1)	N (0.0 ± 0.0)	N (0.2 ± 0.1)
Jointvetch (*Aeschynomene americana*)	unspecified	P (1.5 ± 0.4)	P (1.1 ± 0.4)	N (0.4 ± 0.2)	N (0.5 ± 0.4)	N-P (0.8 ± 0.4)	N (0.0 ± 0.0)
Hairy vetch (*Vicia villosa*)	unspecified	S (4.8 ± 0.1)	S (4.2 ± 0.2)	S (4.9 ± 0.1)	S (5.0 ± 0.0)	S (3.8 ± 0.4)	S (5.0 ± 0.0)
Marigold (*Tagetes erecta*)	Cracker Jack	N (0.0 ± 0.0)	N (0.0 ± 0.0)	N (0.0 ± 0.0)	N (0.0 ± 0.0)	N (0.0 ± 0.0)	N (0.0 ± 0.0)
Millet (*Panicum ramosum*)	Browntop	N (0.0 ± 0.0)	N (0.0 ± 0.0)	N (0.1 ± 0.1)	N (0.0 ± 0.0)	N (0.0 ± 0.0)	N (0.0 ± 0.0)
Sesame (*Sesamum indicum*)	Benne	N (0.1 ± 0.1)	N (0.3 ± 0.2)	P (0.7 ± 0.2)	N (0.0 ± 0.0)	N (0.0 ± 0.0)	N (0.0 ± 0.0)
Sorghum-sudangrass (*Sorghum bicolor x S. sudanense*)	Kandy Graz	S (2.9 ± 0.3)	P (1.8 ± 0.2)	P-S (2.2 ± 0.5)	N (0.0 ± 0.0)	N (0.0 ± 0.0)	N (0.0 ± 0.0)
	Sweet Leaf	P-S (1.9 ± 0.5)	P-S (2.8 ± 0.3)	N-P (1.0 ± 0.4)	N-P (0.4 ± 0.2)	N (0.2 ± 0.2)	N (0.0 ± 0.0)
	Tall One	S (3.3 ± 0.3)	S (3.0 ± 0.3)	P (1.0 ± 0.3)	N (0.3 ± 0.2)	N (0.0 ± 0.0)	N (0.0 ± 0.0)
Sunn hemp (*Crotalaria juncea*)	AU Golden	P-S (2.1 ± 0.5)	P-S (1.8 ± 0.5)	P (1.1 ± 0.3)	N (0.0 ± 0.0)	N-P (1.1 ± 0.5)	N (0.0 ± 0.0)
	Tropic Sun	P-S (2.2 ± 0.4)	P (2.0 ± 0.4)	N (0.3 ± 0.1)	N (0.3 ± 0.2)	N (0.0 ± 0.0)	N (0.0 ± 0.0)
	unspecified	P (2.0 ± 0.3)	P-S (2.4 ± 0.5)	P (1.4 ± 0.4)	P (1.5 ± 0.3)	N-P (0.8 ± 0.4)	N (0.0 ± 0.0)
Velvet-bean (*Mucuna pruriens*)	unspecified	N (0.0 ± 0.0)	N (0.0 ± 0.0)	N (0.2 ± 0.2)	N (0.0 ± 0.0)	N (0.0 ± 0.0)	N (0.0 ± 0.0)

Notes: ^{z}susceptible control; ^{y}host status of cover crop was designated as nonhost (N; EI or GI = 0 or 1), poor host (P; EI or GI = 2 or 3), and susceptible host (S; EI or GI = 4 or 5) based on Wilcoxon signed-rank test at the $P \leq 0.05$ significance level. Mean ± SE are within parentheses.

Figures

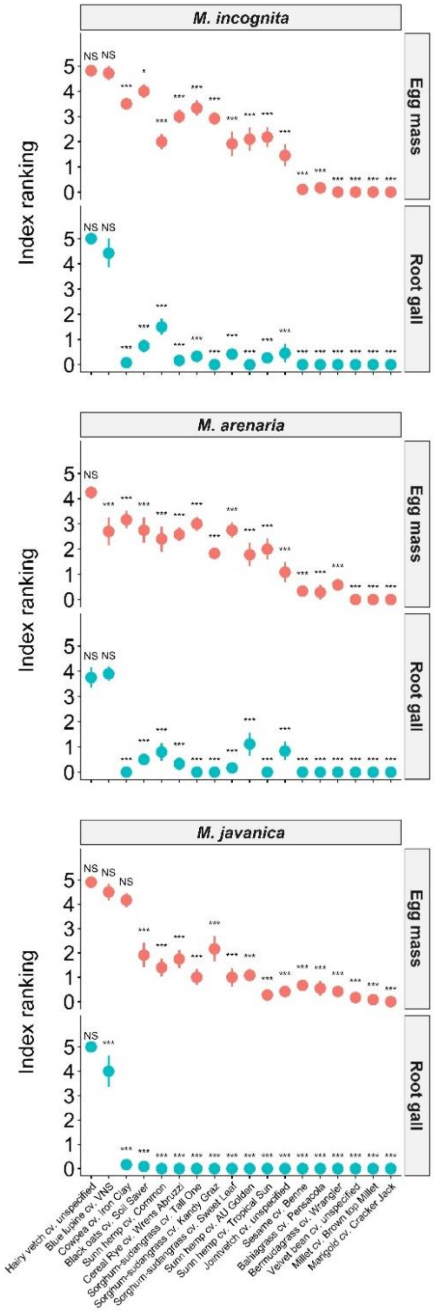

Fig. 1. Mean root gall (red) and egg mass (blue) index (±SE) of 18 different cover crops for three root-knot nematode species. Asterisks indicate a significantly lower index ranking than the susceptible tomato control according to Dunnett’s test. One asterisk indicates $P \leq 0.05$, three asterisks indicate $P \leq 0.001$, and NS indicates no significance.

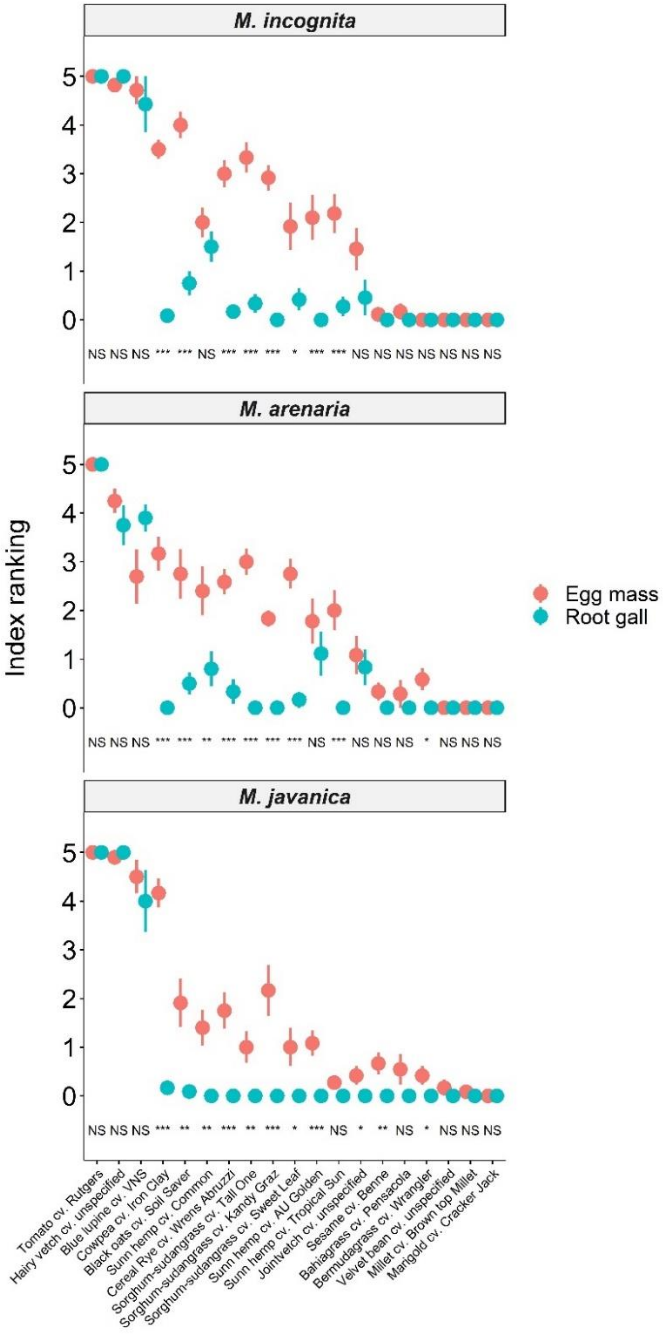

Fig. 2. Mean root gall and egg mass index (±SE) of 18 different cover crops for three root-knot nematode species. Asterisks indicate significantly different rankings between root gall and egg mass index according to a two-sample student's t-test. One asterisk indicates $P \leq 0.05$, two indicate $P \leq 0.01$, three asterisks indicate $P \leq 0.001$, and NS indicates no significance.

CHAPTER 6

SUCCESSIONAL EFFECTS OF COVER CROP-FALLOW COUPLED WITH DEEP TILLAGE ON SUPPRESSION OF SOILBORNE PATHOGENS AND WEEDS[5]

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[5] Marquez, Josiah and Hajihassani, Abolfazl. To be submitted to Journal of Pest Science.

Abstract

Cover crop-fallowing can suppress soilborne pathogens and weeds by serving as a poor host or nonhost to pathogens and producing allelopathic compounds during a fallow period; however, cultural practices can influence their effectiveness. Cover crop-fallow seasons were evaluated against weedy fallow and all-vegetable rotation and their interactions with deep tillage in a 3-season vegetable cropping system for soilborne disease and weed suppression. Field trials in 2019-2021 consisted of tomato in the spring, squash in the fall, and cabbage in the winter. Plots were arranged in a split-plot 2 × 4 factorial randomized complete block design. Whole-plot tillage treatments were shallow tilled or deep tilled. Subplots had two factors of crop rotations: type of fallow (cover crop [Spring or fall sunn hemp or winter rye] or weedy) and season of fallow. Sunn hemp and weedy fallow reduced root-knot nematode (*Meloidogyne incognita*) abundance and root galling in the first subsequent vegetable compared to the all-vegetable rotation ($P < 0.05$) independent of tillage practice but had no effect on fungal pathogens. Fall sunn hemp had higher biomass and reduced root galling in the second subsequent vegetable. Spring and fall sunn hemp improved vegetable yields. Winter rye only reduced ring nematodes (*Mesocriconema* spp.) abundance in the first subsequent vegetable. Deep tillage reduced incidence of fungal pathogens of *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, and abundance of stubby-root nematode (*Nanidorus minor*). Sunn hemp is recommended for *M. incognita* suppression, whereas deep tillage for *R. solani, S. sclerotiorum*, and *N. minor* suppression.

Keywords: sustainable agriculture, integrated pest management, plant-parasitic nematodes, damping-off, Sclerotinia rot of cabbage, *Sclerotinia sclerotiorum*, white mold, *Athelia rolfsii*, crop rotation, crop sequence, allelopathy.

Introduction

Weeds, plant-parasitic nematodes (PPNs), and fungal soilborne pathogens are important challenges to vegetable production (Abawi and Widmer 2000; Hallmann and Meressa 2018; Peachey et al. 2004), resulting in high chemical input costs to control pests and diseases (Abawi and Widmer 2000; Brennan and Smith 2005). Root-knot nematode (RKN; *Meloidogyne* spp.) is the most important PPN in vegetable fields of Georgia (Hajihassani et al. 2020; Marquez et al. 2021). In southeastern United States, soil fumigation with 1,3 dichloropropene (1,3-D) for treating PPNs only or a mixture of 1,3-D and chloropicrin for treating soilborne diseases, PPNs, and weeds is commonly practiced since the phase-out of methyl bromide (Hajihassani 2018; Locascio et al. 1997; Nnamdi et al., 2022). Although fumigants will remain an important control strategy for soilborne pathogens and weeds, robust sustainable management of these pests requires a more holistic long-term management strategy to minimize the risk of harming non-target organisms. Cover crop rotations can serve as an alternative or supplemental PPN management approach with non-host resistance, biotoxin production, enhancing antagonistic flora and fauna, trapping nematodes in the roots (Oka 2010; Wang et al. 2002), and suppression of weeds (Teasdale 1996) and soilborne fungal pathogens (Abawi and Widmer 2000).

Cover crops are typically grown between cash-crop cycles in a fallow season (Snapp et al. 2005) which are incorporated into the soil as green manure (Wang et al. 2001) or remain as surface organic mulch (Quintanilla-Tornel et al. 2016) for the purpose of amending the soil. Cover crops can also be grown during a cash-crop season as a ground cover (Fageria et al. 2005) or between rows known as living mulch (Hartwig and Ammon 2002; Wang et al. 2011). Cover crops have been recommended for vegetable cropping systems (Abawi and Widmer 2000; Gaskin et al. 2018).

In Georgia, common cover crops include cowpea (*Vigna unguiculate*) (Riley et al. 2017), cereal rye (*Secale cereal*), and crimson clover (*Trifolium incarnatum*) (Gaskin et al. 2014).

Tropical sunn hemp (*Crotalaria juncia*) may be an excellent candidate for vegetable rotations, as it fixes nitrogen (Wang et al. 2002), produces a large volume of biomass (McSorley et al. 2009; Schomberg et al. 2007), suppresses soilborne pathogens through production of allelopathic pyrrolizidine alkaloids (Boppré and Thoden 2010), and is a poor-host to RKNs (Wang et al. 2002). Cereal rye is commonly planted in the fall as a forage crop for livestock in Georgia (Johnson and Motsinger 1990) and has been suggested as a cover crop for nematode and weed control due to the production of allelopathic benzoxazinoids (Schulz et al. 2013) and rapid growth, producing large volumes of biomass during the winter season (Reeves 1994). The benzoxazinoids are released from the root exudates (Pérez and Ormeno-Nunez 1993; Pérez and Ormenoñuñez 1991) and the plant shoot tissue with a half-life of 10-12 days (Yenish et al. 1995). It has been reported that suppression of weeds after termination of rye can remain for 4 weeks (Rice et al. 2012; Yenish et al. 1995). However, RKN control using rye is inconsistent in the field (Timper et al. 2006; Timper et al. 2011).

The effectiveness of PPN suppression by cover crops can vary greatly depending on sufficient biomass production. Low biomass can result in ineffective suppression of nematodes (McSorley et al. 2009; Timper et al. 2011). Sunn hemp biomass production can vary among regions and planting dates based on cumulative solar radiation and degree days, in which higher solar radiation and degree days in the May planting season increased biomass more than in the July planting season in South Georgia, USA (Schomberg et al. 2007). Incorporating cover crop biomass into the soil with tillage is also important for PPN suppression (Wang et al. 2002). Although the effect tillage has on controlling PPN is negligible (Collange et al. 2011), the combined effect with cover cropping may reduce PPN abundance. We propose that PPN suppression by cover cropping can be enhanced by the cultural practice of deep tillage and hypothesize that deep tillage will distribute allelopathic compounds from the cover crop shoot biomass at lower soil depths and invert the soil to expose PPN that dwell deeper in the soil to solar radiation and desiccation to provide a planting bed with a low PPN abundance. Since seasons can affect biomass production, this study will also compare the effect of spring and fall sunn hemp- and winter rye-fallowing with shallow tillage and deep tillage on suppression of PPN, fungal soilborne pathogens, and weeds. We hypothesize that 1) the season with the most cumulative degree days would facilitate higher biomass production and greater suppression of PPN, soilborne fungal pathogens, and weeds and 2) soil incorporating cover crops by deep tillage would enhance suppression of nematode and fungal pathogens and weeds by the cover crop.

Materials and Methods

Field trial. Field experiments consisted of a vegetable rotation of tomato, squash, and cabbage, respectively, grown in the spring, fall, and winter of 2019 to 2021 in fields naturally infested with *M. incognita, Mesocriconema* spp., and *Nanidorus minor* (syn., *Paratrichodorus minor*) at the Black Shank research farm, University of Georgia, Tifton, GA. The soil is a fine-loamy, kaolinitic, thermic Plinthic Kandiudults of the Tifton soil series of a sand soil texture class (93% sand, 2.2 % silt, and 4.8% clay). Soil nutrients levels were 38.6 kg/ha of soluble nitrate, 8.8 kg/ha of P, 171.5 kg/ha of K, 57.7 kg/ha of Mg, 94.2 kg/ha of Ca, 842.9 kg/ha of S, 12.3 kg/ha of B, 0.2 kg/ha of Zn, 9.0 kg/ha of Mn, 11.8 kg/ha of Fe and 29.1 kg/ha of Cu. The cation exchange capacity (CEC) was 2 meq/100g of soil with a base saturation of K at 3.5%, Mg at 1.9%, and Ca at 10%. The exchangeable acidity was 53.6% of the CEC with a pH of 6.7. Plots were arranged in a split-plot randomized complete block design with 4 replications in two repeated trials. Tillage practices were whole-plot treatments and consisted of a shallow tillage treatment represented by disking at the 15-cm depth and deep tillage represented by plowing with a moldboard plow at the 45-cm depth. Whole plots were the width of two bare-ground vegetable beds (82.3 m long and 3.7 m wide). Subplot treatments consisted of two factors of crop rotations. The first factor consisted of fallow type by incorporating either a cover crop-fallow or bare ground weedy fallow into a 3-season vegetable rotation of tomato (*Solanum lycopersicum* cv. BHN 602) in the spring, squash (*Cucurbita pepo* cv. Spineless Beauty) in the fall, and cabbage (*Brassica oleracea* var. *capitata* cv. Bravao) in the winter. All vegetables were highly susceptible to RKN. Cover crop-fallow consisted of sunn hemp (cv. Unspecified) in the spring or fall, and rye (cv. Wrens Abruzzi) in the winter. The second factor was the fallow season (spring, fall, or winter). An all-vegetable rotation control (AVRC) treatment served as a normal control without any fallow treatments (Table 1). Subplots were the width of one bed (163.8 m long and 1.8 m wide). Altogether, there were 7 subplots of crop rotation treatments (CRTs) in a 2 × 4 factorial design consisting of 1) AVRC rotations of spring tomato, fall squash, and winter cabbage; 2) spring CRT of spring sunn hemp-fallow, fall squash, and winter cabbage; 3) spring CRT of weedy fallow, fall squash, and winter cabbage; 4) fall CRT spring tomato, fall sunn hemp-fallow, and winter cabbage; 5) fall CRT of spring tomato, fall weedy fallow, and winter cabbage; 6) winter CRT of spring tomato, fall squash, and winter rye-fallow; and 7) spring tomato, fall squash, and winter weedy fallow (Table 1, Fig. S1).

Before initiation of the trials, fields had hairy vetch (*Vicia villosa*) planted in the winter to maintain *M. incognita* and *N. minor* populations over winter. The experiment was initiated on 18 April 2019 and 12 May 2020. Cover crop seeds were planted with a seed drill (3P606NT, Great plains, Salina, Kansas, USA) at a rate of 56 kg/ha for sunn hemp and 84 kg/ha for rye. Rye was top-dressed with 56 kg/ha of nitrogen to insure sufficient biomass. After about 2-4 weeks in seedling trays, tomato, squash, and cabbage were transplanted into vegetable beds. Tomato was grown for 87-88 days, squash for 70 days, and cabbage for 142-164 days. Sunn hemp was grown for 60-70 days and rye over the winter for 142-161 days (Table 2) and terminated at flowering or heading. University of Georgia Cooperative Extension recommendations were followed for control of insects and foliar diseases. All crops were watered with overhead irrigation by farm manager as needed. At the end of each cropping season, cover crops were terminated with a flail mower before tillage. After tillage, each bed was made with a cultivator (Bush hog RTH72 Rotary Tiller, Selma Alabama, USA).

Assessment of cover crops. Cover crop biomass was estimated at the end of each season and after vegetable harvesting by collecting above-ground biomass for each subplot from the mean of three 930 cm^2 quadrants ($n = 3$). Total dry weight biomass was estimated by drying cover crop shoot biomass collected from quadrants in a dryer for 7 days at 60 °C before weighing to estimate total dry weight biomass. Ten roots from each subplot among the 3 quadrants sampled for biomass were also collected for egg mass index consisting of a rank scale of 0-5 with 0 = no egg mass, 1 = 1-2 egg mass, 2 = 3-10 egg mass, 3 = 11-30 egg mass, 4 = 31-100 egg mass, and 5 = >100 egg mass (Taylor and Sasser 1978). Cumulative degree days estimation for each cover crop season was calculated as the sum of daily mean air temperatures subtracted from the base temperature of 9.9 for sunn hemp (Schomberg et al. 2007) and 4.4 for rye (Mirsky et al. 2009). Daily mean air temperatures were retrieved from the University of Georgia Weather Network (http://www.georgiaweather.net/) from Tifton weather station at Bowen research station and were recorded every second with the HMP60 Humidity and Temperature Probe (Vaisala, Vantaa, Finland).

Assessment of plant-parasitic nematodes. Each subplot was sampled after each cropping season and after tillage to assess nematode populations at planting conditions. A total of 5 soil cores per subplot were sampled with a 5-cm diameter Giddings soil probe (Giddings Machine Company, Windsor, Colorado, USA) to the depth of 60 cm in a zig-zag pattern. Each core was divided into 0-30 cm and 30-60 cm strata and combined into a bulk composite sample. Nematodes were extracted by decanting and sieving followed by centrifugal flotation (Jenkins 1964) from a 100 cm^3 sub-sample taken from each composite soil sample. Roots from 5 marked plants were also removed randomly from each plot and assessed for root galling. Root galling was evaluated using a root gall index in which lower levels were rated with Taylor and Sasser (1978) ranks scale mentioned above. Root galls above 100 galls were rated as 5 = 25% of roots are galled; 6 = 50% of roots are galled; 7 = 75% of roots are galled; 8 = roots are completely galled; 9 = roots are completely galled and rotting; and 10 = dead plant (Bridge and Page 1980). PPNs recovered from soil were identified to the genus level based on morphological features (Mai and Mullin 1996; Smart and Nguyen 1988) and counted using an inverted microscope (ZEISS Axio Vert.A1, Oberkochen, Germany) at 10-40x magnification. Identification of stubby-root and root-knot nematodes to the species level was conducted by species-specific PCR described in Hajihassani et al. (2018 and 2022).

Assessment of soilborne fungal pathogens. Initial incidence of southern blight on tomato (*Athelia rolfsii*), damping-off of tomato, squash, and cabbage (*Rhizoctonia solani*), and Sclerotinia rot of cabbage (*Sclerotinia sclerotiorum*) were identified by collecting representative samples from the field and having specimens examined and confirmed by Jason Brock at the University of Georgia Plant Disease Clinic (Tifton, GA). Diagnostic features used to identify pathogens include the right-angled melanized resting hyphae and slight constriction at branching of *R. solani*, the tan to reddish-brown sclerotia and white mycelial fans of *A. rolfsii,* and cabbage head rot and black sclerotia of *S. sclerotiorum* (Erper et al. 2016; Jones et al. 2014; Rimmer 2007). Incidence of southern blight on tomato, damping-off of tomato, squash, and cabbage, and Sclerotinia rot of cabbage was evaluated in bi-weekly intervals starting at the onset of symptoms. Symptoms of southern blight consisted of wilt followed by basal stem lesions and formation of white mycelial mat at the stem base eventually producing sclerotia. Symptoms of damping-off occurred at the beginning of the growing season consisting of wilted seedlings or small plants with crown rot that eventually death of plant. Symptoms of Sclerotinia rot consisted of white mycelium growth on leaves and head of cabbage which led to head rot and formation of black sclerotia. Incidence was represented as a percentage of infected plants from the total number of plants at planting of each subplot. A total of four to eight disease assessments were made with a final incidence rating at the end of the growing season.

Assessment of weed density. Weed density was assessed based on fresh biomass production after final vegetable harvest was completed. Above-ground biomass of broadleaf and grass weeds was quantified from three randomly placed 930-cm^2 quadrants for each subplot (Curran et al. 2011). Biomass was weighted with a portable scale (PBW-3000, intelligent weighing technology, Camarillo, CA) in the field.

Assessment of soil chemical properties. At initiation of field trials, samples at the 0-30 cm stratum were analyzed by Waters Agriculture Laboratories (Camilla, GA) for soil texture, pH, soluble nitrate (NO_3), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sulfur (S), boron (B), zinc (Z), manganese (Mn), iron (Fe), and copper (Cu). Soil texture was measured from 50 g (air dry) of soil with the Bouyoucos hydrometer method (Bouyoucos 1936). Soil pH was measured with 25 g of soil with a hydrogen probe at a 1:1 ratio of soil and water (McLean 1983).

The double acid extraction method was used for Mehlich-1 extractable P, K, Mg, Ca, S, B, Z, Mn, Fe and Cu and was determined with inductively coupled argon plasma spectrometry (ICAP) from 5 g of soil (Mehlich 1953). Soil nitrate was measured with 5 g of soil by the KCl-Cadium reduction method by flow injection analysis (Dahnke 1990). Soil organic matter was measured with 2.5 g of soil by loss on ignition method (Davies 1974).

Assessment of crop response. Vegetable crops were harvested in each subplot from the same 5 plants selected for root galling index. Tomato and cabbage were harvested only once at the end of the cropping season; however, squash had 3-4 harvest periods (Table 2). The biomass of tomato and squash fruits and cabbage heads were taken for yield estimation. Weights were taken with a 60-kg crane scale (CDR-132-N, Chatillon & Sons, Largo, FL) in the field. Plant height was also taken from the same plants from the soil surface to the top portion of the plant.

Data analysis. Since the proximity of the fallow season with the season of assessment (current or subsequent seasons) is confounded, data from the current, first and second subsequent vegetable seasons (SVSs) from the same subplots were analyzed separately and allowed for observation of successional patterns. Data that are dependent on assessments of the subsequent vegetable crops (*i.e.*, root galls, fungal disease incidence, and crop response parameters) do not have current season data. Direct comparisons between CRT seasons could not be made since fallow season and vegetable crop are confounded. Instead, all CRT comparisons were made within each fallow season. To determine if whole-plot tillage treatments, CRT, stratum, and interactions had a significant effect on nematode counts, a 3-way analysis of variance (ANOVA) was conducted. Stratum was treated as a repeated measure, while trials and blocks were considered a random effect. A 2-way ANOVA was conducted to determine the effect whole-plot tillage treatments, subplot crop rotation treatments, and interactions had on crop yield, height, stand count, weed biomass, incidence of fungal diseases, and root gall index. PROC GLIMIX by SAS® 9.4 (SAS Inc. Cary, NC) was used for all ANOVA analyses. All data sets for each season were tested for normality by PROC UNIVARATE and transformed by log(x+1) when needed. If the data was skewed, predictions were linked with a Poisson or negative binomial distribution family based on the best fit. Mean separations were performed using Tukey's honesty significance difference test at $\alpha = 0.05$ unless indicated at $\alpha = 0.1$.

Results

Initiation of the field trial. RKN was the most abundant and prevalent PPN in the experimental plots and did not significantly differ between subplot treatments with a mean abundance of 70 ± 12 second-stage juveniles (J2) per 100 cm^3 of soil. Other PPNs detected include *Nanidorus*, *Mesocriconema* spp., *Tylenchorhynchus* spp., and *Belonalaimus* spp., at a mean abundance of 9, 7, 1, and <1 per 100 cm^3 of soil, respectively. RKN and stubby-root nematodes were identified as *M. incognita* and *N. minor* with species-specific PCR.

Cover crop biomass, cumulative degree days, and egg mass index between seasons. Fall planting of sunn hemp had greater **(**$P < 0.05$) biomass production (14.1 ± 2.5 t/ha dry wt.) than the spring planting (6.7 ± 1.1 t/ha dry wt.**)**, while winter rye maintained the highest biomass **(**26.2 ± 4.2 t/ha dry wt.**;** Fig. 1**)**. Total cumulative degree days averaged among the two trials were 1168, 1070, and 1625 degree days for the spring, fall, and winter cover crop seasons, respectively. Although the spring season had numerically higher total degree days than the fall season, cumulative degree days were numerically higher in the fall sunn hemp season than the spring sunn hemp season for the early growing period of 0-48 and 61-63 days after planting (DAP) with a mean (±SE) daily difference of 35.8 ± 3.0 degree days (Fig. S2). Average cumulative degree days were numerically lower in the fall sunn hemp season than the spring sunn hemp season for the later growing period of 49-60 DAP with a mean daily difference of -11.9 ± 2.3 degree days. Both sunn hemp seasons did not have daily minimum air temperatures reaching below the base temperature (*i.e.*, 9.9 °C; Fig. 2); however, the winter planting season had 43 days of minimal mean air temperature reaching below the base temperature (*i.e.*, 5.5 °C) from 21-131 DAP. Tillage treatments did not affect sunn hemp or winter rye biomass. Winter rye had a mean *M. incognita* egg mass index of 0.11 ± 0.84, whereas egg mass on sunn hemp was only found in the fall season with a mean egg mass index of 0.04 ± 0.8 (Data not shown).

Effect of tillage on nematode abundance among soil strata. Lower *N. minor* abundance was found from deep-tilled winter CRT subplots sampled at all seasons of assessments, the spring CRT subplots sampled at the first SVS, and the fall CRT subplots sampled at the second SVS. Deep tillage had no effect on reducing root galling and *M. incognita* and *Mesocriconema* spp. abundance (Table S1). *M. incognita*, *Mesocriconema* spp., and *N. minor* abundance were consistently greater in the 0-30 cm stratum compared to the 30-60 cm stratum ($P < 0.05$; Table S2), but there was an interaction between tillage practices and strata for all comparisons except winter CRT subplots sampled at the second SVS and all subplots sampled in the spring. Those with a significant interaction showed either that PPN abundance was lower in the top 0-30 cm stratum in deep tilled plots than at the same stratum for shallow tilled plots or was greater in the lower 30-60 cm strata in deep tilled plots than at the same strata in shallow tilled plots ($P < 0.05$; Table S3). *Nanidorus minor* abundance had an interaction between tillage practices and the fallow treatments. The abundance of this nematode was lower in the deep tilled plots than the shallow tilled plots in AVRC of the spring CRT subplots from the current SVS. Lower *N. minor* abundance was also found in spring AVRC subplots from the first SVS ($P < 0.05$; Table S4).

Effect of tillage and fallowing on soilborne pathogens, vegetables, weeds, and soil properties. Deep tillage was successful in reducing the incidence of damping-off of squash caused by *R. solani* in the spring CRT subplots from the first SVS **(**$P < 0.05$**)** and Sclerotinia rot of cabbage caused by *S. sclerotiorum* in the spring fallow from the second SVS ($P < 0.1$; Table S5). Higher yields, height, and stand counts were found in deep-tilled whole plots compared to the shallow-tilled (Table S6). Specifically, higher yield (kg/plot) ($P < 0.1$), height, and stand count/plot were found in deep tilled whole plots in the winter CRT subplots from the second SVS ($P < 0.05$); higher yield (kg/plant) were found in the winter CRT subplots from the first SVS ($P < 0.05$); and higher stand count/plot were found in the spring CRT subplots from the first SVS ($P < 0.1$). Conversely, higher stand (count/plot) were found in shallow tilled whole plots compared to deep tilled whole plots in winter CRT subplots from the second SVS ($P < 0.1$).

Total weed, grass, and broad leaf biomass were increased by deep tillage (Table S7). Specifically, spring CRT subplots from the current SVS had higher total ($P < 0.05$) and grass biomass ($P < 0.1$); winter CRT subplots from the first SVS had higher broad leaf biomass ($P < 0.05$); and winter CRT subplots from the second SVS had higher total ($P < 0.05$) and broad leaf ($P < 0.1$) biomass in the deep tillage whole plots. Deep tillage did not affect nitrate and organic matter in the soil nor was there any significant interaction between tillage practices and CRTs.

Few interactions were found between tillage practices and CRTs among damping-off incidence, crop height, and weeds. Damping-off of cabbage in the winter CRT subplots had a lower incidence of *R. solani* damping-off in CRT subplots that were deep-tilled than shallow-tilled ($P < 0.05$). Tomato height in the spring CRT subplots from the second SVS was shorter in the fallow shallow-tilled subplots compared to any other CRT ($P < 0.05$).

The difference among weeds between tillage practices and CRTs was found in the spring and fall CRT subplots from the current and first SVS (Table S8). In the spring SRT subplots, deep tillage plots maintained higher grass biomass in the sunn hemp and weedy fallow CRT subplots from the current SVS ($P < 0.05$). After two vegetable seasons, shallow tillage maintained the lowest grass ($P < 0.05$) and broad leaf ($P < 0.1$) biomass in the AVRC and weedy fallow CRT subplots.

Suppression of soilborne pathogens from fallowing. Sunn hemp reduced *M. incognita* abundance from AVRC ($P < 0.05$, Table 3). Specifically, spring and fall sunn hemp-fallow CRT subplots had lower *M. incognita* abundance than AVRC after the termination of the current SVS. Fall sunn hemp also reduced *M. incognita* abundance from AVRC after the termination of the first SVS. Spring weedy fallow CRT subplots also reduced *M. incognita* in the current and first SVS. Spring sunn hemp reduced *N. minor* abundance from AVRC after the termination of the current SVS ($P < 0.1$). Winter rye reduced *Mesocriconema* spp. abundance from AVRC after termination of the first SVS. Winter weedy fallow had lower *N. minor* abundance than the rye or AVRC subplots after the termination of the current SVS. Differences among other PPNs were not found.

Spring sunn hemp reduced root galling from AVRC in the first SVS ($P < 0.05$, Fig. 2). Fall sunn hemp reduced root galling from AVRC in the first and second SVS ($P < 0.1$). Spring weedy fallow reduced root galling from AVRC in the first SVS ($P < 0.05$), but not fall weedy fallow. Sunn hemp and weedy fallow also suppressed the incidence of damping-off from *R. solani* (Table S9). Specifically, spring sunn hemp reduced damping-off incidence from AVRC and weedy fallow in the first SVS. Fall weedy fallow reduced damping-off incidence from AVRC in the first SVS ($P < 0.1$).

Soil fertility and crop response to different seasons of fallowing. Cover crops were successful in enhancing crop growth (Table 4). Spring sunn hemp CRT subplots had higher yields per plot from the first SVS than both the weedy fallow and AVRC ($P < 0.05$), while fall sunn hemp and weedy fallow CRT subplots had higher yields per plot from AVRC in the first SVS. Yield per plant was higher in the fall weedy fallow than sunn hemp and AVRC ($P < 0.05$), while winter weedy fallow had higher yields per plant than rye ($P < 0.1$) in the first SVS. Spring and fall sunn hemp and winter rye enhanced crop height. Specifically, spring sunn hemp CRT subplots had higher plants than weedy fallow in the first SVS ($P < 0.1$). Fall sunn hemp CRT subplots had greater crop height than weedy fallow in the second SVS ($P < 0.05$). Winter rye CRT subplots had greater crop height than weedy fallow and ACRC in the first SVS ($P < 0.05$). Spring and fall sunn hemp enhanced stand counts of the subsequent vegetables. Specifically, spring and fall sunn hemp CRT subplots had higher stand counts than AVRC in the first SVS ($P < 0.05$). Fall weedy fallow CRT subplots also had higher stand counts than AVRC in the first SVS ($P < 0.05$). Spring sunn hemp CRT subplots also enhanced stand counts of the second SVS with higher stand counts than AVRC ($P < 0.05$). Spring and fall sunn hemp CRT subplots had greater organic matter content from fallow and comparable levels to the AVRC in the first SVS ($P < 0.05$; Table 5). In the second SVS, spring sunn hemp CRT subplots had higher nitrate levels than AVRC. Winter rye fallow CRT had the lowest soil nitrate levels for both the first and second SVS ($P < 0.05$).

Suppression of weeds from fallowing. Grass weeds consisted of Texas panicum (*Panicum texanum*), large crabgrass (*Digitaria sanguinalis*), bahiagrass (*Paspalum notatum*), common bermudagrass (*Cynodon dactylon*), and bristly starbur (*Acanthospermum hispidum*). Broadleaf weeds consisted of yellow nutsedge (*Cyperus esculentus*), palmer amaranth (*Amaranthus palmeri*), morning glory (*Ipomoea purpurea*), Florida beggarweed (*Desmodium* *tortuosum*), wild radish (*Raphanus raphanistrum*), tropic croton (*Croton capitatus*), common cocklebur (*Xanthium strumarium*), common ragweed (*Ambrosia artemisiifolia*), prickly sida (*Sida spinosa*), Florida pusley (*Richardia scabra*), common lambsquarters (*Chenopodium album*), black medic (*Medicago lupulina*), corn spury (*Spergula arvensis*), cutleaf evening primrose (*Oenothera laciniata*), and cutleaf geranium (*Geranium dissectum*). Cover crops maintained lower weed biomass compared to AVRC, except for spring sunn hemp, which had higher grass biomass than AVRC (Table S10); however, they did not successfully suppress weed biomass in the SVSs. Specifically, fall sunn hemp had higher grass biomass than AVRC in the second SVS. Spring weedy fallow also had higher grass biomass compared to AVRC in the first SVS.

Discussion

Cover crop effect on soilborne pathogens. Successful suppression of soilborne pathogens by cover crops with allelopathic compounds is influenced by biomass production (Timper 2017) and soil incorporation (Wang et al. 2002). This study confirms that sunn hemp biomass production is important for suppression of RKN, as higher biomass from fall sunn hemp resulted in an extended suppressive effect on root galling to the second subsequent vegetable crops in this study. Wang et al. (2012) proposed that sunn hemp should produce at least 11.2 t/ha of dry weight biomass for RKN suppression in Hawaii's tropical agroecosystem. Fall planting of sunn hemp produced sufficient biomass, while spring planting produced biomass below this rate. Nevertheless, *M. incognita* abundance and root galling were reduced by spring sunn hemp, yet only in the first SVS. Fall's higher cumulative degree days in the early growing period may have contributed to its higher biomass production since shoot growth is dominated by the early growing period (Cherr et al. 2006). However, total cumulative degree days were less in the fall than the spring season towards the end of the cropping season, as temperatures began to decrease in the fall and increase in the spring. Schomberg et al. (2007) found later plantings of sunn hemp in the summer months of June and July in Georgia to have slowed down in growth when temperatures decreased. In this study, fall sun hemp was planted in either July or August, suggesting, that these later months provide sufficient biomass for *M. incognita* suppression, despite lower degree days at the end of the season. Probably, the end of the season temperatures had little effect on biomass production as growth stops at flowering.

Sunn hemp's poor-host status could also contribute to the reduction of *M. incognita* abundance in the SVS. Nearly no egg masses were found on the roots of sunn hemp for both seasons, suggesting that the reduction in *M. incognita* abundance could also be explained by the starvation of *M. incognita* J2s. Weedy fallow was comparable to sunn hemp-fallowing as it was able to reduce *M. incognita* abundance and root galling from the AVRC. Although many common weeds are known hosts of *M. incognita* (Rich et al. 2009), fallowing in the spring lowered the abundance of *M. incognita* and root galling from the AVRC, yet only for one SVS. Overall, weedy fallow had less weed biomass than the AVRC and could have resulted in less RKN reproduction from weeds. Cover crop and weedy fallow rotations had some effects on fungal soilborne pathogens. *Rhizoctonia solani* damping-off incidence was affected by spring sunn hemp-fallowing and fall weedy fallowing, with lower incidence than the AVRC on the first SVS. Winter rye did not suppress fungal soilborne disease. Rye serving as an organic mulch is known to reduce the incidence of *S. sclerotiorum* (Pethybridge et al. 2020) and *R. solani* (Wen et al. 2017). However, when rye is soil incorporated, soil pathogens are either stimulated or suppressed (Schulz et al. 2013). The fungal pathogens observed in this study may not be sensitive to the allelopathic compounds released by soil-incorporated rye, although successful suppression of *R. solani* on soybean has been observed with soil-incorporated rye (Wen et al. 2017).

Weeds can be suppressed using sunn hemp or rye (Schulz et al. 2013; Soti and Racelis 2020), yet they were not suppressed in this study by cover crop-fallowing and weedy fallowing. Cover crop-fallowing and weedy fallowing can maintain lower weed densities during the fallow season but did not reduce the weed seed bank for the following seasons. Particularly, grass weed biomass was greater in the spring weedy fallow and fall sunn hemp than the AVRC for the subsequent vegetable seasons, suggesting that grass seeds may have been dormant during the fallow seasons and germinated when conditions were favorable under vegetable cultivation. It is also possible that the near proximity of plots allowed weed seeds contamination to mask the effects of fallowing. In addition, these cover crops were soil incorporated. Using cover crops as a layer of organic mulch for the subsequent cash crop could provide an extra level of weed suppression by reducing light transmittance, while simultaneously releasing allelopathic compounds for a longer period than soil incorporation (Schulz et al. 2013).

Despite top-dressing rye to ensure high biomass which exceeded that of sunn hemp, it did not reduce the abundance of *M. incognita*. Other studies have also observed the inconsistency of winter rye in suppressing *M. incognita* (Johnson and Motsinger 1989; Timper et al. 2011). There are a few reasons for inadequate suppression of *M. incognita* by rye. Timper et al. (2011) suggest that the concentration of benzoxazinoids released by the rye shoot tissue incorporated in the soil may not be sufficient for *M. incognita* suppression, as higher concentrations of benzoxazinoids are required to affect *M. incognita* eggs on plant roots in the soil than eggs in bioassays. Rice et al. (2005) found concentrations of benzoxazinoids to decline with age. Levels of benzoxazinoids may have lessened in concentration as the shoot tissue age after 5 months and started to head. RKN reproduction may have also occurred in rye since some egg masses were observed on the roots at termination. A greenhouse study found rye cv. Wrens Abruzzi to range from a susceptible to poor host for Georgia's *M. incognita*, *M. arenaria*, and *M. javanica* populations (Marquez et al. 2022). A long 5-month season over the winter may have also allowed enough reproduction for abundance to be maintained with two life cycles (Timper et al. 2006). Overwintering sunn hemp for 5-11 months also had no effect on reducing abundance in Georgia and Brazil (Khanal et al. 2021). RKN eggs overwinter and provide a source of new infective J2 for the following spring season (Starr and Jeger 1985). Overwintering *M. incognita* eggs may have been more tolerant to benzoxazinoids. However, rye was successful at reducing *Mesocriconema* spp. abundance in the spring.

Deep tillage was successful at inverting the soil as redistribution of nematode populations was observed. Yet, even after tilling every season (3x/year), deep tillage did not cause enough disruption to reduce the abundance of *M. incognita* and root galling on vegetables. There was also no interaction between cover cropping and tillage practices, suggesting that deep tillage does not enhance the incorporation of cover crops into the soil for *M. incognita* suppression. However, deep tillage reduced the abundance of *N. minor*, which has a longer life span and lower reproduction rate than RKN (Schneider and Ferris 1987), and therefore is more sensitive to disturbance as its populations cannot rebound as quickly (Bongers 1990). Incidence of soilborne fungal pathogens, damping-off (*R. solani*) of squash in the fall and Sclerotinia rot of cabbage (*S. sclerotiorum*) in the winter were also reduced by deep tillage. These pathogens are known to be sensitive to tillage (Gill et al. 2001; Mueller et al. 2002). It has been suggested that deep tillage may be spatially isolating the pathogen and could risk returning inoculum back to the surface with repeated plowing (van Bruggen et al. 2016). However, repeated tillage in this study did not increase the incidence of these fungal pathogens. As these pathogens are sensitive to anaerobic soil disinfestation (van Bruggen et al. 2016), inversion of the soil may have also suppressed these aerobic pathogens by moving them into a more anaerobic environment beneath the soil surface. Despite evidence of deep tillage suppressing weeds (DeVore et al. 2013), deep tillage did not reduce weeds.

Although sunn hemp reduced *M. incognita* abundance and root galling of vegetables from a continuous vegetable rotation, differences were minimal as vegetables still had significant galling. However, cover crops provide other benefits, for example protecting soil from erosion, reducing soil compaction, improving infiltration (Blanco-Canqui and Ruis 2020), increasing organic matter content, amending soil with nitrogen via nitrogen fixation, and reducing leaching of nitrogen via nitrogen scavenging (Dabney et al. 2001). In this study, spring and fall sunn hemp improved yields of vegetables, while rye improved crop height from the AVRC. Leguminous sunn hemp fixes nitrogen resulting in crop residue with lower carbon to nitrogen (C/N) ratio of <20:1 and leads to rapid mineralization (Mansoer et al. 1997). Sunn hemp-fallow plots had plant available nitrogen (*i.e.*, soil nitrates) comparable to the fertilized AVRC and could have contributed to the yield increase. Winter rye, with a much higher C/N ratio (Clark et al. 1994) was successful in enhancing soil organic matter, yet soil nitrates were lower for the subsequent vegetables, probably from N immobilization. Although not in all the subplot treatments, deep tillage improved crop growth in yields and height in winter fallow subplots and reduced the incidence of *R. solani* damping-off in the fall and Sclerotium cabbage rot in the winter. It is possible that improved crop growth is also related to the reduction of fungal soilborne diseases which can impede growth and yields.

Conclusion

This study shows that sufficient degree days in the early period of sunn hemp growth are required for effective biomass production and *M. incognita* suppression; however, higher biomass production from winter rye did not contribute to *M. incognita* suppression. Spring weedy fallowing was also comparable to sunn hemp-fallowing as it reduced root galling and abundance of *M. incognita* and incidence of *R. solani* in the subsequent vegetable crop. This study also indicated that deep tillage had little effect on *M. incognita* but was effective in reducing *N. minor* abundance. No interaction between cover cropping and tillage practices suggests that deep tillage does not enhance cover crop-fallow's suppressive effect on soilborne pathogens. Although spring sunn hemp-fallowing had some effect on reducing the incidence of *R. solani*, the effect of deep tillage alone reduced the incidence of damping-off in the fall on squash Sclerotinia cabbage rot in the winter. This study suggests a short (60 days) sunn hemp rotation in a season with higher degree days in the early growing period (Fall for Georgia) is best for *M. incognita* suppression. In addition, deep tillage was mainly effective in suppressing fungal soilborne pathogens and *N. minor*. Finally, winter rye was not effective for the management of most soilborne pathogens, only suppressing the abundance of *Mesocriconema* spp.

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Tables

Table 1. Subplot crop rotation treatments in 2 (fallow type) x 4 (fallow season) factorial design.

Factors		Crop rotation sequence		
Fallow type[z]	Fallow season[y]	Spring	Fall	Winter
Cover crop	No season[x]	Tomato	Squash	Cabbage
Cover crop	Spring	Sunn hemp	Squash	Cabbage
Cover crop	Fall	Tomato	Sunn hemp	Cabbage
Cover crop	Winter	Tomato	Squash	Winter rye
Weedy	No season	Tomato	Squash	Cabbage
Weedy	Spring	Weedy	Squash	Cabbage
Weedy	Fall	Tomato	Weedy	Cabbage
Weedy	Winter	Tomato	Squash	Weedy

[z] Factor 1 test the effect of cover crop-fallowing or weedy fallowing in a rotation sequence, [y] Factor 2 is the season the cover crop or weedy fallow rotation is implemented. [x] All-vegetable rotation control.

Table 2. Field experiment timeline of planting, harvest, and termination of vegetables and cover crops in repeated trials.

			Vegetable					Cover crop		
				Harvest		Termination			Termination	
Year	Vegetable	Cover crop	Planting date	Date	Days[z]	Date	Days	Planting date	Date	Days
2019-2020										
	Tomato	Sunn hemp	18-Apr-19	27-Jun-19	70	15-Jul-19	88	24-Apr-19	26-Jun-19	63
	Squash	Sunn hemp	17-Jul-19	August 22 to September 12, 2019y	51, 57, 90	25-Sep-19	70	24-Jul-19	25-Sep-19	63
	Cabbage	Rye	18-Oct-19	16-Jan-20	90	30-Mar-20	164	21-Oct-19	30-Mar-20	161
2020-2021										
	Tomato	Sunn hemp	12-May-20	21-Jul-20	70	7-Aug-20	87	28-May-20	6-Aug-20	70
	Squash	Sunn hemp	13-Aug-20	September 12 to October 7, 2020	36, 43, 48, 55	22-Oct-20	70	17-Aug-20	16-Oct-20	60
	Cabbage	Rye	9-Nov-20	15-Mar-21	126	31-Mar-21	142	9-Nov-20	31-Mar-21	142
	Tomato	-	13-Apr-20	25-Jun-21	73	-	-	-	-	-

[z] Days from planting date. [y] Squash was harvested 3 times.

Table 3. The successional effect of fallow rotations on nematode abundance (per 100 cm^3 of soil) at different assessment seasons.

			Meloidogyne incognita			*Mesocriconema* spp.			*Nanidorus minor*		
Season of assessment	Fallow season	Harvest season	Cover crop-fallow[z]	Weedy fallow	All-vegetable rotation	Cover crop-fallow	Weedy fallow	All-vegetable rotation	Cover crop-fallow	Weedy fallow	All-vegetable rotation
Current											
	Spring	Spring	68 ± 20 b	78 ± 27 b	110 ± 18 a	71 ± 20 a	37 ± 10 a	57 ± 12 a	10 ± 2 b*	7 ± 1 a*	9 ± 1 ab*
	Fall	Fall	30 ± 11 b	56 ± 12 a	87 ± 14 a	51 ± 21 a	80 ± 31 a	74 ± 19 a	7 ± 2 a	9 ± 3 a	6 ± 1 a
	Winter	Winter	46 ± 15 a	45 ± 16 a	44 ± 10 a	9 ± 2 a	12 ± 4 a	24 ± 6 a	7 ± 1 a	5 ± 1 b	6 ± 1 a
First subsequent											
	Spring	Fall	62 ± 14 ab	36 ± 8 b	87 ± 14 a	66 ± 24 a	32 ± 11 a	74 ± 19 a	4 ± 1 a	7 ± 1 a	6 ± 1 a
	Fall	Winter	31 ± 12 b	25 ± 9 ab	44 ± 10 a	14 ± 4 a	11 ± 3 a	24 ± 6 a	5 ± 1 a	6 ± 1 a	6 ± 1 a
	Winter	Spring	91 ± 19 a	144 ± 51 a	110 ± 18 a	13 ± 5 b	30 ± 16 ab	57 ± 12 a	12 ± 2 a	5 ± 1 b	9 ± 1 a
Second subsequent											
	Spring	Winter	21 ± 9 a	26 ± 11 a	44 ± 10 a	14 ± 4 a	14 ± 4 a	24 ± 6 a	6 ± 1 a	6 ± 1 a	6 ± 1 a
	Fall	Spring	61 ± 14 a	189 ± 61 a	110 ± 18 a	33 ± 9 a	50 ± 16 a	57 ± 12 a	8 ± 1 a	7 ± 1 a	9 ± 1 a
	Winter	Fall	70 ± 15 a	97 ± 20 a	87 ± 14 a	33 ± 11 a	85 ± 36 a	74 ± 19 a	9 ± 2 a	9 ± 2 a	6 ± 1 a

All means (±SE) between crop rotation treatments (cover crop-fallow, weedy fallow, and all-vegetable rotation) across rows with the same letter by each nematode, season of assessment, and fallow seasons are not significantly different ($P < 0.05$) according to Tukey HSD test, unless indicated by * ($P < 0.1$). [z]Cover crop-fallow = sunn hemp in the spring and fall and rye in the winter and all-vegetable rotation = tomato in the spring, squash in the fall, and cabbage in the winter.

Table 4. The succession effects of fallow rotations on vegetables at different assessment seasons.

Season of assessment	Fallow season	Harvest season	Crop rotation treatment	Yield (kg/plot)	Yield (kg/plant)	Height (cm)	Stand count/plot
First subsequent	Spring	Fall	Cover crop-fallow	25.7 ± 6.1 a	1.6 ± 0.4 a	42.0 ± 1.3 a*	30.3 ± 1.6 a
			Weedy fallow	17.9 ± 4.6 b	1.2 ± 0.3 a	37.9 ± 0.9 b*	27.5 ± 1.6 ab
			All-vegetable rotation	18.5 ± 3.5 b	1.3 ± 0.2 a	38.8 ± 1.1 ab*	26.5 ± 1.0 b
	Fall	Winter	Cover crop-fallow	47.9 ± 7.5 a	2.1 ± 0.3 b	24.9 ± 1.8 a	52.8 ± 0.6 a
			Weedy fallow	53.0 ± 9.6 a	2.5 ± 0.4 a	24.6 ± 2.1 a	52.6 ± 1.0 a
			All-vegetable rotation	39.9 ± 4.8 b	1.9 ± 0.2 b	23.8 ± 1.3 a	50.0 ± 0.7 b
	Winter	Spring	Cover crop-fallow	10.5 ± 4.0 a	1.1 ± 0.4 b*	64.6 ± 2.2 a	14.2 ± 1.9 a
			Weedy fallow	12.4 ± 3.5 a	1.5 ± 0.4 a*	56.3 ± 1.2 b	12.8 ± 1.9 a
			All-vegetable rotation	11.0 ± 2.1 a	1.2 ± 0.2 ab*	58.4 ± 1.2 b	13.9 ± 1.5 a
Second subsequent	Spring	Winter	Cover crop-fallow	40.4 ± 7.8 a	1.9 ± 0.3 a	24.5 ± 1.7 a	53.1 ± 0.9 a
			Weedy fallow	39.9 ± 6.9 a	1.9 ± 0.2 a	23.9 ± 1.6 a	51.6 ± 1.3 ab
			All-vegetable rotation	39.9 ± 4.8 a	1.9 ± 0.2 a	23.8 ± 1.3 a	50.0 ± 0.7 b
	Fall	Spring	Cover crop-fallow	9.5 ± 2.8 a	1.1 ± 0.3 a	61.0 ± 2.1 a	15.1 ± 1.8 a
			Weedy fallow	9.1 ± 2.8 a	1.3 ± 0.4 a	55.5 ± 1.7 b	12.9 ± 1.4 a
			All-vegetable rotation	11.0 ± 2.1 a	1.2 ± 0.2 a	58.4 ± 1.2 ab	13.9 ± 1.5 a
	Winter	Fall	Cover crop-fallow	20.4 ± 5.1 a	1.5 ± 0.3 a	40.2 ± 1.6 a	26.0 ± 1.9 a
			Weedy fallow	21.9 ± 5.2 a	1.4 ± 0.3 a	39.9 ± 1.0 a	27.9 ± 1.6 a
			All-vegetable rotation	18.5 ± 3.5 a	1.3 ± 0.2 a	38.8 ± 1.1 a	26.5 ± 1.0 a

All means (±SE) between crop rotation treatments along columns with the same letter by season of assessment and fallow season are not significantly different ($P < 0.05$) according to Tukey HSD test, unless indicated by * ($P < 0.1$). ^{z}Cover crop-fallow = sunn hemp in the spring and fall and rye in the winter and all-vegetable rotation = tomato in the spring, squash in the fall, and cabbage in the winter.

Table 5. The successional effect of fallow rotations on soil nitrate and organic matter at different assessment seasons.

			Nitrate (ppm)			Organic matter (%)		
Season of assessment	Fallow season	Harvest season	Cover crop-fallow[z]	Weedy fallow	All-vegetable rotation	Cover crop-fallow	Weedy fallow	All-vegetable rotation
First subsequent								
	Spring	Fall	4.0 ± 1.1 a	3.8 ± 1.1 a	4.4 ± 0.8 a	2.8 ± 0.6 a	0.8 ± 0.1 b	1.8 ± 0.2 a
	Fall	Winter	3.8 ± 0.4 a	2.8 ± 0.5 b	4.1 ± 0.4 a	0.3 ± 0.0 a	0.3 ± 0.0 a	0.3 ± 0.0 a
	Winter	Spring	3.0 ± 0.3 c	3.9 ± 0.2 b	7.2 ± 0.6 a	0.6 ± 0.1 a	0.5 ± 0.1 a	0.6 ± 0.1 a
Second subsequent								
	Spring	Winter	6.6 ± 0.9 a	6.2 ± 1.3 ab	4.1 ± 0.4 b	0.3 ± 0.0 a	0.3 ± 0.0 a	0.3 ± 0.0 a
	Fall	Spring	7.1 ± 1.2 a	7.7 ± 0.7 a	7.2 ± 0.6 a	0.6 ± 0.1 a	0.5 ± 0.1 a	0.6 ± 0.1 a
	Winter	Fall	1.5 ± 0.4 b	2.2 ± 0.5 b	4.4 ± 0.8 a	1.8 ± 0.4 a	1.4 ± 0.3 a	1.8 ± 0.2 a

All means (±SE) between crop rotation treatments (cover crop-fallow, weedy fallow, and all-vegetable rotation) along columns with the same letter by season of assessment and fallow season are not significantly different ($P < 0.05$) according to Tukey HSD test, unless indicated by * ($P < 0.1$). [z]Cover crop-fallow = sunn hemp in the spring and fall and rye in the winter and all-vegetable rotation = tomato in the spring, squash in the fall, and cabbage in the winter.

Figures

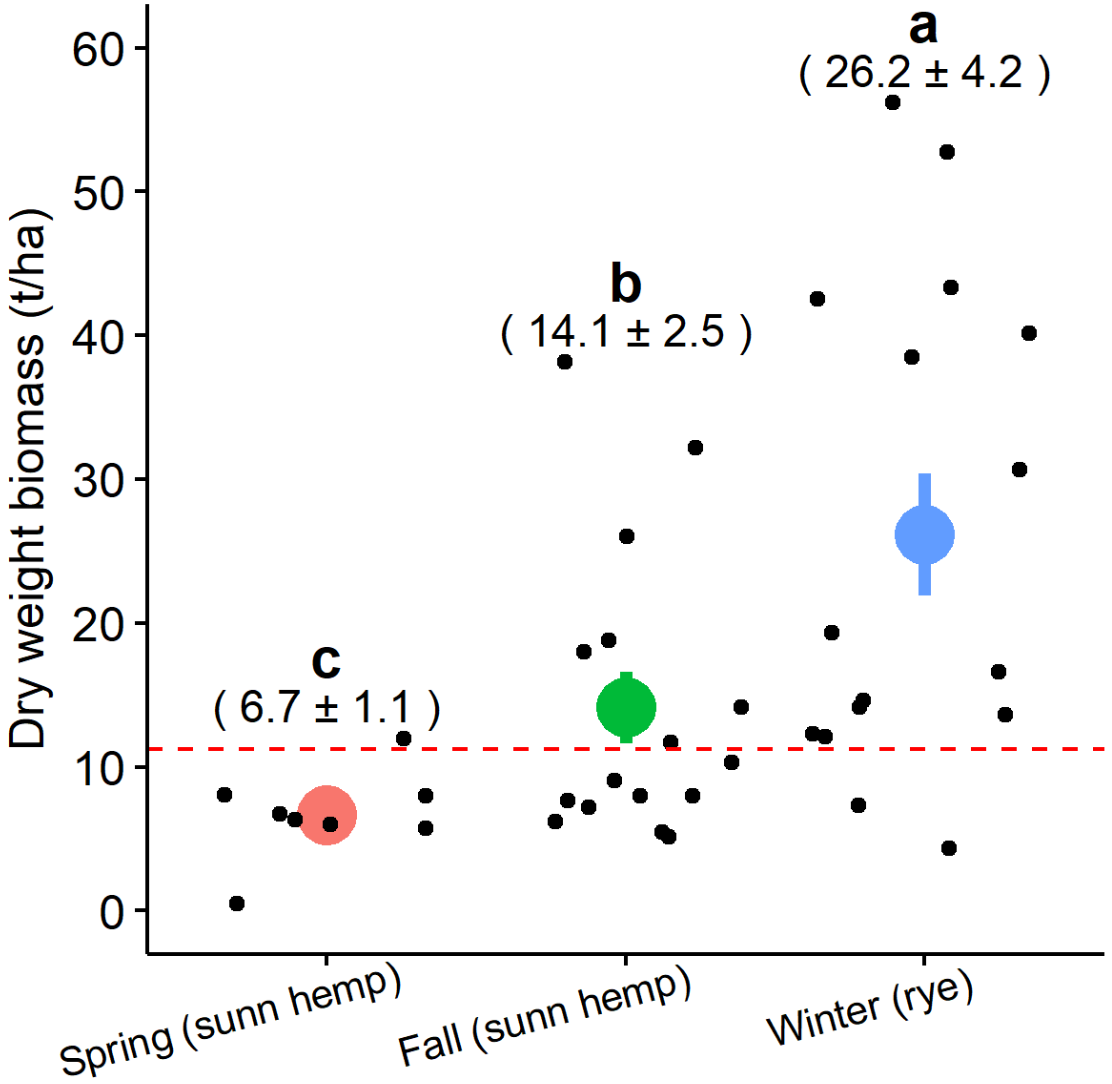


Fig. 1. Mean (± SE) cover crop biomass dry weight (kg/ha) indicated by colored dots for each cropping season. Means above each colored dote with standard error bars followed by the same letter(s) are not different according to Tukey HSD test (*P* < 0.05). Raw data are displayed as black dots. The red dotted line indicates the recommend minimal dry weight biomass (11.2 t/ha) for effective suppression of PPNs by sunn hemp (Wang et al. 2012).

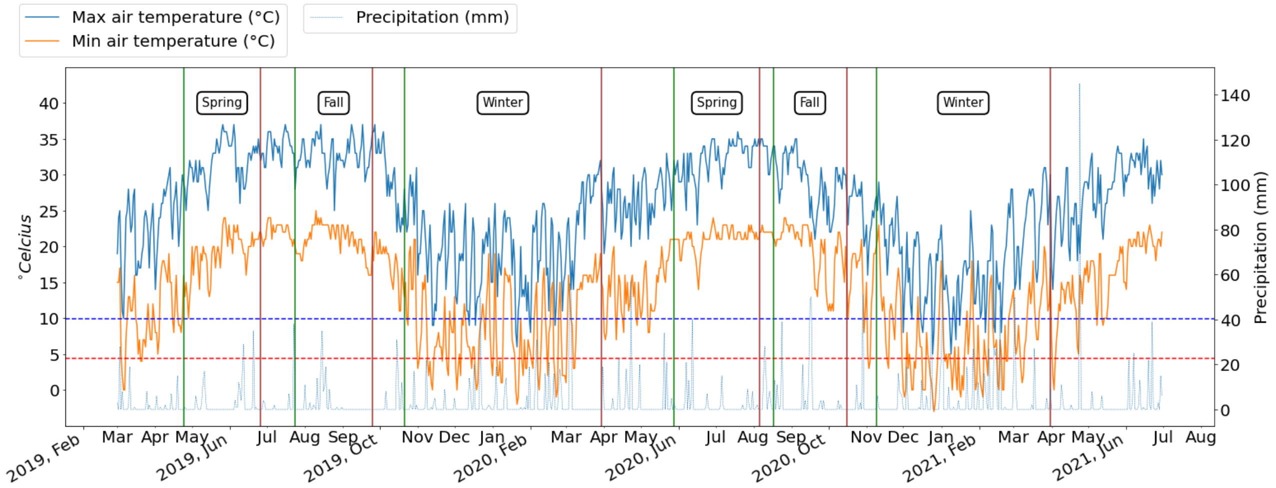


Fig. 2. The daily maximum and minimum air temperatures and precipitation during the spring, fall and winter planting seasons of 2019 and 2020. The horizontal dashed blue line represents the base temperature of sunn hemp (9.9 °C; Schomberg et al. 2007), whereas the red horizontal dashed red line represents the base temperature of rye (9.9 °C; Mirsky et al. 2009). The green vertical line represents planting date of cover crops, whereas the brown vertical line represents termination date of cover crops.

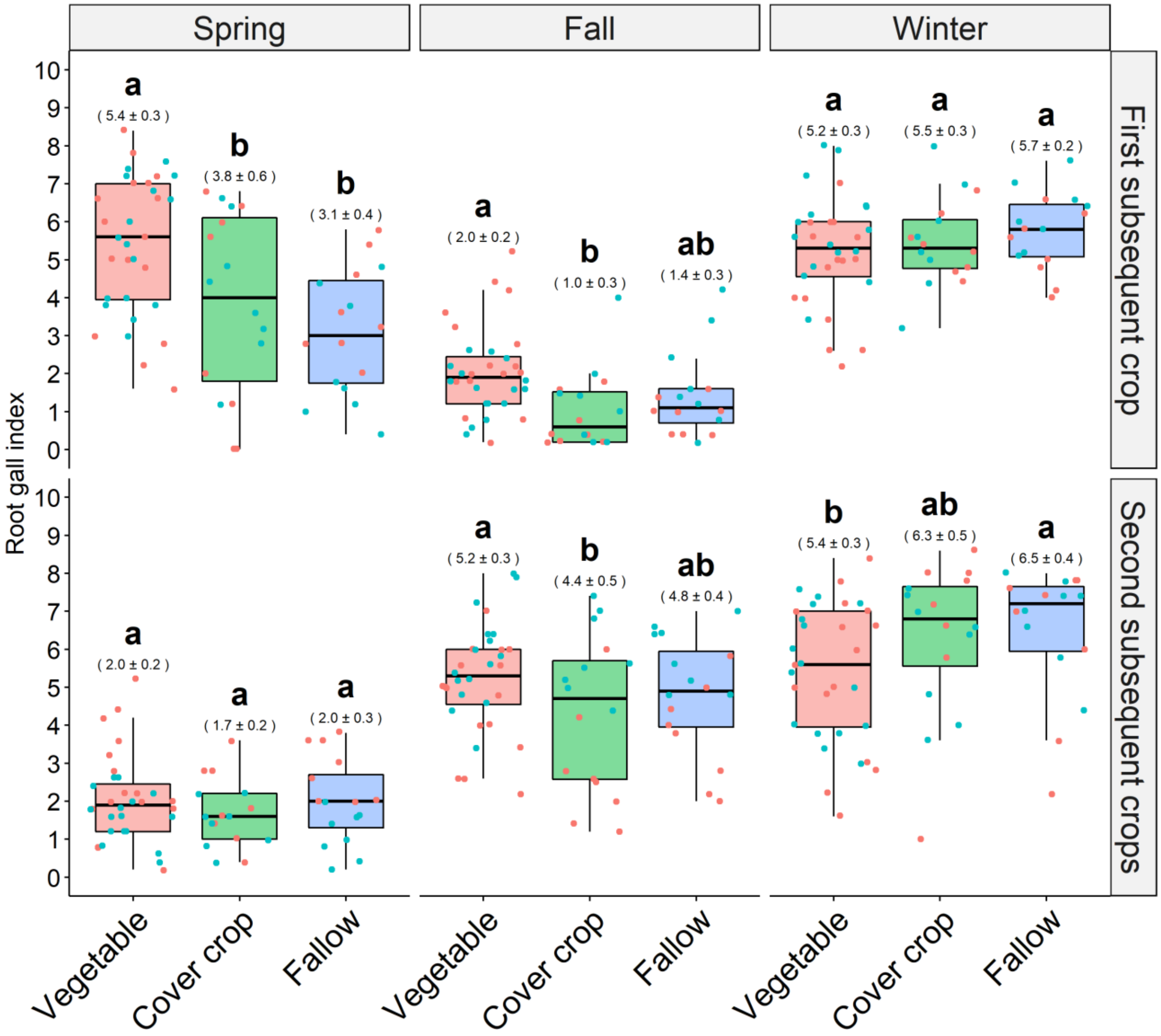


Fig. 2. Boxplot of *Meloidogyne incognita* root gall index (0-10) from crop rotation treatments (cover crop-fallow, weedy fallow, and all-vegetable rotation) by each fallow season assessed from the first or second subsequent vegetable crop. Means (± SE) above boxplot followed by the same letter(s) are not different according to Tukey's HSD test ($P < 0.05$). Raw data are displayed as either red (Trial 1) or blue (Trial 2) dots.

Supplemental Tables

Table S1. The effect of tillage on nematode abundance (per 100 cm^3 of soil) on crop rotation treatment subplots at different assessment seasons.

Season of assessment	Fallow season	Harvest season	*Meloidogyne incognita* Shallow tillage	*Meloidogyne incognita* Deep tillage	*Mesocriconema* spp. Shallow tillage	*Mesocriconema* spp. Deep tillage	*Nanidorus minor* Shallow tillage	*Nanidorus minor* Deep tillage
Current								
	Spring	Spring	114 ± 21 a	69 ± 13 a	66 ± 12 a	45 ± 10 a	10 ± 1 a	8 ± 1 a
	Fall	Fall	79 ± 14 a	50 ± 9 a	83 ± 23 a	57 ± 13 a	10 ± 2 a	4 ± 1 a
	Winter	Winter	50 ± 11 a	39 ± 10 a	23 ± 6 a	11 ± 2 a	7 ± 1 a	5 ± 1 b
First subsequent								
	Spring	Fall	76 ± 13 a	60 ± 10 a	71 ± 19 a	52 ± 13 a	8 ± 1 a	4 ± 1 b
	Fall	Winter	33 ± 7 a	39 ± 10 a	23 ± 6 a	13 ± 2 a	7 ± 1 a	4 ± 1 a
	Winter	Spring	119 ± 20 a	108 ± 26 a	48 ± 12 a	30 ± 8 a	11 ± 1 a	6 ± 1 b
Second subsequent								
	Spring	Winter	28 ± 5 a	39 ± 11 a	25 ± 6 a	13 ± 3 a	7 ± 1 a	5 ± 1 a
	Fall	Spring	167 ± 34 a	68 ± 12 a	57 ± 12 a	41 ± 9 a	10 ± 1 a	6 ± 1 b
	Winter	Fall	87 ± 15 a	84 ± 12 a	90 ± 25 a	44 ± 10 a	11 ± 1 a	4 ± 1 b

All means (±SE) across rows with the same letter by each nematode, season of assessment, and fallow season are not significantly different ($P < 0.1$) according to Tukey HSD test.

Table S2. Changes in nematode abundance (per 100 cm^3 of soil) among strata of crop rotation treatment subplots at different assessment seasons.

			Meloidogyne incognita		*Mesocriconema* spp.		*Nanidorus minor*	
Season of assessment	Fallow season	Harvest season	0 - 30 cm	30 - 60 cm	0 - 30 cm	30 - 60 cm	0 - 30 cm	30 - 60 cm
Current								
	Spring	Spring	81 ± 15 a	13 ± 3 b	65 ± 13 a	20 ± 7 b	8 ± 1 a	7 ± 1 b
	Fall	Fall	110 ± 13 a	18 ± 5 b	123 ± 24 a	16 ± 3 b	9 ± 1 a	4 ± 0 b
	Winter	Winter	37 ± 7 a	11 ± 2 b	35 ± 8 a	7 ± 2 b	6 ± 1 a	2 ± 0 b
First subsequent								
	Spring	Fall	120 ± 13 a	15 ± 3 b	104 ± 20 a	18 ± 5 b	7 ± 0 a	4 ± 0 b
	Fall	Winter	29 ± 6 a	7 ± 1 b	37 ± 8 a	9 ± 2 b	6 ± 0 a	2 ± 0 b
	Winter	Spring	188 ± 29 a	39 ± 7 b	65 ± 13 a	12 ± 3 b	9 ± 1 a	7 ± 1 b
Second subsequent								
	Spring	Winter	28 ± 6 a	10 ± 2 b	38 ± 8 a	10 ± 2 b	6 ± 0 a	2 ± 0 b
	Fall	Spring	199 ± 33 a	35 ± 7 b	81 ± 12 a	16 ± 4 b	11 ± 1 a	5 ± 0 b
	Winter	Fall	131 ± 17 a	24 ± 5 b	155 ± 38 a	18 ± 4 b	9 ± 1 a*	6 ± 1 b*

All means (±SE) across rows with the same letter by each nematode, season of assessment, and fallow season are not significantly different ($P < 0.05$) according to Tukey HSD test, unless indicated by * ($P < 0.1$).

Table S3. The effect of tillage on nematode abundance (per 100 cm^3 of soil) among strata of crop rotation treatment subplots at different assessment seasons.

			Meloidogyne incognita				*Mesocriconema* spp.				*Nanidorus minor*			
			Shallow tillage		Deep tillage		Shallow tillage		Deep tillage		Shallow tillage		Deep tillage	
Season of assessment	Fallow season	Harvest season	0 - 30 cm	30 - 60 cm	0 - 30 cm	30 - 60 cm	0 - 30 cm	30 - 60 cm	0 - 30 cm	30 - 60 cm	0 - 30 cm	30 - 60 cm	0 - 30 cm	30 - 60 cm
Current														
	Spring	Spring	211 ± 34 a	16 ± 5 c	98 ± 21 b	38 ± 10 c	100 ± 19 a	30 ± 13 b	64 ± 17 ab	26 ± 9 b	13 ± 1 a	6 ± 1 a	9 ± 1 a	5 ± 1 a
	Fall	Fall	143 ± 19 a	15 ± 10 b	78 ± 16 a	22 ± 5 b	151 ± 42 a	14 ± 5 b	94 ± 23 a	19 ± 5 b	14 ± 2 a*	4 ± 1 b*	4 ± 0 ab*	3 ± 1 b*
	Winter	Winter	93 ± 20 a	6 ± 1 b	55 ± 17 b	22 ± 8 b	42 ± 10 a	4 ± 1 b	14 ± 2 b	7 ± 2 b	11 ± 1 a	3 ± 0 b	6 ± 1 b	2 ± 0 b
First subsequent														
	Spring	Fall	144 ± 19 a	7 ± 2 c	96 ± 17 a	23 ± 5 b	126 ± 35 a	16 ± 6 b	82 ± 22 a	21 ± 10 b	10 ± 1 a*	4 ± 1 b*	3 ± 0 b*	3 ± 0 b*
	Fall	Winter	60 ± 12 a	5 ± 1 c	65 ± 19 b	11 ± 3 bc	42 ± 10 a	4 ± 1 c	17 ± 3 ab	9 ± 3 bc	10 ± 1 a	3 ± 0 b	6 ± 1 b	2 ± 0 b
	Winter	Spring	218 ± 30 a	20 ± 6 c	158 ± 49 a	57 ± 13 b	88 ± 22 a	8 ± 2 b	43 ± 13 a	16 ± 6 b	13 ± 1 a	9 ± 2 a	6 ± 1 a	5 ± 1 a
Second subsequent														
	Spring	Winter	48 ± 9 a	7 ± 3 b	64 ± 21 b	13 ± 3 b	44 ± 10 a	5 ± 1 c	16 ± 3 b	9 ± 3 c	10 ± 1 a	2 ± 0 b	7 ± 1 b	3 ± 0 b
	Fall	Spring	307 ± 58 a	26 ± 7 c	91 ± 19 b	45 ± 12 c	105 ± 20 a	8 ± 2 b	58 ± 14 a	24 ± 9 b	14 ± 1 a	5 ± 1 a	7 ± 1 a	4 ± 0 a
	Winter	Fall	167 ± 21 a	5 ± 1 a	126 ± 17 a	40 ± 11 a	165 ± 45 a	13 ± 4 a	75 ± 17 a	11 ± 2 a	15 ± 2 a	6 ± 1 a	3 ± 0 a	4 ± 1 a

All means (±SE) across columns with the same letter by each nematode, tillage treatment, season of assessment, and fallow season are not significantly different ($P < 0.05$) according to Tukey HSD test, unless indicated by * ($P < 0.1$).

Table S4. The successional effect of fallow rotations and tillage practice on nematode abundance (per 100 cm^3 of soil).

Season of assessment	Fallow season	Harvest season	Crop rotation treatment	Tillage	*Meloidogyne incognita*	*Mesocriconema* spp.	*Nanidorus minor*
Current							
	Spring	Spring	Cover crop-fallow	Shallow tillage	83 ± 36 a	72 ± 29 a	9 ± 3 ab*
				Deep tillage	53 ± 18 a	70 ± 28 a	11 ± 3 b*
			Weedy fallow	Shallow tillage	92 ± 47 a	57 ± 18 a	7 ± 1 ab*
				Deep tillage	64 ± 28 a	18 ± 4 a	8 ± 2 ab*
			All-vegetable rotation	Shallow tillage	141 ± 31 a	67 ± 19 a	12 ± 2 a*
				Deep tillage	79 ± 19 a	47 ± 15 a	6 ± 1 b*
	Fall	Fall	Cover crop-fallow	Shallow tillage	50 ± 21 a	62 ± 37 a	7 ± 3 a
				Deep tillage	10 ± 3 a	39 ± 18 a	6 ± 2 a
			Weedy fallow	Shallow tillage	69 ± 20 a	76 ± 51 a	13 ± 5 a
				Deep tillage	43 ± 13 a	85 ± 36 a	4 ± 1 a
			All-vegetable rotation	Shallow tillage	100 ± 23 a	97 ± 34 a	9 ± 2 a
				Deep tillage	74 ± 16 a	52 ± 16 a	3 ± 1 a
	Winter	Winter	Cover crop-fallow	Shallow tillage	57 ± 25 a	11 ± 3 a	9 ± 2 a
				Deep tillage	35 ± 16 a	6 ± 1 a	6 ± 2 a
			Weedy fallow	Shallow tillage	66 ± 30 a	17 ± 8 a	5 ± 2 a
				Deep tillage	24 ± 8 a	6 ± 2 a	5 ± 2 a
			All-vegetable rotation	Shallow tillage	39 ± 10 a	32 ± 11 a	8 ± 2 a
				Deep tillage	49 ± 17 a	16 ± 4 a	4 ± 1 a
First subsequent							
	Spring	Fall	Cover crop-fallow	Shallow tillage	66 ± 17 a	49 ± 29 a	6 ± 1 a
				Deep tillage	58 ± 23 a	82 ± 38 a	2 ± 1 a
			Weedy fallow	Shallow tillage	38 ± 14 a	43 ± 20 a	7 ± 2 a
				Deep tillage	33 ± 10 a	22 ± 7 a	7 ± 2 a
			All-vegetable rotation	Shallow tillage	100 ± 23 a	97 ± 34 a	9 ± 2 a
				Deep tillage	74 ± 16 a	52 ± 16 a	3 ± 1 a
	Fall	Winter	Cover crop-fallow	Shallow tillage	25 ± 13 a	18 ± 6 a	5 ± 1 a
				Deep tillage	36 ± 21 a	10 ± 5 a	4 ± 2 a
			Weedy fallow	Shallow tillage	29 ± 14 a	10 ± 5 a	7 ± 2 a
				Deep tillage	21 ± 11 a	13 ± 4 a	5 ± 2 a
			All-vegetable rotation	Shallow tillage	39 ± 10 a	32 ± 11 a	8 ± 2 a
				Deep tillage	49 ± 17 a	16 ± 4 a	4 ± 1 a
	Winter	Spring	Cover crop-fallow	Shallow tillage	85 ± 32 a*	11 ± 7 a	17 ± 3 a
				Deep tillage	97 ± 22 a*	15 ± 7 a	6 ± 1 bc
			Weedy fallow	Shallow tillage	110 ± 40 a*	49 ± 31 a	4 ± 2 c
				Deep tillage	178 ± 95 a*	11 ± 4 a	6 ± 2 c
			All-vegetable rotation	Shallow tillage	141 ± 31 a*	67 ± 19 a	12 ± 2 ab
				Deep tillage	79 ± 19 a*	47 ± 15 a	6 ± 1 c
Second subsequent							
	Spring	Winter	Cover crop-fallow	Shallow tillage	15 ± 4 a	13 ± 5 a	7 ± 2 a
				Deep tillage	27 ± 19 a	15 ± 6 a	5 ± 2 a
			Weedy fallow	Shallow tillage	19 ± 7 a	22 ± 7 a	5 ± 1 a
				Deep tillage	33 ± 21 a	5 ± 2 a	7 ± 2 a
			All-vegetable rotation	Shallow tillage	39 ± 10 a	32 ± 11 a	8 ± 2 a
				Deep tillage	49 ± 17 a	16 ± 4 a	4 ± 1 a
	Fall	Spring	Cover crop-fallow	Shallow tillage	64 ± 22 a	38 ± 15 a	8 ± 2 a
				Deep tillage	58 ± 18 a	28 ± 9 a	7 ± 2 a
			Weedy fallow	Shallow tillage	320 ± 114 a	56 ± 27 a	8 ± 2 a
				Deep tillage	57 ± 19 a	44 ± 18 a	6 ± 2 a
			All-vegetable rotation	Shallow tillage	141 ± 31 a	67 ± 19 a	12 ± 2 a
				Deep tillage	79 ± 19 a	47 ± 15 a	6 ± 1 a
	Winter	Fall	Cover crop-fallow	Shallow tillage	54 ± 17 a	34 ± 17 a	12 ± 2 a
				Deep tillage	86 ± 24 a	32 ± 13 a	6 ± 2 a
			Weedy fallow	Shallow tillage	94 ± 33 a	131 ± 70 a	12 ± 3 a
				Deep tillage	100 ± 26 a	39 ± 17 a	5 ± 1 a
			All-vegetable rotation	Shallow tillage	100 ± 23 a	97 ± 34 a	9 ± 2 a
				Deep tillage	74 ± 16 a	52 ± 16 a	3 ± 1 a

All means (±SE) across columns with the same letter by each nematode, season of assessment, fallow season, crop rotation treatment and tillage treatment are not significantly different ($P < 0.05$) according to Tukey HSD test, unless indicated by * ($P < 0.1$).

Table S5. The effect of tillage on fungal soilborne diseases among tillage treatments of crop rotation treatment subplots at different assessment seasons.

					Incidence (%)					
			Gall index		Damping-off *(Rhizoctonia solani)*		Southern blight *(Athelia rolfsii)*		Scerotinia cabbage rot *(S. sclerotiorum)*	
Season of assessment	Fallow season	Harvest season	Shallow tillage	Deep tillage	Shallow tillage	Deep tillage	Shallow tillage	Deep tillage	Shallow tillage	Deep tillage
First subsequent										
	Fall	Spring	4.2 ± 0.4 a	4.6 ± 0.4 a	30.0 ± 3.4 a	22.1 ± 3.2 b	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	Winter	Fall	1.8 ± 0.2 a	1.4 ± 0.2 a	8.5 ± 1.4 a	6.0 ± 1.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	12.6 ± 1.9 a	18.7 ± 3.0 a
	Spring	Winter	5.7 ± 0.2 a	5.1 ± 0.2 a	17.3 ± 1.7 a	17.8 ± 2.1 a	43.1 ± 3.8 a	46.5 ± 4.8 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Second subsequent										
	Winter	Spring	2.1 ± 0.2 a	1.8 ± 0.2 a	9.4 ± 1.6 a	6.4 ± 1.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	12.2 ± 1.9 b*	19.7 ± 3.2 a*
	Spring	Fall	5.2 ± 0.3 a	4.6 ± 0.3 a	17.9 ± 2.2 a	17.3 ± 1.7 a	45.9 ± 3.8 a	43.5 ± 4.3 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	Fall	Winter	5.7 ± 0.4 a	6.1 ± 0.3 a	35.9 ± 4.3 a	21.1 ± 2.7 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a

All means (±SE) across columns with the same letter by each fungal soilborne disease, season of assessment, and fallow season are not significantly different ($P < 0.05$) according to Tukey HSD test, unless indicated by * ($P < 0.1$).

Table S6. The effect of tillage on vegetables from crop rotation treatment subplots at different assessment seasons.

			Yield (kg/plot)		Yield (kg/plant)		Height (cm)		Stand count/plot	
Season of assessment	Fallow season	Harvest season	Shallow tillage	Deep tillage	Shallow tillage	Deep tillage	Shallow tillage	Deep tillage	Shallow tillage	Deep tillage
First subsequent										
	Spring	Fall	16.0 ± 3.4 a	24.3 ± 3.8 a	1.1 ± 0.2 a	1.6 ± 0.2 a	37.9 ± 1.0 a	40.9 ± 0.8 a	26.1 ± 1.0 b*	29.3 ± 1.1 a*
	Fall	Winter	41.5 ± 4.8 a	48.9 ± 6.1 a	2.0 ± 0.2 a	2.2 ± 0.2 a	24.0 ± 1.1 a	24.6 ± 1.5 a	50.6 ± 0.8 a	52.1 ± 0.5 a
	Winter	Spring	8.2 ± 1.8 a	14.1 ± 2.8 a	0.9 ± 0.2 b	1.7 ± 0.3 a	60.1 ± 1.4 a	58.8 ± 1.3 a	14.7 ± 1.3 a*	12.8 ± 1.5 b*
Second subsequent										
	Spring	Winter	35.8 ± 4.3 a	44.3 ± 5.4 a	1.8 ± 0.2 a	2.0 ± 0.2 a	23.5 ± 1.0 a	24.5 ± 1.4 a	50.4 ± 0.9 a	51.9 ± 0.6 a
	Fall	Spring	7.3 ± 1.6 a	13.0 ± 2.3 a	0.8 ± 0.1 a	1.6 ± 0.3 a	57.2 ± 1.4 a	59.5 ± 1.2 a	14.0 ± 1.3 a	13.9 ± 1.3 a
	Winter	Fall	15.9 ± 3.2 b*	23.7 ± 3.7 a*	1.1 ± 0.2 a	1.6 ± 0.2 a	37.2 ± 1.0 b*	41.7 ± 0.8 a*	24.4 ± 1.2 b	29.0 ± 0.9 a

All means (±SE) across rows by crop growth parameter, season of assessment, and fallow season with the same letter are not significantly different ($P < 0.05$) according to Tukey HSD test, unless indicated by * ($P < 0.1$).

Table S7. The effect of tillage on weed biomass (g/ft^2) among crop rotation treatment subplots at different assessment seasons.

			Total		Grass		Broad leaf	
Season of assessment	Fallow season	Harvest season	Shallow tillage	Deep tillage	Shallow tillage	Deep tillage	Shallow tillage	Deep tillage
Current								
	Spring	Spring	10441.9 ± 886.6 b	12383.7 ± 1479.5 a	623.5 ± 356.7 b*	1024.6 ± 333.0 a*	5715.7 ± 643.9 a	8373.6 ± 1399.2 a
	Fall	Fall	3947.8 ± 766.6 a	5709.0 ± 824.2 a	272.3 ± 109.8 a	524.5 ± 170.5 a	2561.2 ± 784.4 a	4433.6 ± 908.1 a
	Winter	Winter	2379.0 ± 540.5 a	1988.6 ± 556.9 a	0.0 ± 0.0 a	0.0 ± 0.0 a	2379.0 ± 540.5 a	1988.6 ± 556.9 a
First subsequent								
	Spring	Fall	5997.8 ± 939.9 a	7401.8 ± 824.1 a	775.1 ± 247.8 a	1290.3 ± 385.7 a	3811.7 ± 994.2 a	5274.2 ± 956.5 a
	Fall	Winter	3875.8 ± 616.7 a	3211.4 ± 610.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	3875.8 ± 616.7 a	3211.4 ± 610.1 a
	Winter	Spring	12921.5 ± 745.7 a	15092.9 ± 1473.8 a	331.0 ± 146.5 a	312.4 ± 101.3 a	7989.2 ± 829.0 b	10917.6 ± 1470.6 a
Second subsequent								
	Spring	Winter	3822.6 ± 533.6 a	2962.1 ± 556.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	3822.6 ± 533.6 a	2962.1 ± 556.1 a
	Fall	Spring	12131.4 ± 712.9 a	15085.9 ± 1252.6 a	1135.5 ± 449.8 a	261.5 ± 139.7 a	7066.7 ± 722.4 a	11001.6 ± 1288.5 a
	Winter	Fall	5812.1 ± 809.3 b	7129.2 ± 784.2 a	566.5 ± 197.0 a	738.5 ± 218.7 a	3894.3 ± 831.5 b*	5674.7 ± 875.4 a*

All means (±SE) across columns with the same letter by weed biomass, season of assessment, and fallow season are not significantly different ($P < 0.05$) according to Tukey HSD test, unless indicated by * ($P < 0.1$).

Table S8. The successional effect of fallow rotations and tillage practice on weed biomass (g/m^2).

Season of assessment	Fallow season	Harvest season	Crop rotation treatment	Tillage	Total	Grass	Broad leaves
Current	Spring	Spring					
			Cover crop-fallow	Shallow tillage	7853.1 ± 1897.1 a	1039.8 ± 485.3 a	4446.9 ± 1023.3 a
				Deep tillage	7915.7 ± 2179.6 a	0.0 ± 0.0 b	3989.5 ± 976.2 a
			Weedy fallow	Shallow tillage	8958.5 ± 2180.1 a	2326.4 ± 765.4 a	3710.8 ± 1363.9 a
				Deep tillage	9336.4 ± 1859.2 a	1666.9 ± 1110.2 ab	4324.0 ± 1797.7 a
			All-vegetable rotation	Shallow tillage	16361.6 ± 2229.0 a	70.9 ± 36.8 b	12131.6 ± 2136.0 a
				Deep tillage	12257.8 ± 927.9 a	222.8 ± 106.6 b	7187.6 ± 720.1 a
	Fall	Fall					
			Cover crop-fallow	Shallow tillage	2785.4 ± 1034.2 a	250.6 ± 53.5 a	1287.8 ± 882.6 a
				Deep tillage	2214.0 ± 924.9 a	294.1 ± 141.0 a	1490.0 ± 947.4 a
			Weedy fallow	Shallow tillage	4449.1 ± 1145.3 a	356.7 ± 223.0 a	3582.2 ± 1181.7 a
				Deep tillage	2237.5 ± 501.3 a	162.0 ± 126.4 a	919.7 ± 427.3 a
			All-vegetable rotation	Shallow tillage	7800.8 ± 1273.7 a	736.5 ± 311.0 a	6145.9 ± 1478.3 a
				Deep tillage	5669.9 ± 1332.5 a	319.1 ± 222.9 a	3783.6 ± 1362.9 a
	Winter	Winter					
			Cover crop-fallow	Shallow tillage	719.2 ± 394.8 a	0.0 ± 0.0 a	719.2 ± 394.8 a
				Deep tillage	534.5 ± 274.9 a	0.0 ± 0.0 a	534.5 ± 274.9 a
			Weedy fallow	Shallow tillage	387.1 ± 97.7 a	0.0 ± 0.0 a	387.1 ± 97.7 a
				Deep tillage	684.6 ± 242.3 a	0.0 ± 0.0 a	684.6 ± 242.3 a
			All-vegetable rotation	Shallow tillage	3424.0 ± 983.1 a	0.0 ± 0.0 a	3424.0 ± 983.1 a
				Deep tillage	4148.5 ± 870.9 a	0.0 ± 0.0 a	4148.5 ± 870.9 a
First subsequent	Spring	Fall					
			Cover crop-fallow	Shallow tillage	5966.9 ± 1447.5 a	2457.0 ± 1249.3 ab	3470.0 ± 1595.8 ab*
				Deep tillage	7814.6 ± 2632.9 a	232.9 ± 123.7 ab	5557.5 ± 2854.8 ab*
			Weedy fallow	Shallow tillage	8038.7 ± 1607.6 a	1485.5 ± 871.4 ab	5218.4 ± 1870.0 ab*
				Deep tillage	4836.8 ± 641.1 a	1626.8 ± 523.6 a	2122.0 ± 744.0 b*
			All-vegetable rotation	Shallow tillage	7800.8 ± 1273.7 a	736.5 ± 311.0 ab	6145.9 ± 1478.3 a*
				Deep tillage	5669.9 ± 1332.5 a	319.1 ± 222.9 b	3783.6 ± 1362.9 ab*
	Fall	Winter					
			Cover crop-fallow	Shallow tillage	3467.5 ± 1247.6 a	0.0 ± 0.0 a	3467.5 ± 1247.6 a
				Deep tillage	3447.2 ± 958.4 a	0.0 ± 0.0 a	3447.2 ± 958.4 a
			Weedy fallow	Shallow tillage	2530.1 ± 897.4 a	0.0 ± 0.0 a	2530.1 ± 897.4 a
				Deep tillage	3759.0 ± 1587.1 a	0.0 ± 0.0 a	3759.0 ± 1587.1 a
			All-vegetable rotation	Shallow tillage	3424.0 ± 983.1 a	0.0 ± 0.0 a	3424.0 ± 983.1 a
				Deep tillage	4148.5 ± 870.9 a	0.0 ± 0.0 a	4148.5 ± 870.9 a
	Winter	Spring					
			Cover crop-fallow	Shallow tillage	14664.5 ± 3456.3 a	331.5 ± 195.6 a	10593.0 ± 3508.7 a
				Deep tillage	15489.3 ± 1662.2 a	568.9 ± 439.1 a	9316.2 ± 2271.4 a
			Weedy fallow	Shallow tillage	12983.7 ± 2028.8 a	632.5 ± 242.5 a	8814.3 ± 2318.7 a
				Deep tillage	11681.1 ± 1452.9 a	239.5 ± 239.5 a	8265.5 ± 2084.9 a
			All-vegetable rotation	Shallow tillage	16361.6 ± 2229.0 a	70.9 ± 36.8 a	12131.6 ± 2136.0 a
				Deep tillage	12257.8 ± 927.9 a	222.8 ± 106.6 a	7187.6 ± 720.1 a
Second subsequent	Spring	Winter					
			Cover crop-fallow	Shallow tillage	3122.2 ± 941.7 a	0.0 ± 0.0 a	3122.2 ± 941.7 a
				Deep tillage	3597.2 ± 1003.0 a	0.0 ± 0.0 a	3597.2 ± 1003.0 a
			Weedy fallow	Shallow tillage	1878.0 ± 465.4 a	0.0 ± 0.0 a	1878.0 ± 465.4 a
				Deep tillage	3396.3 ± 850.7 a	0.0 ± 0.0 a	3396.3 ± 850.7 a
			All-vegetable rotation	Shallow tillage	3424.0 ± 983.1 a	0.0 ± 0.0 a	3424.0 ± 983.1 a
				Deep tillage	4148.5 ± 870.9 a	0.0 ± 0.0 a	4148.5 ± 870.9 a
	Fall	Spring					
			Cover crop-fallow	Shallow tillage	13450.9 ± 1289.0 a	692.0 ± 406.6 a	8706.0 ± 1872.4 a
				Deep tillage	11427.1 ± 1640.0 a	1722.0 ± 1066.1 a	6419.9 ± 1584.1 a
			Weedy fallow	Shallow tillage	14169.4 ± 1967.3 a	32.3 ± 32.3 a	11037.0 ± 2287.1 a
				Deep tillage	12582.9 ± 1586.9 a	2197.4 ± 1252.0 a	7391.0 ± 2119.2 a
			All-vegetable rotation	Shallow tillage	16361.6 ± 2229.0 a	70.9 ± 36.8 a	12131.6 ± 2136.0 a
				Deep tillage	12257.8 ± 927.9 a	222.8 ± 106.6 a	7187.6 ± 720.1 a
	Winter	Fall					
			Cover crop-fallow	Shallow tillage	6692.2 ± 1669.6 a	1275.5 ± 622.8 a	5283.3 ± 1767.1 a
				Deep tillage	7145.8 ± 1134.2 a	577.8 ± 394.7 a	4357.7 ± 1278.6 a
			Weedy fallow	Shallow tillage	6222.9 ± 937.5 a	313.9 ± 124.2 a	5182.4 ± 1137.5 a
				Deep tillage	4762.9 ± 1513.1 a	1106.0 ± 525.0 a	3652.2 ± 1615.2 a
			All-vegetable rotation	Shallow tillage	7800.8 ± 1273.7 a	736.5 ± 311.0 a	6145.9 ± 1478.3 a
				Deep tillage	5669.9 ± 1332.5 a	319.1 ± 222.9 a	3783.6 ± 1362.9 a

All means (±SE) across columns with the same letter by each weed biomass, season of assessment, fallow season, crop rotation treatment and tillage treatment are not significantly different ($P < 0.05$) according to Tukey HSD test, unless indicated by * ($P < 0.1$).

Table S9. The successional effect of fallow seasons on fungal soilborne diseases.

				Final Incidence (%)^{x}		
Season of assessment	Fallow season	Harvest season	Crop rotation treatment	Damping-off *(Rhizoctonia solani)*	Southern blight *(Athelia rolfsii)*	Sclerotinia cabbage rot *(S. sclerotiorum)*
First subsequent						
	Spring	Fall	Cover crop-fallow	19.2 ± 3.0 b	0.0 ± 0.0 a	0.0 ± 0.0 a
			Weedy fallow	28.5 ± 5.2 a	0.0 ± 0.0 a	0.0 ± 0.0 a
			All-vegetable rotation	28.2 ± 3.6 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	Fall	Winter	Cover crop-fallow	6.0 ± 1.4 ab*	0.0 ± 0.0 a	13.1 ± 3.4 a
			Weedy fallow	5.4 ± 1.5 b*	0.0 ± 0.0 a	13.9 ± 2.7 a
			All-vegetable rotation	8.8 ± 1.4 a*	0.0 ± 0.0 a	17.8 ± 2.9 a
	Winter	Spring	Cover crop-fallow	16.1 ± 2.2 a	42.8 ± 5.9 a	0.0 ± 0.0 a
			Weedy fallow	19.3 ± 3.2 a	45.7 ± 6.8 a	0.0 ± 0.0 a
			All-vegetable rotation	17.4 ± 1.8 a	45.4 ± 4.2 a	0.0 ± 0.0 a
Second subsequent						
	Spring	Winter	Cover crop-fallow	6.5 ± 1.6 a	0.0 ± 0.0 a	13.6 ± 3.4 a
			Weedy fallow	7.5 ± 2.3 a	0.0 ± 0.0 a	14.6 ± 4.0 a
			All-vegetable rotation	8.8 ± 1.4 a	0.0 ± 0.0 a	17.8 ± 2.9 a
	Fall	Spring	Cover crop-fallow	15.8 ± 3.3 a	43.2 ± 6.0 a	0.0 ± 0.0 a
			Weedy fallow	19.8 ± 2.7 a	44.8 ± 5.1 a	0.0 ± 0.0 a
			All-vegetable rotation	17.4 ± 1.8 a	45.4 ± 4.2 a	0.0 ± 0.0 a
	Winter	Fall	Cover crop-fallow	30.0 ± 6.3 a	0.0 ± 0.0 a	0.0 ± 0.0 a
			Weedy fallow	27.7 ± 5.2 a	0.0 ± 0.0 a	0.0 ± 0.0 a
			All-vegetable rotation	28.2 ± 3.6 a	0.0 ± 0.0 a	0.0 ± 0.0 a

All means (±SE) across columns with the same letter by fungal disease, season of assessment, fallow season, and crop rotation treatment are not significantly different ($P < 0.05$) according to Tukey HSD test, unless indicated by * ($P < 0.1$).

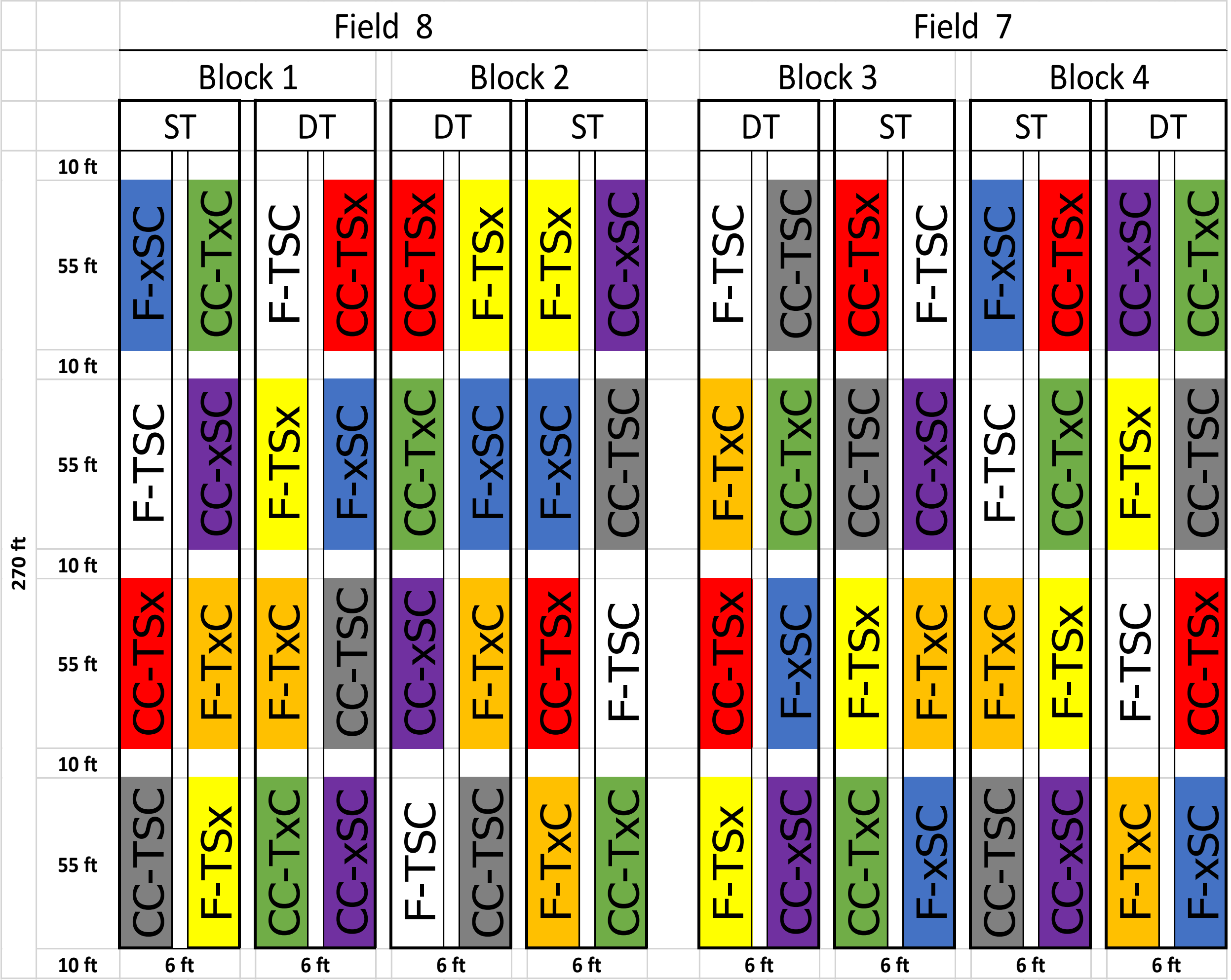
Table S10. The successional effect of fallow season on weeds biomass (g/m^2).

Season of assessment	Fallow season	Harvest season	Crop rotation treatment	Total	Grass	Broad leaves

Current						
	Spring	Spring	Cover crop-fallow	7884.4 ± 1395.8 a	577.7 ± 314.3 a	4203.0 ± 683.6 b
			Weedy fallow	9147.4 ± 1384.9 a	2018.6 ± 640.7 a	4017.4 ± 1087.3 b
			All-vegetable rotation	14309.7 ± 1243.5 a	150.2 ± 59.3 b	9659.6 ± 1194.3 a
	Fall	Fall	Cover crop-fallow	2499.7 ± 674.2 b	270.7 ± 68.1 a	1388.9 ± 618.0 b
			Weedy fallow	3343.3 ± 668.0 a	259.3 ± 125.1 a	2251.0 ± 697.6 b
			All-vegetable rotation	6735.3 ± 926.7 a	565.7 ± 205.9 a	4926.7 ± 1009.6 a
	Winter	Winter	Cover crop-fallow	626.9 ± 233.6 b	0.0 ± 0.0 a	626.9 ± 233.6 b
			Weedy fallow	535.8 ± 131.9 b	0.0 ± 0.0 a	535.8 ± 131.9 b
			All-vegetable rotation	3786.3 ± 649.3 a	0.0 ± 0.0 a	3786.3 ± 649.3 a
First subsequent						
	Spring	Fall	Cover crop-fallow	6890.8 ± 1470.8 a	1345.0 ± 698.3 ab*	4583.4 ± 1661.3 a
			Weedy fallow	6437.7 ± 932.6 a	1560.9 ± 474.7 a*	3670.2 ± 1051.2 a
			All-vegetable rotation	6735.3 ± 926.7 a	565.7 ± 205.9 b*	4926.7 ± 1009.6 a
	Fall	Winter	Cover crop-fallow	3457.3 ± 759.9 a	0.0 ± 0.0 a	3457.3 ± 759.9 a
			Weedy fallow	3144.6 ± 894.9 a	0.0 ± 0.0 a	3144.6 ± 894.9 a
			All-vegetable rotation	3786.3 ± 649.3 a	0.0 ± 0.0 a	3786.3 ± 649.3 a
	Winter	Spring	Cover crop-fallow	15076.9 ± 1855.6 a	470.0 ± 261.5 a	9954.6 ± 2025.7 a
			Weedy fallow	12332.4 ± 1217.0 a	501.5 ± 182.4 a	8539.9 ± 1507.9 a
			All-vegetable rotation	14309.7 ± 1243.5 a	150.2 ± 59.3 a	9659.6 ± 1194.3 a
Second subsequent						
	Spring	Winter	Cover crop-fallow	3359.7 ± 667.4 a	0.0 ± 0.0 a	3359.7 ± 667.4 a
			Weedy fallow	2637.2 ± 507.8 a	0.0 ± 0.0 a	2637.2 ± 507.8 a
			All-vegetable rotation	3786.3 ± 649.3 a	0.0 ± 0.0 a	3786.3 ± 649.3 a
	Fall	Spring	Cover crop-fallow	12439.0 ± 1040.9 a	1167.4 ± 533.9 a	7639.2 ± 1236.8 a
			Weedy fallow	13376.2 ± 1238.0 a	1410.1 ± 840.9 ab	9214.0 ± 1578.0 a
			All-vegetable rotation	14309.7 ± 1243.5 a	150.2 ± 59.3 b	9659.6 ± 1194.3 a
	Winter	Fall	Cover crop-fallow	6919.0 ± 976.7 a	856.9 ± 341.5 a	4820.5 ± 1060.3 a
			Weedy fallow	5492.9 ± 880.2 a	665.9 ± 263.8 a	4417.3 ± 974.5 a
			All-vegetable rotation	6735.3 ± 926.7 a	565.7 ± 205.9 a	4926.7 ± 1009.6 a

All means (±SE) across columns with the same letter by weed biomass, season of assessment, and fallow season are not significantly different ($P < 0.05$) according to Tukey HSD test, unless indicated by * ($P < 0.1$).

Supplemental figures



		Field 8								Field 7							
		Block 1				Block 2				Block 3				Block 4			
		ST		DT		DT		ST		DT		ST		ST		DT	
270 ft	10 ft																
	55 ft	F-xSC	CC-TxC	F-TSC	CC-TSx	CC-TSx	F-TSx	F-TSx	CC-xSC	F-TSC	CC-TSC	CC-TSx	F-TSC	F-xSC	CC-TSx	CC-xSC	CC-TxC
	10 ft																
	55 ft	F-TSC	CC-xSC	F-TSx	F-xSC	CC-TxC	F-xSC	F-xSC	CC-TSC	F-TxC	CC-TxC	CC-TSC	CC-xSC	F-TSC	CC-TxC	F-TSx	CC-TSC
	10 ft																
	55 ft	CC-TSx	F-TxC	F-TxC	CC-TSC	CC-xSC	F-TxC	CC-TSx	F-TSC	CC-TSx	F-xSC	F-TSx	F-TxC	F-TxC	F-TSx	F-TSC	CC-TSx
	10 ft																
	55 ft	CC-TSC	F-TSx	CC-TxC	CC-xSC	F-TSC	CC-TSC	F-TxC	CC-TxC	F-TSx	CC-xSC	CC-TxC	F-xSC	CC-TSC	CC-xSC	F-TxC	F-xSC
	10 ft	6 ft		6 ft		6 ft		6 ft		6 ft		6 ft		6 ft		6 ft	

Fig. S1. Map of whole plots and subplots arrangement. Whole-plot treatments consist of shallow tillage (ST) and deep tillage (DT). Subplots consist of two factors in a three-vegetable crop rotation: 1) fallow type (cover crop-fallow or weedy fallow) and 2) fallow season (Spring, Fall, and Winter. Spring and fall cover crop-fallow had sunn hemp planted, while winter had rye. CC = cover crop-fall, F = weedy fallow, T = spring tomato, S = fall squash, C = winter cabbage, and x = fallow season.

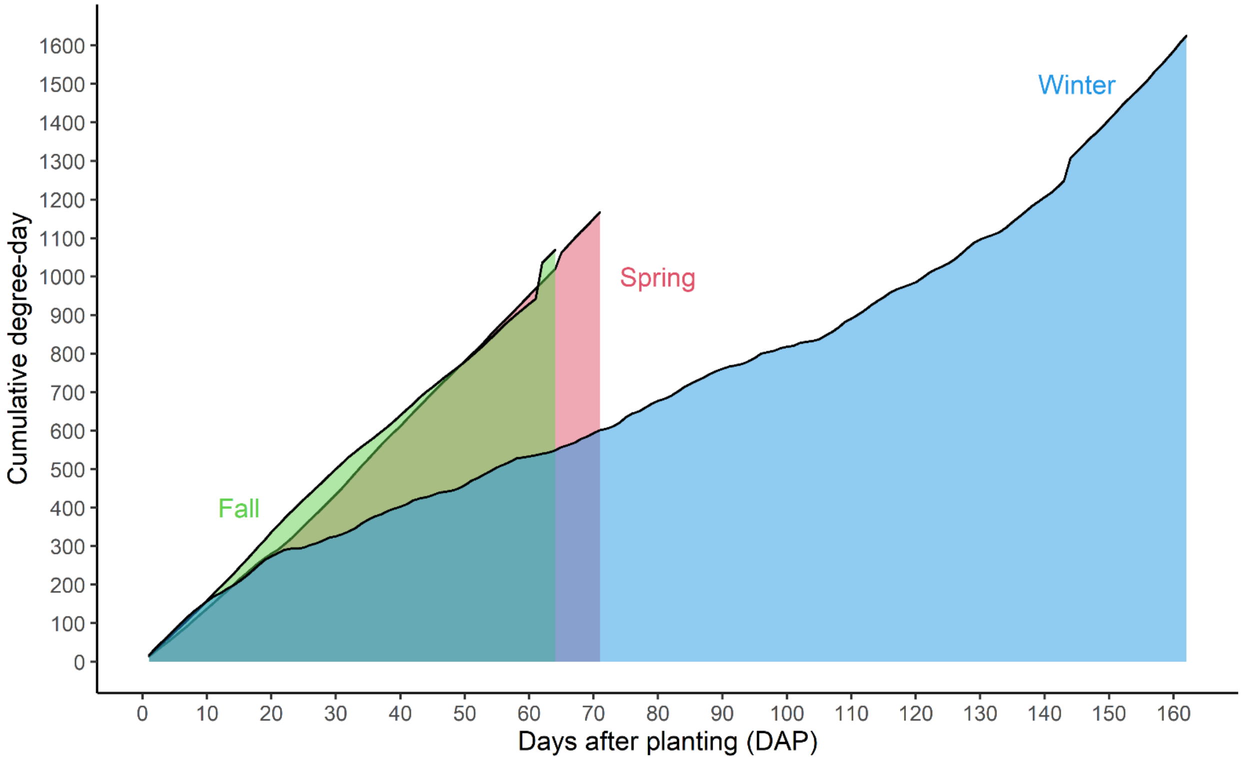


Fig. S2. Cumulative degree days for the spring, fall, and winter cover crop seasons. Degree-day was calculated for each cover crop season as the sum of daily mean air temperatures subtracted by the base temperature of 9.9 for sunn hemp (Schomberg et al. 2007) and 4.4 for rye (Mirsky et al. 2009).

CHAPTER 7

CONCLUSION

Georgia's vegetable industry significantly impacts the nation's supply of vegetables and is consistently ranked 3rd or 4th in utilized vegetable production followed by the states of California, Florida, Arizona, and North Carolina. To assess plant-parasitic nematode (PPN) threats to Georgia's vegetable industry, Chapter 1 investigated the occurrence and distribution of PPNs in vegetable fields comprising of 29 vegetable producing counties in 436 vegetable fields in the spring and fall of 2018. This study validated that root-knot nematode (RKN; *Meloidogyne* spp.) is the most important PPN in vegetable fields of south Georgia. We found cultural practices to have a greater effect on PPN population dynamics than the environment (precipitation, soil temperature, soil moisture, soil texture, soil pH and soil EC). Specifically, *Meloidogyne* spp. were more associated at lower latitudes in the south region, where plasticulture cropping systems are more frequent. Other PPN were associated with bare ground cropping systems in the north region, where more row crops are rotated in vegetable fields. These results suggest that other PPNs may not necessarily be associated with vegetables but associated with other row crops rotated in these fields and/or weeds associated with bare ground beds.

Because *Meloidogyne* spp. was the most important PPN found in vegetable fields, chapter 2 determined the prevalence of different *Meloidogyne* species with molecular diagnostic methods. Samples were collected throughout the vegetable producing regions of south Georgia consisting of a total of 292 *Meloidogyne*-infested fields. DNA samples from individual nematode females were identified with species-specific PCR and mitochondrial haplotype-based identification by

DNA sequencing fragments of the cytochrome c oxidase subunit II, intergenic spacer, tRNA-His, and the large subunit 1S rRNA genes. Five *Meloidogyne* species were detected (*M. incognita, M. arenaria, M. javanica, M. floridensis* and *M. haplanaria*). *M. floridensis* and *M. haplanaria* are new species to be detected in Georgia. The most prevalent species in vegetable fields of Georgia was *M. incognita* followed by *M. arenaria*.

Deep-dwelling PPNs can contribute to inconsistency in fumigant/nematicide efficacy and detection. Moreover, the relationship between PPN vertical distribution and the seasonal fluctuations in edaphic factors was not addressed in the surveillance study in chapter 2, which failed to find any strong relationships between PPN community and edaphic factors. Therefore, in chapter 4, I pursued a study on seasonal fluctuations in population of PPNs' vertical distribution by sampling 6 vegetable fields in south Georgia among 5 different counties at 5 different depths at monthly intervals. We conclude that the illuvial zone in the north region is the main factor in regional differences in vertical distribution of PPN abundance. PPN abundance increased from mid-spring to late winter as PPNs completed multiple life cycles during crop rotations and stop reproduction during the winter fallow period. High RKN second-stage juvinile (J2) abundance in the winter were problably not active, but overwintering J2s. We suggest the optimal time for fumigant/nematicide application would be the summer for RKN management, as most RKN are above the common depth of application (10-35 cm).

Cover crop rotation remains an important cultural practice for PPN management and is recommended for vegetable production systems in the southeastern USA to circumvent high weed, insect, and disease pressure associated with a warm climate. Because *Meloidogyne* spp. have a broad host range and high incidence in vegetable fields, the correct cover crop selection is critical when root-knot nematode is an issue. Therefore, in chapter 5 we conducted a study to evaluate the host status of cover crop candidates for suppression of Georgia’s major root-knot nematode populations and compare them with cover crops identified as the most suppressive from previous studies. Two greenhouse trials tested the host status of 14 species and 18 cultivars with the addition of a tomato cv. Rutgers (*Solanum lycopersicum*) against *M. incognita* race 3, *M. arenaria* race 1, and *M. javanica*. Although all cover crops, except hairy vetch and blue lupine, had little to no galls, some had a significant number of egg mass that suggest these crops are either poor-host or susceptible. Cultivars of sorghum-sudangrass (*Sorghum bicolor* x *S. sudanense*) and sunn hemp (*Crotalaria juncea*) varied in being a susceptible host or non-host, possibly due to variability in genetic resistance. This study suggests that many cover crops do not show obvious root-galling, but some may be considered susceptible or poor-host based on egg mass production.

The aim of chapter 6 was to determine the best cover crop-fallow season and tillage practices in a 3-season vegetable cropping system for nematode, fungal soilborne pathogen, and weed suppression. We concluded that sunn hemp can suppress *M. incognita*, whereas deep tillage suppresses *Rhizotonia solani*, *Sclerotinia sclerotiorum*, and *Nanidorus minor*. We found spring and fall sunn hemp to be successful at RKN suppression and improving vegetable yeilds, but not cover crop-fallowing with winter rye. However, winter rye reduced *Mesocriconema* spp. abundance in the first subsequent vegetable. Fall sunn hemp had higher biomass than spring sunn hemp and reduced root galling even in the second subsequent vegetable.

In conclusion, these findings highlight RKN as an important threat to vegetable production in Georgia as it is the most dominate PPN in vegetables fields. Moreover, new RKN species detected are potentially a major threat, as they can reproduce on resistant vegetable cultivars. Our study also provides new insights to managing RKN and other PPNs. Specifically, in chapter 4, we find that summer could be the optimal time of fumigants/nematicides application, as this season is at the least risk of deep-dwelling RKN escapees. In chapter 5, we find that host-status of cover crops may range between a susceptible to poor host, and needs to be carefully considered before growing in a RKN-infested field. In chapter 6, we find fall sunn hemp suppresses RKN more effectively than in the spring, while winter rye does not suppress. We also found tillage did not enhance cover crop-fallowing but was successful at suppressing fungal soilborne diseases. Taken all together, these findings lay the groundwork for nematologists and vegetable growers to develop programs of sustainable management of PPNs in vegetable fields.